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REVIEW

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Physical Sciences

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Levels of Total Petroleum Hydrocarbons (TPH) and Heavy Metals in Shrimp Waste Meal Supplemented Broiler Feeds and Droppings

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The study determined the levels of cadmium, lead and total petroleum hydrocarbons (TPH) in shrimp waste meal supplemented feeds and droppings of the broiler birds. The samples were collected randomly over a period of two months. Cadmium and lead were determined by atomic absorption spectrophotometry, while total petroleum hydrocarbons (TPH) were determined gravimetrically by following standard methods. The results show for cadmium 0.61 ± 0.48 µg / g and 0.81 ± 0.32 µg / g, respectively; 9.49 ± 2.24 µg / g and 11.49 ± 1.55 µg / g for lead; 369.00 ± 108.40 µg / g and 330.20 ± 59.03 µg / g for TPH, respectively for the starter and finisher feeds. The statistical analysis of variance reveal significant differences at 95% confidence level for lead and TPH compared with control samples. The droppings of the broilers were also collected and analysed with statistically significant difference existing for lead.

Keywords: Shrimp waste meal, Total petroleum hydrocarbons (TPH), Cadmium, Lead, Contamination.

Introduction

Industrialization and urbanization especially in developing countries have gradually led to the accumulation of heavy metals and petroleum hydrocarbons in the environment (Adeniyi 1996; Bamgbose and Osibanjo 1998; Byomi *et al* 1999; Manay *et al* 1999; Ngodigha *et al* 1999; Yamasoe *et al* 2000; Adeniyi and Afolabi 2002). Due to the high cost and sometimes non-availability of conventional feeds, poultry farmers and researchers have in recent years resorted to a large scale usage of unconventional feeds for broiler rations (Islam *et al* 1994; Fanimo *et al* 1996).

Substituted items for fish meal in conventional feeds like shrimp waste meal and maggot meal, have been reported to be of high nutritive value (*Fanimo et al* 2000; Oduguwa *et al* 2000).

Shrimp waste meal has been identified as an animal protein source. The availability of shrimp both in off-shore water and Lagos Lagoon amounts to about 300,000 ton per year and they form the larger part of the catches of artisan fishermen and commercial shrimp trawlers. The increase in shrimp farming and production of shrimp waste meal, which is basically the dried waste of the industry, consist of the heads, appendages and exoskeleton (Fanimo *et al* 1996).

Despite, the increasing wide usage of shrimp waste meal in unconventional broiler rations nothing has been reported on the levels of potentially toxic heavy metals and total petroleum hydrocarbons (TPH) in these feeds. These contaminants may accumulate in the poultry products at levels, which may threaten public health (Kan 1994; Tsuji *et al* 1999).

Poultry droppings is a useful agricultural by-product but its potential for environmental pollution requires attention (Van der Watt *et al* 1994; Ihnat and Fernandes 1996; Martinez *et al* 2000).

This study was designed to determine the levels of cadmium, lead and total petroleum hydrocarbons (TPH) in the shrimp waste meal supplemented rations with a view to ascertain the level of safety of these feeds considering the fact that accumulation of these contaminants in the resultant poultry products may not be ruled out.

Materials and Methods

*Materials.*The samples used for this study were collected from the College of Animal Sciences Farm, University of Agriculture, Abeokuta between September and November 2000. The shrimp waste supplemented and control feeds were classified into starter and finisher mash according to the physiological stage of growth of the chicks. The control diets contained fish meal as the only protein source while in the supplemented feeds, shrimp waste meal was used to replace the fish meal in the control diets. The diets were formulated to contain the required levels of protein and energy for the respective stages viz for starter feed and for finisher feed.

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The feed samples were collected randomly from the chicks plastic feeding trough. The birds droppings were also collected at the fourth week (end of the starter phase) and the eight week (end of the finisher phase) of the experiment. The feeds and droppings were dried in an oven (55°C-60°C) for four days and homogenised following the method described earlier (Hanczakowski *et al* 1995; Adeniyi 1996).

Determination of cadmium and lead. Two stocks of eight feeds/dropping sub-samples along with a "blank" containing reagent and no feeds/droppings were digested for analysis using conc. HNO_3 as described earlier (Van der Watt *et al* 1994; Ihnat and Fernandes 1996). Cadmium and lead were determined using a Buck model 200 A Atomic Absorption Spectrophotometer. This was operated according to the manufacturer's recommended conditions in replicates while pure elemental standard solutions were used for calibration.

Determination of total petroleum hydrocarbons (TPH). The total petroleum hydrocarbons (TPH) levels in the samples were determined gravimetrically using 10 - 20g dried feed/droppings, 0.6g KOH with reflux for two and half hour in 50 cm^3 n-hexane. The crude extracts were cleaned up with short silica gel columns (Kieselgel 60 F_{254}, 70 - 230 mesh) using petroleum ether as described in (IOC 1982; Hewari *et al* 1995; Onianwa 1995; Adeniyi and Afolabi 2002).

Statistical analysis. Statistical significant differences in the samples were analysed using ANOVA as described by Pentecost (1999).

Results and Discussion

Cadmium, lead and total petroleum hydrocarbons (TPH) levels shown in (Table 1) reveal varying degrees of burden in the feeds and droppings samples. The mean cadmium levels (μg/g) of 0.61 ± 0.48 and 0.81 ± 0.32, respectively for starter and finisher feeds compare favourably with the levels found in the control (0.38 ± 0.29 μg/g and 1.05 ± 0.53 μg/g, respectively). These values were however, found to be non-significant at 95% confidence level (Table 1). Nevertheless, the mean levels of cadmium in the droppings of the poultry birds fed on the control and supplemented feeds were 1.72 ± 0.36 μg/g and 1.44 ± 0.24 μg/g for starter and finisher birds fed on control feeds; 0.83 ± 0.96 μg/g and 1.45 ± 0.36 μg/g for starter and finisher birds fed on the supplemented feed, respectively. A similar trend has been observed before (Van der Watt *et al* 1994; Ihnat and Fernandes 1996; Schuler *et al* 1997; Gruszeck *et al* 2000). However, human exposure to heavy metals through accumulated metals in plant and animal tissues is of growing concern (Gardiner *et al* 1995; Adeniyi 1996; Freedman 1996; Nyobo *et al* 1996; Kugonic *et al* 1999; Hensbergen *et al* 2000). It is noteworthy that only a fraction of the total cadmium ingested are assimilated into the tissues of birds as observed by (Rambeck and Weiser 1992; Kan 1994). There are also possibilities of droppings contamination with cadmium from sources other than dietary source (Freedman 1996).

Lead has the highest mean value of 11.49 ± 1.55 μg/g in the supplemented finisher feed and statistically significant 95% confidence level (Table 1). This may be taken as an indication of lead contamination (Adeniyi 1996). The relatively high values of lead in the supplemented feed samples may not be unconnected to poor handling, particularly exposure of ingredients and products to exhaust from the Mill's diesel engine power generating plant (Lawani and Abdulmukaila 1984; Wrzesien *et al* 1999). The mean lead levels in between 4.81 ± 5.82 μg/g and 12.80 ± 4.55 μg/g for the droppings (Table 1) were generally higher than the feeds samples. This trend has been observed before (Van der Watt *et al* 1994; Ihnat and Fernandes 1996), this probably suggest contributions from aerial sources (Falahi-Ardakani 1984; Nybo *et al* 1996; Adeniyi 1996; Yamasoe *et al* 2000).

The mean TPH values for the feeds (Table 1) ranged between 209.20 ± 128.93 μg/g and 369.00 ± 108.40 μg/g. The control sample values were generally lower than the supplemented feeds samples. The starter and finisher feed samples values of 256.00 ± 152.60 μg/g and 209.20 ± 128.93 μg/g for control and 369.00 ± 108.40 μg/g and 330.20 ± 59.03 μg/g, respectively for supplemented feeds (Table 1) were found to be statistically significant at 95% confidence level. The relatively high values of TPH in the feed samples (Table 1) may be the result of contamination from exhaust fumes in the feed mill, poor handling and or through long-range air pollution of the ingredients (Schroll *et al* 1994; Lorber 1995; Larsen 1995; Douben *et al* 1997; Wiedinmyer *et al* 2000). Similarly, the mean TPH values for the droppings (Table 1) which ranged between 373.00 ± 209.78 μg/g and 605.00 ± 185.35 μg/g are generally higher than the corresponding feeds values (Table 1). Among others, this observation may not be unconnected with the burning of heavy oils (engine oil, black oil and diesel) in the vicinity of the poultry farm to keep soldier ants (*Oecaphylla longinoda*) at bay. The TPH values in the droppings, were however, found not to be significant (Table 1) at 95% confidence level.

The good and high nutritive values of these supplements as well as their affordability not withstanding (Fanimo *et al* 1996; Oduguwa *et al* 2000). There is the urgent need to pay more attention to the level of hygiene as regards the source of ingredients and handling procedures adopted in compounding the rations. This is imperative in the coming years in other to realise the full potential of these emerging new and novel feed ingredients. They no doubt have the potential to reduce the

Table 1
Concentrations (μg/g) ± SD of heavy metals and total petroleum hydrocarbons (TPH) in shrimp waste supplemented feeds and poultry droppings

S.No.		Control		Supplemented		
		Starter	Finisher	Starter	Finisher	$F_{0.05}$
1.	Cd	0.38 ± 0.29	1.05 ± 0.53	0.61 ± 0.48	0.81 ± 0.32	2.63
		(1.72 ± 0.36)	(1.44 ± 0.24)	(0.83 ± 0.96)	(1.45 ± 0.36)	1.81
2.	Pb	6.69 ± 4.03	10.41 ± 1.50	9.49 ± 2.24	11.49 ± 1.55	3.40*
		(12.16 ± 1.95)	(12.80 ± 4.55)	(4.81 ± 5.82)	(12.14 ± 1.03)	3.87*
3.	TPH	256.00 ± 152.60	209.20 ± 128.93	369.00 ± 108.40	330.20 ± 59.03	38.34*
		(377.25 ± 103.64)	(500.50 ± 198.46)	(373.00 ± 209.78)	(605.00 ± 185.35)	1.53

Notes: * significantly differences between metals and TPH in the control and supplemented feeds/droppings at $p < 0.05$. Values in parentheses are for the droppings.

cost of livestock feed production. This is coupled with its attendant multiplier effect in our quest to provide high quality animal protein to the greatest number of our citizen without compromising their future health (Beck *et al* 1994; Minissi *et al* 1998; Tchernitchin 1998; Manay *et al* 1999).

The values of heavy metals and TPH (Table 1) in the control commercial feeds, though relatively low, are equally of concern because these contaminants are known to bioaccumulate as they journey through the biological system. (Albers 1995; Mlcachlan 1996; Nybo *et al* 1996; He Yang and Cha 2000). This may have serious health implications as poultry products are important links in the complex food chain.

References

Adeniyi A A 1996 Determination of cadmium, copper, iron, lead, manganese and zinc in water leaf *(Talinum triangulare)* in Dumpsites. *Environ Inter* **22** 259 - 262.

Adeniyi A A, Afolabi J A 2002 Determination of total petroleum hydrocarbons (TPH) and heavy metals in soils within the vicinity of facilities handling refined petroleum products in Lagos metropolis. *Environ Inter* **28** 79 - 82.

Albers P H 1995 Petroleum and Individual Polycyclic Aromatic Hydrocarbons In: Handbook of Ecotoxicology. Haffman, D. T, Rattner, B. A. Burton, G. A. Cairns, J (Eds.), London, UK.

Bamgbose O, Osibanjo O 1998 Comparative assessment of three methods for chlorinated hydrocarbon determination in fish. *Nig J Sc* **32** 169 - 174.

Beck H, Dross A, Mathar W 1994 PCDD and PCDF exposure and levels in humans in Germany. *Environ Health Perspective* **102** 173 - 185.

Byomi A M, Hassan A M, Hassan M M, Saleh R S, El-Amrousi S A, Youssef M S, Aamer A A 1999 Pollution of water and animal feed with some heavy metals and their implications in raw buffalo milk. In: *Proceedings of the 5th Scientific Congress Egyptian Soc. for Cattle diseases. Assiut Egypt* 28 - 30, 226 - 230.

Douben P E T, Alcock R E, Jones K C 1997 Congener specific transfer of PCDD/Fs from air to Cow's milk. An evaluation of current modelling approaches. *Environ Pollut* **95** 333 - 344.

Falahi-Ardakani A1984 Contamination of environment with heavy metals emitted from automotives. *Ecotoxicol Environ Saf* **8** 152 - 161.

Fanimo A O, Mudama E, Umukoro T O, Oduguwa O O 1996 Substitution of shrimp waste meal for fish meal in broiler chicken rations. *Trop Agric* (Trinidad) **73** 201 - 205.

Fanimo A O, Oduguwa O O, Onifade A O, Olutunde T O 2000 Protein quality of shrimp - waste meal. *Bioresource Tech.* **72** 185 - 188.

Freedman B 1996 *Environmental Ecology (The Impacts of Pollution and Other Stresses on Ecosystem & Function).* Academic Press, New York, USA, 54 - 62.

Gardiner D T, Miller R W, Badamchian B, Azzari A S, Sisson D R 1995 Effects of repeated sewage sludge applications on plant accumulation of heavy metals. *Agric Ecosystems and Environ* **55** 1 - 6.

Gruszecki T M, Lipiec A, Klewiec J, Lipecka C, Junkuszew A and Gabryszolk M 2000 *50 Yrs. of the National Research Institute of Animal Production, Safe Food as a Challenge to Animal Sciences,* Balice, Poland, 24 May, 2000. Supplement **8** 72 - 75.

Hanczakowski P, Szymczyk B, Wawrzynski M 1995 Composition and nutritive value of sewage-grown duckweed (*Lemna minor* L.*)* for rats. *Animal feed Science and Technology* **52** 339 - 343.

He M, Yang J, Cha Y 2000 Distribution, removal and chemical forms of heavy metals in polluted rice seed. *Toxicol Environ Chem* **125** 17 - 24.

Hensbergen P J, Velzen M J M, Mugroho R A, Donker M H, Straalen N M, Van-Velzen M J M 2000 Metallothionein-bound cadmium in the Insect *Orhesella cincta* (collembola) in relation to dietary cadmium exposure. *Comp Biochem Physiol Pharmacol & Endocrin* **125** 17 - 24.

Hewari J, Beaulieu C, Qullete D, Pontbriand Y, Halsaz A M, Vanata H 1995 Determination of petroleum hydrocarbons in soil: SFE vs. Soxhlet and water effect on recovery. *Inter J Environ Anal Chem* **60** 123 - 137.

Ihnat M, Fernandes L 1996 Trace elemental characterization of composted poultry manure. *Bioresource Tech* **57** 143 - 156.

Inter-governmental Oceanographic Commission (IOC) 1982 The Determination of Petroleum Hydrocarbons in Sediments. *Manuals and Guidelines of the IOC,* Paris, France **11** 11 - 15.

Islam M A, Hossain M D, Balbul S M, Howlinder M A R 1994 Unconventional feeds for broilers. *Indian Vet J* **71**, 775 - 780.

Kan C A 1994 Factors affecting absorption of harmful substances from the digestive tract of poultry and their level in poultry products. *World's Poultry Sci J* **50** 39 - 53.

Kugonic N, Grcman H, Vilhar B, Grill D, Gettenberger H 1999 The accumulation of Cd, Pb and Zn in different vegetables from Zasavje (Slovenia). In: *Proceedings on 2nd Slovenian Symposium on Plant Physiology with International Participation*, Gozd Martuljek, Slovenia, Sept. 30 - Oct. 2 1998. *Phyton Horn* **39** 161 - 165.

Larsen J 1995 Levels of pollutants and their metabolites: exposures of organic pollutants. *Toxicol* **101** 11 - 27.

Lawani S A, Abdulmukaila M T 1984 Contributions to the cadmium, lead and mercury contents of diets by using grinding stones. *J Sci Food Agric* **35**, 698 - 700.

Lorber M N 1995 Development of an air-to-leaf vapour phase transfer factor for dioxins & furans. *Organohalogen Compds* **24** 179 - 189.

Manay N, Pereira L, Cousillas Z 1999 Lead contamination in Uraguay. *Rev Environ Contam Toxicol* **159** 25 - 39.

Martinez J, Bozec G L, Le-Bozec G 2000 Pig manure and environmental concerns in europe.*Cahiers Agrics* **9** 181 - 190.

Minissi S, Caccese D, Passafiume F, Grella A, Eleonora C, Rizzoni M 1998 Mutagenecity (Micro-nucleus test in *Vicia faba* root tips), polycyclic aromatic hydrocarbons and heavy metals content of sediments collected in tiber River and its tributaries within the Urban area of Rome. *Mutat Res* **420** 77 - 84.

Mlcachlan M 1996 Bioaccumulation of hydrophobic chemicals in agricultural food chains. *Environ Sci Tech* **30** 252 - 259.

Ngodigha E N, Olayimika F O, Oruwari B M, Ekweozor E K E, Whekhe S N 1999 Toxic effects of crude oil in organs and blood cells of west Africa dwarf goat, *Nig Vertinary J* **20** 82 - 91.

Nyobo S, Fjeld P E, Jerstad K, Nissen A 1996 Long-range air pollution and its impact on heavy metal accumulation in *Dippers Cinclus Cinctus* in Norway. *Environ Pollut* **94** 31 - 38.

Oduguwa O O, Fanimo A O, Onifade A A, Arije A 2000 Nutritive value of shrimp waste-meal and fly larva-meal (maggotmeal) in growing albino rats. *Indian J Animal Sci* **70** 70 - 72.

Onianwa P C 1995 Petroleum hydrocarbon pollution of Urban top soil in Ibadan City, Nigeria. *Environ Inter* **21** 341 - 343.

Polley J W, Kim D E, Vigniswarin W T, Berkozitz R, Charbel F 1994 Heavy metal. *Beware J Craniofac Surg* **7** 460 - 464.

Rambeck W A, Weiser H 1992 Reduction of tissue cadmium retention by L-Ascorbic Acid. In: *Proceedings of 19th World's Poultry Congress* **2** 259 - 261.

Schroll R, Bierling B, Cao G, Dorfler U, Lahaniati M, Langenbach T, Scheunet I 1994 Uptake pathways of organic chemicals from soil by agricultural plants. *Chemosphere* **28** 297 - 303.

Schuler F, Schmid P, Schlatter C 1997 Transfer of airborn polychlorinated dibenzo-*p*-dioxins and dibenzofurans into dairy milk *J Agric Food Chem* **45** 4162 - 4167.

Tchernitchin N N, Villagra A, Tchernitchin A N 1998 Antiestrogenic activity of lead. *Environ Toxicol & Water Qual* **13** 43 - 53.

Tsuji L T S, Nieboer E, Karagatzides J D, Hanning R M, Katapatuk B 1999 Lead shot contamination in edible portions of game birds and its dietary implications. *Ecosystem Health* **5** 183 - 192.

Van der Watt H V H, Sumner M E, Cabrera M L 1994 Bioavailability of copper, manganese and zinc in poultry litter. *J Environ Qual* **23** 43 - 49.

Wiedinmyer C, Strange I W, Estes M, Yarwood G, Allen D T 2000 Biogenic hydrocarbon emission estimates for north central texas, *Atmospheric Environ* **34** 3419 - 3435.

Wrzesien R, Budzinska - Wrzesien E, Chudzkka M 1999 Accumulation of selected heavy metals in hare in connection with their feeding conditions. *Agriculture* **36** 245 - 250.

Yamasoe M A, Artaxo P, Miguel A H, Allen A G 2000 Chemical composition of aerosol particles from direct emissions of vegetation fires in the amazon basin. *Water Soluble Species and Trace Elements. Atmospheric Environ* **34** 1641 - 1653.

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PRODUCTION OF BIOGAS AT MESOPHILIC AND THERMOPHILIC TEMPERATURES

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Experiments were accomplished for the production of biogas using a slurry comprising 50% fresh buffalo dung and 50% water at ambient and elevated temperatures. After 2 weeks, observations for the release of biogas were noted. It was observed that with the increase of temperature, the rate of generation of gas was enhanced. A slurry containing 200 g fresh buffalo dung and 200 g water produced 121.5 ml gas / day at an average ambient temperature of 35˚C, whereas at 45˚C, 50˚C, 55˚C and 60˚C, the average rates of gas generation were noted as 152.0, 221.5,292.0 and 354.0 ml / day. The volume of 232.5 ml of biogas in excess was produced with the temperature difference of 25˚C (60˚C - 35˚C). The input of 42000 joules of energy for heating 400 ml of slurry produced gas of 5196.375 joules. It shows that there is a loss of 36803.625 joules of heat which makes the process, thermally, not viable. Hence, ambient temperature is recommended for the production of biogas for domestic plants.

Keywords: Buyffalo dung, Fermentaion, Biogas, Thermophilic temperatures.

Introduction

Biogas generation is one of the techniques for the utilization of animal, agricultural and industrial wastes, combating deforestation and better environment by disposing of wastes beneficially. A voluminous work has been done on biogas generation to improve the technology and to make it economical (Christopher *et al* 1973; Govinda 1983; Narasimhamurty and Purush 1986; Ismat Ali 1987a; 1993b; and 1994c; Kishore 1989; Kasali 1989; Liachena 1989; Qureshi 1989). Biogas, generated from digestion of animal excreta, is composed of methane (60 - 65%) and carbon dioxide (35 - 40%) and traces of hydrogen sulphide (H_2S) of heating value 22.35 MJ / $m^{3.}$ Usually, it is being produced at ambient temperature when the slurry containing cow / buffalo dung is kept in sealed vessel at ambient temperature. The biogas produced is an ideal domestic fuel for cooking and lighting. The gas is smokeless, inexpensive and environment friendly as the raw material (animal refuse) is abundantly, available in the villages of developing/underdeveloped countries either free or at very reasonable cost. On comparison, the cost of biogas with other domestic fuels, it is found lowest / cheapest. The cost of biogas is approximately Rs.100/- per Giga joule, whereas, the cost of Liquefied Petroleum Gases (LPG), kerosene, charcoal, wood are Rs.750/=, 400/=, 250/=, 180/=, for one Giga joule, respectively. The cost of these fuels is constantly increasing day by day. The only drawback of this technology is the low rate biogas generation. It is 0.25 unit per unit of slurry per day (Ismat Ali 1993). Attempts have been made to produce biogas at elevated temperature to examine the enhancement in the rate of biogas generation.

Since, the technology of biogas generation is simple and straight forward, it does not need any technical skill to operate and maintain biogas plants. Hence, this technology is recommended for such areas (particularly villages) where natural gas is not available to meet domestic fuel requirements. This paper describes the methods of biogas generation at ambient and elevated temperatures (45˚C, 50˚C, 55˚C, 60˚C) with a view to examine its economic feasibility.

Experimental

Material. Buffalo dung and water.

Procedure. In laboratory, 400 g slurry (buffalo dung + water in equal proportion) was confined in one litter amber colored reagent bottle stoppered and connected them with polythene tubes to 10 litter aspirators as shown in Fig 1 (a & b). The main aim of connecting the aspirators was to collect the gas by displacing water and the displaced water was collected in graduated cylinder. The amount of water collected in the cylinder was assumed equal to the amount of the gas produced per day. Five such sets of experiements were designed to examine the rate of gas generation. One of the set of experiments was kept at ambient temperature, whereas, other four sets of experiments were controlled at 45˚C, 50˚C, 55˚C, 60˚C (Fig 1 a & b).

Analysis of animal excreta. Normally, animal excreta (buffalo dung) contains 80% moisture when fresh. It is in emul-

* Authors for correspondence

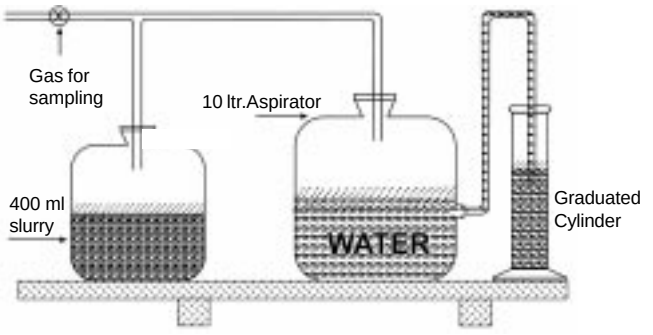

Fig 1(a). Experimental setup for biogas generation at ambient temperatures

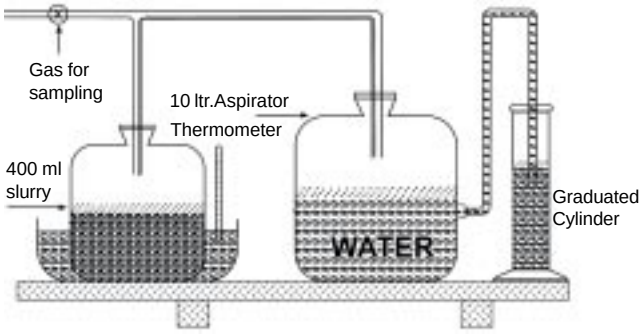

Fig 1(b). Experimental setup for biogas generation at elevated teperatures

Table 1
Proximate analysis of buffalo dung (Aerial and oven dried)

Constituents	%	% on dry basis
Water	74.4	
Moisture (corrected on Mac - 400)	5.4	
Total water	79.6	
Volatile matter 650˚C	15.6	76.47
Ash (Residue)	4.8	23.53

Table 2
Ultimate (elemental) analysis buffalo dung on CHN-600, LECQ, USA (dry ash free basis)

Elemental	Percent
C	37.5
H	6.8
N	1.4
O	54.3
Total	100.0

Table 3
Gas production from 400 ml slurry (buffalo dung + water) at ambient and elevated temperatures (45˚C, 50˚C, 55˚C, 60˚C)

Days	Ambient temperature (ml)	45˚C (ml)	50˚C (ml)	55˚C (ml)	60˚C (ml)
01	124	150	226	290	350
02	120	154	220	294	256
03	122	152	220	290	354
04	122	150	220	296	360
05	124	154	224	290	354
06	120	150	220	300	352
07	120	150	220	290	350
08	124	150	220	290	360
09	124	158	230	296	354
10	120	150	220	286	352
11	122	150	220	286	350
12	124	152	222	296	350
13	120	154	220	290	360
14	120	152	220	292	354
15	120	152	220	292	352
Mean	121.5	152	221.5	292	354

sion form and is problematic to remove its great amount by heating as there was loss of gases too. In the present study, 100 g of dung was first aerial dried in a partially covered petri dish for two days noting down weight loss for every four hours in the day time. The results of water content of dung were further cross checked by Dean and Stark method. It was noted that moisture content of dung varies with the passage of time due to atmospheric evaporation of water. The proximate analysis of dried dung was carried out on Mac - 400 LECO. USA and Ultimate (elemental) analysis was carried out on CHN - 600, LECO, USA. Results are presented in Table 1 & 2. Carbon and nitrogen ratio is one of the most important parameters which palys an important role in the degradation of cellulosic material (Almassi and Dunn 1974).

Results and Discussion

Table 1 and 2 describe proximate and ultimate (elemental) analysis of buffalo dung, respectively. Data on production of biogas from 400 ml slurry (buffalo dung + water) at ambient temperature and 45˚C, 50˚C, 55˚C, 60˚C are summarized in Table 3 and 4.

The only drawback of biogas generation from fermentation of dung is its slow rate of degradation of carbohydrates / cellulosic material to methane (CH_4) and carbon dioxide (CO_2) (Barker 1956). Incubation period varies from 2 - 6 weeks depending upon the nature of dung (fresh or otherwise). The release of biogas, in the present studies, is observed 0.3 unit per unit of slurry at ambient conditions. The ideal conditions

Table 4

Cumulative gas production from 400 ml slurry (buffalo dung + water) at ambient and elevated temperatures (45˚C, 50˚C, 55˚C, 60˚C)

Days	Ambient temperature (ml)	45˚C (ml)	50˚C (ml)	55˚C (ml)	60˚C (ml)
1	122	150	226	290	350
2	244	304	446	584	706
3	366	456	666	874	1060
4	488	606	886	1170	1420
5	612	760	1110	1460	1774
6	732	910	1330	1760	2126
7	852	1060	1550	2050	2476
8	976	1210	1770	2346	2836
9	1000	1368	2000	2632	3196
10	1220	1518	2220	2918	3542
11	1342	1668	2440	3214	3892
12	1466	1820	2662	3504	4244
13	1586	1974	2882	3796	4604
14	1606	2126	3102	4088	4958
15	1826	2278	3322	4380	5310

for the degradation of organic waste /cellulosic material for the production of biogas are total solid content (7 - 9%) total volatile of the total solid (70 - 80%), pH (6.8 - 7.4), carbon / nitrogen ratio (25 - 30), temperature range (27 - 71˚C) (Amassi and Dunn 1974). The entire biochemistry of conversion of cellulosic wastes to combustible gas is not completely understood. However, it is assumed that the following reactions take place during the anaerobic digestion of cellulosic material.

Cellulosic material $\longrightarrow$ Carbohydrate $\longrightarrow$ $nCH_4 + nCO_2$

Above mentioned reaction, following two routes are suggested:

$C_6H_{12}O_6 \longrightarrow 3CH_3COOH$ (Carbohydrate from organic wastes)

The route of the above mentioned reactions is likely to be:

$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2$ (Carbohydrate from digestion of organic waste)

$2C_2H_5OH + 2CO_2 \longrightarrow 3CH_3COOH$

$3CH_3COOH \longrightarrow 3CH_4 + 3CO_2$

For the preparation of biogas in the laboratory, the proximate and ultimate analyses of buffalo dung were accomplished. The results are shown in Table 1and 2. The proximate analyses of buffalo dung based upon aerial and oven dried conditions show moisture as 79.6%, whereas volatile matter at 650˚C and ash (residue) are 15.6% and 4.8% respectively.

Further, the ultimate analyses of the dung indicate C = 37.5%, H = 6.8%, N = 1.4%. These estimations show that buffalo dung may be used for the production of biogas, the heating values of different fuels were also taken into consideration. The heating value of different fuels comparing with the heating value of the biogas. Coke oven gas and retort coal gas

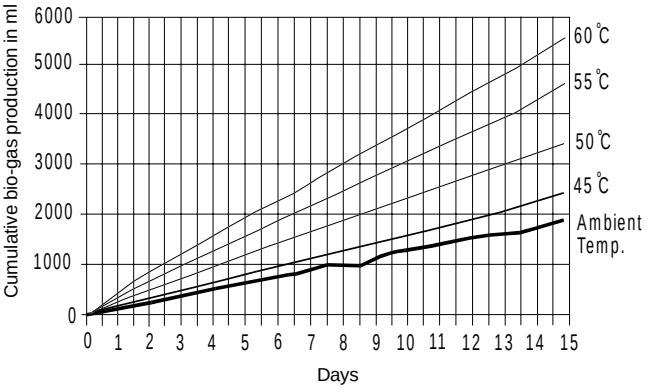

Fig 2. Cumulative gas production from 400 ml slurry (buffalo dung + water) at ambient and elevated temperature (45˚C, 50˚C, 55˚C and 60˚C)

show heating values as 21.90 and 21.42 MJ/m^3 respectively, whereas, the heating values of carbureted water gas is 19.67 MJ/m^3. Blue water gas and water gas have similar heating values 12.1 and 11.47 MJ/m^3. Among these fuels, biogas is the only one which shows maximum heating value as 22.35 MJ/m^3.

Tables 3 - 4 describe the data obtained for the gas production from 400 ml slurry (buffalo dung + water) at ambient and elevated temperatures (45˚C, 50˚C, 55˚C, 60˚C). These results show that the release of biogas increased with increase in temperature. At ambient temperature of 35˚C, the average rate of gas generation was 121.5 ml / day, whereas, at temperatures 45˚C, 50˚C, 55˚C, and 60˚C the average rate of gas generation was 152, 221.5, 292 and 354 ml / day, respectively from the same composition of slurry, 232.5 ml of gas in excess was produced with the temperature difference of 25˚C (60 - 35˚C). The graph shown in Fig 2 shows that the rate of release of biogas is enhanced with the rise in temperature.

It is very important to examine the energy balance due to change in temperature. Assuming that there is no loss of heat and entire energy from the water bath was absorbed by 400 ml (m = 400 ml = 0.4 kg) slurry and specific heat of slurry is c = 4200 J/kg ˚C (as slurry contains 90% water). The energy input due to change in temperature (Δt = 25˚C) is calculated as:

$\Delta E = mc\ \Delta\tau$

[m = mass of slurry, c is the specific heat of slurry and $\Delta\tau$ denotes temperature difference]

ΔE_{input} = (0.4) x 4200 x 25 = 42000 joules.

The heat potential of excess gas produced 232.5 ml/day (232.5 x 10^{-6} m^3/d) due to change in temperature from 35˚C to 60˚C can be calculated from the heating value of biogas i.e. 22.35 mega joules/m (22.35 x 10^{+6} J/m^3)

Heat potential of 232.5 x 10^{-6} m^3 biogas = Heat Output = 22.35 x 10^{+6} x 232.5 x 10^{-6} = 5196.375 J (1 m^3 biogas has heat potential = 22.35 x 10^{+6} J).

Heat input = 42000 J.

Heat output = 5196.375 J.

Loss = 36803.625 J.

Now, we see that the input of 42000 joules of heat for heating 400 ml of slurry produces heat potential as 5196.375 joules. It shows that there is a loss of 36803.625 (42000 - 5196.375) joules of heat. It is therefore, obvious from above calculations that the production of the biogas at elevated temperature is thermally not favorable.

As far as the use of dung cake as such for cooking purposes is concerned, there will be a loss of manure potential. The heat potential of the dried dung is 16 MJ/kg of total solid. The maximum conversion efficiency is reported as high as 90% (John Twidell and Tony 1980).

In brief, warming of slurry from external source of energy (heat) for the production of biogas is thermally not favorable i.e. at elevated temperature as energy input is more than the energy output. It is, therefore, on large scale production of biogas, the ambient temperature is the only suitable temperature. It will be very simple and economical and there will be no problem of maintaining temperature. Furthermore, conversion of biomass into combustible gas through anaerobic fermentation increases the potential of nitrogen rich fertilizer available/applicable to nearby farms (Mahajan 1987).

It is also concluded that biogas technology, still, needs more research and development work for the increase of energy output from the biomass and to enhance rate of gas generation by the addition of chemicals / inoculum so as the cost of the plant is reduced to a level that a common man can afford to install biogas plant for meeting his energy requirements.

References

Almassi M, Dunn P D 1974 *Generation of Methane from Farm Waste Materials - Methane Colloquinum*. Imperial College, London, UK, pp 1 - 8.

Barker H A 1956 *Bacterial Fermentation.* John Wiley and Sons Inc. New York, USA, pp 151 - 174.

Chirstopher B, Slene B, Derel D, Patrick K 1973 *Methane: Fuel of the Future an Assessment.* Andrew Singa, Brok Publisher & Printer Botthisham Park Mill, Botthisham Cambridge Stere, UK.

Govinda P D 1983 Biogas Research and development in Nepal. In: *Procceding on International Workshop on Renewable Energy Source*, Lahore, Pakistan, March 3 - 22, 1983.

Hobson P N, Bousfield S, Summers R 1986 *Energy from Wastes Series Methane Production for Agriculture and Domestic wastes.* Applied Science Publications Ltd. Ist ed, pp 238 - 239.

Ismat A 1987 High pressure biogas plant - a Pakistani design, Science Technology & Development. *A Journal of Pakistan Council for Science and Technology* **6** (6) 13 - 18.

Ismat A 1993a *Biogas: An Energetic Fuel for Rural Areas Energy World.* Institute of Energy, UK, NO 213, pp 16 - 18.

Ismat A 1993b Biogas: An alternate, efficient and clean fuel for remote areas of Pakistan. *Journal of Science and Technology in the Islamic World* **11** (2) 89 - 94.

Ismat A 1993c Biogasification *Engineering Horizons* **4** (11), 27 - 28.

Ismat A 1994 Some studies on fermentation of cowdung for biogas generation, *In: Recent Trends in Bio Chemical Research in Pakistan*, eds Qasim H, Isahq, Azhar. University of Karachi, Pakistan, 47 - 54.

John T, Tony W 1980 *Renewable Energy Resources.* E & F.N.S, NewYork, USA, pp 302 - 307.

Kasali G B, Semor E 1989 Effect of temperature and moisture in the anaerobic digestion of refuse. *J Chem Technology Bio Technol* **44** (1) 31 - 14.

Khandenal K C, Mahdi S S 1986 *Bio Gas Technology*. A Practical Hand Book. Tata Mc Graw Hill Publishing Co. Ltd., New Delhi, India, p 2.

Kishore V V 1989 A heat transfer analysis of fixed - dome biogas plants. *Biology Wastes* **30** (3), 199 - 215.

Laichena J K 1989 Rural energy in Kenya, Is there is a future of biogas. *A Energy Exploration and Exploitation* **7** (2) 116 - 127.

Mahajan S S 1987 Biogas from agricultural and kitchen garden waste. *Invention Intelligence* **22** (5) 188-192.

Narasimhamurty G S, Purush O A 1986 Biogas generation from Bagasse. *India Chem Engg* **28** (3) 54 - 56.

Qureshi M A, Karbanda V P 1983 Choice of technology in China & India. The case of biogas. *Journal of Science and Industrial Research*, (42) 597 - 601.

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A Family of Iteration Formulas for the Determination of the Zeros of a Polynomial

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We present a class of globally monotonically convergent iterative methods for the determination of zeros of a polynomial. The proposed method uses the well-known Newton's second order method as a basic ingredient to generate this class of methods, following the approach of Petkovic and Trickovic as supported by Cauchy Schwartz inequality from which come in hand three methods of fourth order. The obtained methods can be used to provide tight inclusion conditioning bounds separating the sought zeros. This means that they always provide good numerical approximations within the theoretical conditioning bounds. It is found that one of the fourth order methods so obtained competes most favourably with any known methods for finding zeros of a polynomial.

Key words: Newton's methods, Halley's methods, Chevbyshev's method, Polynomial zeros.

Introduction

When a zero seeking algorithm has local convergency properties, the iteration if it converges will display a doubling phenomenon at each iteration. The point at which an iterative sequence starts experiencing a contraction immediately an iterative process is began called a point of attraction or else we say that the iterative sequence diverges which is known as point of repulsion. Of major importance in this paper is the derivation of two new methods through the technique of Petkovic and Trickovic (1995) as supported by the concept of Cauchy - Schwartz inequality $\left(\sum \frac{1}{Z - Z_i}\right)^2 \leq n \sum \left(\frac{1}{Z - Z_i}\right)^2$ where, $Z_1, Z_2 \ldots Z_n$ are the roots of polynomial of degree n for the case of real simple zeros, given by: $P(Z) = \sum_{j=0} a_j Z^j$, $a_j \in \subset$.

It is found that one of the two new methods obtained competes favourably with the popular Chevbyshev third order method for the extraction of zeros of a polynomial.

In a note of passing we shortend notation by omitting the argument Z from function such as P(Z), and write simply P. We also denote the first three derivatives of a function P by P′,P″ and P‴, respectively. It is also supposed that the functions discussed in this paper has what ever number of C^{n+1} necessary on the interval.

Experimental

Derivation of the class of methods. The class of methods under consideration is a particular case of a general one point iterative formula considered in Petkovic and Trickovic (1995) as well as Milovanovic (1974) which is as follows, we define:

$$Z_i^{(k+1)} = g'(Z^{(k)}), (k = 0, 1...) \qquad (2.1)$$

where; $g(Z) \rightarrow Z_i - \Phi(Z_i)$ as a one point method.
Observe that $\Phi(Z_i)$ is a rational map which converges such that: $Z^{k+1} - Z^k \rightarrow 0$.

For $m \geq 2$ an interative method of order m could be modified (see, Petkovic and Trickovic (1995) for more details) such that:

$$Z_i^{(k+1)} = Z_i^{(k)} - \left(\Phi(Z^{(k)}) \left(1 + \frac{1}{m} g'(Z_i^{(k)})\right)\right| \qquad (2.2)$$

In this paper, starting from Newton's Second order method we shall generate such higher methods which are structurally similar to those obtained by Jarrat (1968). Method (2.2) is a major plank in which our derivation heavily leans on.
Define Newton's formula as:

$$Z_i^{(k+1)} = Z_i^{(k)} \frac{p'}{p'}, (k = 0, 1....) \qquad (2.3)$$

In view of (2.1) and (2.2) we write (2.3) in the form:

$$\begin{aligned}
\hat{Z}_i &= Z_i - \frac{p}{p'} \left(1 + \frac{1}{2} g'(Z)\right) \\
&= Z_i - \frac{p}{p'} \left\{1 + \frac{1}{2} \frac{d}{dz}\left(Z_i - \frac{p}{p'}\right)\right| \\
&= Z_i - \frac{p}{p'} \left(1 + \frac{1}{2} \frac{(p'^2 - p'^2 + pp'')}{p'^2}\right) \\
&= Z_i - \frac{p}{p'} \left(1 + \frac{pp''}{2p'^2}\right) \qquad (2.4)
\end{aligned}$$

Method (2.4) is the Chevbyshev third order method and consider method of Jarrat (1968) for more details.

Next, by setting $\Phi(Z_i) = \frac{p}{p'}\left(1+\frac{pp''}{2p'^2}\right)$, and using as before the procedure described above we write:

$$\hat{Z} = Z_i - \Phi(Z_i)\left(1+\frac{1}{3}(g'(Z))\right) \qquad (2.5)$$

We differentiate g (Z) as follows:

$$g'(Z) = \frac{d}{dz}\left(Z-\left(\frac{p}{p'}+\frac{p''}{2p'}\left(\frac{p}{p'}\right)^2\right)\right) =$$

$$1-\left(\frac{p'^2-pp''}{p'^2}+\frac{p''}{2p'}\frac{d}{dz}\left(\frac{p}{p'}\right)^2+\left(\frac{p}{p'}\right)^2\frac{d}{dz}\frac{p''}{2p'}\right)$$

$$= 1-\left(\frac{p'^2-pp''}{p'^2}+\frac{pp''}{P'^2}\left(\frac{p'^2-pp''}{p'^2}\right)+\frac{1}{2}\frac{p^2}{p'^2}\frac{p'p'''-p''^2}{(p')^2}\right)$$

After some calculations we shall obtain:

$$1-\frac{2p'^4-4p'^2pp''+3p^2p''^2-p^2p''p'''}{2p'^4} \qquad (2.6)$$

Where, the term - $4p'^2 pp''$ is introduced into the numerator part of (2.6) to compensate for the term $3p^2p'^2 - p^2p'p'''$.

However, the choice of this extral term - $4p'2\,pp''$ is arbitary which is also found to be optimal in some sense.
In view of (2.6), method (2.5) takes the form:

$$\hat{Z}_i = Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\left(1-\frac{2p'^4-4p'^2pp''+3p^2p'^2-p^2p'p'''}{2p'4}\right)\right) \qquad (2.7)$$

Simplifying we have:

$$\hat{Z}_i = Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{4p'^2-3p^2p''^2+p^2p'p'''}{2p'^4}\right)\right) \qquad (2.8)$$

Following carefully some selected ideas in Hansen and Partick (1977), it is easy to see that:

$$\frac{p\,p''-p'^2}{p^2} = -\sum_i\left(\frac{1}{Z-Z_i}\right)^2$$

By Cauchy - Schwartz inequality, we have that:

$$\left(\sum_i\frac{1}{Z-Z_i}\right)^2 \le n\sum_i\left(\frac{1}{Z-Z_i}\right)^2 \qquad (2.9)$$

It follows that:

$$p'^2-p\,p'' = p^2\sum\left(\frac{1}{Z-Zi}\right)^2 \ge \frac{p^2}{n}\left(\sum\frac{1}{Z-Zi}\right)^2$$

Deduce then immediately that:

$$(n-1)\,p'^2 - n\,p\,p'' \ge 0 \qquad (2.10)$$

In view of (2.10), we shall obtain two new methods out of method (2.8) which is precisely our aim.

We shall categorize our procedures of derivation under two cases:

Case I: $p\,p'' \le \frac{n-1}{n}\,p'^2$ (2.11)

We now rewrite (2.8) by subsituting the right hand side of (2.11) for pp'' into the resulting expressing for $g'(Z)$:

$$\hat{Z}_i = Z_i - \Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{pp''(4p'^2-3\,pp'')+p^2p'p''}{2\,p'^4}\right)\right)$$

$$= Z_i - \Phi(Z_i)\left(1+\frac{1}{3}\frac{\left(\frac{n-1}{n}\right)p'^2\left(p'^2-3\left(\frac{n-1}{4n}\right)p'^2+p^2p'p'''\right)}{2p'^4}\right)$$

$$= Z_i - \Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{(n-1)}{n}p'^4\frac{\left(1-3\frac{(n-1)}{4n}\right)+p^2p'p'''}{2p'^4}\right)\right) \qquad (2.12)$$

We simplify the term as follows:

$$\frac{(n-1)}{n}p'^4\left(1-3\frac{(n-1)}{4n}\right) = \frac{n-1}{4n}p'^4\left(\frac{4n-3n+3}{4n}\right) = \frac{n-1}{n}p'^4\left(\frac{n+3}{4n}\right)$$

$$= \frac{(n-1)(n+3)p'^4}{4n^2} \qquad (2.13)$$

Substitute (2.13) into (2.12), we have:

$$\hat{Z}_i = Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{(n-1)(n+3)p'^4}{8n^2p'4}+\frac{p^2p'p'''}{2P'^4}\right)\right)$$

$$= Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{(n-1)(n+3)}{8n^2}+\frac{p^2p'p'''}{2P'^4}\right)\right)$$

$$\hat{Z}_1 = Z_i-\Phi(Z_i)\left(\frac{p^2p'p'''}{6p'^4}+\frac{(n-1)(n+3)}{24n^2}+1\right) \qquad (2.14)$$

Case II: $p'^2 \ge \frac{n}{n-1}pp''$ (2.15)

As before, we have from:

$$\hat{Z}_1 = Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{pp''\left(p'^2\,\frac{3}{4}\,pp''\right)+p^2p'p'''}{2p'^4}\right)\right) \qquad (2.16)$$

Substitute the right hand side of (2.15) for p'^2 in method (2.16), we have:

$$\hat{Z}_1 = Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\frac{\left(\frac{n-1}{n}\right)\left(p'^2-\frac{3(n-1)}{4\,n}p'^2\right)+p^2p'p'''}{2p'^4}\right) \qquad (2.17)$$

Simplifying (2.17), we have:

$$\hat{Z}_1 = Z_i-\Phi(Z_i)\left(1+\frac{1}{3}\left(\frac{(n-1)}{n}p'^2\left(\frac{4n-3n+3}{4n}\right)+p^2p'p'''\right)\right)$$

Table 1

No. of Iteration	Proposed method 2.14	Proposed method 2.18	Proposed method 2.19	Halley's method	Chevbyshev's method
0	-4.500000000	-4.5000000000	-4.500000000	-4.500000000	-4.500000000
1	-4.036653964	-4.0569632550	-4.010442833	-4.033284200	-4.056976693
2	-3.998106190	-4.0031990970	-4.047858808	-4.000019194	-4.000212678
3	-4.000101004	-3.9999994955	-4.001058584	-4.000012796	-4.000000000
4	-3.999994613	-4.0000000000	-4.000000008	-3.999997013	-
5	-4.000000285		-3.9999999869	-3.999996018	-
6	-3.999999670	-	-	-3.9999994692	-
7	-	-	-	-4.000000002	-
8	-	-	-	-4.0000000000	-

$$= Z_i - \Phi(Z_i)\left(1 + \frac{1}{3}\left(\frac{\left(\frac{(n-1)(n+3)}{4n^2}\right)p'^2 + p^2 p' p'''}{2p'^4}\right)\right)$$

$$= Z_i - \Phi(Z_i)\left(1 + \frac{(n-1)(n+3)\,p'^2 + 12n^2 p^2 p' p'''}{12n^2 \times 2p'^4}\right)$$

$$= Z_i - \Phi(Z_i)\left(\frac{p^2 p' p'''}{2p'^4} + \frac{(n-1)(n+3)\,p'^2}{24n^2 p'^4} + 1\right) \qquad (2.18)$$

Now, if we ignore the extract term $-4p'^2 pp''$ added to method (2.8) we shall obtain directly an iterative method of the form:

$$\hat{Z}_i = Z_i - \Phi(Z_i)\left(1 + \frac{p^2 p' p''' - 3p'^2 p''^2}{6p'^4}\right) \qquad (2.19)$$

Methods (2.14), (2.18) and (2.19) are of fourth order of convergence, in the sense of Hansen and Patrick (1977) as well as Traub (1964) and Petkovic and Herceg (1992). In the next section, we shall discuss our methods numerically in comparison with Halley's method, Davies and Dawson (1975), (Hansen and Patrick (1977), Chevbyshev's method, Petkovic and Herceg (1992).

Results and Discussion

The iterative methods discussed earlier can be used for any polynomial of degree $n \geq 3$. Our scalar test problem is a polynomial of degree 5 given by:

$$p(Z) = Z^5 - 6Z^4 - 20Z^3 + 120Z^2 + 64Z - 384 = 0$$

The initial inclusion zeros is $Z^{(0)} = -4.5$

We used the results from our methods (2.14) and (2.18) to compare with results from Halleys method and Chevbyshev's method. The Halley's and Chevbyshev's methods are given by:

$$\hat{Z}_1 = Z_i - \frac{p}{p' - \frac{pp''}{2p'}}$$

(Halley's method)

$$\hat{Z}_1 = Z_i - \frac{p}{p'}\left(1 + \frac{pp''}{2p'^2}\right)$$

(Chevbyshev's method)

We present our results in ordinary real floating arithmetic.

All results are presented below in Table 1.

It can be seen from the Table 1 that Halley's third order method performs worst than any of the four methods. It can also be seen that our method (2.18) has high and extremely fast convergency properties as Chevbyshev's third order method. It is also noticed that our method (2.14) is converging but at a rate less than both of our methods, (2.18), (2.19) and Chevbyshev's method.

The actual zero of the polynomial problem is - 4.

One striking thing about our methods (2.14) and (2.18) is that, convergence to the desired zeros is not affected by the degree of polynomial.

All our tested problems are polynomial with real simple zeros. The methods can be adapted for polynomial with real multiple zeros but this has not been studied in details in this paper.

As, in Patrick and Hansen (1992) iterative method. it is known that Cauchy - Schwartz inequality can be used to prove that:

$$p'^2 - \left(\frac{1}{n-1} + 1\right)pp'' > 0$$

This was a major plank in which our methods were obtained. Since convergence is monotonic for all real zeros satisfying the methods described above, the method with fastest convergence is one with the largest step $Z^{(k+1)} - Z^{(k)}$. As observed in our calculations, it is seen that the

factors $\frac{p^2 p' p'''}{2p'^4}$ and $\frac{p^2 p' p'''}{6p'^4}$ decay to zero number at a very rapid rate. It is also hoped that if multiple precision arithmetic is used in the implementation of the methods, the rate of convergency may require fewer steps in our calculations.

Local existence. We set forth to prove that our methods conform with fixed point theorem.

Theorem. Each of our methods (2.14), (2.18) and (2.19) converges monotonically towards the desired zero.

Proof. Since each has a Lipschitz constant less than 1 it is self verifying that each of the methods does not possess normal structure. It follows that the sequence $\{Z_i^{(k)}\} \rightarrow \xi$ where, all $Z_i^{(k)}$ and ξ all lie in the interval disk of desired zero. It holds good that in the limit as $k \rightarrow \infty$ $p(Z_i^{(k)}) \rightarrow 0$. This shows that the distance topology is $\|Z_i^{(k)} - Z_i^{(k+1)}\| \rightarrow 0$. In addition, the triangular inequality holds for the set $\{Z_i^{(k)}\}$. Therefore, we conclude that our methods are feasible and endorsed fixed point which converges to a limit point.

References

Davies M, Dawson B 1975 On the global convergence of Halley's iteration formula. *Numer Math* 24, 133 - 135.

Hansen E, Patrick M 1977 A family of root finding methods. *Numer Math* 27, 257 - 269.

Jarratt P 1968 The use of comparison series in analyzing iteration functions. *Computer Journal* **11**(3) 314 - 316.

Milovanovic G V 1974 A method to accelerate iteration process in Banach Space. Univ. Beograd Publ. *Elektrotehn Fak Ser Mat Fiz* 461 - 497.

Petkovic M S, Herceg D 1992 High - order iterative methods for approximating zeros of analytic functions. *J Comput Appl Math* 39, 234 -258.

Petkovic M S, Trickovic S 1995 On zero - finding methods of fourth order. *J Comput Appl Math* 64, 291 - 294.

Traub J F 1964 *Iterative Methods for the Solution of Equations*. Englewood Cliffs N. J., Prentice Hall Inc., USA.

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THE EFFECT OF FRESH AND AGED *CASSAVA* PROCESSING EFFLUENT ON THE PHYSIO - CHEMICAL PROPERTIES OF SOIL

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Fresh *Cassava* processing effluent was obtained from a *Cassava* processing mill in Ekpoma, Edo State. One half of the fresh effluent was used to pollute top soil while the second half was aged for 7 days before use. The relative effects of the fresh and aged *Cassava* effluents on the physio - chemical properties of soil were determined. The effects of pollution varied with the soil/effluent contact period and the nature of the effluent. The result showed increase in the levels of pH, organic carbon, phosphorus, sodium, potassium; and decrease in calcium, magnesium and nitrogen in the soil after treatment with the effluents. There were no marked differences in the particle size distribution nature of the soil and the level of the exchangeable acidity after treatment with the effluents. The results showed that the disposal on the top soil of fresh and aged *Cassava* processing effluent could have diverse effects on the nutrient availability in the soil.

Key words: *Cassava*, particle size, Dicotyledon plant, *Euphorbiaceae.*

Introduction

Cassava (*Manihot esculenta* Crantz) is a dicotyledon plant belonging to the family Euphorbiaceae. Its tuberous roots are valuable source of cheap calorie,especially in developing countries where calorie deficiency and malnutrition are wide spread. *Cassava* is well - cultivated in all the tropical regions of the world (Nestel 1973).

Two main types of *Cassava* are cultivated in Nigeria; namely, the "sweet" variety, which has low content of cyanogenic glycosides, and the "bitter" variety, which has high cyanide content. The utilization of *Cassava* roots for both human and animal nutrition appears to be limited by the presence of the cyanogenic glycosides. As a result a number of traditional methods (Rosling 1988) have been used to process *Cassava* tubes in order to reduce toxicity and improve palatability.

Garri, the form in which *Cassava* is most widely consumed in Nigeria, is derived from the roots by peeling away the back of the *Cassava* tuber, grating the tuber, dewatering and fermentation of the grated pulp and then roasting of the resultant mash. Garri is probably the most important single traditional stable food in West Africa, and it is estimated that about 70% of the total *Cassava* grown in Nigeria are channeled into garri production (Olayide *et al* 1972). The annual output of garri in Nigeria has been estimated as 1.5 - 2 million metric tones, amounting to a monetary turnover of N150 - 200 million (Ngoddy and Kaplinsky 1968). The large volume of wastewater generated in the processing of *Cassava* is often discharged untreated into the environment. In this study, the relative effects of fresh and aged *Cassava* processing effluents on the physio-chemical properties to top soil are examined.

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Experimental

Collection of soil sample and Cassava effluent. The soil sample used for this study was collected from a fallow land in Ekpoma. Composite topsoil (0 - 15cm) sample was collected, air - dried, crushed, mixed, sieved through 2mm sieve and stored in a plastic container. The soil sample was characterized in terms of pH, organic carbon, exchangeable bases and acidity, nitrogen, phosphorus and particle size distribution.

Fresh *Cassava* processing effluent was collected at a *Cassava* processing mill in Ekpoma. One half of the *Cassava* effluent was aged (allowed to stand) for 7 days before use. The fresh and aged samples of the effluents were characterized in terms of pH, organic carbon, nitrogen, phosphorus, exchangeable bases and cyanide content.

Treatment of the soil sample with Cassava effluent. Three hundred g of the soil samples were separately weighed into ten plastic containers. Five of the weighed soil specimens were mixed thoroughly with 100ml of fresh effluent and air - dried for 5,10,15,20, and 25 days respectively. The samples were designated F_5, F_{10},F_{15},F_{20} and F_{25}. Remaining five samples were treated in the same manner with aged *Cassava* effluent. The samples were designated A_5, A_{10}, A_{15}, A_{20}, and A_{25}. The fol-

Table 1
Characteristics of the fresh and aged *Cassava* effluents

Parameters tested	Fresh *Cassava* effluent	Aged *Cassava* effluent
pH	6.30	6.00
Carbon(%)	0.46	0.52
Nitrogen(%)	0.27	0.36
Phosphorus(ppm)	192.00	168.20
Sodium (meq / 100 soil)	1.90	0.54
Potassium ″	2.70	1.45
Calcium ″	0.68	0.92
Magnesium ″	0.85	0.87
Cyanide (meq / 1)	2.56	1.98

lowing determinations were carried out using the various soil samples.

pH of soil sample in water. Twenty g of the soil sample was weighed into a 50ml beaker; 20ml of distilled water was added, stirred and allowed to stand for 30 min. The pH of the mixture was then determined using a Bechman pH meter.

Soil organic carbon. The organic carbon contents of the soil samples were determined by Walkely Black method (Black 1965).

Exchangeable bases (calcium magnesium, potassium and sodium). Calcium and magnesium in the soil samples were determined using the EDTA complexometric method (Jackson 1958), while the potassium and sodium were determined by the flame photometry (Jackson 1958).

Exchangeable acidity (hydrogen and aluminium). The exchangeable acidity of the soil samples was determined by titration of the KCl extract of the sample with 0.01M NaOH (Jackson 1958). The exchangeable acidity in the soil samples were expressed in meq/100 of soil.

Phosphorus. The phosphorus contents of the soil samples were determined using the Bray PI method (Bray and Kurtz 1945).

Total nitrogen. The total nitrogen content of the soil samples was determined using the Macro - Kjeldhal digestion - distillation method (Bremner 1960).

Physical properties of the soil sample by mechanical analysis (Davidson 1953). Fifty g of air-dried soil was weighed into a shaking bottle. 50ml of distilled water was added, mixed with a stirring rod and allowed to set for 30 min. The soil suspension was then stirred with a mechanical stirrer for 30 min.

Table 2
Physio - chemical characteristics of the untreated soil sample

Parameters tested	Composition
pH	6.60
Carbon(%)	0.38
Nitrogen(%)	0.46
Phosphorus(ppm)	5.30
Sodium (meq / 100 soil)	0.06
Potassium ″	0.10
Calcium ″	7.92
Magnesium ″	0.88
Hydrogen ″	0.20
Aluminium ″	9.26
ECEC	9.26
Clay (%)	2.30
Silt (%)	4.40
Sand (%)	93.30

The suspension was transferred to the mechanical analysis glass cylinder and made up to mark with distilled water. The cylinder was shaken by inverting it several times until all the soil went in suspension. The cylinder was placed on a flat surface and the time was noted. A hydrometer was slowly slided into the suspension and the first reading on the hydrometer was recorded after 60 sec to obtain the percentage sand in the soil. The second reading of the hydrometer was taken after 2 h to obtain the percentage clay. The difference in the hydrometer readings gives the percentage silt.

Characterisation of Cassava processing effluents. The fresh aged *Cassava* processing effluents were characterised to determine their composition. The pH, the carbon, nitrogen and phosphorus contents and the exchangeable bases were determined.

The cyanide contents of the effluents were determined by titrating 25ml of the *Cassava* effluent with 0.1N silver nitrate using diphenyl carbazine as indicator (Vogel 1978).

Results and Discussion

The results obtained from the analysis of the fresh and aged *Cassava* effluents and the various soil samples are shown in Table 1 to 4. The *Cassava* effluents were found to be rich in phosphorus, potassium and sodium. The fresh effluent contained 192 ppm phosphorus, 2.70 meq /l potassium and 1.90 meq /l potassium and 0.54 meq / l sodium as compared to the untreated soil which contained 5.30 ppm phosphorus, 0.10 meq potassium and 0.06 meq sodium per 100 g soil.

Table 3
Physio - chemical characteristics of soil samples treated with fresh *Cassava* effluent

Parameters tested	F_5	F_{10}	F_{15}	F_{20}	F_{25}
pH	7.30	7.70	7.90	8.00	8.00
Carbon (%)	0.96	0.90	0.77	0.70	0.65
Nitrogen (%)	0.27	0.22	0.19	0.14	0.12
Phosphorus (ppm)	60.40	66.70	70.60	71.90	70.80
Sodium (meq/100 soil)	0.27	0.21	0.15	0.13	0.11
Potassium ″	0.57	0.54	0.53	0.49	0.47
Calcium ″	7.12	7.20	7.28	7.52	7.76
Magnesium ″	2.64	2.56	1.60	0.88	0.16
Hydrogen ″	0.20	0.20	0.20	0.30	0.20
Aluminium ″	0.00	0.00	0.00	0.00	0.00
ECEC	10.80	10.61	9.76	9.32	8.70
Clay (%)	2.30	1.80	2.30	2.30	2.30
Silt (%)	3.40	2.90	3.40	3.40	3.40
Sand (%)	94.30	95.30	94.30	94.30	94.30

Table 4
Physio - chemical characteristics of soil samples treated with aged *Cassava* effluent

Parameters tested	F_5	F_{10}	F_{15}	F_{20}	F_{25}
pH	7.40	7.80	8.20	8.20	8.30
Carbon (%)	1.18	1.12	1.09	0.90	0.76
Nitrogen (%)	0.44	0.42	0.38	0.27	0.26
Phosphorus (ppm)	42.40	43.50	51.50	51.50	52.00
Sodium (meq/100 soil)	0.09	0.08	0.09	0.10	0.11
Potassium ″	0.20	0.20	0.22	0.25	0.29
Calcium ″	8.82	8.88	8.40	8.08	7.20
Magnesium ″	2.55	1.58	0.96	0.64	0.40
Hydrogen ″	0.20	0.20	0.20	0.20	0.10
Aluminium ″	0.00	0.00	0.00	0.00	0.00
ECEC	11.86	10.94	9.87	9.27	9.27
Clay (%)	2.30	1.80	2.30	2.30	2.30
Silt (%)	3.40	2.90	3.40	3.40	3.40
Sand (%)	94.30	95.30	94.30	94.30	94.30

The results in Table 3 and 4 show that there was no marked difference in the particle size distribution nature of the soil, before and after treatment with the effluents. The untreated soil sample was found to contain 93.30% sand, 4.40% silt and 2.30% clay. Treatment with fresh *Cassava* effluent increased the sand content to 95.30% and reduced the silt content to 2.90% after the soil/effluent contact period of 10 days. Pollution did not show any appreciable effect on the clay content of the sample. Similar results were obtained when aged *Cassava* effluent was used instead of the fresh effluent.

The pH of the untreated soil, the fresh and aged effluents were 6.60, 6.30 and 6.00, respectively. The pH levels of the soil samples treated with aged effluents ranged from 7.30 to 8.00 while those of samples treated with aged effluents ranged from 7.40 to 8.30 as the contact period increased from 5 to 25 days. The results show that *Cassava* processing effluents (fresh or aged) increase the pH of the soil making it alkaline. However, the increase in pH was more pronounced when the aged effluents were used. Soil alkalinity is mainly caused by the presence of OH^- and HCO_3^- anions. These anions are generally produced by the hydrolysis of carbonates in the soil, particularly sodium carbonate. The increase in the pH of the soil samples following treatment with *Cassava* effluents could be due to the fermentation of the effluents during which CO_2 is released. The increase in pH of the soil from 6.60 to 8.30 could have a negative effect on the growth of plants, as it is known that plants survive in a pH range of 5.70 to 7.80 (Egharevba and Mayah 2001).

The organic carbon content of the soil increases form 0.38% for the untreated soil to 0.96% when the soil was treated with fresh effluent for a period of 5 days. Further increases in the contact period reduced the organic carbon content to 0.65 (25 days contact period). When the aged effluent was used, the percentage of carbon contents was generally higher than the fresh effluent. The organic carbon increased to 1.18% at the contact period of 5 days and decreased to 0.76 at the contact period of 25 days. The general increase in the levels of organic carbon of the soil can be attributed to the contribution from the effluents.

The nitrogen contents of the soil samples were depleted when the fresh and fermented effluents were used to pollute the soil samples. However, the negative effect produced by the fresh effluent was more than the fermented effluent.

The nitrogen content decreased from 0.46 % to 0.12 and 0.26% after treating for 25 days with fresh and aged effluents respectively. Adequate nitrogen in the soil is important for the optimum growth of plants since all the vital processes in plants are associated with the presence of nitrogen. It has been reported that nitrogen functions effectively in the structure of proteins, vitamins, hormones and chlorophyll (Dvayi and Ekong 1981). Fresh *Cassava* effluent would in particular render the soil infertile. The decrease in the level of nitrogen in the soil after treatment with the effluents can be attributed to volatilization of the gaseous nutrient from the surface of the soil as well as denitrification (Ulysses 1982). Of the nutrients that plants normally require nitrogen is the most easily lost as ammonia particularly in soil that is poorly drained at a pH of about 7 (Ulysses 1982). The pH of the soil sample treated with fresh and aged effluents varied from 7.30 - 8.00 and 7.40

- 8.30 respectively when the soil / effluent contact period varied from 5 to 25 days. At a pH of about 7, some nitrogen could have been lost as ammonia. In the process of denitrification, nitrogen can be converted by anaerobic bacteria present in the effluents to gaseous nitrogen, which is lost to the atmosphere.

The phosphorus content of the soil was markedly increased from 5.30 ppm for the unpolluted soil to 71.90 ppm and 52.00 ppm when the soil samples were treated with fresh and aged effluents for 25 days respectively. This was justified because the analysis of the *Cassava* effluents revealed a high phosphorus content of 192.00 ppm and 168.20 ppm for the fresh and aged effluents respectively. Phosphorus is a highly predominant mineral in *Cassava* tuber. It is important in the plant system for carbohydrate breakdown for energy release, cell division and stimulation of early root growth and development. It also enhances early maturation of seeds and fruits (Jones 1982). This study has shown that polluting the soil with *Cassava* effluent could enrich the soil with phosphorus.

The results obtained for the exchangeable bases show that the levels of sodium and potassium generally increases when the soil is treated with the fresh and aged effluents. The level of calcium in the soil was reduced when treated with fresh effluent, but it increased from 7.92 to 8.88 meq /100g soil when treated with aged effluent at a contact period of 10 days. The levels of magnesium decreased from 0.88 meq per 100g soil to 0.16 meq and 0.40 meq per 100g soil at a contact period of 25 days respectively, when the soil samples were treated with fresh and aged *Cassava* effluents. The decrease in the levels of calcium and magnesium can be attributed to their precipitation as insoluble phosphates (Egharevba and Mayah 2001).

The exchangeable bases are important plant nutrient elements in the soil. Treatment of soil with fresh or aged effluent did not produce any marked effect on the available hydrogen and aluminium in the soil sample.

The Effective Cation Exchange Capacities (ECEC) of the soil samples increased from 9.26 meq /100g soil for the untreated soil to 10.80 and 11.86 meq/100g soil respectively, when the soil samples were treated with fresh and aged effluents for 5 days. The values decreased to 8.70 and 9.27 after 25 days of treatment with fresh and aged effluent.

This study shows that polluting the top - soil with fresh and fermented *Cassava* effluents lead to a diverse effect on the physio - chemical properties of the soil. The increase in pH and the depletion of nitrogen and calcium when fresh *Cassava* effluent was used to pollute the soil could have a negative effect on the fertility of the soil. The deteriorating effect of pollution on the nitrogen content of the soil was less pronounced when the aged effluents were used. Also, the calcium contents of the soil was enhanced when the aged effluent was used to pollute the soil. It can therefore, be recommended that effluents from *Cassava* processing mills should be stored in a tank for a suitable period of time before it is discharged into the environment.

References

Black C A 1965 Methods of soil analysis. *Amer Soc Agronomy, Madison,* **2** (9) 6.

Bray R H, Kurtz L T 1945 Determination of total organic and available forms of phosphorus in soils. *Soil Sci* **37**, 29 - 39.

Bremner J M 1960 Determination of nitrogen in soils by Kjeldahl method. *J Agric Sci* **55** 11 - 13.

Davidson D T 1953 Mechanical analysis of soils, *Lowa Engineering Experiment Station,* Report No. 21 p 23.

Dvayi E A, Ekong E E 1981 *General Agriculture and Soils.* Cassell Publishers Ltd. London, UK, pp 53 - 63.

Egharevba F, Mayah DA 2001 The effect of *Cassava* peel extract on the chemical properties of soil. *Nig J App Sci* **19** 99 - 100.

Jackson M L 1958 *Soil Chemical Analysis.* Prentice - Hall, Inc., Eaglewood Cliffs, New Jersey, USA, P 10,14, 126.

Jones J G 1982 Effect of gross pollution by kerosine hydrocarbons on soil. *Nature* 227.

Nestel B L 1973 Chronic *Cassava* toxicity. In: *Proceedings of an Interdisciplinary Workshop* London 29 - 30 January 1973, Ottawa, International Development Research Centre, Ottawa, P 5.

Ngoddy P O, Kaplinsky R 1968 Technical Review on Food. International Technical Cooperation Centre. *Tel Aviv* **5** p 56.

Olayide S O, Olatunbosun D, Idusogie E O, Abiagon J D 1972 *A Report of Qualitative Analysis of Food Requirements, Supplies and Demands in Nigeria* 1965 - 1985. Federal Department of Agriculture, Longman, Nigeria, P 12.

Rosling H 1988 *Cassava Toxicity and Food Security. A Review of Health Effects of Cyanide Exposure from Cassava and Ways to Prevent these Effects.* A Report for UNICEF African Household Food Security Programme, 2nd ed., Tryck Kontakt, Uppsala, Sweden.

Ulysses J S 1982 *Fertilizers and Soil Fertility.* Reston Publishing Co. Inc Reston, Virginia, USA 2nd ed, p 27 - 28.

Vogel A I 1978 *Textbook of Quantitative Inorganic Analysis.* Longman, Group Ltd., England 4th ed., p 325.

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GEOCHEMICAL AND FLOTATION STUDIES OF COPPER ORE OF NORTH WAZIRSTAN AGENCY

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Important copper deposits of North Wazirstan Agency are confined to Shinkai and Degan Paikhel areas Miran Shah Town. The copper mineralization in these areas is of native and sulphide types as veins, veinlets, stringers and rims around fragments and associated with cupriferous breecia. The cupriferous massive sulphides occur in basalts belonging to obducted ophiollite complex. An average copper contents of 17 ore samples is 0.45%. The flotation results show that maximum liberation of chalcopyrite with gangue minerals occurs at - 63 (240 mesh) size. The complex nature of valuable minerals with gangue does not hinder the separation of copper from the gangue materials. Results show that the copper contents of the ore were enhanced from 0.5% to 24.0% in the concentrate. For ensuring better results about grade and recoveries, further studies on second and third stage flotation are needed.

Key words: Copper mineralization, Sulphide, Ophiolite complex, Froth flotation.

Introduction

The Miran Shah is the head quarter of North Wazirstan in the North West Frontier Province. Important copper deposits have been found at Shinkai near Boya located (longitude 69°, 55° E and altitude 32°, 57° N) at a distance of 19 km from Miran Shah and in the adjoining areas of Deggan and Khaddar Khel (Badshah 1983). A number of chromite and manganese deposits have also been reported from the North Wazirstan Agency. Important occurrences of copper are confined to Shinkai and Deggan Paikhel areas near Miran Shah Town. The mineralization in these areas is characterized as copper native and its sulphides are generally associated with breecia and occurs as veins, veinlets, stringers and rims around fragments (Khan 1998). Present work deals with geochemical and flotation studies of Shinaki copper ore and its associated rocks to assess the economic potential of these deposits.

Geological setting. The Wazirstan plateau overlooks the vast Bannu - D.I. Khan predominant plane in the east, which is part of the desert fringe zones of Indus basin. The eastern abutment of Wazirstan plateau is a thousand feet thick deltaic mass where thin interbedded Cretaceous limestone, shale,siltstone, Eocene limestone and shale are encountered . These rocks are intensely folded and thrust eastward on younger Muree Siwalik - Molsse Group of rocks. The western part of Wazirstan constitutes a complex igneous belt characteristic of tectonic activity at plate scale. The belt represents collision suture zone between Indo - Pakistan plate to the east and Kabul plate to the west. The intense folding of Cretaceous rock at the eastern contact of the igneous complex is indicative of allochthonous emplacement of ophiolites (Badshah 1983).

The igneous belts extend from north east to south west and consists of ultramafic masses, mafic to acidic injections and ultrabasic to basic volcanics. The ultramafic rocks consist of harzbergite, pyoxenite, peridotite and dunite which are generally altered and serpentinized. The intrusives, subordinate on occurrence comprise of diorite, quartz diorite, micro - quartz diorite, granodiobite and gabbro. Volcaic rocks include fine grained porphyritic pillow basalts and andesites with subordinate breecia and minor dacites, tuffs and agglomerates (Badshah 1983).

The ophiolites are generally dismembered and have a complex geological configuration (Afridi *et al*, 1991). On a regional scale, however, the ophiolites can be roughly identified into a basal,part of ultrabasic, an intermediate zone of sheeted dykes or sills and a zone of basaltic and andestie pillow lavas. Presence of jasperites is considered to represent the pelagic sediments hosting manganese oxide deposits. Chromite deposits occur in serpentinized dunites, manganese oxide deposits occur in sediments and cupriferous massive sulphides are hosted in basalts which occur in obducted ophiolitie complex and ophiolitic melange along the collisional suture zone in the Indian and Eurasuian plates.

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Table 1
Chemical evaluation of copper ore (15 samples) of North Wazirstan Agency for major elements % composition of major elements

SiO_2	$[Fe_2O_3]$*	Al_2O_3	CaO	MgO	L.O.I	Total
52.52	14.50	06.20	08.08	10.20	08.60	100.10
60.00	10.30	04.20	08.50	10.25	06.80	100.05
53.20	20.50	07.20	02.50	07.50	08.90	99.80
48.36	25.20	08.70	02.60	08.00	07.10	99.90
60.24	15.30	08.40	01.68	08.00	06.40	100.02
54.40	20.30	09.20	01.68	07.80	06.23	99.61
60.20	17.00	06.20	04.20	06.40	05.80	99.80
58.24	18.40	09.80	08.60	02.40	02.40	99.84
41.04	06.30	07.90	01.12	29.30	13.90	99.56
0.62	00.80	06.90	50.40	00.00	41.05	99.77
04.70	14.30	06.20	23.00	12.40	13.50	100.10
50.98	14.00	08.00	11.20	05.60	10.10	93.88
64.54	14.40	06.30	01.65	08.00	05.00	99.58
54.60	21.10	10.00	01.68	08.40	04.80	99.58
62.40	15.20	09.80	03.36	02.80	06.25	99.58

* Fe_2O_3 as total iron
L.O.I Loss on Ignition at 100˚C

Table 2
Determination of copper content in the ore (28 samples) North Wazirstan Agency

% Composition of elements							
Cu	Co	Mn	Ni	Pb	Zn	Ag	Au
0.150	0.010	0.100	0.050	0.0005	0.060	0.050	<0.001
0.180	0.010	0.150	0.030	0.0000	0.140	0.003	<0.001
0.070	0.000	0.050	0.020	0.0004	0.180	0.002	<0.001
0.160	0.010	0.090	0.010	0.0003	0.020	0.003	<0.001
0.200	0.010	0.180	0.010	0.0000	0.040	0.004	<0.001
0.000	0.010	0.000	0.020	0.0002	0.020	0.001	<0.001
0.000	0.010	0.000	0.010	0.0000	0.020	0.002	<0.001
0.100	0.010	0.000	0.010	0.0000	0.020	0.005	<0.001
0.000	0.010	0.000	0.010	0.0000	0.020	0.005	<0.001
0.100	0.020	0.000	0.270	0.0005	0.020	0.000	<0.001
0.320	0.000	0.130	0.000	0.0001	0.010	0.002	<0.001
0.500	0.010	0.250	0.010	0.0005	0.030	0.000	<0.001
0.060	0.000	0.000	0.000	0.0000	0.020	0.000	<0.001
0.080	0.010	0.000	0.030	0.0003	0.010	0.000	<0.001
0.000	0.010	0.000	0.000	0.0005	0.070	0.001	<0.001

Experimental

Standard procedures (Furman 1963) were applied for chemical evaluation of the representative 15 samples of crude copper ore from North Wazirstan Agency for their major elements. The data obtained (Table I) indicate that the contents of silica (SiO_2) vary from 0.62 to 64.54%, Fe_2O_3 (total) 0.80 to 25.20%, Al_2O_3 4.20 to 9.80%, CaO 1.12 to 23.00% and MgO 0.00 to 29.30%, whereas loss on ignition at 100˚C is in the range of 2.40 to 13.90%. Flame Atomic Absorption Spectrophotometer (Model Z - 8000, Hitachi, Japan) detection limit 0.004 ppm, sensitivity 0.03 ppm and working standards 0.5 ppm to 4.0 ppm was used for the determination of copper in copper ore (Table 3). The copper contents of ore samples range from 0.12 to 0.98% and the average copper content is 0.50%. The minor elements such as Co, Mn, Ni, Pb, Zn, Ag, and Au were also determined using Atomic Absorption Spectrophotometric methods (Table 2).

The X - ray diffraction (XRD) analysis shows that the ore samples contain chalcopyrite, pyrite, and magnetite as major valuable metallic minerals, and brochanite, jasperite, chamosite, namite and quartz as gangue minerals.

The particle size determination of major valuable metallic minerals chalcopyrite, pyrite and magnetite are given in (Table 4). Other minor metallic minerals were found with very low contents. Liberation studies were conducted to obtain liberated chalcopyrite with gangue minerals. The maximum liberation of chalcopyrite with gangue minerals occurs at - 63 microns (240 mesh) size. After undertaking chemical analysis of 28 samples for their copper content, all these samples were combined (28 kg) and crushed in jaw crusher to obtain a suitable feed for grinding. The grinding and powdering was carried out using a laboratory rod mill (Model WEDAG Westfalia, Bochum) and electric sieve shaker (Endecott, Model 2 MK II, London). Regrinding method was carried out to achieve the desired mesh of liberation of chalcopyrite in the ground product.

A series of flotation tests were carried out to determine the effect of collector, potassium propyl xanthate (kpx) concentration and activator sodium sulphide (Table 5). The commercially available kpx was recrystallized twice from acetone by addition of petroleum ether and dried under vacuum to avoid any atmospheric oxidation. Analytical reagent grade sodium sulphide was used to study the effect of activator in flotation. Solutions were made with distilled water (Khan 1998). All the flotation tests were carried out by using laboratory flotation machine (Denver, England) in a litre cell at 25% (weight / volume) pulp density. The impeller speed was kept constant at 1200 rmp throughout the flotation tests. 25 g powdered (240 mesh) copper ore was conditioned prior to its flotation with 60 g / ton sodium sulphide activator for five minutes and subsequently with collector for five minutes. The effect of various concentrations of collector are given in Table 5. The desired value of pH (9 - 10) was adjusted with lime water and was kept constant throughout the flotation tests, 2 - 3 drops of frother (cynamide, aero froth 65) was used for flotation. Time

Table 3
Determination of copper content in the ore (28 samples) of North Wazirstan Agency

S. No	Sample No.	Cu (%)	Rock / ore
1	P3 - 1	0.03	Associated rock
2	P3 - 2	0.05	Associated rock
3	P3 - 3	0.04	Associated rock
4	P3 - 4	0.02	Associated rock
5	P3 - A1	0.27	Ore
6	P3 - A2	0.33	Ore
7	P3 - A3	0.07	Associated rock
8	P3 - A4	0.09	Associated rock
9	P3 - B1	0.94	Ore
10	P3 - B2	0.41	Ore
11	P3 - B3	0.05	Associated rock
12	P3 - B4	0.64	Ore
13	P3 - C1	0.59	Ore
14	P3 - C2	0.30	Ore
15	P3 - C3	0.40	Ore
16	P3 - C4	0.49	Ore
17	P3 - D1	0.22	Ore
18	P3 - D2	0.21	Ore
19	P3 - D3	0.19	Ore
20	P3 - D4	0.17	Ore
21	P3 - E1	0.98	Ore
22	P3 - E2	0.42	Ore
23	P3 - E3	0.05	Associated rock
24	P3 - E4	0.12	Ore
25	P3 - F1	0.25	Ore
26	P3 - F2	0.08	Associated rock
27	P3 - F3	0.38	Ore
28	P3 - F4	0.10	Associated rock

batch method was used during each flotation test and the products were obtained after 5 minutes each . The flotation time was normally noted to be less than 15 min. The copper concentrate, middling and tailing obtained were dried and assayed for copper contents by atomic absorption spectrophototmetric technique (Rao *et al* 1976; Xiang and Yen 1998).

Results and Discussion

The copper content in the North Wazirstan Agency copper ore ranges from 0.12 to 0.98% and the average copper content of 28 samples is 0.48%. Other minor elements are not present in significant quantity. The major valuable metallic minerals are chalcopyrite, pyrite and magnetite, other minerals such as galena, sphalerite etc. and found with low contents. Therefore, size determination was made only on chalcopyrite, pyrite and

Table 4
Results of particle size determination of principal minerals

Average size	Chalcopyrite %		Pyrite %		Magnetite %	
µm	Ind.	Cum	Ind.	Cum	Ind.	Cum
1.286	2.330	-	-	-	4.26	-
1.000	5.620	7.95	7.88		7.95	12.21
0.503	13.230	21.18	17.73	25.61	8.49	20.70
0.253	18.950	40.13	31.49	57.10	11.89	32.59
0.128	17.200	57.33	20.96	78.06	20.72	53.31
0.060	24.700	82.03	16.01	94.07	27.23	80.54
0.020	14.490	96.52	5.18	99.25	14.89	95.43
0.005	3.480	100.00	0.75	100.00	4.57	10.00

Table 5
Effect of potassium propyl xanthate concentration on the flotation studies of copper ore (containing 0.48% Cu)

Collector dosage g / ton	Flotation fraction	%Cu	% Recovery	Cum. % recovery	Cum. % grade
	Conc. 1	14.00	52.00	42.00	14.00
50	Conc. 2	9.34	23.56	65.56	10.50
	Middling	6.00	7.44	73.00	9.70
	Tailing	0.56	17.00	100.00	1.70
	Conc. 1	19.00	54.36	36.36	19.00
100	Conc. 2	9.10	22.25	58.61	12.02
	Middling	5.00	10.24	78.85	8.35
	Tailing	0.43	13.15	100.00	1.47
	Conc. 1	20.00	56.25	34.24	20.00
150	Conc. 2	16.00	21.09	85.33	17.00
	Middling	5.52	11.79	87.12	10.00
	Tailing	0.30	10.88	100.00	1.93
	Conc. 1	21.00	47.23	47.23	21.00
200	Conc. 2	18.00	25.88	73.11	15.25
	Middling	6.10	18.08	91.19	11.00
	Tailing	0.20	6.31	100.00	2.00
	Conc. 1	22.92	58.03	58.03	22.91
250	Conc. 2	9.40	11.26	69.29	15.72
	Middling	6.20	8.72	78.01	12.82
	Tailing	0.40	21.99	100.00	1.71
	Conc. 1	24.00	51.65	61.65	24.00
300	Conc. 2	15.00	39.06	90.71	17.87
	Middling	3.70	4.00	94.71	14.21
	Tailing	0.11	5.29	100.00	1.82
	Conc. 1	23.00	66.22	66.22	23.00
400	Conc. 2	1.48	21.19	86.45	1.35
	Middling	3.30	4.98	91.30	12.89
	Tailing	0.24	8.61	100.00	2.31
	Conc. 1	20.15	45.20	45.20	20.15
600	Conc. 2	12.23	18.09	63.29	15.94
	Middling	4.85	5.82	69.11	12.61
	Tailing	1.19	30.89	100.00	3.18

magnetite. Particle size of chalcopyrite is found in a rather wide range with 0.060 mm fraction accounting for 82.03% - 0.06 mm fraction about 18%. If one stage grinding was used to liberate all chalcopyrite particles, a considerable part of chalcopyrite and other minerals would be overground, which would not only need more equipment and energy consumption but also cause difficulties in its flotation. Therefore, a stage grinding with coarser one to discard tailing followed by regrinding of rough concentrate has been recommended.

Sulphide minerals are among the simplest to float but commercially very important. There were the earliest group of minerals treated in this way and have been the subject of numerous researchers (Leija 1982; Critechley and Riaz 1991; Riaz *et al* 2001). The North Wazirstan Agency copper deposits are porphyry copper sulphide ore, containing predominantly chalcopyrite and pyrite. Therefore, a lime circuit was used in these studies to keep the pH in the range of 9 - 10. Use of lime was preferred as it acts as depressant for iron sulphide gangue minerals.

A series of flotation tests were conducted with different collector dosages, keeping all other flotation parameters constant. The increase of collector concentration upto 300 g / ton of the ore increases the copper content in concentrate with improved percent recovery and grade. Best flotation results were obtained with 300 g / ton of collector concentration with enhanced copper content from 0.5% to about 24%. The increased concentration of collector over this value affects the chalcopyrite flotation adversely. The results obtained by froth flotation technique indicate that it is possible to obtain good separation of chalcopyrite from the gangue materials. Despite the complex nature of the ore, the upgradation is quite satisfactory. For obtaining better grade and recoveries, further studies for two stage flotation are recommended.

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References

Afridi M I, Badshah M S, Ihsanullah M 1991 Copper occurrences in Wazirstan In: *Proceedings of First SEGMITE Symposium*, Peshawar, Pakistan. pp 70 - 73.

Badshah M S 1983 Geology and breccia pipe primary and secondary copper mineralization in Waziristan. In: *Proceedings of 2nd National Seminar on Development Mineral Resources*, Peshawar, Pakistan. pp 1 - 15.

Critechley J K, Riaz M 1991 Study of synergism between xanthate and dithiocarbamate collectors in flotation of heazlewoodite. *Trans International Mineral Metal* (section C: London UK) 100, 55 - 57.

Furman H N 1963 Standard Methods of Chemical Analysis. **1** 6th ed. D.Van Nostrand Company Inc. Princeton, New York, USA, pp 395 - 417.

Khan K 1998 Hydrometallurgical methods for extraction and recovery of metals of industrial importance with tertiary amines. Ph.D thesis, Department of Chemistry, University of Peshawar, Pakistan.

Leja Ian 1982 *Surface Chemistry of Froth Flotation*. A Plenum Press, New York, USA, pp 607 - 609.

Riaz M, Khan A R, Mumtaz, Khan K, Jan A 2001 Synergistic effect between thiol collectors in reaction with sulphide minerals. *Pak J Sci Ind Res* **44**(5), 257 - 272.

Rao S R, Moon K S, Leja I 1976 Effect of grinding media on the surface reaction (and flotation) of heavy metal sulfides. Memorial M.C. Fuerstenau, AIME, *Am. Inst. Min. Metal Engg.* New York, USA, **1** pp 509 - 527.

Xiang H W, Xen X 1998 The effect of grinding media and environment on the surface properties and flotation behavior of sulphide minerals. *International Journal of Mineral Processing* **7**, 49 - 79.

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SYNTHESIS AND CHARACTERIZATION OF SILYL - GROUP - CONTAINING FLUORENES

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Cyclization reactions of biphenyl with dichloropropyltrichlorosilane and dichlorobutyltrichlorosilane in the presence of anhydrous aluminum chloride catalyst gave 9 - (2 - trichlorosilylethyl)fluorene and 9 - methyl - 9 - (2 - trichlorosilylethyl) fluorene along with isomeric uncyclized products. All the compounds were structurally identified by GC / MS, ^{1}H and ^{13}C - NMR spectroscopy.

***Key words*:** Cyclization of biphenyl, Friedel - Crafts alkylation, Chlorosilyl - ethyl fluorenes.

Introduction

The alkylation of biphenyl was first carried out in the presence of BF_3 catalyst (Romadane 1957, 1959; Romadane *et al* 1957). Based on their work in the 1950s, the products were mixtures of 4 - alkyl - and 4, 4 - dialkylbiphenyl. Although, various fluorenyl - silanes, i.e. dimethylbis (9 - fluorenyl) silane (Patsidis *et al* 1996; Resconi *et al* 1996) and dimethyl (9 - fluorenyl) cyclopentadienylsilane (Silaghi *et al* 1995) are known to be prepared from lithiation reaction of fluorene, the cyclization of biphenyl with alkyl halides containing silyl groups has never been reported.

The present paper describes synthesis of new fluorenyl derivatives containing silicon metal as precursors for blue - light - emitting polyfluorenes. 9 - (2 - trichlorosilylethyl)fluorene and 9 - methyl - 9 - (2 - trichlorosilylethyl)fluorene were synthesized through cyclization reactions of biphenyl with dichloropropyltrichlorosilane and dichlorobutyltrichloro - silane, in the presence of anhydrous aluminum chloride. All these compounds were structurally identified by GC / MS, ^{1}H and ^{13}C - NMR spectroscopy.

Experimental

All reactions were carried out under nitrogen atmosphere. All glassware used was flame - dried or oven - dried before use. Solvents were purified using standard purification procedures (Perrin *et al* 1980). Anhydrous aluminum chloride, trichlorosilane, H_2PtCl_6 (0.1M solution in IPA), triphenylphosphine, $PdCl_2$ and 1,3 - butadiene were purchased from Aldrich Chemical Co. and used without further purification. Biphenyl was purchased from Yakari Pure Chemical Co. while 3 - chloropropyltrichlorosilane and trichlorosilyl - 2 - butene were prepared by the reported procedure (Tsuji *et al* 1974). Dichloropropyltrichlorosilane and dichlorobutyltrichlorosilane were prepared by passing chlorine gas through 3 - chloropropyltrichlorosilane and trichlorosilyl - 2 - butene respectively.

All air - sensitive liquids and dried solvents were transferred by standard syringe or double - tipped - needle technique. Reaction products were analysed by GLC using a capillary column (SE - 30, 30m) or a packed column (10% OV - 101 on 80 - 100 mesh Chromosorb W / AW, 1 / 8 in x1.5 in) on a Varian 3300 gas chromatograph equipped with a flame ionization detector or a thermal conductivity detector. The samples for characterization were purified by preparative GLC using a Varian aerograph series 1400 gas chromatograph with a thermal conductivity detector and a 2m by 1 / 8in. stainless steel column packed with 20% OV - 101 on 80 - 100 mesh chromosorb P / AW. Mass spectra were obtained using a Hewlett - Packard 5890 Series II gas chromatograph equipped with a 5972 mass selective detector.

NMR spectra were recorded on a Varian spectrometer using samples in $CDCl_3$ solution, ^{1}H - NMR spectra at 300 MHz and ^{13}C NMR at 75.4 MHz.

Syntheses. 9 - (2 - Trichlorosilylethyl) fluorene (1). Biphenyl, 80g (519 mmol) and anhydrous aluminum chloride, 4.8g (36 mmol) were charged into a 500c.c. 2 - neck flask attached with a reflux condenser. Biphenyl was allowed to melt under N_2 atmosphere. While it was stirring, dichloropropyl - trichlorosilane, 64g (259.7 mmol) was added very slowly via a syringe. HCl evolution was noticed. The reaction mixture was kept at 130°C for 2 h after that it was worked up by deactiva-ting the $AlCl_3$ with $POCl_3$, 6.6g (43.2 mmol). The reaction mixture was then extracted with dry n - hexane. Low boilers were removed using bulb to bulb distillation, while high boilers were distilled out under vacuum. The product along with the isomers were collected at a b.p. of 130 ~ 134°C / 0.2 torr. Pure 9 - (2 - trichlorosilylethyl)fluorene, 38.28g was obtained through recrystallization from dry Et_2O in 45% yield, based on silane used.

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^1H - NMR. δ 0.93 - 0.99 (m, 2H,CH_2), 2.44 - 2.51 (m, 2H, CH_2), 4.17 - 4.20 (t, 1H, CH), 7.36 - 7.89 (m, 8H, aromatic).
^{13}C - NMR. δ 18.57 (CH_2), 24.71 (CH_2), 47.73 (CH), 120.16, 124.16, 127.36, 127.59, 141.74, 145.41 (aromatic C).
GC / MS (m / e). calcd. for $C_{15}H_{13}SiCl_3$, (M^+) 326, found 326.

9 - Methyl - 9 - (2 - trichlorosilylethyl)fluorene (2a). Eight g (51.88 mmol) biphenyl and 0.33g (2.475 mmol) $AlCl_3$ (anhydrous) were taken into a 50cc, 2 - neck flask connected with a reflux condenser and flushed with N_2. 3.38 Gram (12.97 mmol) of dichlorobutyltrichlorosilane was added via a syringe. The reaction mixture was heated at 100°C for 1 h. After that time the reaction mixture which was purple in colour, was worked up by deactivating the catalyst with $POCl_3$, 0.46g (2.97 mmol). It was extracted with dry n - hexane. Low boilers were removed through bulb to bulb distillation at atmospheric pressure, while high boilers were distilled out at reduced pressure. Yellow oily distillate was obtained at a temperature of 140 ~ 145°C / 0.2 torr which contained a mixture of 3 isomers. The yield of the major product i.e. 9 - methyl - 9 - (2 - trichlorosilylethyl)fluorene was 33% as obtained from GLC analysis. Preparative GC was performed to separate the product which was later on fully characterized by GC / MS, ^1H and ^{13}C - NMR. The other two isomers were characterized as substituted biphenyls as represented by structures (2b) and (2c).

^1H - NMR (2a). δ 0.60 - 0.66 (m, 2H, CH_2), 1.54 (s, 3H, 9 - methyl), 2.24 - 2.30 (m, 2H, CH_2), 7.36 - 7.77 (m, 8H, aromatic).
^{13}C - NMR (2a). δ 19.05 (CH_2), 26.48 (CH_3), 32.56 (CH_2), 51.0 (C_9), 120.13, 122.61, 127.52, 127.64, 141.74, 150.41 (aromatic).
GC / MS (m / e) (2a). Calcd. for $C_{16}H_{15}SiCl_3$ (M^+), 340, found 340.

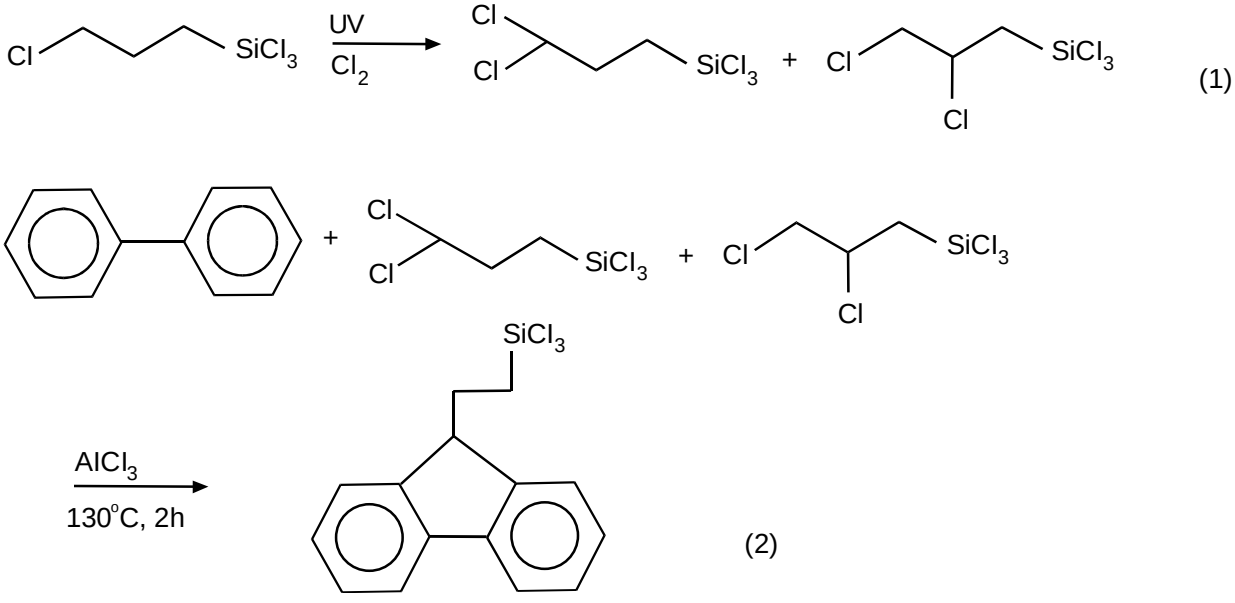
^1H - NMR (2b). δ 1.34 - 1.36 (d t, 5H, CH_3, CH_2), 1.87 - 1.95 (q, 2H, CH_2), 2.78 - 2.85 (h, 1H, CH), 7.27 - 7.62 (m, 9H, aromatic).
^{13}C - NMR (2b). δ 21.03 (CH_3), 21.92 (CH_2), 30.41 (CH_2), 41.33 (CH), 126.78, 126.89, 127.03, 127.16, 139.14, 144.21 (aromatic).
GC / MS (m / e) (2b). Calcd. for $C_{16}H_{17}SiCl_3$ (M^+), 342, found 342.
^1H - NMR (2c). δ 1.33 - 1.35 (d, t, 5H, CH_3, CH_2), 1.85 - 1.94 (q, 2H, CH_2), 2.76 - 2.83 (h, 1H, CH), 7.25 - 7.59 (m, 9H, aromatic).
^{13}C - NMR (2c). δ 21.03 (CH_3), 21.92 (CH_2), 30.41 (CH_2), 41.33 (CH), 126.78, 126.89, 127.03, 127.16, 139.14, 144.21 (aromatic C).
GC / MS (m / e) (2c). Calcd. for $C_{16}H_{17}SiCl_3$ (M^+), 342,found 342.

Results and Discussion

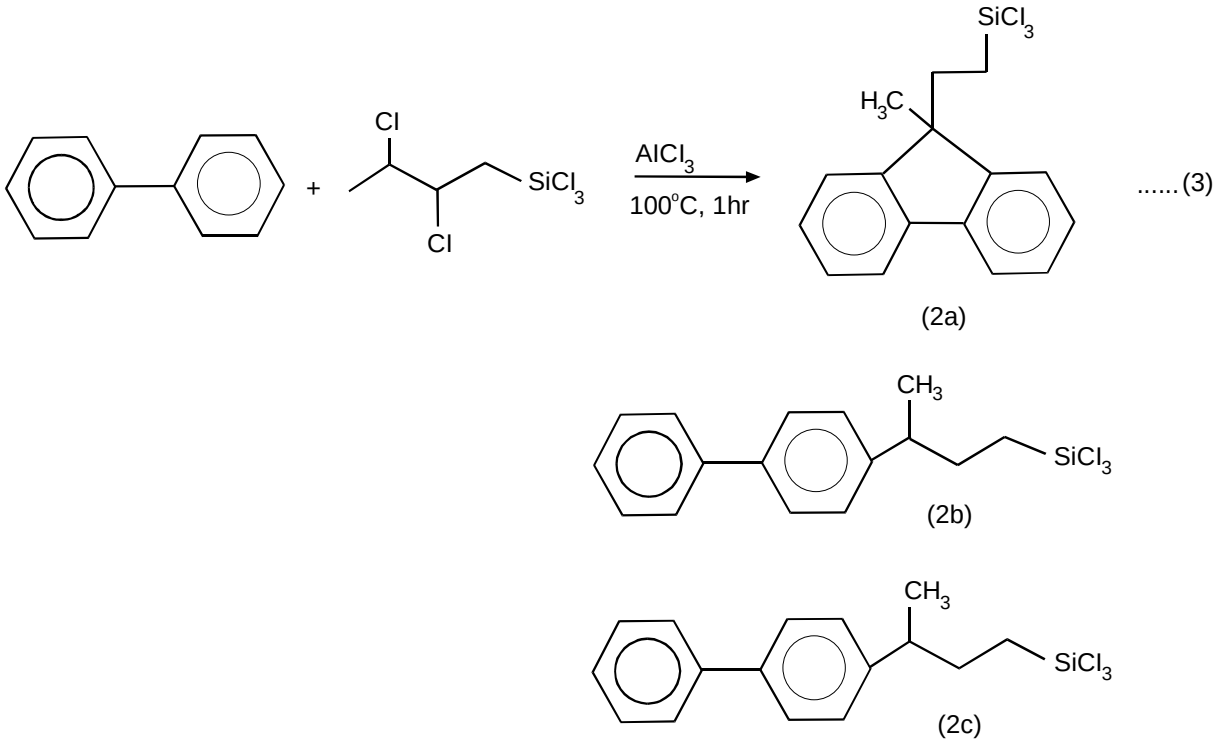
Alkylation reactions. Biphenyl was alkylated with dichloropropyltrichlorosilane in the presence of anhydrous aluminum chloride in order to get 9 - (2 - trichlorosilylethyl)fluorene (1). Dichloropropyltrichlorosilane was obtained by chlorinating 3 - chloropropyltrichlorosilane under UV - irradiation. During the chlorination reaction two isomers of equal ratio i.e. 3, 3 - and 2, 3 - dichloropropyltrichlorosilanes were produced both of which were later on used for the alkylation reaction (rtn. 1 and 2). (1) was obtained in 45% yield, using the molar ratio of 2 : 1 (biphenyl : silane).

The reaction also gave some polymeric material which could not be identified.

Reaction 2 suggests that the γ - C of the dichloropropyltrichlorosilane takes part in the cyclization reaction, forming 9th carbon of the fluorene ring and shifting proton at γ position of 2, 3 - dichloropropyltrichlorosilane to β position after dechlorination.



Scheme 1



Scheme 2

Similarly, 9 - methyl - 9 - (2 - trichlorosilylethyl)fluorene (2a) was synthesized from reaction of biphenyl with 2,3 - dichlorobutyltrichlorosilane (rtn. 3). When biphenyl was reacted with 2, 3 - dichlorobutyltrichlorosilane in molar ratio of 4:1 (biphenyl to silane) for 1 h, 9-methyl-9-(2-trichlorosilylethyl)fluorene (2a) was obtained in 33% yield based on silane used. Two uncyclized side products (2b) and (2c) were also produced in lower yields.

Structural elucidation. The ^{1}H - NMR spectrum of (1) shows two multiplets each of relative area 2 and one triplet (rel. Area 1) in aliphatic region. One multiplet at δ 0.93 - 0.99 ppm corres-ponds to the protons at silicon coordinated carbon while the 2nd multiplet at δ 2.44 - 2.51 ppm is attributed to the protons of the 2nd carbon of the ethyl group. The triplet at δ 4.17 - 4.20 ppm is due to the single proton at the 9th carbon of the fluo-rene ring.

In aromatic region, two sets of peaks are originating. The multiplet at 7.36 - 7.89 corresponds to the six protons (1, 2, 3, 6, 7, 8) and peaks at δ 7.82 and 7.84 ppm are due to protons at 4 and 5 position of the fluorene ring.

The ^{13}C - NMR spectrum shows three resolved peaks in aliphatic region while six peaks are observed in aromatic region and is a good coincidence with the ^{1}H - NMR spectrum.

Similarly, structures for 2a, 2b and 2c were deduced from the ^{1}H and ^{13}C - NMR data.

References

Patsidis K, Alt H G, Milius W, Palackal S J 1996 The synthesis, characterization and polymerization behavior of onsa cyclopentadienylfluorenyl complexes; the X-ray structures of the complexes $[(C_{13}H_8)SiR_2(C_5H_4)]ZrCl_2$(R = Me or Ph). *J Organomet Chem* **509**(1) 63 - 71.

Perrin D D, Armarego W L F, Perrin D R 1980 *Purification of laboratory chemicals*. Pergamon Press, New York, USA, pp 1 - 200.

Resconi L, Jones R L, Rheingold A L, Yap G P A 1996 Highmolecular weight atactic polypropylene from metallocene catalysts. 1 - $Me_2Si(\eta - 9 - Flu)_2ZrX_2$(X = Cl, Me). *Organometallics* **15**(3) 998 - 1005.

Romadane I A 1957 Alkylation of naphthalene with normal alcohols in the presence of boron trifluoride under pressure. *Zhur Obshchei Khim* **27** 1833 - 1835.

Romadane I A 1957 Alkylation of naphthalene with iso alcohols in the presence of boron trifluoride. *Zhur Obshchei Khim* **27** 1939 - 1941.

Romadane I A 1959 Alkylation of naphthalene with a molecular compound of ethyl alcohol with boron trifluoride under pressure. *Zhur Obshchei Khim* **29** 102.

Romadane I A, Dokuchaeva V M 1975 Alkylation of naphthalene with secondary and isobutyl alcohols. Latv PSR Zinat Akad Vestis, *Kim Ser* (1) 61 - 65.

Romadane I A, Shuikin N I, Egorov Ya P 1957. Determination of purity of 1and 2 - butylnaphthalenes by ultraviolet spectra. Izvest Akad Nauk SSSR, *Otd Khim Nauk* 648 - 649.

Silaghi D L, Haiduc I, Escudie J, Couret C, Stage J 1995 Investigations in the field of group 14 difluorenyl compounds. *Synth react inorg metorg chem* **25**(4) 575 - 90.

Tsuji J, Hara M, Ohno K 1974 Organic synthesis by means of noble metal complexes. XCII. Palladium catalysed hydrosilation of monoenes and conjugated dienes. *Tetrahedron* **30**(14) 2143 - 2146.

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Biochemical Changes in Chickpea Roots after Inoculation with Virulent and Hypovirulent. Isolates of *Fusarium oxysporum* f. sp. *Ciceris*

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Total phenolic contents increased in the roots of susceptible and resistant varieties of chickpea after inoculation with the virulent as well as hypovirulent isolates of *Fusarium oxysporum* f. sp. *ciceris* (FOC). Highest increase was observed against the highly virulent isolate 9718 in the roots of Aug - 424 and CM 98. While least increase was found against less virulent isolates viz. 2002 and 2014. Increases in total phenols were more in resistant variety as compared to susceptible one. Inhibition of total phenols was observed in the roots of both varieties nine days after germination by the isolates 2012, 9718 and 2002. No reduction in phenols of both varieties was observed against less virulent isolate 2014. The biautography of the developed TLC by using *Cladosporium cucumerinum* revealed that two inhibition zones were produced by both the varieties against all the FOC isolates. Highest expression of total phenols in chickpea roots was found by the most virulent isolate 9718 followed by 2012 and 2002, reduction was more in CM 98 as compared to Aug - 424. The results suggest that the virulent and hypovirulent isolates produced non-specific elicitors while specific suppressors were produced by the isolates 9718, 2012 and 2002.

Key words**:** *Fusarium oxysporum* f. sp. *ciceris*, Virulent, Phenols, Elicitor, Suppressor.

Introduction

Fusarium oxysporum f. sp. *ciceris* is the most devastating disease resulting in 10 - 50% crop loss every year in Pakistan. The fungus is seed borne as well as soil borne and can survive in the soil for more than five years. Moreover, it has some symptom - less carriers like lentil and peas (Saxena and Singh 1987) and it is impracticable to control the disease by using fungicides and through crop rotation. Use of resistant varieties is the best way to control the disease. But due to absence of true resistance in chickpea against wilt disease and a continuous problem of the occurrence / development of new pathogenic races (Jimenez - Diaz *et al* 1989) it has become difficult to overcome the yield losses.

Isolates of the pathogen induce yellowing or wilt syndromes as a result of vascular infections and both pathotypes showed varying degree of pathogenicity towards chickpea lines. Seven races of FOC have been identified by their differential interactions with chickpea lines (Haware and Nene 1982; Trapero - Cases and Jimenez - Diaz 1985; Jimenez - Diaz *et al* 1989). The underlying mechanism for varying pathogenicity / virulence is still unclear. Several biochemical phenomenons have been reported in plant - microbe interactions where phytotoxins (Yoder 1980; Alam and Iftikhar 1996), fungal enzymes including pectic enzymes, cutinase enzymes etc. Kollattukudy (1985); Artes and Tena (1990) or suppressors (Hiramatsu *et al* 1986) have been found as pathogenicity / virulence factor. Elicitor's production by the pathogen has also been referred as a virulence factor in many cases (Deverall and Deakin 1985; De Wit *et al* 1985). A great deal of work has been done on the production of elicitor / suppressor of *Ascochyta rabiei* causing blight of chickpea (Kessmann and Barz 1986) but no reports are available about FOC.

The objectives of the present studies were to identify the possible mechanisms regarding elicitor / suppressor production by the hypovirulent / virulent isolates of FOC.

Materials and Methods

Chickpea material. Two chickpea varieties Aug - 424 (susceptible) and CM 98 (resistant) were used in this study.

Fungi. The two virulent strain 2012 (virulent) and 9718 (highly virulent) and two hypovirulent isolates 2002 and 2014 of *Fusarium oxysporum* f. sp. *ciceris* (Isolated from the diseased chickpea samples collected from Thal, Punjab, Pakistan, during a survey in 2000), were used in this study.

Estimation of total phenols. Wilt, sick soil was prepared as described by Nene *et al* (1981) and filled in small plastic pots (4″x4″x4″). Ten seeds of each variety were sown in one pot in three replicates. The fresh roots of both varieties were

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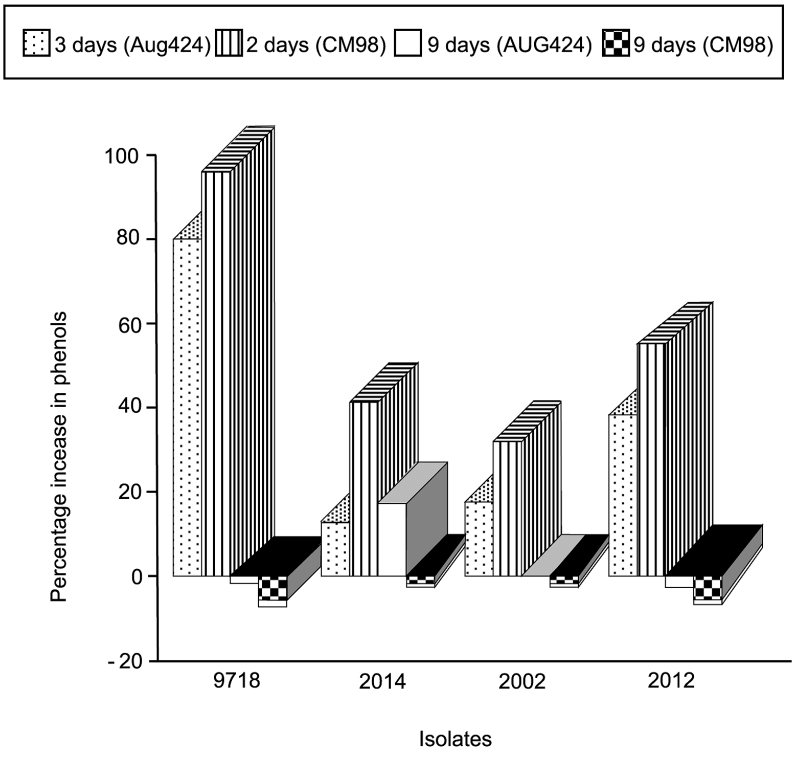

Fig 1. Percentage increase of total phenols in chickpea roots of two varieties at three days and nine days after germination when inoculated with virulent / hypovirulent isolates of *F. oxysporum* f. sp. *ciceris*.

collected after 3 and 9 days of germination in three replicates, washed in distilled water and dried in filter paper. The roots (0.5 gm) were grinded with pestle and mortar in 2.0 ml of acidified methanol (80% with 0.1% HCI) and the material was filtered through buchner funnel using suction pump. The solvent was evaporated on rotary evaporator at 40˚C and finally dissolved in 0.5 ml of methanol. Total phenols were estimated in the fresh roots of these lines by the Folin reagent (Simson and Ross 1971). Total phenols were also estimated in healthy (control) roots of these varieties.

Detection of antifungal compounds. Methanol extract of each sample (50 µl) were spotted on thin layer chromatographic (TLC) plate (0.5 mm thick silica gel 60 GF_{254} plates). The plates were developed in chloroform - methanol (97:3) solvent system. The developed TLC plates were placed in hot incubator at 40˚C for 3 h to evaporate the solvents. The TLC plates were bioautographed against test fungus *Cladosporium cucumerinum* as described by Sibtain *et al* (2002). The developed TLC plates were sprayed with Folin Ciocalteu reagent to identify the nature of these compounds.

Results and Discussion

Total phenolic contents increased in the roots of susceptible and resistant varieties against the virulent as well as hypo virulent FOC isolates as compared to the control (non inoculated plants) three days after germination. Highest increase (80 and 96%) was observed against the highly virulent isolate 9718 in the roots of Aug - 424 and CM 98, respectively. While lowest increase was produced against less virulent isolates viz. 2002 and 2014. The isolate 2012 induced 37.4 and 54.00% increase of total phenols in susceptible and resistant varieties. The percentage increases of total phenols were more in resistant variety as compared to susceptible one Fig 1. The isolated 2012, 9718 and 2002 inhibited total phenols in the roots of both varieties nine days after germination as compared to their control. No reduction in phenols of both varieties was observed against less virulent isolate 2014.

The decrease in total phenols was highest in both varieties against the most virulent isolate 9718 followed by 2012 and 2002. No significant reduction was produced by less virulent isolate 2014. Percentage reduction in phenols was higher in CM 98 as compared to Aug - 424 (Fig 1). Aug - 424 completely wilted at 15 days of germination and the resistant variety CM 98 wilted at 25 - 27 days after germination.

The bioautography of the developed TLC by using *Cladosporium cucumerinum* revealed that two inhibition zones at Rf values 0.85 and 0.88 were produced by both the varieties against all the FOC isolates (Fig 3). Healthy plants (control)

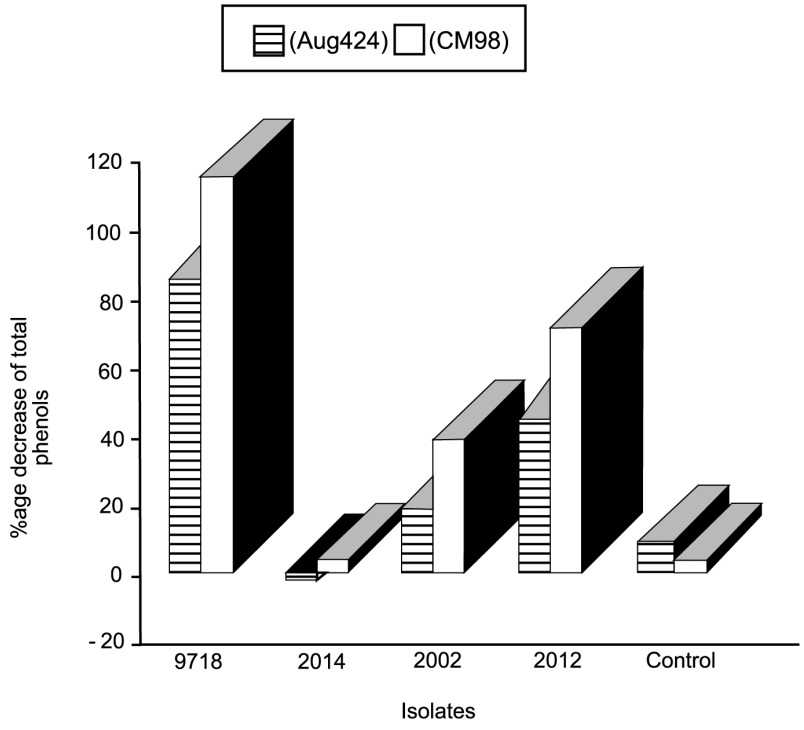

Fig 2. Percentage of total phenols decreased in chickpea roots at 9 days vs. 3 days after germination when inoculated with virulent / hypovirulent isolates of f. *oxysporum* F. sp. *ciceris*.

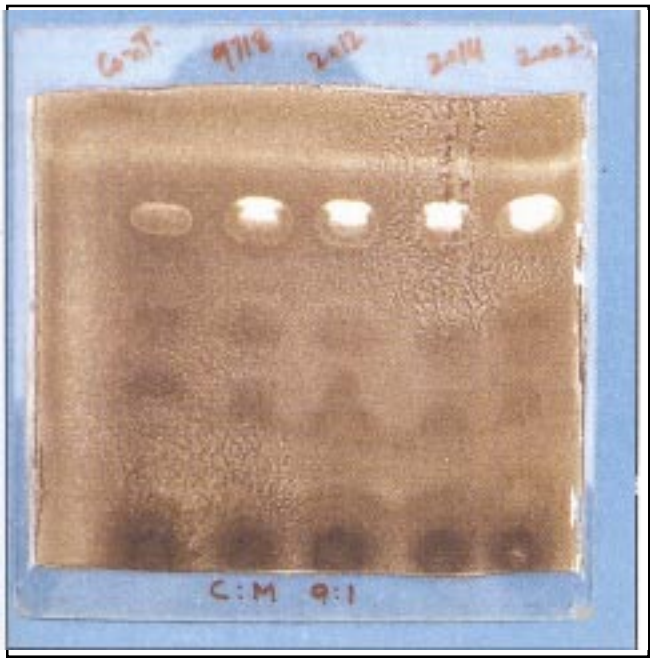

Fig 3. Bioautography of the TLC plates against *Cladosporium cucumerinum* showing antifungal compounds produced by the roots of chickpea.

did not produce these antifungal compounds which revealed that the antifungal compounds were phytoalexins and only produced after fungal attack. No difference was observed in the samples taken at three days and nine days after germination. The antifungal compounds produced blue color after spraying with Folin reagent, which confirmed that the compounds were phenolic in nature.

The results suggested that the phenomenon of elicitor production by the less virulent isolates (production of specific elicitors) might not be operating in FOC isolates because the highly virulent isolates induced higher amount of phenolic contents in both varieties, (three days after germination) as compared to the less virulent isolates. But non - specific elicitor would be produced by all isolates that might be the cause of accumulation of phenolic compounds in chickpea roots. Endopolygalacturonase enzymes and fragments of pectin have been reported to elicit phytoalexins in plants (Stekoll and West 1978; Roberston 1986). The virulent / hypovirulent isolates of FOC produced polygalacturonase and pectin lyase enzymes (Artes and Tena 1990) and in our studies the highly virulent isolate 9718 produced highest polygalacturonase activity (unpublished results). So, the enzyme itself or its degradation products might be eliciting the accumulation of phenols in chickpea roots three days after germination.

The highest percentage decrease of total phenols was produced by the highly virulent isolate 9718 (Fig 2) followed by the virulent isolate 2012 indicating that these isolate would be

producing suppressors. As, there was no difference in the production of anifungal compounds against all the isolates (Fig 3) even during nine days. So, the suppressors produced by the virulent isolates would be specific and might be eliminating or decreasing the effect of non - specific elicitors and resulting compatible interaction. Similar results were observed by Doke and Tomiyama (1980) when they isolated high molecular weight non - specific elicitor and a specific low molecular weight glucan suppressor from *Phytopthora infestans*. The results also indicated that less virulent isolate 2014 is not producing specific suppressor.

Conclusion

The virulent and hypovirulent isolates of *F. oxysporum* produced non - specific elicitors while specific suppressors were only produced by virulent isolates.

References

Alam S S, Iftikhar A K 1996 Phytotoxins from *Fusarium oxysporum* f. sp. *ciceri* involved in wilt of chickpea. *Pak J Phytopathology* **8** 132 - 135.

Artes P E, Tena M 1990 Purification and characterization of pectic enzymes from two races of *Fusarium oxysporum* f. sp. *ciceris* differing in virulence to chickpea (*Cicer arietimum* L.) Physiological and Molecular Plant Pathology **37** 107 - 124.

Deverall B J, Deakin A L 1985 Assessment of Lr 20 gene - specificity of symptom elicitation by intercellular fluid from leaf rust - infected wheat leaves. *Physiological Plant Pathology* **27** 99 - 107.

De Wit P J G M, Hofman J E, Velthuis G C M, Kuc J A 1985 Isolation and characterization of an elicitor of necrosis isolated from intercellular fluids of compatible interactions of *Cladosporium fulvum* (syn. *Fulvia fulva*) and tomato. *Physiological Plant Pathology* **77** 642 - 647.

Doke N, Tomoyama K 1980 Suppression of the hypersensitive response of potato tuber protoplasts to hyphal wall components by water - soluble glucans isolated from *Phytopthora infestans*. *Physiological Plant Pathology* **16** 177 - 186.

Haware M P, Nene Y L 1982 Races of *Fusarium oxysporum* f. sp. *ciceri*. *Plant Disease* **66** 809 - 810.

Hiramatsu M, Ichinose Y, Shiraishi T, Oku H, Ouchi S 1986 Regulation of pisatin biosynthesis in pea leaves by elicitor and suppressor produced by *Mycosphaerella pinods*. *Ann Phytopthol Soc Jpn* **52** 53 - 58.

Jimenez - Diaz R M, Trapero - Casas A, Cabrera de la Colina J 1989 Races of *Fusarium oxysporum* f. sp. *ciceris* infecting chickpea in Southern Spain. In: Vascular Wilt Diseases of Plants. eds. Tjamos E C & Beckman C H, Berlin, Germany, Springer - Verlag, NATO ASI SER Vol H 28, 515 - 520.

Kessmann H, Barz W 1986 Elicitation and suppression of phytoalexins and isoflavone accumulation in cotyledons of *Cicer arietinum* L. as caused by wounding and by polymeric components from the fungus *Ascochyta rabiei* **117** 321 - 335.

Kollattukudy P E 1985 Enzymatic penetration of plant cuticle by fungal pathogens. *Annu Rev Phytopathol* **23** 223 - 250.

Nene Y L, Haware M P, Reddy M V 1981 Chickpea diseases resistance - screening techniques. Information bulletin No.10. Patancheru 502 324, Andhra Pardesh, India. International Crops Research Institute for the Semi - Arid Tropics.

Robertson B 1986 Elicitors of the production of lignin - like compounds in cucumber hypocotyls. *Physiol Mol Plant Pathol* **28** 137 - 148.

Sexena M C, Singh K B 1987 The chickpea CAB. International. The International Center for Agric Research in Dry areas. pp 250 - 252.

Sibtain M, Ilyas M B, Iftikhar A K, Khan I A, Alam S S 2001 Screening of chickpea germplasm against fusarium wilt in pot soil, water culture and cultural filtrate. *On Line J Biol Sci* **1** 229 - 231.

Simson J T, Ross A F 1971 Changes in phenol metabolism associated with induced systemic resistance to tobacco mosaic virus in Samsun NN tobacco. *Phytopathology* **61** 1261 - 1265.

Stekoll M, West C A 1978 Purification and properties of an elicitor of castor bean phytoalexin from culture filtrates of the fungus *Rhizopus stolonifer*. *Plant Physiology* **61** 38 - 45.

Trapero - Cases A, Jimenez - Diaz R M 1985 Fungal Wilt and root rot diseases of chickpea in Southern Spain. *Phytopathology* **75** 1146 - 1151.

Yoder O C 1980 Toxins in pathogenesis. *Annu Rev Phytopathol* **18** 103 - 129.

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ANTIBACTERIAL ACTIVITY OF PAKISTANI *RHAZYA STRICTA*

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The crude ethanolic extract of *Rhazya stricta* Dcne; (Family, Apocynaceae) was tested, on the basis of medicinal and folklore reports for antimicrobial activity against a wide range of gram - positive and gram - negative organisms. Leaves extract was found to be more active as compared to other parts exhibiting 69.23% and 66.66% activity against gram - positive and gram - negative organisms respectively. Seeds extract exhibited maximum inhibitory activity ("A" category zone) i.e. 23.07% and 16.66% against gram - positive and gram - negative organisms respectively as compared to other parts of *Rhazya stricta*. Reference standard i.e. co-trimoxazole exhibited only 7.69% "A" category zones against gram - positive organisms only.

Key words: *Rhazya stricta*, Apocynaceae, Antibacterial activity.

Introduction

Recognition, importance, necessity and potentiality of medicinal plants in present day practice cannot be overlooked. They are still being used as a source of medicament for a variety of diseases, not only by rural population but also by socio - cultural system of people with fair amount of success having no scientific information.

Rhazya stricta Dcne., commonly known as Sundwar, Sewar or Gandera, belongs to the family Apocynaceae - an alkaloids bearing family. It comprises of 300 genera and 200 species. Found abundantly in Pakistan and is an endemic flora of Sind (Hooker 1875, Salimuzzaman *et al* 1966, Baqar 1967 & 1989 and Khan *et al* 1979). The plant is well known for its antitumor activity (Watt 1892 & Sarfaraz *et al* 1972). In indigenous system of treatment, the plant is used as an effective remedy for boils, eruption, syphilis, sore throat, fever, rheumatism, pain and painful affections and in general debility (Watt 1892, Kirtikar *et al* 1933 & Chopra *et al* 1956).

Earlier studies indicates that the plant has been a subject of number of chemical investigations, which have revealed the presence of a large number of indole alkaloids whose structure have been elucidated using modern spectroscopic techniques (Chatterjee *et al* 1961, Salimuzzaman 1966, Mukhopadhayay *et al* 1981, 1983 and Atta -ur- Rahman *et al* 1982, 1989 & 1995; Gerasimenko *et al* 2001, Stockigt *et al* 2002). These alkaloids are reported to exhibit hypotensive, antispasmodic, antidiabetic (Tanira *et al* 1996 and Ali *et al* 1997) anti-inflammatory (Wasfi *et al* 1995) antimicrobial (Mariee *et al* 1988, Bashir *et al* 1992 & 1994) and antineoplastic (Mukhopadhyay *et al* 1981 & 1983) activities.

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Keeping in view, the reported medicinal properties related to this plant, it was thought to exploit *Rhazya stricta* plant biologically for its antimicrobial activity. The present work involves the antibacterial activity of different parts i.e. leaves, roots, fruits and seeds of *Rhazya stricta* plant. This plant is also available in other parts of the world. On the other hand, it will also give a new challenge to strike new sources of medications as claimed by folklore practitioners on firm footed scientific evaluations.

Materials and Methods

Collection and identification of plant material. The plant material was collected from the suburbs of Karachi. Properly identified by a taxonomist and a voucher specimen was deposited in the herbarium of Applied Biology Research Centre, PCSIR Laboratories Complex, Karachi.

Preparation of extract. Each part of the collected plant material i.e. leaves, roots, fruits and seeds were individually washed and dried in oven at 45˚C ± 5˚C. The dried and milled part of *Rhazya stricta* was extracted with 95.00 % ethyl alcohol (100 g per 1.25 lit) at room temperature. Solvent was allowed to remain in contact with each part of plant material for 48 h with continuous agitation for 6 h per day, then decanted and pooled. Process was repeated thrice in order to obtain the maximum quantity of the extract. Pooled solvent was removed under vacuum at 40˚C ± 1˚C. This afforded a crude ethanolic extract. Percentage yield of each part i.e. leaves, roots, fruits and seeds was calculated as 7.40%, 5.20%, 3.20% and 2.10% respectively.

Preparation of solution. All extracts were dissolved in an aqueous ethanolic solution (25.00%) with 2.50% Triton as an

emulsifier to give a strength of 100 mg / ml. Co-trimoxazole was used as a reference standard due to its wide range of effectivity against gram - positive and gram - negative group of organisms.

Micro-organisms used. The said activity was assessed against gram - positive (13) and gram - negative (24) organisms. All the test organisms used in the present study were clinical isolates and obtained from the Department of Microbiology, University of Karachi. The organisms were maintained on Tryptic soya agar slants (Merck) at 4°C prior to testing. Test culture inocula was prepared by using Tryptic soya broth culture medium maintained at 37°C ± 1°C for 24 h.

Antibacterial activity. Antibacterial was carried out by hole plate diffusion method using 0.5 ml of the inoculum containing 10^5 bacterial cells, respectively. The inoculum was thoroughly mixed with 25 ml of melted sterile liquid Tryptic soya agar and poured into pre - sterilized petri dishes respectively. Plates were left to set at 4°C for one h. Holes of 6mm diameter were made from the centre of each seeded plate. Holes were then filled aseptically with 0.2 ml (20mg) of test solution (various parts of plant extracts), reference standard and negative control (solvent only) respectively and marked accordingly. All plates were then incubated at 37°C ± 1°C for 24 h and zones of inhibition were measured and recorded accordingly. The above experiment was repeated thrice.

Results and Discussion

Alcoholic extracts of various parts of *Rhazya stricta* showed a varied degree of antibacterial activity against wide range of gram - positive and gram - negative organisms as shown in Table 1 and 2, while Table 3 represent a comparative data on different parts of *Rhazya stricta* according to the zone of categorization in percentage. Negative control exhibited no activity against gram - positive and gram - negative organisms. Extracts exhibiting zone of inhibition less than 9mm were taken as negative.

The study gives a preliminary account of antibacterial substances present in different parts i.e. leaves, roots, fruits and seeds of *Rhazya stricta* plant. The considerable difference observed in the potency of activity amoung different parts of *Rhazya stricta*, clearly indicates that there exists a marked difference in active component in various parts of the plant. In general, leaves extracts exhibited a pronounced maximum activity i.e. 66.66% against gram - negative group and 69.23% against gram - positive groups, while root extract exhibited miximum activity i.e. 33.33% against gram - negative and 23.07% against gram - positive organisms respectively. Seed portion exhibited 46.15% and 54.16% and fruit 46.15% and 41.66% activity respectively against gram - positive and gram-negative organisms. Negative control was found to be non-active against both gram positive and negative group of orga-

Table 1

Comparative antibacterial study of different parts of *Rhazya stricta* (leaves, roots, fruits & seeds) and co-trimoxazole against gram - positive organisms according to zone of inhibition (mm)

S.No.	Name of organism	Zone of inhibition					
		Rhazya stricta				Standard	Negative
		Leaves	Roots	Fruits	Seeds	Co - trimoxazole	control
1.	*Bacillus subtilis*	B	-	-	-	-	-
2.	*Cornybacterium diptheriae*	C	-	-	-	B	-
3.	*Cornybacterium xerosis*	-	-	-	-	-	-
4.	*Micrococcus roseus*	-	-	-	-	C	-
5.	*Micrococcus variance*	A	-	-	-	A	-
6.	*Staphylococcus aureus*	D	-	-	-	B	-
7.	*Staphylococcus aureus*	B	D	C	B	B	-
8.	*Staphylococcus aureus*	-	-	C	C	-	-
9	*Staphylococcus epidermidis*	-	-	-	-	C	-
10.	*Staphylococcus saprophyticus* (coagulase + ve)	C	-	B	D	C	-
11.	*Streptococcus faecalis*	C	-	B	A	C	-
12.	*Streptococcus pyogenes*	B	B	B	A	C	-
13.	*Streptococcus viridans*	B	B	A	A	C	-
Total	Percent Activity	69.23%	23.07%	46.15%	46.15%	76.92%	

Categorization of zone of inhibition in mm: A = 40 - 49; B = 30 - 39; C = 20 - 29; D = 10 - 19.
Zone of inhibition from 6 - 9 mm were considered as negative.

Table 2

Comparative antibacterial study of different parts of *Rhazya stricta* (leaves, roots, fruits & seeds) and co - trimoxazole against gram - negative organisms according to zone of inhibition (mm)

S.No.	Name of organism	Zone of Inhibition					
		Rhazya stricta				Standard	Negative
		Leaves	Roots	Fruits	Seeds	Co - trimoxazole	control
1.	*Acinetobacter calcoaceticus*	-	C	B	C	-	-
2.	*Aeromonas hydrophilia*	-	D	D	C	-	-
3.	*Actinobacillus lignierersii*	-	-	-	-	-	-
4.	*Branhamella catarrhalis*	B	B	B	B	-	-
5.	*Citrobacter diversican*	-	-	-	A	-	-
6.	*Citrobacter freundii*	B	-	-	B	D	-
7.	*Escherichia coli (Communior)*	D	-	-	-	D	-
8.	*Escherichia coli (Communis)*	-	-	-	-	D	-
9	*Klebsiella pneumoniae*	A	B	B	A	-	-
10.	*Klebsiella ozaenae*	B	-	-	-	-	-
11.	*Proteus mirabilis*	B	B	B	A	C	-
12.	*Proteus vulgaris*	-	-	-	-	D	-
13.	*Pseudomonas aeroginosa*	A	D	D	C	B	-
14.	*Pseudomonas mallei*	-	-	-	-	-	-
15.	*Pseudomonas maltophilia*	A	C	C	A	-	-
16.	*Salmonella para typhi A*	B	-	C	D	-	-
17.	*Salmonella para typhi B*	C	-	-	-	B	-
18.	*Salmonella typhi*	C	-	-	-	B	-
19.	*Shigella bodydii*	C	-	-	-	B	-
20.	*Shigella dysenteriae*	C	-	-	D	C	-
21.	*Shigella flexaneriae*	C	B	-	-	C	-
22.	*Shigella sonnei*	-	-	D	B	C	-
23	*Vibrio cholera eltor*	B	-	-	-	D	-
24.	*Vibrio cholera inaba*	B	-	-	-	D	-
Total	Percent activity	66.66	33.33	41.66	54.16	54.16	Nil

Categorization of zone of inhibition in mm A= 40 - 49; B = 30 - 39; C = 20 - 29; D = 10 - 19.
Zone of inhibition from 6 - 9 mm were considered as negative.

nisms. Reference standard exhibit 76.92% and 54.16% activity against gram - positive and gram - negative group of organisms respectively (Table 1 & 2).

According to Table 3, based on zone of categorization, the highest activity was exhibited by seeds extract i.e. 23.07% and 16.66% against gram - positive and gram - negative organisms respectively, while reference standard i.e. co-trimoxazole fails to exhibit 'A' category activity against gram - negative group of organisms and 7.69% against gram - positive group of organisms. It is evident from the collected data that activity of various parts of *Rhazya stricta* plant when categorized according to the zone of inhibition falls mostly in A and B category while on the other hand reference standard i.e. co-trimoxazole mostly in "C" category (Table 3).

The difference in activity among test i.e. *Rhazya stricta* and reference standard i.e. co - trimoxazole is basically due to the fact that most of the organisms are gradually becoming less sensitive due to wide spread use of antibiotics while on the other hand, various parts of *Rhazya stricta*, present a good potency of antibacterial activity. Furthermore, the antibacterial activity assessed also confirms the folklore use of the plants and its various parts against various infectious diseases such as boils, eruption, fever, sore throat, inflammation and in anti - neoplastic diseases. (Watt 1892, Kirtikar *et al* 1993

Table 3

Comparative study of different parts (leaves, roots, fruits and seeds) of *Rhazya stricta* against gram - positive and gram - negative organisms according to zone of categorization.

Categorization index	% Zone of inhibition									
	Gram positive					Gram negative				
	Leaves	Roots	Fruits	Seeds	Ref.Std.	Leaves	Roots	Fruits	Seeds	Ref.Std.
A	7.69	-	7.69	23.07	7.69	8.33	-	-	16.66	-
B	30.76	15.38	23.07	7.69	23.07	29.16	16.66	16.66	12.50	12.50
C	23.07	-	15.38	7.69	46.15	25.00	8.33	12.50	16.66	16.66
D	7.69	7.69	-	7.69	-	4.16	8.33	12.50	8.33	25.00
Total activity (%)	69.21	23.07	46.14	46.14	76.91	66.65	33.32	41.66	54.15	54.16

and Chopra *et al* 1956). The antibacterial activity exhibited can be attributed due to the wide spread occurrence of alkaloids, tannins, chrysophenol and trace metal contents present in *Rhazya stricta* plant (Philipson *et al* 1984; Atta - ur - Rehman *et al* 1989 and Kaneez *et al* 1999). It can be concluded that different parts of *Rhazya stricta* may be used as a good antibacterial agent drug in the treatment of infectious diseases, provided it is found effective and safe in *in - vivo* study.

References

Ali B H 1997 The effect of plasma glucose, insulin and glucagoen levels of treatment of diabetic rats with the medicinal plant *Rhazya stricta* and with glibenclamide alone and in combination. *J Pharm Pharmacol* **49**(10) 1003 - 1007.

Atta - ur - Rehman 1989 Isolation and structural studies on new natrural products of potential biological importance. *Pure Appl Chem* **61**(3) 453 - 456.

Atta - ur - Rahman and Fatima K 1982 Alkaloids of *Rhazya stricta* Decaisne. *Pak J Chem Soc* No.4, 121 - 127.

Atta - ur - Rahman, Qureshi M M, Zaman K, Malik Sand Ali S S 1989 The alkoloids of *Rhazya stricta and Rhazya orientalis*, A review. *Fitoterapia* **60**(4) 291 - 322.

Atta - ur - Rahman, Abbas S, Zaman K, Quershi M M, Nighat F, Muzaffar A 1995 Saifine and N - methyl leuconolam New indole alkaloids from *Rhazya stricta*. *Nat Prod Lett* **5** 245 - 253.

Baqar R S 1989 *Medicinal and Poisonous Plants of Pakistan.* Printas Karachi, Pakistan, 379.

Baqar R S, Tasnif M 1967 *Medicinal Plants of Southern West Pakistan.* PCSIR Publication Karachi, 43.

Bashir A K, Abdallah A A, *et al* 1994 " Phytochemical and antimicrobial studies on the leaves of *Rhazya stricta* growing in United Arab Emirates" *Fitoterapia*, **65** (1) 84 - 85.

Bashir A K, El Syed S Hassan, Amire M A, Abdallah A, Abdallah A I, Wasfi A 1992 Antimicrobial activity of certain plants used in the folk medicine of United Arab Emirates. *Fitoterapia*. **LXII** (4) 371 - 375.

Chatterjee A, Ghosal C R, Aditya Chaudhury N, Ghosal S 1961 "Alkaloids of *Rhazya stricta*. Decaisne *Chemistry & Industry* **(8)** 1034 - 1035.

Chopra R N, Nayyar S I, Chopra I C 1956 *Glossary of Indian Medicinal Plants*, CSIR, New Delhi, 212.

Fatima K, Shireen F K, Kalhoro M A, Qadiruddin M, Badar Y 1999 Trace Elements in Indigenous Medicinal Plants *Rhazya stricta, Vinca rosea and Pagonia cretica*. *Pak J Sci Ind Res* **42**(4) 182 - 183.

Gerasimenko I, Sheludko Y, Unger M, Stockigt J 2001 Development of an efficient system for the separation of indole alkalids by high performance liquid chromatography and its applications *Phytochem Anal* **12**(2) 96 - 93.

Hooker J D 1875 *"Flora of British India"* Reeve & Co. London, UK, Vol.**III** 640.

Khan A A, Ashfaq M, Ali M N 1979 Pharmacognostic studies of selected indigenous plants of Pakistan. *Pakistan Forest Institute*, Peshawar, Pakistan, 75.

Kirtikar K R, Basu B D 1933 *"Indian Medicinal Plants"*. Allahabad, India, Vol **II** 1557.

Mariee N K, Khalil A A, Naseer A A, Al - Hiti M M, Ali W M 1988 Isolation of the antimicrobial alkaloids stemmadenine from Iraqi *Rhazya stricta. J Nat Prod* **51**(1) 186 - 187.

Mukhopadhyay S, Handy G A, Furayama S, Cordell G A 1981 Anticancer indol alkaloids of *Rhazya stricta. J Nat Prod* **44**(6) 696.

Mukhopadhyay S, El - Syed A, Handy G A, Cordell G A 1983 *Cathranthus* alkaloids XXXVII.16 - *Epi* - Z - isositsirikine, a monomeric indole alkaloid with antineoplastic activity from *Catharanthus roseus* and *Rhazya stricta. J Nat Prod* **46** 409.

Philpson J D, Linda A A 1984 Pharmacological active compounds in herbal remedies. *The Pharmaceutical J* **14** 44.

Salimmuzzaman S, Yusuf A, Baig M I 1966 Studies on *Rhazya stricta* Dcne. *Pak J Sci Ind Res* **9**(2) 97 - 102.

Stockgist J, Sheludk Y, Unger M, Gerasimenko I, Warzecha H and Stockigt D 2002 High Performance liquid chromatographic, capillary electrophoretic and capillary electrophoretic - electrospray ionization mass spectrometric analysis of selected alkaloid groups. *J Chromatogr A*, **16** 967(1) 85-113.

Sarfaraz S and Bukhari A Q S 1972 "Leukopenic effects of *Rhazya stricta*". *Nature* **18** 393.

Tanira M O M, Ali B H, Bashir A K, Wasfi I A, Chandranath I 1996 Evaluation of the relaxant activity of some united Arab Emirates Plants on Intestinal smooth muscle. *J Pharmacol* **48**(5) 545 - 550.

Wasfi I A, Bashir A K, Abdalla A A, Banna M R, Tanira M O M 1995 Antiinflammatory activity of some medicinal plants of United Arab Emirates, *Int J Pharmacogn* **33** (2) 124 - 128.

Watt G M 1892 "*Dictionary of the Economic Products of India.* W H Allen and Company London, UK, Vol **VI**, Part I, 443.

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RECYCLING OF SUGARCANE INDUSTRIAL WASTE AS A BIOFERTILIZER THROUGH COMPOSTING

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About 500 tons of industrial wastes (liquid and solid) being discharged daily from sugar factory during crushing season and presently dumped in vicinity of the sugar factory. The quantity of wastes, however, depends on the crushing capacity of sugar mills. Studies for recycling and composting of sugarcane industrial waste press mud, boiler ash and distillery waste water, as a biofertilizer, was carried out at Habib Sugar Mills Ltd., Nawabshah, Sindh, Pakistan. Samples of waste were collected for analysis from dumping ground. By mixing the heaps of press mud and boiler ash, 3:1 ratio was formed. Each heap was sprinkled with distillery waste water for two months with regular interval of one day. Two manual turning of heaps were done to maintain the temperature during the curing period and thereafter allowed for 3 months for decomposition and humus formation. Based on physico-chemical analysis of finished product, it is estimated that each ton of biofertilizer contains value-added nutrient of Rs.2897/= when compared with chemical fertilizer other than the soil amendment characteristic. The cost effectiveness of the biofertilizer for millers and farmers were about Rs.1:4 and Rs.1:12, respectively.

Key words: Sugarcane industrial waste, Compost, Biofertilizer, Press mud, Bioler ash, Distillery waste water.

Introduction

Urbanization, modernization and industrialization have increased solid waste generation, one of the major environmental problems being faced by the world today. This situation leads to solid waste management to cope with the situation that generally starts with the possible reduction of waste generation at source, then to reuse and recycle waste materials if possible. The left over material, at the end, is subjected to disposal. Generally, solid waste is disposed off in three ways i.e. incineration, sanitary landfill and composting.

Composting is a process in which biodegradable organic material is converted into soil conditioner that can be used to improve soil fertility. Developing countries like Pakistan can afford landfill and composting techniques to dispose off solid waste. Preferably, the biodegradable solid waste should undergo composting that results not only into the solid waste disposal but also provides useful product that can improve soil condition and will reduce expensive chemical fertilizer use. Use of chemical fertilizer has number of environmental problems that can be limited in this way (Khan 1996). To improve the soil fertility, the use of organic manure has a vital importance, but unfortunately farmers rarely applied the natural manure mostly due to unavailability and lack of knowledge.

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In Pakistan, 74 sugar manufacturing industries are involved in sugarcane crushing and produced 3.2 million tons of sugar during 1998 - 1999 (Anonymous 1999). It was reported that the average amount of nutrient, i.e. N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu removed by 100 MT of cane were 148.00, 123.00, 238.00, 42.00, 39.00, 38.00, 7.50, 4.12, 0.50, and 0.10 kg, respectively (Calcino 1995). Since millions of tons of sugarcane industrial wastes (filter cake, boilers ash and distillery waste water) is being discharged each year and dumped in the vicinity of factories. These wastes carry sufficient amount of macro and micronutrients (Subba 1985). These nutrients could be effectively recycled for soil improvement (Hussein and Anjum 1999). Organic materials not only improve the soil fertility but also lessen the demand of chemical fertilizer. The major factor behind this low yield is seemed to be unbalanced fertilizer. Since last couple of decades growers mostly depend on NPK chemical fertilizers for crop production. This indiscriminate use of chemical fertilizer has led to massive depletion of organic matter, accumulation of deleterious salts and increased the soil pH as well (Arain *et al* 2000).

Sugarcane is one of the major cash crops of Pakistan. The total area under cultivation of sugarcane during 1998 - 1999 was 1.155 million hectare and the estimated yield was 55.19 million tons (Anonymous 1999). In this way, per hectare yield is about 48 tons / ha. The yield is quite low as compared to

other developing countries (Khushk 1999). The present study leads to develop a procedure for recycling of sugarcane industrial waste and to sustain the soil and nutrient requirement of sugarcane and other crops. The study also aims to determine the quantity of macro and micronutrients in the finished product or compost.

Materials and Methods

Analysis of industrial wastes. Studies were carried out at Habib Sugar Mills (Pvt) Ltd, Nawabshah from November 1998 to March 1999 to evaluate the physico-chemical properties of the press mud, boiler ash and distillery waste water. This sugar mill is equipped with distillery unit and boiler ash collection facilities. Approximately, 300 - 500 tons of industrial waste (solid and liquid) is being discharged daily, depending upon the crushing capacity of the factory. Fifty primary samples of each having weighed about one kg were collected randomly from the dumping ground. The samples were mixed together thoroughly and three laboratory samples were taken by a divider. These laboratory samples were kept in plastic bags, tagged and sent to the laboratory for analysis. The reference samples of the lots were also taken to maintain the record of physico-chemical contents. The sampling of industrial waste were taken four times during crushing season by adopting same procedure.

The nitrogen content was determined by oxidizeable carbon and organic matter content and C/N ratio were determined by dichromatic method (Rowell 1994). The fumigation and extraction method were used for determination of microbial population (Rowell 1994). The phosphorous content in the waste were extracted with sodium carbonate solution (Olson method) and phosphate content of solution by spectrophotometric method, potash and sodium by flame photometric method while calcium, magnesium and other micronutrients were measured by atomic absorption spectrophotometric method (Rowell 1994). The pH of industrial wastes was measured by using Orion meter (Model 201). The temperature of the heaps was recorded by installing the thermocouple leads in the bottom center and peripheral layers to ensure the proper composting. The Telethermameter Model 441/D series 63/18 was used for this purpose.

Following two equations can be used to compute moisture content and carbon - nitrogen ratio for three components system:

$$G = \frac{M1 \times Q1 + M2 \times Q2 + M3 \times Q3}{Q1 + Q2 + Q3} \quad (1)$$

Where Q = mass of material
G = required moisture in the pile (%)
M= moisture (%)

1. $R = Q1(C1x (100 - M1)) + Q2(C2x (100 - M2)) + Q3(C3x (100 - M3))$

   $Q1(N1x (100 - M1)) + Q2(N2x (100 - M2)) + Q3(N3x (100 - M3)$

Where

R = Required C/N ratio in the pile
C = Carbon %
N = Nitrogen %

Contents in the industrial wastes. The data presented in the Table 1 indicated the contents of industrial wastes.The filter cake contained 33.65 organic matter with 30:1 C/N ratio. The average nitrogen, phosphorous, potash, calcium and sulfur content were found to be 1.50, 2.68, 2.25, 2.72 and 1.47%, respectively. The micronutrient such as magnesium, iron, zinc were also present but relatively in less quantity. The pH of filter cake was 7.46 and moisture content was 53.85%. Filter cake as a manure in combination with chemical fertilizer sustained the soil fertility and crop productivity has been cited by earliers (Yadava 1991). Singh and Solomon (1995) showed an equal yield of sugarcane with application of 10 tons/ha press mud with 75 kg/ha as compared to 150 kg N/ha alone. Filtered press mud plays positive role in reclamation of saline alkaline and saline sodic soils and successfully decreases the soil pH. Approximately, millions of tons of filter cake is being produced annually in Asia and Pacific region having potential to recycle about 0.90 million tons of N, 1.08 million tons of phosphorous and substantial quantity of other essential minerals(Yadava 1991).

Table 1 also shows the composition of boiler ash; an average of 31.330% and 12.000% of silica and black carbon major content, respectively were recorded in boiler ash while calcium and magnesium were 2.880, 1.650 and 0.950%, respectively in ash. The other inorganic contents were found in relative less quantity. Boiler ash also contained sodium, aluminum and manganese in small quantity. Silica, the major component of boiler ash is an essential nutrient for increasing yields of sugarcane and sugar content. Application of 15 tons of silica increases the sugarcane productivity 70 and 125%, respectively in plant and ratoon crop (Gardner *et al* 1985). Lakshmikantham has reported that a ton of cane can remove 11.2 kg of silica as nutrient from the soil (Lakshmikantham 1983). In Australia, it was observed that in soil application of 150 tons/ha boiler ash provides 435 kg calcium, 390 kg potassium, 225 kg magnesium, 120 kg phosphorous and 60 kg nitrogen/ha (Calcino 1995).

The distillery waste water contains potash, calcium and sulfate 1.26400, 1.12400 and 1.16000%, respectively

Table 1
Physico - chemical contents of sugarcane industrial wastes

Parameters	Industrial wastes		
	Press mud	Boiler ash	Distillery waste water
Physico - chemical factors			
pH	7.46 ± 0.60	-	7.12000 ± 0.1300
Moisture (%)	53.85	49.47	92.50000
Organic matter	33.65	-	1.65000 ± 0.1900
C/N ratio	30:1	-	-
Nutrients (%)			
Nitrogen	1.50 ± 0.03	-	0.97000 ± 0.3600
Phosphorous (P_2O_5)	2.68 ± 0.26	-	0.66000 ± 0.0130
Potash (K_2O)	2.25 ± 0.76	2.88 ± 0.15	1.12640 ± 0.1600
Calcium	2.72 ± 0.41	1.65 ± 0.18	1.12400 ± 0.1600
Sulfur	1.47 ± 0.24	-	1.16000 ± 0.0300
Magnesium	0.30 ± 0.11	0.95 ± 0.05	0.47000 ± 0.0160
Iron	0.43 ± 0.10	-	0.12000 ± 0.0150
Zinc	0.33 ± 0.06	-	0.00340 ± 0.0003
Sodium	0.82 ± 0.18	-	0.00160 ± 0.0010
Mangnese	-	0.66 ± 0.07	0.00260 ± 0.0001
Aluminum	-	0.02 ± 0.00	0.00830 ± 0.0001
Silica	-	0.54 ± 0.44	0.00160 ± 0.0200
Boron	-	33.33 ± 0.57	0.00016 ± 0.0005
Cobalt	-	-	0.00010 ± 0.0000
Boiler ash %	-	31.330	-
Silica %		12.000	
Black carbon %	-	12.000	-

(Table 1). The other nutrients like nitrogen, phosphorous, magnesium and iron were found in waste water. The distillery waste water also have some other important micronutrients, zinc, sodium, manganese, aluminum, silica, boron and cobalt in very low quantities with other dissolved organic contents. During fermentation of molasses and ethanol distillation processes, large quantity of distillery waste water (13 liter of water/liter of ethanol) is generally discharged (Liu *et al* 1991). The distillery waste water possesses high manural, soil conditioning properties and also within this range distillery waste water was sprinkled on the heaps for two months to add more nutrients at a regular interval of one day. During this period, two turning was done manually to ensure proper mixing of ingredients. The heaps were then left over for three months for further decomposition and humus formation. Moreover, this will also help in the development of microbial population within the formulated biofertilizer (Maynard 1998).

Temperature. The temperature was recorded daily from different layers of the composting heaps to ensure microbial activity and composting process. Since, all microorganisms have an optimum temperature range for their growth and development. For composting, this range is between 32°C and 60°C (Maynard 1997).

Biological process. Microorganisms have the preferences for the type of organic material they consume. When the organic molecules that they require are not available, they may become dormant or die. In this process, the humic acid end product resulting from the metabolic activity of one generation or type of microorganisms may be used as a food or energy source by another generation or type of microorganisms. This chain of succession of different types of microbes continues until there is little decomposable organic material remaining (Maynard 1997). At this point, the organic material remaining is termed compost. It is made up largely of microbial cells, microbial skeletons and by products of microbial decomposition and un-decomposed particles of organic and inorganic origin. Decomposition may proceed slowly at first because of smaller microbial populations, but as populations grow in the first few hours or days, they rapidly consume the organic materials present in the feedstock (Breslin 1995).

Carbon contents of organic material can be determined on the basis of its volatile solid contents. Volatile solids are the components (largely carbon, oxygen and nitrogen) which are burnt from a dry sample in a laboratory at 500 - 600°C, leaving only the ash (largely calcium, magnesium, phosphorus, potassium, and other mineral elements that do not oxidize). For most of the biological materials, the carbon content is between 45 to 60% of the volatile solids. Assuming 55%, the formula to calculate the carbon content is given below:

%C = %VS x 0.55 where %VS = 100 - %Ash

Results and Discussions

Data presented in Table 2 represent the various physico-chemical properties of biofertilizer. The macronutrients were also calculated on dry weight basis while the nitrogen, phosphorus and potash were found 2.6000, 4.1700 and 7.2500% respectively, similarly the calcium, sulfur, silica and manganese contents were 10.8500, 5.1700, 5.2000 and 2.3300%. Iron, copper, zinc, magnesium and aluminum content were recorded as 0.6000, 0.5000, 0.4700, 0.4200 and 0.3200% respectively. The sodium content of biofertilizer was found to be 2.0000%. This is being due to saline soil in the area. The chemical environment is largely determined by the composition of material to be composted. In addition, several modifications can be made during the composting process to create an ideal chemical environment for rapid decomposition of organic materials. Several factors determine the chemical environment for composting, especially (a) the presence of an adequate carbon (food) energy source (b) a balanced amount of nutrients (c) the correct amount of water (d) adequate oxygen (e) appropriate pH, and (f) the absence of toxic constituents that could inhibit microbial activity (Maynard 1994a). Among the plant nutrients (nitrogen, phosphorus, and potash), nitrogen is of greatest concern. The ratio of carbon to nitrogen is considered critical in determining the rate of decomposition. In general, an initial ratio of 30:1 carbon: nitrogen is considered ideal. Higher ratios tend to retard the process of decomposition, while ratios below 25:1 may result in odour problems. Finished compost should have ratio of 15:1 to 20:1. To lower the carbon : nitrogen ratios, nitrogen rich materials such as yard trimmings, animal manures, or bio-solids are often added. Adding partially decomposed or composted materials (with a lower carbon : nitrogen ratio) as inoculumn may also lower the ratio. Attempts to supplement the nitrogen by using commercial fertilizers often create additional problems by modifying salt concentrations in the compost pile, which in turn impedes microbial activity (Maynard 1993a). As temperatures in the compost pile rise and the carbon nitrogen ratio falls below 25:1, the nitrogen in the fertilizer is lost in a gas form (ammonia) to the atmosphere. This ammonia is also a source of odours (Maynard 1993b).

The final moisture content of the compost was maintained at 40% so that the microbial population counts could be managed (Table 2). A moisture content of 50 to 60% of total weight is considered ideal (Maynard 1993b). The moisture content should not be great enough, however, to create excessive free flow of water and movement caused by gravity. Excessive moisture and flowing water from leachate create a potential liquid management problem and potential water pollution and odour problems. Excess moisture also impedes oxygen transfer to the microbial cells. Excessive moisture can increase the possibility of anaerobic conditions developing and may lead to rotting and obnoxious odours (Maynard and Hill 2000). Microbial processes contribute moisture to the compost pile during decomposition. While moisture is being added, however, it is also being lost through evaporation. Since the amount of water evaporated usually exceeds the input of moisture from the decomposition processes, there is generally a net loss of moisture from the compost pile. In such cases, adding moisture may be necessary to keep the composting performing at its peak. Evaporation from compost piles can be minimized by controlling the size of piles (Maynard and Hill 1994). Piles with larger volumes have less evaporating surface per unit volume than smaller piles. The water added must be thoroughly mixed so that all portions of the organic fraction in the bulk of the material are uniformly wetted and composted under ideal conditions. A properly wetted compost has the consistency of a wet sponge (Maynard 1998).

The compost also contained 58.62% organic matter with C/N ratio of 20:100, the microbial population in biofertilizer was 218×10^8 per gram and fungal count 125×10^5 per gram (Table 2). The final moisture content was kept at 40% so that the microbial population counts could be maintained. At this point, the organic material remaining is termed compost. It is made up largely of microbial cells, microbial skeletons and by pro-ducts of microbial decomposition and un-decomposed parti-cles of organic and inorganic origin (Maynard 1994b). Mic-roorganisms in the compost process are like microscopic plants; they behave more or less the same nutritional needs (nitrogen, phosphorus, potassium, and other trace elements) as the larger plants. There is one important exception, however, compost microorganisms rely on the carbon in organic material as their carbon/energy source instead of carbon dioxide and sunlight, which is used by higher plants (Maynard 1993a). The carbon contained in natural or human made organic materials may or may not be biodegradable. The relative

Table 2
Physico - chemical analysis of the finished compost by mixing filter cake, boiler ash and distillery water in 3:1:3 ratio and after curing of three months

Factors	Biofertilizer	
	Wet Basis	Dry basis
Physico - chemical factors		
pH	-	7.1000+0.250
Moisture content(%)	58.6200	40.0000
Organic matter	-	35.1700
C / N ratio	-	20:1
Microbial population	-	$218x10^8$/gm
Fungal count	-	$125x10^5$/gm
Nutrients (%)		
Nitrogen	2.6000	1.5600+0.2400
Phosphorous	4.1700	2.5000+0.1200
Potash	7.2500	4.3500+0.4600
Calcium	10.8500	6.5100+0.6500
Sulfur	5.1700	3.1100+0.2500
Manganese	2.3300	1.4000+0.0500
Iron	0.6000	0.3600+0.0020
Copper	0.5000	-
Zinc	0.4700	0.2800+0.0200
Aluminum	0.3200	0.1900+0.0700
Sodium	2.0000	1.2000+0.0200
Magnesium	0.4200	0.2500+0.0060
Silica	5.2000	3.1200+0.1300
Boron	0.0030	0.0020+0.0010
Cobalt	0.0002	0.0001+0.0000

ease with which a material is biodegraded depends on the genetic makeup of the microorganisms present and the makeup of the organic molecules that the organisms decomposes (Maynard 1998). For example, many types of microorganisms can decompose the carbon into sugars, but far fewer types can decompose the carbon into lignin (binding material in wood) and the carbon into plastics may not be biodegradable by any microorganisms (Maynard 1997). Because most municipal and agricultural organics and yard trimmings contain adequate amounts of biodegradable forms of carbon, carbon is typically not a limiting factor in the composting process. As the more easily degradable forms of carbon are decomposed, a small portion of the carbon is converted to microbial cells, and a significant portion of this carbon is converted to carbon dioxide and lost to the atmosphere. As the composting process progresses, the loss of carbon decreases the weight and volume of the feedstock. The less easily

Table 3
Nutritional value of the compost per ton

Nutrient	On dry weight basis quantaty of nutrient (kg / ton)	Market Price of nutrients (Rs.)
Macronutrient		
Nitrogen	15.60	234/=
Phosphorus (P_2O_5)	25.00	350/=
Potash (K_2O)	43.50	435/=
Sulfur	31.10	311/=
Calcium	65.10	65/=
Magnesium	14.00	140/=
Micronutrient		
Iron	3.60	43/=
Manganese	2.50	425/=
Silica	31.00	62/=
Zinc	2.80	392/=
Copper	0.40	155/=
Aluminium	1.90	285/=
Sodium	10.00	-
Total	-	Rs. 2897/=

decomposed forms of carbon will form the matrix for the physical structure of the final product - compost (Maynard 1995).

Microorganisms are responsible for the degradation of organic material. Peak performance by microorganisms requires that their biological, chemical and physical needs be maintained at ideal level throughout all stages of composting (Poincelot 1975). Microorganisms such as bacteria, fungi, and actinomycetes play an active role in decomposing the organic material. Larger organisms such as insects and earthworms are also involved in the composting process, but they play a less significant role compared to the microorganisms (Maynard 1996). As microorganisms begin to decompose the organic material, the carbon in it is converted to by products like carbon dioxide and water, and a humic end product - compost. Some of the carbon is consumed by the microorganisms to form new microbial cells as they increase their population . Heat is released during the decomposition process (Poincelot 1975).Decomposition may proceed slowly at first because of smaller microbial populations, but as populations grow in the first few hours or days, they rapidly consume the organic materials present in the feedstock (Breslin 1995).

The pH of composted material was found to be about 7.1000 (Table 2). The pH between six and eight is considered optimum. The pH affects the amount of nutrients available to the microorganisms, the solubility of heavy metals, and the overall

metabolic activity of the microorganisms. While the pH can be adjusted upward by addition of lime or downward with sulfur, such additions are normally not necessary. The composting process itself produces carbon dioxide, which, when combined with water, produces carbonic acid. The carbonic acid could lower the pH of the compost. As the composting process progresses, the final pH varies depending on the specific type of feed stocks used and operating conditions. Wide swings in pH are unusual. Because organic materials are naturally well buffered with respect to pH changes, down swings in pH during composting usually do not occur. Increasing cost of commercial fertilizer emphasizes the need to find out some alternatives resources and methodology for the improvement of the efficiency of added fertilizer (Twyford 1994). The use of biofertilizer for increasing crop yeild, will help the farmer's community belonging to low income group (Hussain and Anjum 1999). The biofertilizer helps in loosening of clay soil and binding of sandy soil, increasing the availability of oxygen, nutrients at rhizosphere and increases the water holding capacity (Bhatti 1999).

The temperature during composting ranges 50˚C to 60˚C. However, it markedly varies over composting period indicating the biological decomposition. For each group of organisms, as the temperature increases above the ideal maximum, thermal destruction of cell protein kills the organisms. Likewise, temperatures below the minimum required for a group of orga-nisms, affects the metabolic regulatory machinery of the cells. Although, composting can occur at a range of temperatures, the optimum temperature range for thermophilic microorga-nisms is preferred, for two reasons i) to promote rapid com-posting, ii) and to destroy pathogens and weed seeds. Larger piles build up and conserve heat, build up and conserve heat is better than smaller piles. Temperatures above 65˚C are not ideal for composting. Temperatures can be lowered, if needed by increasing the frequency of manual turning. Mixing or mechanical aeration also provides air for the microbes. Pathogen destruction is achieved when compost is at a temperature of greater than 55˚C for at last three days. It is important that all portions of the compost material be exposed to such temperatures to ensure pathogen destruction throughout the compost (Maynard 1995). At these temperatures, weed seeds are also destroyed. After the pathogen destruction is completed, temperatures may be lowered and maintained at slightly lower levels 51˚C to 55˚C (Maynard 1996).

Nutritional value added cost of compost. Table 3 reflects the quantities of different macro and micro nutrients in one ton of compost. It was estimated that with each ton of compost contain nutrients worth Rs. 2897/= that could be added to the soil. This cost does not include the other benefits, like enrichment of organic matter, increase in microbial population, lowering of soil pH, improvement of the soil physical properties and also increase in the availability of nutrient to plants etc.

Table 4
Cost effectiveness of biofertilizer

a)	*Farmers cost on application of biofertilizer*		
1.	Cost of 3 tons of biofertilizer per acre @ Rs.150/= per ton	=	Rs.450.00/=
2.	Transport cost per ton @ Rs.100/= per ton within 20km.	=	Rs.300.00/=
	Total Cost	=	Rs.750.00/=
b)	*Cost incurred on prepration of one ton of biofertilizer by miller*		
1.	Operational cost (includes staff salary, machinery, POL, 16% interest on capital etc.)	=	Rs.17.55/=
2.	Waste collection and shifting cost	=	Rs. 5.10/=
3.	Publicity and advertisement	=	Rs. 7.29/=
4.	Land rent	=	Rs. 2.91/=
5.	Miscelleneous	=	Rs. 7.15/=
		Total =	Rs.40.00/=
c)	*Cost-benefit ratio*		
1.	In respect to biofertilizer application per acre of land (Rs. 8691/= as cost of nutrients, Rs. 750 as transport cost incurred by the farmer)	=	Rs.750 : 8691 or 1:11.60
2.	Cost in respect to millers	=	Rs.120 : 450 or 1:3.75

It is likely to reduce the 20 to 30 % demand of chemical fertilizer initially (Singh and Solomon 1995). They also reported that continuous use of biofertilizer increases the sugarcane yield by 20% with an additional income of Rs. 3500/=. Mineralization of biofertilizer in the soil improves the population of ammonifying bacteria. The decomposition of biofertilizer in the field supplements the demand of farmyard manure (Bhatti 1999). The population of *Azobacter*, *Azospirillum* and ammonifying bacteria increases several fold with the continuous use of biofertilizer in the agricultural fields (Singh and Solomon 1995). They also recommended that the application of biofertilizer to the soil provokes the development of soil microbial life, it maintains the soil pH, increases the cation exchange capacity increase the availability NPK and improves the structural stability of soil. Biofertilizer also regulates the release of NPK fertilizer and provides nutrient to sugarcane and other crops as per their requirement (Bhatti 1999). During decomposition of filter cake, heat is generated and may catch fire. Mixing of boiler ash and distillery water

that exerts a sponging and buffering effect and allows free exchange of heat and gases can prevent this. The heat generated during decomposition released in air and moist condition enhanced the microbial activity to produce good biofertilizer. The by-products of sugar industry are bagasse molasses, press mud, distillery effluents (Paturou 1982); (Zende and Patil 1988).

Cost economics. Table 4 summarizes the cost economics of recycling of sugar industrial waste into biofertilizer. Keeping in view, the recycling cost at commercial scale it was calculated that each ton costs around Rs.40/= to the millers if the sale price is fixed at Rs.150/= per ton. The cost benefit ratio is about 1:4 in favour of the millers. However, the farmer will have to spend Rs.750/= (Rs.450/= for 3 ton/acre as cost of biofertilizer and Rs.300/= as transport charges) for each acre of the land. By spending Rs.750/= the farmer adds macro and micronutrients worth Rs.8691/= to each acres of his land besides harvesting other important benefits discussed before, therefore, cost effectiveness for farmer is about 1:12. However, the mill owner can give more relaxation to farmers in cost of biofertilizer for improvement of soil health and other environmental and mutual benefits.

Conclusion

Disposal of sugarcane industrial wastes is a big challenge for industrialist because it requires larger area for dumping, as it produces foul smell environmental pollution and health hazard problems in the vicinity. Since synthesis of sucrose in sugarcane involves CO_2 and H_2O, through metabolic process. The huge amount of nutrient transported from soil with sugarcane crop may cause rhizosphere depletion of essential macro and micro nutrients in the field, therefore, the vitality and success of sugar industry were found in recycling sugarcane industrial wastes as biofertilizer. The only use of chemical fertilizer can not solve the nutrient depletion problem of Pakistani soil. Since, chemical fertilizer was developed as nutrient correction factor but it was adopted as a solution for soild fertility. It is, therefore, recommended that each sugar industry should develop sugarcane industrial wastes recycling facilities for sustainable agriculture.

References

Anonymous 1999 Agriculture Monthly Economic Letter, National Bank of Pakistan **26**(8) 3 - 4.

Arian M A, Mubarik A, Muzaffar A 2000 Some physico-chemical characteristic of soil in sugarcane cultivated area of Nawabshah, Sindh. *Pak J Botany* **32**(1) 93 - 98.

Bhatti M A R 1999 Self sufficiency in food through soil improvement - use of biofertilizer. *Pak Food and Agri Review* **1** 12 - 14.

Breslin V T 1995 Use of MSW compost in commercial sod production. *Bio Cycle* **36**(5) 68 - 193.

Calcino D V 1995 *Australian Sugarcane Nutrition Manual*. Published by Sugarcane Research and Development Corporation (SRDC), pp 60.

Gardner F P, Pearce R B, Mitchell R L 1985 *Physiology of Crop Plant*, Iowa State University Press, USA, pp 327.

Hussain T, Anjum S 1999 Role of Biofertilizer in Boosting Agriculture. "Daily Dawn" Economic & Business Review, October 4 - 10, pp 3.

Khan A A 1996 Soil depletion, a major threat on our agriculture. *Pak Food and Agri Review* **I** (XI - XII) 10 - 13.

Khushak A M 1999 Sugarcane Production and Sugar Industry. "Daily Dawn", Economic and Business Review. February 1 - 7, pp 3.

Lakshmikantham M 1983 *Technology in Sugarcane Growing*. Oxford and IBH Publishing Company, New Delhi 110028, India, pp 259.

Liu Y T, Kuo Y C, Wu G D, Li L B 1991 Organic compound fertilizer from ethanol distillery slop. *Taiwan Sugar Research Institute Journal* 358 - 362.

Maynard A A 1993a Nitrate leaching from compost amended soils. *Compost Science and Utilization* **1**(2) 65 - 72.

Maynard A A 1993b Evaluating the suitability of MSW compost as a soil amendment in field-grown tomatoes. *Compost Science and Utilization* **1**(2) 34 - 36.

Maynard A A, Hill D E 1994 Impact of compost on vegetable yields. *Bio Cycle* **35**(3) 66 - 67.

Maynard A A 1994a Sustained vegetable production for three years using composted animal manures. *Compost Science and Utilization* **2**(1) 88 - 96.

Maynard A A 1994b Effect of annual amendments of compost on nitrate leaching in nursery stock. *Compost Science and Utilization* **2**(3) 54 - 55.

Maynard A A 1995 Cumulative effect of annual additions of MSW compost on the yield of field-grown tomatoes. *Compost Science and Utilization* **3**(2) 47 - 54.

Maynard A A 1996 Cumulative effect of annual additions of undecomposed leaves and compost on the yield of field-grown peppers. *Compost Science and Utilization* **4**(2) 81 - 88.

Maynard A A 1997 Cumulative effect of annual additions of undecomposed leaves and compost on the yield of tomatoes. Compost: The Process and Research 1313 tomatoes and peppers. *Compost Science and Utilization* **5**(1) 38 - 48.

Maynard A A 1998 Utilization of MSW Compost in Nursery Stock Production. *Compost Science and Utilization* **6**(4) 38 - 44.

Maynard A A, Hill D E 2000 Cumulative effect of leaf compost

on yields and size distribution in onions. *Compost Science and Utilization* **8**(1) 12 - 18.

Paturou J M 1982 By products of cane sugar industry. *Taiwan Sugar Corp Taipei*, pp 241 - 244.

Poincelot R P 1975 The biochemistry and methodology of composting. *Ct. Agric. Exp. Sta.* Bulletin 754, pp 18.

Rowell D L 1994 *Soil Science Methods and Applications*. Longman group U.K. Limited, Longman House Burnt Mill Harlow. CM20 2JE England, UK, pp 350.

Singh B B, Solomon S 1995 *Sugarcane Agro - Industrial Alternatives*. Published by Vijay for Oxford and IBH Publishing Company, 66 Janpatt New Delhi (13), India, pp 556.

Stentiford E I 1993 Diversity of composting systems. Science and Engineering of Composting : Design, Environmental, Microbiological and Utilization Aspects. In: *Proceedings of an International Composting Research Symposium,* Ohio State University 1992 pp 95 - 110.

Subba R B 1985 Sugar Factory and Allied Industrial Wastes. *New Facu* pp 68 - 75.

Tchobanoglous G H, Theisen S V 1993 *Integrated Solid Waste Management*. McGraw - Hill, Book Co., USA, pp 70 - 71, 689 - 691.

Twyford I T 1994 Fertilizer Use and Crop Yield. In: *Proc.4th, National Congress Soil Science, Islamabad*, Pakistan pp 47 - 71.

Yadava R L 1991 *Sugarcane Production Technology*. Constraints and potentialities. Oxford and IBH Publishing Co., 66 Janpatt New Delhi, 110001, India, pp 291.

Youshida S, Forno D A, Cock J H, Gomez K A 1976 *Laboratory Manual for Physiological Studies of Rice*. pp 83.

Zende N A, Patel A S 1988 *Pro. Nat. Seminar on Industrial Wastes*. A new focus Tech. Session III, pp 37 - 42.

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TRACE ELEMENTS IN INDIGENOUS MEDICINAL DIURETIC PLANTS IN HUMAN HEALTH AND DISEASE (*CYMBOPOGON CITRATUS* (DC) STAPF., *RAPHANUS SATIVUS* LINN. AND *ZEA MAYS* LINN.)

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Three indigenous medicinal plants reported to be diuretic have been selected for the study of trace elements and their possible role in human health. Twelve trace elements (Cu, Zn, Mn, Fe, Cu, Ni, Cd, Pb, Cr, Ag, Na and K) have been detected and estimated in ash of various parts (leaves of *Cymbopogon citratus* (DC) Stapf., seeds of *Raphanus sativus* Linn. and corn silk of *Zea mays* Linn.).

Key words: Diuretic medicinal plants, Trace elements, *Cymbopogon citratus, Raphanus sativus, Zea mays*.

Introduction

Trace elements are widely distributed in nature in variable proportions and they play a vital role in the growth, health and maintenance of human body, in the same way as the proteins, vitamins, and other essential nutrients do. While the trace elements are of great interest and importance, for their therapeutic efficacy, their total or partial lack may result in characteristic pathological deficiency signs and symptoms. Therefore, daily intake of trace elements should be such that their lack does not lead to any diseases / disturbance (Bukhari *et al* 1987).

Medicinal plants play an important and vital role in traditional medicine. In most developing countries, most of the flora remain virtually unexplored from point of view of the medicinal utilization through traditional eastern system of medicine. In the present studies, twelve trace elements Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Cr, Ag, Na and K have been estimated in the following three indigenous medicinal plants.

1. *Cymbopogon citratus* (DC) Stapf.
   Family Gramineae (Chopra *et al* 1958)
2. *Raphanus sativus* Linn.
   Family Cruciferae (Nadkarni 1954; Chopra *et al* 1958; Watt *et al* 1962)
3. *Zea mays* Linn.
   Family Gramineae (Watt *et al* 1962)

These plants are used as a diuretic and also used for the treatment of cancer, cardiovascular diseases and hypercholesterolemia (Nadkarni 1954; Chopra *et al* 1958; Watt *et al* 1962).

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Materials and Methods

Seeds of *Raphanus sativus* Linn. (Voucher No. 501) and stigma (corn silk) of *Zea mays* (Voucher No. 502), one kg each, were purchased from local market and leaves of *Cymbopogon citratus* (DC) Stapf. (Voucher No. 499) were taken (cultivated in PCSIR Laboratories Complex, Karachi and identified by the Pharmacognosy Section of this laboratory). The sample leaves of *Cymbopogon citratus* (DC) Stapf., seeds of *Raphanus sativus* Linn. and corn silk (stigmata) of *Zea mays* Linn. were separated, washed, oven dried, powdered, ignited in muffle furnace at 550˚C to obtain ash of various parts and then used for the analysis.

1. *Instruments.* i. Hitachi Z-8000 Atomic Absorption Spectrophotometer equipped with Zeeman background correction and a data processor was used for elemental analysis of the samples. ii. Sodium and potassium were estimated by flame photometer (Corning Model 410).

2. *Procedure*. 1.0 gm of ash was digested with 10.0 ml concentrated nitric acid (Analar) (HNO_3) in acid washed pyrex tube at 120˚C till the solution was clear and volume was reduced to about 1.0 ml. The solution was made upto 10.0 ml with double distilled water. Similarly, a blank sample was also prepared. Estimations were made using standard addition technique. The dilutions were made such as to keep the concentration of different elements within the linear range of absorbance (Williams 1984). Sodium and potassium were estimated by flame photometer (William 1984).

3. *Calibration.* Calibration curves were prepared for each element keeping in view their linear working ranges.

Table 1

Trace elements in indigenous medicinal diuretic plants *Cymbopogon citratus* (DC) Stapf. (leaves), *Raphanus sativus* Linn. (seeds) and *Zea mays* Linn. (corn silk)

Sr. No.	Name of Plant	Trace elements											
		Cu	Zn	Mn	Fe	Co	Ni	Cd	Pb	Cr	Ag	Na	K
1.	*Cymbopogon citratus* (DC) Stapf. (leaves)	64.77 ± 0.01	59.09 ± 3.00	31.82 ± 2.00	594.30 ± 4.00	-	1.14 ± 0.10	-	51.14 ± 2.00	-	-	2727.27 ± 1.50	10795.45 ± 0.80
2.	*Raphanus sativus* Linn. (seeds)	51.55 ± 0.02	524.07 ± 4.00	326.47 ± 5.00	1348.84 ± 1.00	2.58 ± 0.40	12.89 ± 0.60	1.72 ± 0.50	4.30 ± 1.00	Traces	3.44 ± 1.00	17612.30 ± 1.00	13209.20 ± 1.20
3.	*Zea mays* Linn. (corn silk)	50.85 ± 0.02	417.73 ± 0.55	217.91 ± 5.00	1153.29 ± 2.00	2.72 ± 0.50	7.26 ± 0.70	0.91 ± 0.20	28.15 ± 2.00	11.81 ± 0.70	4.54 ± 0.80	19070.10 ± 1.30	122593.50 ± 1.00

Mean value ± S.D. (n=5)

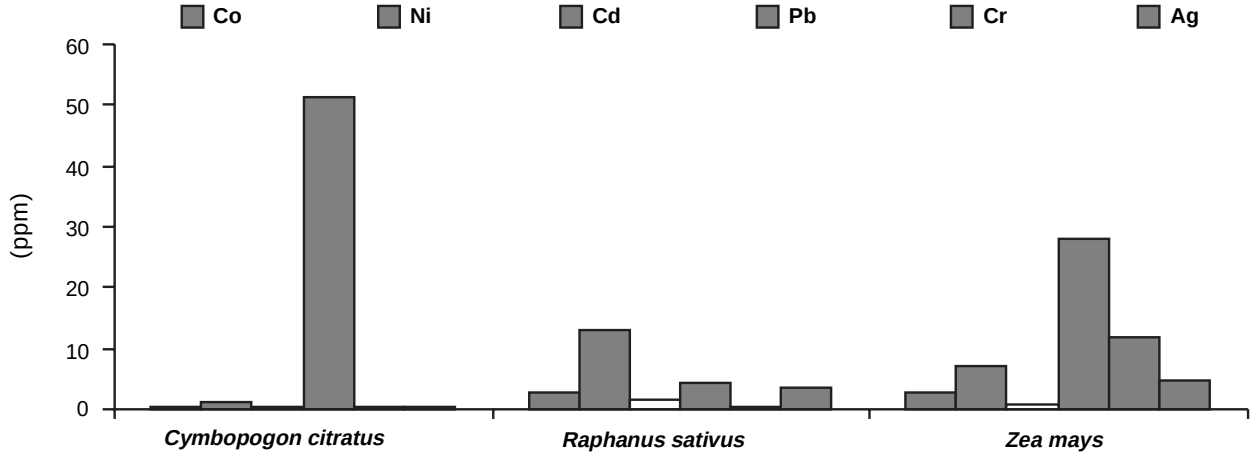

Fig. 1 Trace elements in indigenous medicinal diuretic plants *Cymbopogon citratus* (DC) Stapf. (leaves), *Raphanus sativus* Linn. (seeds) and *Zea mays* Linn. (corn silk).

4. *Calculation.* Amount of different elements in ppm in the sample solutions were obtained from the data processor (Table 1).

Results and Discussion

Medicinal plants were analysed for their trace elements contents. Onawunmi *et al* (1984) reported that *Cymbopogon citratus* commonly known as lemon grass had been used for medicinal purposes in West Africa. It is used for the treatment of nervous and gastrointestinal disturbances (Carlini *et al* 1986). Mirza *et al* (2001) had shown that the lemon grass tea is as safe as other widely used green tea. It was found that the leaves of *Cymbopogon citratus* contain Fe, Mn, Zn, Cu, Ni and Pb but Co, Cr and Ag are not present in the leaves, whereas Na and K contents are very high but low as compared to two other plant contents *Raphanus* and *Zea mays* (Table 1).

Cymbopogon citratus has a β-sitosterol, it acts as an anticholestemic agent (Wang *et al* 1979). The presence of Mn may be correlated with therapeutic properties i.e. antidiabetic and for cardiovascular diseases. Cu and Zn being chemical antagonist and both have an important role in controlling lipid level. The content of Cu is high in *Cymbopogon* as compared to two other plants contents.

It was observed that *Raphanus sativus* Linn. seeds contain Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Ag, Na and K. Chromium is also present in trace amounts. The contents of Zn, Mn, Fe and Ni are very high as compared to *Cymbopogon* and *Zea mays* (Table 1).

The seeds of *Raphanus sativus* Linn. have been used in the treatment of cancer (Quisumbing 1951) and also show diuretic and lithotryptic activity (Watt *et al* 1962).

Zea mays (corn silk) is a chemical complex medicinal plant, having valuable properties. The substances found in it are sitosterol, fatty and volatile oils, saponins, a bitter glucosidic substance, vitamin C and vitamin K. Corn silk acts as a diuretic agent (Bobrayshev 1962). It is also used for kidney and bladder complaints (Watt *et al* 1962).

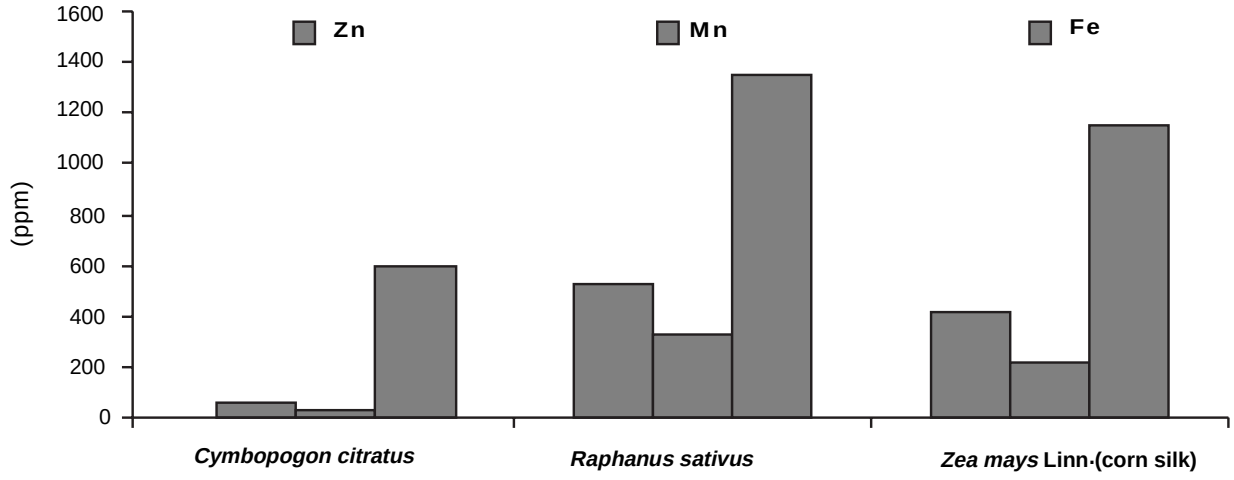

Fig. 2 Trace elements in indigenous medicinal diuretic plants *Cymbopogon citratus* (DC) Stapf. (leaves), *Raphanus sativus* Linn. (seeds) and *Zea mays* Linn. (corn silk).

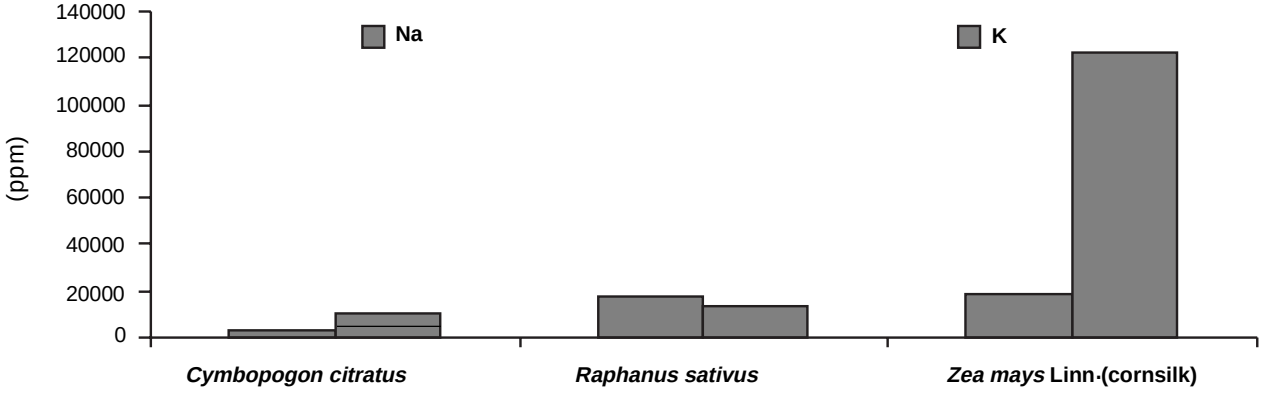

Fig. 3 Trace elements in indigenous medicinal diuretic plants *Cymbopogon citratus* (DC) Stapf. (leaves), *Raphanus sativus* Linn. (seeds) and *Zea mays* Linn. (corn silk).

Zea mays (corn silk) contains Cu, Zn, Mn, Fe, Co, Ni, Cd, Pb, Cr, Ag, Na and K. The contents of Na, K and Cr are very high as compared to two other plant contents (Table 1 and Fig. 1- 3).

Sodium and potassium are the major ions in the body fluids. The regulation of the proper concentration of these ions in the extracellular and intracellular fluid is critical for homeostasis (Montgomery *et al* 1996).

Trace elements in one form or in another form play an important role in the field of medicine in combating disease as a curative or preventive agent. It is concluded from the above study that trace elements content in these plants may play an important role in human health.

References

Bobryshev N E 1962 Corn a medicinal plant. *Kukuruza* **59** (9) *Biol Abstr* 1963 **62**(4) 15513.

Bukhari A Q S, Ahmad S, Mirza M 1987 The role of trace elements in health and disease. *Elements in Health and Disease,* Second International Conference, Hamdard Foundation, Pakistan pp 116.

Carlini E A, Contar J de P, Silva-Filho A R, Da Silveira-Filho, N G, Frochlehgarten M L, Bueno O F A 1986 Pharmacology of lemon grass (*Cymbopogon citratus* Stapf). I: Effect of teas prepared from the leaves of laboratory animals. *J Ethnopharmacol*, **17** 37 - 64.

Chopra R N, Chopra I C, Handa K L, Kapur L D 1958 *Chopra's Indigenous Drugs of India.* U.N. Dhur & Sons, Calcutta, India pp 503, 522.

Mirza M, Askari S, Yaqeen Z, Ahmed Z, Qadri R B 2001 Diuretic studies on lemon grass tea from *Cymbopogon citratus* (DC) Stapf in rat. *Pak J Sci Ind Res* **44**(2) 96 - 100.

Montgomery R, Conway T W, Spector A A, Chappell D 1996 Nutrition. In: *Biochemistry*. 6th ed, Mosby - Year Book Inc. USA, pp 120.

Nadkarni A K 1954 *Indian Materia Medica*. Popular Book Depot, Bombay, India, Vol. I, 3rd ed., pp 1049 - 1050.

Onawunmi G O, Yisak W A B, Ogunlana E O 1984 Antibacterial constituents in the essential oil of *C. citratus* (DC). *J Ethnopharmacol* **12** (3) 279 - 286.

Quisumbing E 1951 *Tech Bull Phillip Dep Agric Nat Res*, 16.

Wang C T, Ting K, Wu Y H 1979 Studies on chemical constituents of *Raphanus sativus* seeds. Part I. *Shan-hsi Hsin I Yao* **8**(11) 50, 65.

Watt J M, Maria G, Breyer B 1962 *The Medicinal and Poisonous Plants of Southern and Eastern Africa*. E & S Livingstone Ltd. Edinburgh and London, UK, 2nd ed. pp 334, 489 - 490.

Williams S 1984 *Official Methods of Analysis of the Association of Official Analytical Chemists*.14th ed Association of Official Analytical Chemist, Inc. USA, pp 38 - 40.

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SEED - BORNE MYCOFLORA OF OATS IN THE PUNJAB

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Twenty - eight seed samples of eight oats (*Avena sativa* L.) cultivars were collected from Sargodha, Faisalabad and Bahawalpur districts of the Punjab, Pakistan during 1999 - 2000 and analyzed for seed - borne mycoflora during 2000 - 2001. Four genera and nine species of fungi were identified at different frequencies. *Drechslera avenae* and *D. sorokiniana*, known pathogens causing pre - and post - emergence seedling blight and leaf spot in mature plants were detected in 67.80 and 53.60% of the seed samples with maximum infection of 34.00 and 6.00%, respectively. *Phoma* sp. was found in 46.40% of the sample with a maximum infection of 16.00%. Phoma is a new pathogen recorded on oats in Pakistan. All these fungi were found equally pathogenic and caused 86.00, 67.80 and 86.70% pre - and post - emergence seedling mortality in pathogenicity test. These pathogens produced almost the same type of symptoms on roots and leaves. In two samples *Cephalosporium* sp. was recorded in high frequencies (up to 66.00%) but did not show any pathogenic effect on seeds and seedlings. The observed association of different fungi with oats seeds in the present study indicates the need of thorough survey for these and other pathogenic fungi.

Key words**:** *Avena sativa*, Seed - borne fungi, Seed germination.

Introduction

Fodder crops play a pivotal role in the dairy industry. These crops are equally important for draught - adapted animals such as camels, bulls, horses and mules etc. Generally, these crops are planted as secondary crops. Therefore, little attention is devoted to fertilization, planting density and plant protection measures. Efforts have been made to enhance the yield of fodder by different cultural means such as fertilizer, sowing methods, irrigation etc. However, little attention has been paid to fodder crops diseases that should be considered as one of the important causes for poor vigour and low yield. Oat (*Avena sativa* L.) is one of the major Rabi fodder crops. It is mainly sown around the big cities, at dairy farms and remount depots for feeding of cattle and draught - adapted animals.

Oats are attacked by many diseases, of which seedling blight and leaf spot [*Drechslera avenae* (Eidam) Scharif], seedling blight and root rot [*D. sorokiniana* (Sacc.) Subram and Jain], Victoria leaf blight [*D. victoriae* (Meehan and Murphy) Subram and Jain], snow mould and brown foot rot [*Fusarium nivalae* (Ces. Ex Berl. and Voglino)], leaf blotch [*Septoria avenae* (A. B. Franke)] and loose smut [*Ustilago avenae* (Pers.) Rostr] are of major importance in the world (Neergaard 1979). In Pakistan, efforts have been made to enhance the yield by developing high yielding cultivars and adopting advanced agronomical practices but little attention has been given to the health status of seeds of fodder crops that is one of the main reasons of low yield.

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In the light of the importance of seed - borne pathogens in oats and the limited information on such aspects, present study was initiated to find out the health status of oats seeds in the Punjab and the influence of important fungi on germination of oats seeds.

Materials and Methods

Twenty - eight seed samples (about 1.5 kg) of oats cultivars; Algerian, Avon, Early miller, Fullgrain, Kent, Palestine, PD_2 LV65 and SSII were obtained from the Fodder Research Institute, Sargodha, the Fodder Research Sub Station, Faisalabad and the local markets of Bahawalpur from crops harvest of 1999 - 2000. Four hundred seeds taken randomly from each sample were analyzed for the presence of seed mycoflora by following standard blotter paper method (ISTA 1993) at the Regional Agricultural Research Institute, Bahawalpur. Twenty - five seeds were plated in each petri dish (9 cm dia) containing three layers of blotter paper well moistened with sterilized water. The petri dishes were incubated at 22 ± 2°C for seven days under 12 h alternate cycle of light and darkness. Fungi developing on seeds were examined and identified on the 8th day under the stereoscopic microscope and high magnification of the compound microscope (Ellis 1971; Paul *et al* 1983). The isolates were purified and maintained on potato dextrose agar (PDA) media for further confirmation of identification and pathogenicity studies. Heavily infected seeds of important isolates of *Drechslera avenae*, *D. sorokiniana*, *Phoma* sp. and *Cephalos-*

Table 1
Frequency of fungi recorded on oats seeds by Blotter Method in 28 seed samples

Sr. No.	Fungi	Infected samples (%)	Infection range (%)
1	*Cephalosporium* sp.	53.60	0.25 - 66.00
2	*Drechslera avenae*	67.80	0.50 - 34.00
3	*D. biocolor*	10.70	0.50 - 2.50
4	*D. rostrata*	14.30	0.25 - 1.00
5	*D. sorokiniana*	53.60	0.50 - 6.00
6	*Fusarium equiseti*	25.00	0.25 - 2.00
7	*F. moniliforme*	35.70	0.50 - 6.00
8	*F. semitectum*	64.30	0.50 - 14.00
9	*Phoma* sp.	53.60	0.25 - 16.00

porium sp. were transferred from blotter to pots having sterilized soil to observe the effects of these pathogens on seeds and seedlings of oats. These observations were later compared with inoculated seeds in pathogenicity test.

One sample (cv. Early miller) with lowest visible infection of *D. avenae*, *D. sorokiniana* and *Phoma* sp. was selected. Two hundred seeds pre-treated with 1.00% available chlorine for 5 min were placed for 48 h on top of 10 days old viable pure cultures of each test pathogen. All the seeds were then transferred to test tubes (one seed per test tube) having 1.00% agar in order to examine the effects of these fungi on germination and seed to seedling transmission of the diseases. Surface sterilized seeds plated on water agar at the same time served as check. Data on germination, seedling mortality and shoot and root length were recorded 16 days after placing the seeds in agar slants. The symptoms appeared on seedling were also recorded.

Results and Discussion

From 28 seed samples of oats, *Cephalosporium* sp., *Drechslera avenae*, *D. bicolor*, *D. rostrata*, *D. sorokiniana*, *Fusarium equiseti*, *F. moniliforme*, *F. semitectum* and *Phoma* sp. were recorded in different frequencies (Table 1). *D. avenae* was recorded in 67.80% of the seed samples. The fungus was recovered from 0.50 to 34.00% from five cultivars Algerian, Avon, Fullgrain, Palestine and SSII with a maximum infection of 34.00% on Fullgrain followed by 23.00% in Algerian (Table 2). In most of the cases, the seeds were covered with profuse growth of mycelia and conidia causing lesions on emerging radicals. Small black Pycnidia, the perithecial state of *Pyrenophora avenae*, were also recorded on few seeds. These results are in accordance with those of Linns (1989) who found *D. avenae* associated with 90.00% of 232 samples from 7 oats cultivars from the 1987 harvest and that most of

Table 2
Incidence (%) of important fungi on oats seeds of different cultivars in the Punjab

Cultivar	No.of samples examined	Important isolates	Infection range (%)
Algerian	5	*Cephalosporium* sp.	0.00 - 66.00
		Drechslera avenae	0.50 - 23.00
		D. sorokiniana	0.00 - 4.50
		Phoma sp.	0.00 - 2.50
Avon	6	*Cephalosporium* sp.	0.50 - 6.75
		D. avenae	0.00 - 7.50
		D. sorokiniana	0.00 - 4.50
		Phoma sp.	0.00 - 3.00
Early miller	2	*D. sorokiniana*	0.00 - 0.50
Full grain	6	*D. avenae*	0.00 - 34.00
		D. sorokiniana	0.00 - 1.50
		Phoma sp.	0.00 - 4.50
Kent	1	*Cephalosporium* sp.	8.50
		Phoma sp.	1.75
Palestine	5	*D. avenae*	0.00 - 12.00
		D. sorokiniana	0.00 - 2.50
		Phoma sp.	3.00 - 16.00
PD_2 LV65	2	*Cephalosporium* sp.	1.00 - 6.50
		D. sorokiniana	0.50 - 6.00

the samples were only 1 - 9.00% infected with one sample showing 84.00% infection. Moreover, no differences were reported in infection rate among cultivars (Linns 1989). Langaro *et al* (2001) reported that the fungus *D. avenae* (*Pyrenophora chaetomioides*) the causal agent of *Helminthosporium* leaf spot of oats (*Avena sativa*) survives as mycelia in crop residues and in infected seeds.

The growth intensity of *D. avenae* on individual seeds in the blotter test was directly correlated with the amount of damage to these seeds measured in loss of seed and seedling in the soil. The infected seeds by this fungus when transferred from blotter to sterilize soil in pots, gave 86.00% pre - and post - emergence loss of seedlings (Table 3). The infection of roots and coleoptiles either arrested the germination or caused death of seedlings for 10 - 15 days after emergence. The parts of dead seedlings and rotted seeds from these pots yielded 97.90% *D. avenae* when incubated for 7 days at 22 ± 2°C (Table 3). These results agree with the studies conducted by Ruland *et al* (1989) who reported that infection of *D. avenae* at seedling stage in the field was proportional to the infection of heads and flowers at the time of grain formation. *D. avenae* is of world wide importance and known to cause economic looses to oats in the forms of pre- and post- emergence seedling blight and

Table 3
Percentage of seedling mortality and recovery of pathogens from infected parts of dead seedlings

No.of infected sample	Pathogen	No.of infected seeds transferred from blotter to soil	Pre- and post-emergence seedling mortality (%)	Pathogens recovered from dead seedlings	Recovery (%)
2	*Cephalosporium* sp.	100	3.00	-	-
2	*Drechslera avenae*	100	86.00	*Drechslera avenae* *D. sorokiniana* *D. avenae* + *F. semitectum*	97.90 1.05 1.05
3	*D. sorokiniana*	56	67.80	*D. sorokiniana* *D. avenae* + *Phoma* sp.	83.30 16.70
2	*Phoma* sp. *Phoma* sp. + *D. avenae*	60	86.70	*Drechslera avenae* *D. avenae* + *Phoma* sp. *Phoma* sp.	64.60 12.50 23.00

Table 4
Effect of pathogens on seed germination and seedling of *Avenae sativa* in pathogenicity test (200 seeds)

Pathogen	Germinated seeds (%)	Decrease over control (%)	Average shoot length (cm)	Average root length (cm)	Symptoms on seedlings
Cephalosporium sp.	96.50	1.50	16.00	6.00	No symptoms
Drechslera avenae	84.00	14.30	7.80	3.40	Blighting of coleoptiles and roots with brown flecks with light brown to yellowish white centers on leaves spreading up and down, covering entire leaf blade, roots stunted with brown lesions darkened when specks coalesced
D. sorokiniana	85.00	13.30	6.50	3.00	Shoot appearing weak with brown to black flecks on leaves spread up and down, roots stunted, decayed with brown lesions
Phoma sp.	85.50	12.70	9.10	3.10	Reddish brown flecks with grey cottony mycelium on coleoptiles reddish brown lesions with yellow halos on leaf sheet, roots stunted with brown colour
Un-inoculated seed (check)	98.00	-	15.40	5.60	No symptoms

as leaf spotting of mature plants (De Tempe 1964; Malone and Muskett 1964).

D. sorokiniana was found in 15 seed samples from six cultivars and severity of infection ranged from 0.50 to 6.00%. Maximum severity (6.00%) was recorded in PD_2 LV65 (Table 2). *D. sorokiniana* has a wide host range and causes seedling blight, root rot and blotch of oats, brome grass, barley and wheat (Noble and Richardson 1968). Khan and Bhutta (1994) isolated *D. sorokiniana* in high frequencies as a main pathogen from 1267 wheat seed samples of 25 cultivars collected during 1985 - 1990 in Pakistan. They also recorded *Fusarium moniliforme* (*Gibberella fujikuroi*) and *Cephalosporium acremonium* (Corda) in different frequencies along with some saprophytic fungi. Fifty - six seeds infected with *D. sorokiniana* transferred from blotter to sterilized soil in pots resulted in 76.80% pre - and post - emergence seedling mortality (Table 3). In Brazil, Goulart (1996) studied the transmission of *Bipolaris sorokiniana* [(Sacc.) Shoemaker] from seed of wheat coleoptiles and

found positive and significant correlation between incidence on seed and its transmission to seedling coleoptiles both in field and green house studies. The seeds of wheat cv. Anahuac were found to be 16 - 90.50% naturally infected, and that the seeds with 90.50% infection resulted in 1.1: 1 transmission index. The loss of wheat due to root rot (*B. sorokiniana*) was assessed in field experiments by Zhang *et al* (1999) in Heilongjiang province (China) and reported that the percentage of ear bearing tillers decreased at the seedling stage while grain weight decreased significantly due to the disease causing necrotic leaf spots and head blight at the ripening stage.

Phoma sp. was observed in 46.40% of the samples (Table 1). The recovery of the fungus ranged from 0.25 to 16.00% with one sample showing upto 16.00% infection in the variety Palestine. Most of the seeds were covered with pycnidia. This is a new record in Pakistan on oats. This fungus is responsible for foot rot of flax in most of the European countries where the crop is grown for the fiber. The disease may become epidemic if the crop is grown from infected seed (Malone and Muckett 1964). Bevilaqua and Pierobom (1995) studied the presence of seed - borne fungi in the 1992 - 1993 harvested seeds of *Avena strigosa* in Brazil. Of the 11 fungal genera identified, only *Helminthosporium* sp. and *Phoma* sp. occurred in 29.00 and 22.00% incidence, respectively. High fungal contamination was associated with reduced germination and rate of emergence in the field.

Seeds heavily infected with *D. avenae*, *D. sorokiniana* and *Phoma* sp. resulted in poor germination when transferred from blotter to sterilized soil in pots. Reddish brown spots with a light brown to yellowish white centre were observed covering the leaf in case of *D. avenae* and *Phoma* sp., while *D. sorokiniana* produced brown to black flecks on leaves (Table 4). Most of the seedlings failed to appear above ground while others reached the surface and produced aggregated loss of 76.80 to 86.70%. Isolations made from rotted seeds and diseased parts of the seedling yielded *D. avenae*, *D. sorokiniana* and *Phoma* sp. individually and in combination of these fungi in different frequencies (Table 3).

Two seed samples of the cv. Algerian were heavily infected (up to 66.00%) with *Cephalosporium* sp. (Table 2). In most of the cases, the fungus completely covered the seeds. Seeds infected with *Cephalosporium* sp. produced healthy seedlings and did not show any symptoms even after two months of growth (Table 4).

Pathogenicity test revealed that *D. avenae*, *D. sorokiniana* and *Phoma* sp. lowered the germination by 14.30, 13.30 and 12.70%, respectively, in comparison to the control and reduced the root and shoot length of seedlings. Similar symptoms developed on seedlings regardless of fungi (Table 4).

The present study indicated the need of thorough survey for presently identified pathogens as well as other pathogenic fungi. Such measures will permit the introduction of regulations of seed certification in order to provide healthy seed of improved cultivars to the farmers.

References

Bevilaqua G A P, Pierobom C R 1995 Sanitary and physiological quality of seeds of *Avena strigosa* Schreb from Southern Rio Grande do Sul. *Revista Brasileira de Sementes* **17**(1) 19 - 22.

De Tempe J 1964 *Helminthosporium* spp. in seeds of wheat, barley, oats and rye. *Proc Int Seed Test Assoc* **29** 117 - 140.

Ellis M B 1971 *Dematiaceous Hyphomycetes*, CMI. Kew, Surrey, England, pp 608.

Goulart A C P 1996 Transmission of *Bipolaris sorokiniana* from seeds of wheat coleoptiles. *Summa Phytopathologica* **22**(1) 5 - 9.

ISTA 1993 International Rules for Seed Testing. *Proc Int Seed Testing Assoc*, Zurich, Switzerland, pp 177.

Khan M O, Bhutta A R 1994 Seed - borne fungi of wheat cultivars in Pakistan. *Pak J Sci Ind Res* **37**(9) 397 - 398.

Langaro N C, Reis E M, Floss E L 2001 Detection of *Drechslera avenae* in oats seeds. *Fitopatologia Brasileira* **26**(4) 745 - 748.

Linns H 1989 Detection of oats leaf spot pathogen, *Drechslera avenae* in certified seed in Lippe, Westphalia. *Gesunde Pflanzen* **41**(2) 55 - 59.

Malone J P, Muskett A E 1964 Seed-borne fungi: Description of 77 fungus species. *Proc Int Seed Test Assoc* **29**(2) 179 - 384.

Neergaard P 1979 Economic significance of seed-borne diseases. In:*Seed Pathology*, Vol.I. The Macmillan Press Ltd., London, UK, pp 3 - 38.

Noble M, Richardson M J 1968 An annotated list of seed-borne diseases. *Proc Int Seed Test Assoc* **33** 1 - 191.

Paul E N, Tourson T A, Marasos W F O 1983 *Fusarium Species. An Illustrated Manual for Identification of Fusarium Species.* Pennsylvania State Univ Press, University Park, London, UK, pp 193.

Ruland W, Frahm J, Fehramann H 1989 Disease development of *Drechslera avenae* on oats. *Zeitschrift fiir Pflanzenkrankheiten and Rflanzenschutz* **96**(1) 39 - 46.

Zhang Y H, Chi P, TiRuo L, YanKun L, Zhibin Z, Ying L X, Yong L 1999 Loss of spring wheat caused by wheat root rot in Heilongjiang Province. *Acta Phytopathologica Sinica* **29**(4) 329 - 332.

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EFFECTS OF NITROGEN AND COPPER FERTILIZATION ON RICE YIELD AND FERTILIZER NITROGEN EFFICIENCY: A ^{15}N TRACER STUDY

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A greenhouse experiment was conducted at University Putra Malaysia to evaluate the effects of nitrogen (N) and copper (Cu) fertilizations on rice yield and fertilizer N efficiency using ^{15}N tracer technique. Four rates of N (0, 60, 120 and 180 kg N ha^{-1} and three rates of Cu (0, 5 and 10 kg Cu ha^{-1}) were used in this study. Nitrogen was applied as ^{15}N tracer technique. Four rates of N (0, 60, 120 and 180 kg N ha^{-1}) and three rates of Cu (0, 5 and 10 kg Cu ha^{-1}) were used in this study. Nitrogen was applied as ^{15}N labelled urea. Grain yield increased significantly due to N fertilization up to 120 kg N ha^{-1}. Regression analysis indicated that grain yield response due to N fertilization that was quadratic in nature. Estimated N rate for maximum yield was 158 kg N ha^{-1}. Copper application did not increase grain yield although the soil was deficient in Cu. The ^{15}N atom excess percentage in both grain and straw, and fertilizer N uptake by rice plant increased gradually with increasing N rates. Recovery (%) of fertilizer N was around 40% irrespective of N and Cu rates. The non-significant effect of Cu might be due to higher Cu adsorption in the soil. Plant analysis results indicated that Cu content in the straw was below the critical deficiency level of 6 mg kg^{-1}. These findings indicate that higher rate of Cu fertilizer (above 10 kg Cu ha^{-1}) may be useful in this soil to increase rice yield and fertilizer N efficiency if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Further, research is needed to find out the optimum Cu rate and method of application for this soil.

Key words: Nitrogen, Copper, Rice, ^{15}N Tracer technique.

Introduction

Nitrogen (N) is a primary essential macro nutrient element for all plants including rice. Nitrogen requirement of rice is high (Sahrawat 2000; Choudhury *et al* 2001). Rice plant removes about 16 kg N for the production of 1 tonne rough rice including straw (De Datta 1981). Most of the rice soils of Asia are deficient in Nitrogen. So, fertilizer N application is essential to meet the crop's demand. Copper (Cu) is an essential micro nutrient element for all crops including rice. Rice crop removes about 8 g Cu for the production of 1 tonne rough rice including straw (De Datta 1981). In wetland rice soils, the availability of water soluble Cu decrease in yield (Ambak and Tadano 1991). This problem can be solved by applying proper amount of Cu fertilizer in Cu deficient soils.

The largest rice growing area of Malaysia is located in the Muda irrigation scheme, Kedah that covers an area of about 95,000 ha. Recent investigations showed that there is a tendency of yield decline in many sites of this area due to Cu deficiency (Samy *et al* 1992a). Investigations showed that soils of some locations of this Irrigation Scheme are deficient in Cu (Choudhury and Khanif 2000a). Farmers are applying a single fertilizer dose of $N_{80}P_{30}K_{20}$ (80 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1} and 20 kg K_2O ha^{-1}) in rice fields throughout the irrigation scheme (Samy *et al* 1992b). Indiscriminate application of fertilizers throughout the irrigation scheme caused low yield in many locations due to Cu deficiency. In addition, this N dose (80 kg N ha^{-1}) is not optimum in many locations. It is necessary to determine the appropriate doses of N and Cu fertilizers to increase rice yield and fertilizer N efficiency. The ^{15}N tracer technique is used as the precise method to estimate fertilizer N efficiency (Cao *et al* 1984; Choudhury and Khanif 2001). With this view in mind, the present study was undertaken to evaluate the effects of N and Cu fertilization on rice yield and fertilizer N efficiency using ^{15}N tracer technique.

Materials and Methods

A greenhouse experiment was conducted at University Putra Malaysia to evaluate the effects of N and Cu fertilization on rice yield and fertilizer N efficiency. The study was conducted in two soils (Idris and Tebengau series). In this paper, the findings on one soil (Tebengau series) are discussed. The taxonomy of the soil is Typic endoaquent, very fine clayey, mixed, isohyperthermic, pallid (Paramananthan 1998). The soil was collected from rice growing areas under the Muda

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irrigation scheme, Kedah, about 500 km north of Kuala Lumpur, Malayasia, soil samples were collected from 0 - 15 cm depth. These soil samples were air dried, ground and sieved through 2 - mm sieve. Soil was analysed for organic matter, pH, cation exchange capacity (CEC), total N and available Cu. Organic matter was analysed by potassium dichromate and sulphuric acid (H_2SO_4) digestion method (Walkley and Black 1934). Soil pH was measured by glass electrode (Peech 1965). Total N was determined by sulphuric-salicylic acid digestion method (Bremner and Mulvaney 1982). Cation exchange capacity was determined by ammonium acetate extraction method (Schollenberger and Simon 1945). Available Cu was analysed by 0.05N HCl extraction method (Ponnamperuma *et al* 1981). The soil had a pH of 4.1 with CEC 26.36 cmol kg^{-1}. It contained 5.07% organic matter, 0.18% total N and 0.06 mg kg^{-1} available Cu.

Four N rates (0, 60, 120 and 180 kg N ha^{-1}) and three Cu rates (0, 5 and 10 kg Cu ha^{-1}) were used in the experiment. The experiment was laid out in randomised complete block design with four replications. The soil used for the study was collected from the plough layer of the field and was filled into plastic pots of 15 - liter capacity to 10 cm below the brim of the container. The height and diameter of the pots were 29 cm and 28 cm, respectively. The soil was flooded and preincubated for three weeks to stabilize the physico-chemical properties before seed sowing. Sprouted rice seeds of variety MR 185 were sown. The number of seeds needed per pot was calculated on the basis of surface area of the pot and a sowing rate of 40 kg ha^{-1}. Ten sprouted seeds were equally spaced in the puddle soil of each pot.

Phosphorus (30 kg P_2O_5 ha^{-1} and K (20 kg K_2O ha^{-1}) fertilizers as triple superphosphate (TSP) and muriate of potash (MOP), respectively, were applied as basal dressings to all pots. The N in the form of 15N - labelled urea (8.378% atom excess was applied according to treatments (0, 60, 120 and 180 kg N ha^{-1}). Nitrogen was applied in three splits (1/2 as basal + 1/4 at active tillering stage + 1/4 at panicle initiation stage). Copper was applied as copper sulphate ($CuSO_4.5H_2O$) according to treatments (0, 5 and 10 kg Cu ha^{-1}). Copper application was done all as basal. Nitrogen and copper were applied in solution form. The amount of fertilizers was calculated on the basis of soil surface area of each pot. Rice crop was harvested at maturity. Grain and straw weights (gram per pot) were recorded. Grain and straw yields were calculated as t ha^{-1} considering the surface area of each pot. About 10 g of representative grain and straw samples were ground to pass through a 1 mm sieve and were kept in plastic containers for chemical analyses. Total N of the plant tissue was analysed by H_2SO_4 digestion followed by steam distillation method (Bremner and Mulvaney 1982), and subsequently the ^{15}N content of the plant samples was analysed by using emission spectrometry (Hauck 1982). Total N uptake by grain and straw were calculated from yield and N content data. The fertilizer uptake and recovery percentage of added N by rice plant were calculated following 1N HCl extraction method (Yoshida *et al* 1976). Total Cu uptake by grain and straw was calculated from yield and Cu content data.

Table 1
Effects of N and Cu on grain and straw yields of rice

N rate kg ha^{-1}	Cu rate (kg ha^{-1}) 0	5	10	Mean
	Grain yield (t ha^{-1})			
0	2.12	2.14	2.36	2.21 c
60	3.70	3.94	3.91	3.85 b
120	5.36	4.68	5.16	5.07 a
180	5.03	5.16	4.83	5.01 a
Mean	4.05	3.98	4.07	
	Straw yield (t ha^{-1})			
0	2.89	2.81	3.10	2.93 c
60	4.73	5.52	5.08	5.11 b
120	6.66	6.65	6.62	6.64 a
180	6.92	7.11	6.93	6.99 a
Mean	5.30	5.52	5.43	

Effect of Cu was not significant on either grain or straw yield. Means followed by different letters for a parameter in a column are significantly different at 5% level by Duncan's Multiple Range Test (DMRT).

The data were analysed for of variance (ANOVA) and the means were compared using Duncan's multiple range test (DMRT) where the F - test was significant. Regression analysis was also done using four replicated values where F-test was significant. All the analyses were done following statistical analysis system (SAS Institute Inc. 1987). Nitrogen level for maximum yield was calculated from the equation $Y = a + bx - cx^2$ (Gomez and Gomez 1984) as follows:

$N_y = (b/2c)$, where N_y = N rate (kg ha^{-1}) for maximum yield.

Results and Discussion

Grain and straw yields. Grain yield ranged from 2.12 to 5.36 t ha^{-1} while straw yield ranged from 2.81 to 7.11t ha^{-1} (Table 1). Effect of N on both grain and yields was significant, whereas, the effect of Cu was not significant on either of the parameters. Grain and straw yields increased significantly due to N fertilization up to 120 kg N ha^{-1}. Regression analysis indicated that grain and straw yield responses to N

Table 2
Effects of N and Cu on N contents in grain and straw, and total N uptake by rice plant

N rate kg ha^{-1}	Cu rate (kg ha^{-1}) 0	5	10	Mean
	Grain yield (t ha^{-1})			
0	0.80	0.86	0.88	0.85 c
60	0.95	0.83	0.88	0.89 c
120	1.10	1.06	1.08	1.08 b
180	1.21	1.20	1.19	1.20 a
Mean	1.02	0.99	1.01	
	N content (%) in straw			
0	0.43	0.34	0.39	0.39
60	0.41	0.37	0.40	0.39
120	0.37	0.38	0.42	0.39
180	0.40	0.48	0.47	0.45
Mean	0.40	0.39	0.42	
	Total N uptake (kg ha^{-1}) by whole rice plant (grain + straw)			
0	29.43	27.96	32.81	30.07
60	54.49	50.99	54.44	53.31 c
120	83.08	74.95	80.35	79.46 b
180	93.51	95.60	89.94	93.02 a
Mean	65.13	62.38	64.39	

Effect of N was not significant on N content in straw. Effect of Cu was not significant on any of the three parameters.
Means followed by different letters for a parameter in a column significantly different at 5% level by DMRT

Table 3
Effects of N and Cu on ^{15}N atom excess percentage in grain and straw

N rate kg ha^{-1}	Cu rate (kg ha^{-1}) 0	5	10	Mean
	^{15}N atom excess percentage in grain			
60	3.741	3.643	3.520	3.635 c
120	5.205	5.433	5.350	5.329 b
180	6.255	6.088	6.205	6.183 a
Mean	5.067	5.055	5.025	
	^{15}N atom excess percentage in grain			
60	4.144	3.768	3.670	3.861 c
120	5.233	5.550	5.147	5.310 b
180	6.149	6.184	6.177	6.170 a
Mean	5.175	5.167	4.998	

Effect of Cu on ^{15}N atom excess (%) in either grain or straw was not significant. Means followed by different letters in a column for a parameter are significantly different at 5% level by DMRT.

Table 4
Effects of N and Cu on N uptake and recovery by rice plant

N rate kg ha^{-1}	Cu rate (kg ha^{-1}) 0	5	10	Mean
	Fertilizer N uptake (kg ha^{-1}) by whole plant (grain + straw)			
60	25.44	22.46	23.25	23.72 c
120	51.74	48.73	50.73	50.40 b
180	69.24	69.71	66.95	68.63 a
Mean	48.81	46.97	46.98	
	Fertilizer N recovery (%) by rice plant			
60	42.40	37.44	38.75	39.53
120	43.12	40.61	42.28	42.00
180	38.47	38.73	37.20	38.13
Mean	41.33	38.93	39.41	

Effect of N was not significant on fertilizer N recovery percentage. Effect of Cu was not significant on either fertilizer N uptake or recovery percentage.
Means followed by different letters in a column are significantly different at 5% level by DMRT.

rates that were quadratic in nature (Table 6). The increase in grain and straw yields due to N fertilization was expected as the soil was deficient in N. It is in agreement with previous findings (Shah *et al* 1996; Choudhury *et al* 1997). The estimated N rate (calculated from the regression equation) for maximum yield was 158 kg N ha^{-1}. Farmers are applying single N rate (80 kg N ha^{-1}) throughout the Muda Irrigation Scheme (Samy *et al* 1992b). The present study indicated that this level is not optimum for all soils. In Tebengau series, application of higher N rate (158 kg N ha^{-1}) may be useful to get maximum yield. This should be verified by field experiments before recommendation. Copper application did not increase grain and straw yields although the soil was deficient in Cu. The non-significant effect of Cu was attributed to higher Cu adsorption in this soil (Choudhury and Khanif 2000b). Plant analysis indicated that the Cu content in the straw was below the critical deficiency level of 6 mg kg^{-1} (Yoshida *et al* 1976). This indicates that application of 5 or 10 kg Cu ha^{-1} was not sufficient to increase grain yield in this soil. Higher rates of Cu over these rates might be useful to increase grain yield significantly. Further, research is needed to get optimum Cu rate for this soil.

Nitrogen content and uptake. Nitrogen content in grain ranged from 0.80 to 1.21%, while N content in straw ranged

Table 5
Effects of N and Cu on Cu contents in grain and straw, and total Cu uptake by rice plant

N rate kg ha^{-1}	Cu rate (kg ha^{-1}) 0	5	10	Mean
	Cu content (mg kg^{-1}) in grain			
0	2.76	3.25	3.53	3.18 d
60	3.20	3.67	4.13	3.67 c
120	4.46	4.59	4.78	4.61 b
180	4.61	5.31	5.28	5.07 a
Mean	3.76 C	4.21 B	4.43 A	
	Cu content (mg kg^{-1}) in straw			
0	3.28	4.21	5.38	4.29 c
60	3.79	4.73	5.94	4.82 b
120	4.91	5.44	6.84	5.40 a
180	5.30	5.43	6.58	5.44 a
Mean	4.32 C	4.95 B	5.69 A	
	Total Cu uptake (g ha^{-1}) by whole rice plant (grain + straw)			
0	15.24	18.71	25.12	19.69 c
60	29.79	40.93	46.38	39.03 b
120	56.16	57.92	63.27	59.12 a
180	59.71	65.43	63.55	62.90 a
Mean	40.23 C	45.75 B	49.58 A	

Means followed by different small letters for a parameter in a column, and different capital letters in a row are significantly different at 5% level by DMRT.

from 0.34 to 0.48% (Table 2). Nitrogen content (%) in grain increased significantly due to N fertilization up to 180 kg N ha^{-1}, whereas, Cu effect was not significant. There was no significant effect of N or Cu on N content in straw. Total N uptake by whole plant (grain and straw) ranged from 27.96 to 95.60 kg ha^{-1} (Table 2). Total N uptake increased significantly due to N fertilization up to 180 kg N ha^{-1}, whereas, the effect of Cu was not significant. The increase in total N uptake by whole plant (grain and straw) due to N fertilization was attributed to the increases in grain and straw yields due to N fertilization as well as due to the increase N content in grain and total N uptake increased linearly with increasing N rates (Table 6).

The ^{15}N atom excess. The ^{15}N atom excess (%) in grain ranged from 3.520 to 6.255 while the ^{15}N atom excess (%) in straw ranged from 3.670 to 6.184 (Table 3). The ^{15}N atom excess (%) in grain and straw increased significantly at higher N rates, whereas, Cu effect was not significant. Regression analysis indicated that the ^{15}N atom excess (%) in both grain and straw increased linearly with increasing N rates (Table 6). The increase in ^{15}N atom excess in grain and straw with increasing N rates indicates that fertilizer contributed more amounts in total N uptake at higher N rates.

Table 6
Regression equations and R^2 values relating N rate with different parameters

Parameter	Regression equation	R^2 value
Grain yield	$y = 2.18 + 0.037167 x - 0.000118x^2$	0.9537**
Straw yield	$y = 2.9275 + 0.04525 x - 0.000125x^2$	0.9716**
N content in grain	$y = 0.819 + 0.0021 x$	0.9503**
Total N uptake	$y = 31.715 + 0.3583 x$	0.9849**
^{15}N atom excess in grain	$y = 2.501 + 0.0212x$	0.965**
^{15}N atom excess in straw	$y = 2.8047 + 0.0192x$	0.9788**
Fertilizer N uptake	$y = 2.6733 + 0.3743x$	0.9883**
Cu content in grain	$y = 3.141 + 0.011x$	0.9805**
Cu content in straw	$y = 4.383 + 0.0067x$	0.9129**
Total Cu uptake	$y = 22.727 + 0.2495x$	0.9372**

** = significant at 1% level of probability

Table 7
Regression equations and R^2 values relating Cu rates with Cu content in grain and straw, and total Cu uptake by rice plant

Parameter	Regression equation	R^2 value
Cu content in grain	$y = 3.7983 + 0.067x$	0.9622**
Cu content in straw	$y = 4.3017 + 0.137x$	0.9979**
Total Cu uptake	$y = 40.512 + 0.935x$	0.9892**

** =Significant at 1% level of probability.

Fertilizer N uptake and recovery. Fertilizer N uptake by whole plant (grain and straw) ranged from 22.46 to 69.71 kg ha^{-1} (Table 4) Fertilizer N uptake increased significantly at higher N rates, whereas, Cu effect was not significant. Regression analysis indicated that fertilizer N uptake increased linearly with increasing N rates (Table 6). The increase in fertilizer N uptake by whole plant with increasing N rates was attributed to higher ^{15}N atom excess at higher N rates (Table 3) as well as due to higher total N uptake at higher N rates (Table 2). The increase in fertilizer N uptake at higher N rates is in agreement with previous findings (Guindo *et al* 1994b; Panda *et al* 1995). Fertilizer N recovery percentage (quantified by ^{15}N atom excess) by rice plant is presented in Table 4. Fertilizer N recovery ranged from 37.20 to 43.12%. Effects of N and Cu were not significant on fertilizer N

recovery. In general, the recovery of fertilizer N by rice plant was around 40.00%. It is in agreement with previous findings (Craswell and Vlek 1979; Guindo *et al* 1994a). Copper application did not increase fertilizer N recovery by rice plant significantly. This was due to the non-significant response of rice crop to add Cu. The available Cu content in the soil was below the critical deficiency level of 0.1mg kg^{-1} (Ponnamperuma *et al* 1981), and it was expected that Cu application might increase rice yield and thereby, increase fertilizer N uptake. But due to higher adsorption of Cu was not significant. Higher doses of Cu (above 10 kg Cu ha^{-1}) might be useful to increase grain yield and N uptake. Further, research using various levels of Cu is needed to draw the inference.

Copper content and uptake. Copper content in grain ranged from 2.76 to 5.31 mg kg^{-1} while Cu content in straw ranged from 3.28 to 5.94 mg kg^{-1} (Table 5). Copper content in grain and straw increased significantly due to both N and Cu fertilization enhanced plant growth. This might contribute in increase in Cu content of both grain and straw due to higher Cu absorption capacity of the rice plants. Although Cu content in straw increased significantly due to Cu fertilization, it was below the critical deficiency level of 6 mg kg^{-1} (Yoshida *et al* 1976). This indicates that the applied Cu rates were not enough in this soil to meet the demand of the rice plants. As a consequence, grain yield did not increase due to Cu fertilization . A follow - up laboratory experiment indicated that Cu adsorption capacity of this soil was high (Choudhury and Khanif 2000b). Maximum Cu adsorption capacity (calculated from the Langmuir equation) in this soil was 833 mg kg^{-1} (Choudhury and Khanif 2000b). So, higher rate of Cu (more than 10 kg Cu ha^{-1}) is needed to get response in this soil if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Copper uptake by whole rice plant (grain + straw) ranged from 15.24 to 65.43 g ha^{-1} (Table 5). Copper uptake by rice plant increased significantly due to both N and Cu fertilization. This was attributed to the increases in Cu content in grain and straw, due to N and Cu fertilization. Regression analysis indicated that copper content in grain and straw due to Cu fertilization is in agreement with some other findings (Ambak and Tadano 1991; Choudhury and Khanif 2002).

Conclusion

The present study indicates that application of 80 kg N ha^{-1} is not enough for all soil of the Muda Irrigation Scheme. In Tebengau series, application of higher N rate (158 kg N ha^{-1}) instead of the present farmers' practice (80 kg N ha^{-1}) may be useful to get maximum yield. This should be verified by field experiments before giving recommendation. Copper application did not increase grain yield and fertilizer N efficiency significantly although the soil was deficient in Cu. This was attributed to higher Cu adsorption in this soil. It indicates that application of 5 or 10 kg Cu ha^{-1} was not enough to increase grain yield and fertilizer N efficiency in this soil. Higher rates of Cu over these doses may increase grain yield and fertilizer N efficiency if Cu is applied as basal. Alternately, Cu may be applied as foliar spray on standing crop to avoid Cu adsorption in the soil. Further, research is needed to find out optimum Cu rate and method of application for this soil.

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References

Ambak K, Tadano T 1991 Effect of micronutrient application on the growth and occurrence of sterility in barley and rice in a Malaysian deep peat soil. *Soil Sci Plant Nutr* **37** (4) 715 - 724.

Bremner J M, Mulvaney C S 1982 *Nitrogen - Total. In: Methods of soil analysis,* Part 2, 2nd edition, eds Page A L, Miller R H & Keeny D R. American Society of Agronomy, Inc. and Soil Science Society of American, Inc., Madison, Wisconsin, United States of America, pp 595 - 624.

Cao Z, De Datta S K, Fillery IRP 1984 Effect of placement methods on flood water properties and recovery of applied nitrogen (^{15}N - labelled urea) in wetland rice. *Soil Sci Soc Am J* **48** (1) 196 - 203.

Choudhury A T M A, Khanif Y M 2000a Potassium, magnesium and copper status of some rice soils of Malaysia. *Thai J Agric Sci* **33** (1 & 2) 83 - 88.

Choudhury A T M A, Khanif Y M 2000b Copper adsorption behaviour of three Malaysian rice soils. *Commun Soil Sci Plant Anal.* **31** (5 & 6) 567 - 579.

Choudhury A T M A, Khanif Y M 2001 Evaluation of the effects of nitrogen and magnesium fertilization on rice yield and fertilizer nitrogen efficiency using ^{15}N trace technique. *Journal Plant Nutr* **24** (6) 855 - 871.

Choudhury A T M A, Khanif Y M, Aminuddin H, Zakaria W 2001 Potassium and magnesium adsorption properties of some Malaysian rice soils. In: *Proceedings of the International Conference on Agricultural Science and Technology, Session 2: Sustainable Agriculture,* eds Jingguo W, Bin H, Lihong N & Zhou S. Ministry of Science and Technology, Beijing, Peoples Republic of China

pp 340 - 345.

Choudhury A T M A, Khanif Y M 2002 Effect of nitrogen, copper and magnesium fertilization on yield and nutrition of rice. *Pakistan J Sci Ind Res* **45** (2) 102 - 107.

Choudhury A T M A, Zaman S K, Bhuiyan N I 1997 Nitrogen response behaviour of four rice varieties under wetland culture. *Thai J Agric Sci* **30** (2) 1995 - 202.

Craswell E T, Vlek P L G 1997 Fate of fertilizer nitrogen applied to wetland rice. In: *Nitrogen and rice.* International Rice Research Institute, Los Banos, Philippines, pp 175 - 192.

De Datta S K 1981 *Principles and Practices of Rice Production.* John Wiley and Sons, Inc., New York, United States of America, pp 316 - 332.

Gomez K A, Gomez A A 1984 *Statistical procedures for agricultural research,* 2nd edition. John Wiley and Sons, Inc., New York, United States of America, pp 316 - 332.

Guindo D, Norman R J, Wells B R 1994a Accumulation of fertilizer nitrogen - 15 by rice at different stages of development. *Soil Sci Soc Am J* **58** (2) 410 - 415.

Guindo D, Wells B R, Norman R J 1994b Cultivar and nitrogen rate influence on nitrogen uptake and partitioning in rice. *Soil Sci Soc Am J* **58** (3) 840 - 845.

Hauck R D 1982 Nitrogen - isotope - ratio analysis. In: *Method of soil analysis,* Part 2, 2nd edition, eds Page A L, Miller R H & Keeny D R. American Society of Agronomy, Inc. and Soil Science Society of America, Inc., Madison, Wisconsin, United States of America, pp 735 - 779.

Panda M M, Mohanty S K 1995 Time of application of low dose nitrogen to rainy - season rice *(Oryza sativa)* for increasing N - use efficiency. *Indian J Agric Sci* **65** (4) 283 - 285.

Panda M M, Mosier A R, Mohanty S K, Chakravorti S P, Chalam A B, Reddy M D 1995 Nitrogen utilization by lowland rice as affected by fertilization with urea and green manure. *Fert Res* **40** (3) 215 - 223.

Paramananthane S 1998 *Malaysian soil taxonomy (second approximation): A proposal for the classification of Malaysian soils.* Malaysian Society of Soil Science and Param Agricultural Soil surveys. Kuala Lumpur, Malaysia pp 206 - 251.

Peech M 1965 Hydrogen ion activity. In: *Methods of soil analysis,* Part 2, Ist edition, ed Black C A. American Society of Agronomy, Inc. and Soil Science Society of America, Inc., Madison, Wisconsin, United States of America pp 914 - 926.

Ponnamperuma F N 1972 The chemistry of submerged soils. *Adv Agron* **24** 29 - 96.

Ponnamperuma F N 1985 Chemical kinetics of wetland rice soils relative to soil fertility. In: *Wetland soils: Characterization, classification and utilization.* International Rice Research Institute, Los Banos, Philippines, pp 71 - 89.

Ponnamperuma F N, Cayton M T, Lantin R S 1981 Dilute hydrochloric acid as an extractant for available zinc, copper and boron in rice soils. *Plant Soil* **61** (3) 297 - 310.

Sahrawat K L 2000 Macro and micronutrients removed by upland and lowland rice cultivars in West Africa. *Commun Soil Sci Plant Anal* **31** (5 & 6) 717 - 723.

Samy J, Xaviar A, Lee C S, Rahman A B, Rafeah A R 1992a The importance of copper in rice. In: *Proceedings of International Conference on Fertilizer usage in The Tropics,* ed Aziz B. Malaysian Society of Soil Science, Kuala Lumpur, Malaysia, pp 137 - 147.

Samy J, Zahari A B, Lee C S 1992b Nutrient requirement of rice after two decades of double cropping in Malaysia. In: *Proceedings of International Symposium on Paddy Soils.* Chinese Academy of Science, Beijing, China, pp 283 - 289.

SAS Institute Inc. 1987 SAS / STAT Guide for personal computers. SAS Institute Inc. Carry, North Carolina, United States of America, pp 1 - 1028.

Schollenberger C J, Simon R H 1945 Determination of exchange capacity and exchangeable bases in soil-ammonium acetate method. *Soil Sci* **59** (1 - 6) 13 - 23.

Shah A L, Choudhury A T M A, Rahman M S, Bhuiyan N I 1996 Nitrogen and sulphur interactions in wetland rice. *Bangladesh J Sci Res* **14** (2) 161-168.

Walkley A, Black I A 1934 An examination of Degtijaref method for determination of soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci* **37** 29 - 38.

Yoshida S, Forno D A, Cock J H, Gomez K A 1976 *Laboratory manual for physiological studies of rice.* 3rd edition. International Rice Research Institute, Los Banos, Philippines, pp 7 - 34.

Zaman S K, Choudhury A T M, Bhuiyan N I 1994 Stem cutting *Sesbania rostrata:* an approach of green manure establishment for rainfed lowland rice. *Thai J Agric Sci* **27** (3) 269 - 276.

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INTEGRATED PEST MANAGEMENT OF POTATO CUTWORM, *AGROTIS IPSILON* (HUFNAGEL)

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An experiment was conducted at Regional Agricultural Research Station (RARS), Jamalpur, Bangladesh, during the period from November 2000 to March 2001 to find out the effective management strategy in controlling potato cutworm, *Agrotis ipsilon* in the field. The integration of different cultural, biological and chemical management practices showed significant effect on cutworm infestation than the control plots. The lowest percentage of infested tuber by number and weight was observed from the plots treated with hand picking + two irrigation + one spray of Dursban + perching and its yield was also highest. It might be considered as an integrated pest management (IPM) package for combating the cutworm on potato.

***Key words*:** IPM, Potato cutworm, *Agrotis ipsilon*

Introduction

Potato (*Solanum tuberosum* L.) is one of the important crops in Bangladesh and plays a significant role in the human diet. About 134 thousands hectares of area are cultivated for potato in Bangladesh (Anonymous 1997). One of the major constraints of potato production in Bangladesh is the attack of insect pests resulting in severe damage to the crop (Das 1995). The cutworm, *Agrotis ipsilon* (Hufnagel) is the most common and major insect pest of potato. In the early stage of the crop, the larvae of the cutworm cut the plants at the ground level and feed on the leaves of the standing plants affecting their growth, vigour and yield. The amount of damage by cutting the plants exceeds the amount by actual eating (Cabello and Hernandez 1988). At tuberization, the larvae bore into the tubers and consume the inner contents of tubers reducing the yield and market value of potato to a great extent (Patel *et al* 1990). Cutworm damage to the tubers varied from 5 to 75% in India (Lal 1990). In Bangladesh, practical experience reveals that the tuber damage due to the cutworm ranges from 5 to 73% without any control measure depending upon the location and year.

In Bangladesh, main reliance for the control of this pest is on insecticides which is not only expensive but also its residues left over the crops or in the soil have become a matter of great concern for human health and environmental pollution. Over the years the problems associated with the use of insecticides led the entomologists to find ecologically sound, environmentally safe methods of the pest control. Cultural control as a vital component of IPM can be used in combination with other control measures. The present research work was under taken to study the performance of integration of cultural, biological and insecticide management practices against cutworm infestation in the potato field.

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Materials and Methods

The experiment was conducted at Regional Agricultural Research Station (RARS), Jamalpur, Bangladesh, during 2000 - 2001 potato season with eight treatments following Randomized Complete Block (RCB) design having three replications. The eight treatments were as follows:

T_1 = Hand picking + one irrigation (45 DAP) + perching
T_2 = Hand picking + two irrigation (30 and 45 DAP) + perching
T_3 = Hand picking + no irrigation + two spray of Dursban
T_4 = Hand picking + one irrigation (45 DAP) + one spray of Dursban
T_5 = Hand picking + two irrigation (30 and 45 DAP) + one spray of Dursban + perching
T_6 = Hand picking
T_7 = Spray of Dursban at 15 days interval
T_8 = Control

The unit plot size was 3m × 3m and spacing was 60cm × 25cm. Fertilizers were used as per recommendation (Urea: 350 Kg / ha, TSP: 220 Kg / ha and MP: 260 Kg / ha). Weeding and other cultural managements were done as and when necessary. Hand picking was done in the morning everyday from the first inci-

dence of cutworm infestation. Perching was done by putting bamboo branches in the irrigated plots. One irrigation was given at 45 days after planting (DAP) and two irrigations at 30 and 45 DAP. The insecticide Dursban (Chlorpyrifos) 20EC was sprayed @ 5 ml / L of water. Data on the tuber infestation by the cutworm and yield data were recorded at harvest. All data were analyzed statistically after arcsin transformation and separated by DMRT.

Results and Discussion

The effect of different management practices for the control of cutworm in the potato field showed that the percentage number and weight of infested tubers varied significantly with different treatment combinations ($P < 0.01$) Table 1 - 2. The percentage of infested tubers ranged from 20.05 to 31.90 in the treated plots as compared to 36.02 in untreated plots. The treatment with the combination of hand picking, two irrigation (30 and 45 DAP), one spray of Dursban (Chlorpyrifos) and perching gave better control of the cutworm resulting in 44.34 % reduction of number of tuber infestation. Statistically, the second better treatment combination was hand picking + two irrigation (30 and 45 DAP) + perching (T_2) followed by hand picking + one irrigation (45 DAP) + one spray of Dursban (T_4) and spray of Dursban at 15 days interval (T_7). Only, the treatment hand picking was least effective and reduced the number of tuber infestation by 11.44 %.

Analysis of data on the percentage of weight of the infested tubers also showed that the combination of hand picking + two irrigation (30 and 45 DAP) + one spray of Dursban and perching were most effective and reduced the percentage of infested tubers by 42.59% over control. Statistically, similar effect on the infestation of tuber by weight was observed in the treatment T_2 (hand picking + two irrigation - 30 and 45 DAP + perching), T_3 (hand picking + no irrigation + two spray of Dursban), T_4 (hand picking + one irrigation (45 DAP) + one spray of Dursban) and T_7 (spray of Dursban at 15 days interval).

Results on the yield of potato tubers from different plots with the combination of different management practices showed significant increase ($P < 0.01$) in the yield of healthy tubers (Table 3). The weight of damaged tubers in different treated plots did not show significant difference ($P < 0.05$). However, there was a statistically significant difference in the total yield of potato when treated with the combination of different IPM components.

The highest healthy tuber yield (22.56 ton / ha) was recorded from the plots treated with the combination of hand picking + two irrigation (30 and 45 DAP) + one spray of Dursban + perching (T_5) as against 7.01 ton / ha in the untreated plots followed by 20.83 ton / ha recorded from the plots treated with hand picking + two irrigation (30 and 45 DAP) + perching and hand picking + one irrigation (45 DAP) + one spray of Dursban (18.70 t / ha), hand picking + one irrigation (45 DAP) + perching (14.07 ton / ha), spray of Dursban at 15 days interval (13.26 ton/ ha), hand picking + no irrigation + two

Table 1

Effect of different management practices in reducing the number of infestation of potato tubers by *A.ipsilon*

Treatment	Percentage of infested tubers (No.)	Percentage reduction of infestation over control*
T_1	30.65 [b]	14.91
T_2	24.10 [c]	33.09
T_3	30.33 [b]	15.80
T_4	25.67 [c]	28.73
T_5	20.05 [d]	44.34
T_6	31.90 [b]	11.44
T_7	25.77 [c]	28.86
T_8	36.02 [a]	-
CV (%)	5.56	-

*Percentage of reduction of infestation was calculated from the number of tubers of untreated plot, Data were analyzed after arcsin transformation, Data in a column followed by same letter(s) do not differ significantly.

Table 2

Effect of different management practices in reducing weight (%) in potato tubers by *A. ipsilon*

Treatment	Percentage of infested tubers (Wt.)	Weight (%) reduction in infested tubers over control*
T_1	27.19 [b]	22.80
T_2	24.32 [bc]	30.95
T_3	24.77 [bc]	29.67
T_4	21.87 [c]	37.90
T_5	20.22 [c]	42.59
T_6	30.35 [ab]	13.83
T_7	22.54 [c]	36.00
T_8	35.22 [a]	-
CV (%)	11.14	-

*Percentage of reduction of infestation was calculated from the weight of tubers of untreated plot, Data were analyzed after arcsin transformation, Data in a column followed by same letter(s) do not differ significantly.

Table 3
Effect of different management practices on the yield of potato tubers

Treatment	Weight of healthy tubers (ton / ha)	Weight of damaged tubers (ton / ha)	Yield (ton / ha)
T_1	14.07 [b] (74.52)	4.81 (25.47)	18.88 [ab]
T_2	20.83 [a] (83.81)	4.02 (16.19)	24.85 [a]
T_3	10.33 [c] (66.60)	5.18 (33.40)	15.51 [b]
T_4	18.70 [ab] (79.78)	4.74 (20.22)	23.44 [a]
T_5	22.56 [a] (86.27)	3.59 (13.73)	26.15 [a]
T_6	9.07 [c] (62.00)	5.56 (38.00)	14.63 [bc]
T_7	13.26 [b] (78.18)	3.70 (21.82)	16.96 [b]
T_8	7.01 [c] (53.47)	6.10 (45.84)	13.11 [c]
CV (%)	16.02	23.75	12.63

Data in a column followed by same letter(s) do not differ significantly, Data within the parenthesis represent percentage of healthy and damaged tubers in respective column, Percentage of weight of healthy and damaged tubers were calculated from total yield data, Data were analyzed after arcsin transformation.

spray of Dursban (10.33 ton / ha) and hand picking only (9.07 ton / ha). The treatments T_2 (hand picking + two irrigation - 30 and 45 DAP + perching) and T_5 (hand picking + two irrigation - 30 and 45 DAP + one spray of Dursban + perching) increased 83.81% and 86.27% healthy tuber yield, respectively over total yield.

The cutworm caused a tuber damage of 3.59 ton / ha when treated with hand picking + two irrigation (30 and 45 DAP) + one spray of Dursban + perching and 3.70 ton / ha in the plots treated with spray of Dursban at 15 days interval. Only hand picking was found to be ineffective as the level of damage was similar with that of untreated plots. The total yield was higher (26.15 ton / ha) in the plots treated with hand picking + two irrigation (30 and 45 DAP) + one spray of Dursban + perching. Statistically, similar yield was obtained from the plots treated with hand picking + one irrigation (45 DAP) + perching (18.88 ton / ha), hand picking + two irrigation (30 and 45 DAP) + perching (24.85 ton / ha) and hand picking + one irrigation (45 DAP) + one spray of Dursban (23.44 ton / ha). The results indicated that among the seven treatments, the management practice with the combination of hand picking + two irrigation (30 and 45 DAP) + one spray of Dursban + perching was found most effective in reducing the infestation of cutworm in potato field.

Ram *et al* (2001) reported that Dursban 20 EC (Chlorpyrifos) @ 0.5 kg a.i. / ha / spray application once at earthing and once after 21 to 30 days of first spraying was the best treatment for suppressing the damage of cutworm. Dursban or Chloropyrifos proved as an effective insecticide for the control of plant and tuber damage in potato crops by the cutworm (Mannan *et al* 1998). Nikkhoo and Moiini (1991) reported that *Agrotis ipsilon* was the most dominant pest of vegetables. The most effective control measures were collecting and burning of crop residues, deep ploughing and flood irrigation of infested fields in winter. Prasad *et al* (1987) repoted that irrigation resulted in a significant increase in plant height and in yield. Some important pests were reduced in numbers (*Agrotis* sp) or even eliminated. Esbjerg *et al* (1986) showed that mortality of young larvae (*A. segetum*) increased with increasing soil moisture. The young larvae hide in the top layer of the soil, this behaviour being disturbed by wet soil. The infestation of *A. ipsilon* was lower in the irrigated plots than drought stress (Davis and Pedigo 1991). The findings of the above authors are in partial agreement with the findings of the present study. From the present study, it can be concluded that integration of cultural, mechanical, chemical and biological control could show better performance than the one component of integrated pest management. However, further researches on the effectiveness of different combinations of these treatments are warranted.

References

Anonymous 1997 Potato In: *Statistical Year Book of Bangladesh.* Bangladesh Bureau of Statistics, Bangladesh pp 136.

Esbjerg P, Nielsen J K, Philipsen H, Zethner O, Ogard L 1986 Soil moisture as mortality factor for cutworms, *Agrotis* spp (Lepidoptera: Noctuidae). *J Appl Entomol* **102** (3) 277 - 285.

Cabello T, Hernandez M D 1988 Feeding behaviour of larvae of *Agrotis segetum* (Denis and Schiffermuller) and *A. ipsilon* (Hufnagel) (Lepidoptera: Noctuidae) and levels of damage to maize. *Bol Sanidad Veg Plagas* **14** (2) 295 - 305.

Das G P 1995 In: *A Bibliography of Cutworm, Agrotis spp. Complex*. Tuber Crops Research Centre, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur - 1701, Bangladesh, pp 1 - 2.

Lal L 1990 Insect pests of potato and their status in North-eastern India. *Asian Potato J* **1** 49 - 51.

Mannan M A, Islam K S, Jahan M, Saha K C, Hossain M A 1998 Comparative efficacy of some insecticides against cutworm, *Agrotis ipsilon* (Hufnagel) in potato field. *Progress Agric* **9** (1&2) 43 - 46.

Nikkhoo F, Moiini M 1991 Study of cutworm biology in the vegetable growing areas around Tehran and determination of its control measures. *Appl Entomol and Phytopath* **58** (1 & 2) 1 - 3.

Patel M M, Godhani B G, Patel R L, Patel P K, Vyas H N 1990 Relative bioefficacy of certain insecticides against potato cutworm (*Agrotis ipsilon* Rott.). *Agric Sci Dig* **10** (4) 213 - 216.

Prasad S, Singh K M, Katiyar R N, Singh R N 1987 Influence of irrigation on the crop growth, pest incidence and crop yield of cowpea, *Pisium sativum* Linn. *Indian J Entomol* **49** (3) 330 - 344.

Ram K, Misra S S, Kishore R 2001 Field evaluation of synthetic insecticides and neem cake for the management of cutworm, *Agrotis ipsilon* (Hufnagel) damaging potatoes in different agroclimatic zones of India. *J Entomol Res* **25** (1) 31 - 35.

Davis P M, Pedigo L P 1991 Economic injury levels for management of stalk borer (Lepidoptera: Noctuidae) in corn. *J Econ Entomol* **84** (1) 290 - 293.

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A Study of the oil Content of Nigerian Grown *Monodora Myristica* Seeds for its Nutritional and Industrial Applications

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A study of the oil content of *Monodora myristica* for its potential and industrial applications has been undertaken. The study revealed that *M. myristica* seeds have high oil and protein content (21.79 ± 0.51% and 20.57 ± 0.38%, respectively). The carbohydrate content is quite high, (44.29 ± 1.50%) while it has low crude fibre content (4.70 ± 0.15%). The physico - chemical characteristics of the seed oil show that the oil has high acid value, (14.31 ± 0.32%); peroxide value, (15.90 ± 0.50%) and saponification value, (252.11 ± 2.50%). The iodine value of the seed oil which places the oil in the non-drying group is 85.00 ± 0.50%. Eight nutritionally valuable minerals of the seeds were determined and the result indicates the seeds to be richest in potassium 64.96 ± 1.60 ppm followed by magnesium (8.58 ± 1.50 ppm) and iron (8.40 ± 0.91 ppm). Fatty acid composition of the seed oil shows the oil to be rich in linoleic acid (35.52%) and oleic acid (33.15%). It also contains arachidic acid 9.52%. The other fatty acids present in the oil are palmitic acid, stearic acid, gadoleic acid and linoceric acid. Triacylglycerols (OOO, OPO / POO and OOL) accounted for over 57.70% of the total triacylglycerol content of the oil. In addition, high molecular weight triacylglycerols (containing fatty acid moiety > 18 carbons) was also detected in oil The potential domestic and industrial applicatioins of the oil under study are enumerated.

Key words: *Monodora myristica,* Physico - chemical properties, Mineral element, Fatty acid.

Introduction

Fats and oil belong to a class of compound known as lipids, which can be either simple or complex triacylglycerol. The existence of these as fat and oil depends on their state at room temperature, 30 ± 2°C (fats are solid while oils are liquid at room temperature) (Gurr *et al* 1972).

Fats and oil are indispensable food factors (Tooley 1971) and they are also extensively used for nutritional, cosmetic and industrial purposes (Berdick 1972). They are used for delivering fat soluble vitamins as carriers and contributing flavours to food (Masson 1981) and also for supplying essential fatty acids such as linoleic, linolenic and arachidonic acids which are not made by the body but are required by the body (Triebold and Aurand 1963). They are also used for producing drug dispersants in therapeutics (Oyolu 1971 and Ngoddy and Ihekoronye 1984).

Monodora myristica (Gaertn) Dunal also called African nutmeg or false nutmeg is a tree of evergreen and deciduous forest, up to 35m high by 2m in girth. It is of the family Annonaceae (Unwin 1920). The seeds of *M. myristica* which are embedded in a white sweet-smelling pulp are the most important part of the tree economically. The seeds contain 5 - 9.00% colourless essential oil consisting largely of terpenes and with a pleasant taste and smell (Oliver 1960) and about 35.00 - 36.00% of a reddish-brown fixed oil which contains mainly linoleic acid (46.90%) and oleic acid (35.00%) (Busson 1965).

The bark of *M. myristica* is used in Ivory Coast to treat haemorrhoids, stomachache and febrile pains and mixed with that of *M. tenuifolia,* a collyrium is prepared for use in eye troubles (Bouquet 1974). Dusting or application of the pomade is used to disinfect from fleas and lice. The seeds chewed up are applied to the forehead for headache and for migraine in Gabon. The seeds are also applied on sores and are eaten as anti-emetic operative and tonic in Congo (Burkill 1985).

The world production of fats and oils shows the production of vegetable oils to be higher than fats from animal (International Association 1988). Currently, despite the relatively high oil and seed meal production in the United States, the USDA (U.S. Department of Agriculture) continues to investigate non-conventional seeds. The example set forth by the USDA is worth emulating by the developing countries that are more in need of alternative oil sources. According to Balogun *et al* (1986), lack of information on the composition and utilization of the many and varied oil seeds indigenous to the tropics is more of problems than is the real shortage of oils. There exist already abundant data in literature on the proximate

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composition, mineral content and other characteristics of the more conventional oil seeds but this is lacking on the non-conventional oil seed types. In response to these needs and in continuation of our effort to bring into focus the non-conventional seed oils (Oderinde and Ajayi 1998 and 2000), the oil content of *Monodora myristica* seeds have been studied for its domestic and industrial applications.

Experimental

The seeds of *M. myristica* were purchased from a local market (Ojoo market in Ibadan). The chemical used were supplied by British Drug House (BDH)

Sample preparation. The seeds were cracked to remove the brown-coloured kernels. These were ground to powder with the use of a laboratory mortal and pestle. They were then stored in a polythene bag and kept in the fridge until needed for analysis.

Extraction. Extraction of oil of the seed was carried out in a soxhlet apparatus using purified hexane as the solvent. The oil obtained, after distilling off the hexane, was stored in a labelled flask. All analysis was carried out in the Chemistry Department of Ibadan except for crude protein determination which was carried out in Human Nutrition Department of University of Ibadan.

Physical characterization. Physical characterization of the seeds and kernels was carried out according to the method outlined by Femeni *et al* (1995). Weight, length and width of one seed kernel was determined by taking the mean measure of 50 seeds / kernel.

Proximate properties. Pulp and seed moisture was determined gravimetrically by placing 1g of specimen into an oven at 105˚C for 6h to reach constant weight according to Femenia *et al* (1995). Crude protein (N% x 6.25) was determined by the micro Kjedhal method while analysis for ash and crude fibre were according to the methods of AOAC (1980).

Carbohydrate content was determined by difference [100.00% - (protein + crude fat + ash + crude fibre)] (AOAC 1990; Ajayi *et al* 2002).

Physico - chemical characteristics. Procedures for the determination of iodine value (Wij's method), saponification value, peroxide value and acid value were as those recommended by the AOAC (1984). For iodine value, 0.20g of the oil was taken into a glass-stoppered flask and dissolved in 15ml carbon tetrachloride. 25ml of Wij's solution was added, the flask was stoppered and allowed to stand for 2h in the dark at 25˚C, after which 20ml of 10.00% KI solution was added. The mixture was titrated with 0.2N $Na_2S_2O_3$ using starch indicator. A blank determination was carried out. The iodine value was calculated as follows:

$$\text{Iodine value} = 12.69M \frac{V_2 - V_1}{W}$$

Where, M = Molarity of thiosulphate
V_1 = Volume (in ml) of thiosulphate solution used in test
V_2 = Volume (in ml) of thiosulphate solution in blank
W = Weight of sample

The saponification value of the oil was determined by dissolving 1g of it in 12.50ml of ethanolic KOH and refluxing the mixture for 30 minutes after which 1ml of phenolphthalein indicator was added, the hot soap solution was then titrated with 0.50N HCl. A blank determination was carried out under the same condition and the following equation was used to calculate the saponification value of the oil.

$$\text{Saponification value} = 56.1M \frac{(V_2 - V_1)}{W} \quad \text{(AOAC 1984)}$$

Where, M = Molarity of hydrochloric acid used
V_1 = Volume of hydrochloric acid used in test
V_2 = Volume of hydrochloric acid used in blank
W = Weight of oil taken

For the peroxide value, 1g of the oil was weighed into a 200ml conical flask and 25ml of 2:1 v / v glacial acetic acid and chloroform solvent was added. 1ml of potassium iodide solution was then added and the solution was left in the dark for 1 minute after which 30ml of water was added. The mixture was titrated with 0.20 N thiosulphate solution using 5ml starch as indicator. A blank was determined simultaneously. The peroxide value of the oil was then calculated using the equation below:

$$\text{Peroxide value} = 100 \frac{(V_1 - V_2)}{W} M \text{ meg / kg (AOAC, 1990)}$$

Where, W = Weight of sample
V_1 = Volume (ml) of $Na_2S_2O_3$ used in test
V_2 = Volume (ml) of $Na_2S_2O_3$ used in sample
M = Molarity of $Na_2S_2O_3$

The acid value of the oil was determined by dissolving 0.20g of the oil in 2.5ml of 1:1v / v ethanol and diethylether and titrating each with 0.10M NaOH with swirling using phenolphthalein as indicator. The acid value is calculated as follows:

$$\text{Acid value} = 56.1M \frac{(V_2 - V_1)}{W}$$ (Cock and van Rede 1966; AOAC 1990).

Where, M = Molarity of NaOH used
W = Weight of sample
V_1 = Volume (ml) of NaOH used

Refractive index and specific gravity. The refractive index of the oil was determined with an Abbe refractometer while the specific gravity was measured using the specific gravity bottle. Both parameters were determined at room temperature (30˚C ± 2˚C) and following the procedures described by Pearson (1976).

Metal composition. The metal composition of the seeds was determined according to the method used by Idouraine *et al* (1996). 1g of the seed was completely ashed in a muffle furnace at 600˚C. The ash obtained was digested with 3ml concentrated HNO_3. The digest was filtered carefully into a 100ml volumetric flask and made up to mark with distilled water. A blank solution was also prepared. The metal composition was then determined using an Atomic Absorption Spectrophotometer and following the manufacturer's specifications.

Fatty acid analysis. Fatty acid was determined at the Institute of Organic Chemistry, University of Tübingen, Germany according to the method of Lutz *et al* (1998). To 0.10g of the oil was added 5ml of CH_3OH and 1ml of CH_2Cl_2. The mixture was cooled in ice and 0.6ml of CH_3COCl was added.1ml of the solution was withdrawn into the hydrolysis tube and heated for 1 hour at 110˚C. The solution was cooled and discharged into 10ml of 1.00% NaC1 solution in a separating funnel. The organics were extracted with 3 x 4ml hexane and volume was reduced to 0.5ml using a rotary evaporator. This was eluted on silica gel column successively with 5ml hexane and 4ml CH_2C1_2. The CH_2C1_2 fraction was separated on a DB5 30m x 0.25mm capillary installed on a GC Chrompack 9001 equipped with computer software and Mosaic integration. Flame ionisation detector was used. The temperature was programmed as follows: 35˚C for 3 minutes, temperature was then increased at 20˚C per minute up to 230˚C for 5 min. Heptadecanoic acid was used as an internal standard.

Triacylglycerols. Lipids were first separated on silica gel (20 x 20cm) using petroleum ether: diethylether:acetic acid (80:20:1) as the mobile phase. Details of the procedure have been described in earlier publication (Esuoso and Bayer, 1998). The triacyglycerol fraction was identified, scrapped off and eluted with CH_2Cl_2. The samples and standards (0.1µl) were injected into the gas chromatography (CHROMPACK CP9000) using a Chrompack TAP capillary column, 25m x 0.25mm, film thickness, 0.1µ(J & W Scientific, Köln, Germany). The carrier gas was hydrogen maintained between 95 - 96 kpa. The temperature was programmed as follows; 80˚C for 2 min.; temperature increased to 280˚C at 30˚C per min.; temperature increased to 355˚C at 3˚C per min.

All the standards used for the studies were purchased from the SIGMA Chemical Company. They include: Tripalmitin PPP; 1,2 - Dipalmitoyl - 3 - oleoyl - rac - glycerol PPO; 1,3 - Dipalmitoyl - 2 - oleoyl - rac - glycerol POP; 1,2 - Distearoyl -3 -palmotoyl - rac - glycerol SSP; 1 - Stearoyl - 2 - oleoyl -3 -palmitoyl - rac - glycerol SOP; 1,3 - Dioleoyl - 2 - palmitoyl - rac - glycerol OPO; 1 - Palmitoyl - 2,3 - dioleoyl - rac - glycerol SOO, 1,3 - Dioleoyl - 2 - stearoyl - rac - glycerol OSO; 1,3 - Distearoyl - 2 - oleoyl - rac - glycerol SOS; 1,2 - Distearoyl - 3 - oleoyl - rac - glycerol SSO; Triolein OOO; 1,2 - Dimyristoyl - 3 - lauroyl - rac - glycerol MMLa; 1,2 - Dimyristoyl - 3 -palmitoyl - rac - glycerol MMP; 1,3 - Dipalmitoyl - 2 - Linoleoyl - rac - glycerol PLP; Trolinolein LLL; 1,2 - Dilauroyl - 3 - myristoyl - rac - glycerol LaLaM; 1,2 - Dilinoleoyl - 3 - oleoyl - rac - glycerol LLO; 1 - Palmitoyl - 2 - oleoyl - 3 - linoleoyl - rac - glycerol POL; 1,2 - Dioleoyl - 3 - linoleoyl - rac - glycerol OOL, 1,2 - Linoleoyl - 3 - arachidonyl - rac - glycerol LLA ; 1-oleoyl - 2 - Linoleoyl - 3 - arachidonyl - rac - glycerol OLA ; 1 - stearoyl - 2,3 - diarachidonyl - rac -glycerol SAA ; 1 - oleoyl - 2 - stearoyl - 3 - arachidonyl - rac - glycerol OSA ; 1 - Linoleoyl - 2 - stearoyl - 3 - arachidonoyl - rac - glycerol LSA ; 1,2 - dioleoyl - 3 - arachidonoyl - rac - glycerol OOA

Results and Discussion

The result of the proximate composition of *M. myristica* seed is presented in Table 1. The oil yield of the seed is 21.79 ± 0.51% while the protein content is 20.57 ± 0.38%. The oil yield compare favourably with 21.00% of *C. lanatus* (Chinese) (Al - Khalifa 1996) and it is in the same order with the oil content reported for some conventional oil seeds such as soybeans (19.00 ± 2.00%), olive (22.51 ± 2.50%) and cotton seed (19.50 ± 1.00%). The protein content of the seed is also comparable to those of known oil seeds like sunflower (19.50%), castor seed (18.90%) and cashew nut (12.20%) (Fetuga *et al* 1973). The seed has a low quantity of moisture (5.21 ± 9.24%) which is comparable to the value reported in literature for C. *colocynthis* (Al - Khalifa, 1996). The carbohydrate content of the seed is similar to 45.35 ± 1.10% reported for *E. pursaetha* (Oderinde and Ajayi, 1998).

The oil from *M. myristica* seeds which is reddish-brown in colour is consistently liquid at room temperature. The acid value of the oil is 14.31 ± 0.32% (Table 2), it is lower than the value reported in literature for *C. reticulata varsatsuma* (21.71 ± 0.30%) and *C. tuberosus* tuber oil (17.39 ± 0.75%) (Dagne and Johnson, 1997). This value is high but it can be reduced by alkali refining. The saponification value is 252.11 ± 2.50%, this value is closed to that of *A. cohune* 252 - 256 (Oboh and Oderinde 1998). The high saponification value of the oil suggests that the oil contains high molecular weight fatty acids and also that it will be useful for soap production. The high

Table 1
Proximate properties of *M. myristica* seeds

Parameters	Range (%)	Mean ± S.D
Oil yield	21.28 - 22.30	21.79 ± 0.51
Moisture content	4.97 - 5.45	5.21 ± 0.24
Ash content	3.24 - 3.64	3.44 ± 0.20
Crude protein	20.19 - 20.95	20.57 ± 0.38
Crude fibre	4.55 - 4.85	4.70 ± 0.15
Carbodhydrate content	42.79 - 45.79	44.29 ± 1.50

[a]Mean of triplicate analysis

Table 2
Physico-chemical characteristics of *M. myristica* seed oil

Characteristics	Range (%)	Mean ± S.D
Oil content (%)	21.2800 - 22.3000	21.79 ± 0.510
Saponification value	249.6100 - 254.6000	252.11 ± 21.50
Peroxide value	15.4000 - 16.4000	15.900 ± 0.500
Acid value	13.9900 - 14.6300	14.310 ± 0.320
Iodine value	80.0000 - 90.0000	7.2000 ± 0.320
Free fatty acid (as % oleic)	6.8800 - 7.5200	237.800 ± 2.180
Ester value	235.6200 - 239.9800	1.4400 ± 0.015
Refractive index (25°C)	1.4520 - 1.4550	0.9180 ± 0.026
Specific gravity	0.8428 - 0.8668	
Consistency at room temperature	Liquid	
Colour	Reddish - brown	

[a]Mean of triplicate analysis

ester value 237.80 ± 2.18 is an indication of high level of ester is present in the oil. The specific gravity and refractive index of the oil are 0.8428 ± 0.02% and 1.4400 ± 0.015%, respectively.

The role of trace elements in human nutrition and disease cannot be overemphasized. Even though the mineral elements form a small portion of total composition of most plant materials and of total body weight and they do not contribute to energy value of food, and are of great physiological importance particularly in body metabolism (Schwart 1975). The seeds of *Monodora myristica* are richest in potassium (64.96 ± 1.60 ppm) followed by iron (8.40 ± 0.91ppm) and magnesium (8.58 ± 1.50ppm) and poorest in copper (0.33 ± 0.05ppm). From the potassium content of the seed, it can be deduced that the seed of *M. myristica*, if consumed, could be a good source of potassium and to some extent iron.

Presented on Table 4 is the fatty acid composition of *M. myristica* seed oil. The fatty acid composition of the total seed oil reveals that linoleic acid (35.52%) and oleic acid

Table 3
Mineral element composition of *M. myristica* seeds

Element	Range (%)	Mean ± S.D
Calcium	2.08 - 2.48	2.28 ± 0.20
Magnesium	7.08 - 10.08	8.58 ± 1.50
Potassium	63.36 - 66.56	64.96 ± 1.60
Sodium	1.25 - 1.55	1.40 ± 0.15
Manganese	0.92 - 1.08	1.00 ± 0.08
Iron	7.49 - 9.31	8.40 ± 0.91
Copper	0.28 - 0.38	0.33 ± 0.05
Zinc	0.54 - 0.94	0.74 ± 0.20

Table 4
Fatty acid composition of *M. myristic*a seed oil

Fatty acid (%)	*M. myristica* oil[a]	Groundnut oil[b]	Soybean oil[b]
12: 0	-	0.05	trace
14: 0	-	0.05	0.10
16: 0	5.96	11.15	11.20
18: 0	4.44	0.10	0.10
18:1	33.15	-	4.10
18:2	35.52	-	21.70
18:3	-	3.15	53.90
20:0	9.52	51.75	7.50
20:1	2.96	0.05	0.40
20:2	5.43	1.40	0.20
20:3	2.87	1.20	-
22:0	-	3.25	-
22:1	-	0.15	0.50
22:2	-	1.65	0.10
24:0	0.15	0.15	0.20
Saturated	20.07	20.30	16.40
Unsaturated	79.93	78.70	83.50

[a]Present work; [b]Rossell and Pritchard (1991)

(33.15%) are the predominant unsaturated fatty acids. It is well - known that dietary fats rich in linoleic acid prevents disorders such as coronary heart disease, atherosclerosis and high blood pressure and also linoleic acid derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds (Viles and Gottenbos 1989). The other fatty acids found in *M. myristica* seed oil are palmitic acid (5.96%), stearic acid (4.44%), gadoleic acid (2.96%), arachidic acid (9.52%) and linoceric acid (0.15%). The percentage saturated fatty acid in the oil is 20.07% while the percentage of unsaturated fatty acid is 79.93%. This is of great nutritional significance. This suggests that the oil could serve nutritional purposes. It can be used to lower serum cholesterol and prevent coronary heart disease.

Table 5
Physical characterization of *Monodora myristica* seeds

Property	Range	Mean ± SD
Kernel (% of whole seed)	77.13 - 90.54	83.835 ± 0.671
Weight of 100 seeds	98.20 - 100.13	99.165 ± 0.965
Weight of 100 kernels	69.74 - 76.37	73.050 ± 3.315
Seeds length (mm)	0.52 - 0.80	0.660 ± 0.140
Seeds width (mm)	0.32 - 0.47	0.395 ± 0.075
Kernel length (mm)	0.52 - 0.76	0.640 ± 0.120
Kernel width (mm)	0.30 - 0.45	0.375 ± 0.075

Physical properties of *Monodora myristica* seed and kernel regarding weight, length and width in addition to the kernel percentage of whole seed are listed in Table 5. The result showed that the kernel constitute 77.13% of the seeds. This is higher than the value(22-38g / 100g) reported sweet kernel of apricot by Filsoof *et al* (1976); Hallabo *et al* (1975) and Ermakov (1980). The average length and width of the seeds are 0.660 mm ± 0.140 and 0.395 ± 0.075 mm, respectively while those for the kernel are 0.640 ± 0.120 mm and 0.375 ± 0.075 mm, respectively.

The comparison of the cost of production reported in Table 6 revealed that the cost of production of conventional oil such as soybean is slightly lower than and our unconventional oil (*Monodora myristica*) used for the present studies.

The composition of the triacylglycerols in the oil is presented in Fig 1. OOO, OPO / POO and OOL accounted for over 57.70% of the total triacylglycerol in the oil. High molecular weight triacylglycerols (containing fatty acid moiety > 18 carbons) was also detected in oil. SAA, OSA and LLA accounted for about 5.00% of the total triacylglycerol. The search for vegetable oil and fats as alternatives for cocoa butter in chocolate and confectionery products have been a major focus of research for decades now. The terms cocoa butter equivalents (CBEs); cocoa butter substitutes (CBSs) and cocoa butter replacers (CBRs) are strong for technological and economic reasons (Lipp and Anklam 1998). From the composition of the triacylglycols of the oil, it appears our *M. myristica* oil could serve as cocoa butter equivalents (CBEs)

Conclusions

Monodora myristica seeds could be utilized as a source of edible oil and protein for human consumption. In addition, the seeds could be considered as a good source of dietary fibre. The seeds have high content of unsaturated fatty acids and therefore, could serve as substitute for highly unsaturated fatty oils. Finally, the triacylglyreol content of the oil revealed that the oil could serve cocoa butter equivalents.

Table 6
Comparison of the cost of production of the present oil (*Monodora myristica*) with a conventional oils (Soybean and groundnut oils)

Seed	Yield/kg	Residue/kg	Cost of seed/kg ($)	Selling price ($) Oil	Residue	% profit
M.myristica	217.90	782.10	2.00	2.50	0.70	60.00
Groundnut	400.00	600.00	1.50	2.00	0.50	66.70
Soybean	200.00	800.00	20.00	2.80	0.50	65.00

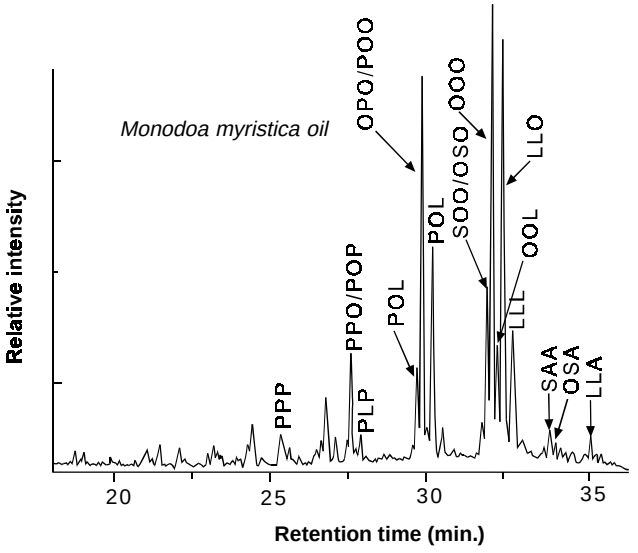

Fig.1 Triacylglycerols of *M. myristica* oil

References

Al - Khalifa A S 1996 Physico - chemical characteristics, fatty acid composition and lipoxygenases activity of crude pumpkin and melon seed oil. *J. Agric Food Chem* **44** (4) 964 - 966.

AOAC 1980 *Official Methods of Analysts*, Association of Official Analytical Chemists, 14th ed. Arlington, USA **67** 503 B 515.

AOAC 1984 *Official Methods of Analysts*, Association of Official Analytical Chemists Washington, DC, USA **63** 2.

AOAC 1990 *Official Methods of Analysts*, Association of Official Analytical Chemists, 14th ed. Washington DC, USA.

Balogun A M, Fetuga B L, Osibanjo O 1990 Nutrient composition of the kernel of *Terminalia catappa* (Tropical almond) *Nig J Sci* **24** 206-209.

Berdick M 1972 The role of fats and oils in cosmetics. *JAOCS* **49** (7) 406 - 407.

Bouquet A, Debray M 1974 *Plantes medicinale de la Cote d'ivoire* Trav. Doc ORST OM 32.

Burkill H M 1985 *Useful Plants of West Africa:* Families A - D Royal Garden Kew, 2nd ed pp 119 - 120.

Busson F 1965 *Plantes alimentaires de l'ouset African, Leconte, Marseilles.*

Dagne K and Johnson A 1997 Oil content and fatty acid composition of seeds of *Cmizzotia cass* (Compositae) *J Sci Food. Agric* ***73*** **(2)** 274 - 278.

Ermakov A I 1980 Composition and comparative fatty acid content in the oils of apricot and peach seeds. *Tri Prikl Bot Genet Sel* **6** 6120 - 6127.

Esuoso, K O and Bayer, E 1998 Chemical composition and potentials of some underutilised tropical biomass II: *Adenopus breviflorus* and *Cucumeropsis edulis. Rivista Italiana Delle Sostanze Grasse*, **75** 191 - 196.

Femina A, Rosells C, Mulet A, Canellas J 1995 Chemical composition of bitter and sweet apricot kernels. *J Agric Food Chem* **43** (2) 356 - 361.

Fetuga B L, Babatunde G M, Oyenuga V A 1973 Composition and nutritive value of cashew nut to rat. *J Agric Food Chem* **22** (4) 67 - 682.

Filsoof M, Mehran M and Farrohi F 1976 Determination and comparison of oil characteristics in Iranian almond, apricot and peach nuts. *Fette, Seifen Anstrichim* **4** 150 - 151.

Gurr M I, James A T 1972 *Lipids Biochemistry, an introduction.* Cornell University Press, Ithaca, New York, USA pp 1.

Hallabo S A S, El - Wakeil F A and Khairy S 1975 Chemical and physical properties of apricot kernel oil and almond kernel oil Egyptian. *J Food Sci* **3** 1 - 5.

Idouraine A, Kohlehepp E A, Webber C W 1996 Nutrient constituents from eight lines of naked seed squash (*Curubita pepo* L*). J Agric Food Chem* **44** (3) 721 - 724.

International Association of seed crushers 1998 *Congress Proceedings* Rome, New Delhi, India, Vancouver.

Lipp M and Anklam E 1998 Review of cocoa butter and alternative fats for use in chocolate - Part A. Composition data. *Food Chemistry* **62** 73 - 97.

Lutz H, Esuoso K Kutubuddin M, Bayer E 1998 Low temperature conversion of sugar cane by - products, *Biomass and Bioenergy* **15** (2) 155 - 162.

Masson L 1981 Relative nutritional values of various dietary fats and oils *JAOCS* **28** (3) 767.

Ngoddy P O, Ihekoronye A I 1964 Integrated food science and technology for the tropics. In: *Baileys Industrial Oil and Fat Produced Swern,* USA 3rd ed. pp. 1103.

Oderinde R A, Ajayi I A 1998 Metal and Oil composition of *Terminalia catappa. La* Riv Ital *Delle Sostanze Grasse* **75** 361 - 362.

Oderinde R A Ajayi I A 1998 Composition of *Entada pursaetha* seed and seed oil grown in Nigeria *La Rivista Ital Sostanze Grasse* **75** 457 - 459.

Oderinde R A, Ajayi I A 2000 Physico - chemical and metal composition of *Calophyllum inophyllum* seed and seed oil *Pak J Sci Ind Res* **43** (6) 357 - 358.

Oboh F O J, Oderinde R A 1998 Development of *Tucum* pulp oil fractions for possible edible and industrial use. *La Riv Delle Sostanze Grasse* **75** 209 - 211.

Oliver B 1960 *Medicinal plants in Nigeria.* Nigerian College of Arts, Science and Technology 231.

Oliver C 1971 A qualitative and quantitative study of seed types in Egusi (*Colocynthis citrulus* L.*) Trop. Sci* **19** (1) 55 - 56.

Pearson D 1976 The Chemical Analysis of Food, Sheck Wah Tong pp 10 - 18.

Rossell, J B and Pritchard J I R 1991 Analysis of oil seeds, fats and fatty foods. Elsevier Science Publishers Ltd.,The Netherlands pp 305 - 308.

Schwart M K 1975 Role of trace elements in cancer *Cancer Research* **35** 3481 - 3484.

Tooley P 1971 The Chemistry in Industry, *Fats, Oils and Waxes.* John Murrey, London, UK pp 16.

Triebold H O, Aurand L W 1963 *Food Composition and Analysis.* D Van Nostrand Co. Inc. Princeton, NJ, USA.

Unwin A H 1920 *West African Forests and Forestry* Unwin, London, U.K.

Viles R O, Gottenbos J J 1989 *Nutritional characteristics and food uses of vegetable oils in:* Oil crops of the World, Robblen G., Downey RK and Ashri A. McGraw Hill, New York, USA, 36 - 86.

Short Communication

Pak. J. Sci. Ind. Res. 2004 ***47****(1) 66 - 68*

PHYSICO - CHEMICAL CHARACTERISTICS OF COMMONLY CONSUMED LEGUMES AFTER DOMESTIC PROCESSING

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Food legumes, widely grown and consumed throughout the world are excellent sources of proteins (20 - 25%) and carbohydrates (50 - 60%). They are also fairly good sources of dietary fibre, minerals and vitamins. However, presence of tannins, phytic acid and other anti-nutritional substances reduce the availability of protein and other nutrients in legumes (Morrow 1991; Van der Poel *et al* 1991; Stanley 1992). Most of the nutrients and anti-nutrients are lost during soaking and cooking processes (De-Leon *et al* 1992). Physical characteristics of certain legumes are associated with these soaking and cooking processes (Phirke *et al* 1982; Attia *et al* 1994). However, digestibility of starch and protein of the legumes is not well documented in literature. This paper reports the effect of cooking on nutrients, anti-nutrients and digestibility of protein and starch of commonly used legumes. Physical characteristics of these legumes were also studied after soaking them in simple water.

Raw form of five legumes (black grams, chick-peas, lentils, red and white kidney beans) were obtained from Ayub Agriculture Research Institute, Faisalabad (Pakistan). Physical characteristics including water absorption capacity (Sefa-Dedh and Stanly 1979), swelling capacity (Akinyele *et al* 1986), seed density (Phirke *et al* 1982), and cooking time (Singh *et al* 1991) of the legumes were determined after soaking in water for 4 h. The ash, protein, soluble sugars, starch, tannins, phytic acid, protein and starch digestibility was estimated before and after cooking the pre-soaked legumes (AOAC 1990).

Table 1 summarized the physical characteristics of raw legumes. Apparent seed density of the legumes were found to be from 0.48 to 1.85g/ml. Cooking time of unsoaked whole seeds of these five legumes showed wide variations ranging from 16 - 130 min depending upon the size and hardness of seeds. Cooking time was reduced by 34.61 to 43.75%, as a result of soaking in water for 4 h. Reduction in cooking time could be the result of absorption of sufficient water from the soaking media which ultimately decreased hardness of legumes.

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Table 1
Physical characteristics of raw legumes

Legumes	Legumes apparent seed density (g /ml)	Water absorption capacity (g /100g)	Swelling capacity (g /100g)	Cooking time (min)
Black gram	1.16	25.60	73.00	110
Chick-peas	1.85	23.80	84.53	75
Lentils	0.48	15.42	32.72	16
Red kidney beans	1.23	34.74	74.39	130
White kidney beans	1.10	27.80	66.07	45

Black grams, chick-peas, lentils, red and white kidney beans contained 19.43 - 26.00% protein, 2.73 - 3.73% minerals, 5.50 - 8.46% soluble sugars and 37.62 - 47.00% starch (Table 2). These nutrients were lost to various extents during cooking process. About 14.78 to 21.83% proteins were lost from these five legumes on cooking. These losses in proteins are attributed to partial removal of certain essential as well as non essential amino acids along with other nitrogenous compounds which were formed as a result of chemical degradation of complex molecules of proteins into simple water soluble amino acids due to high temperature and pressure. About 18.99 to 39.50% minerals, 26.70 - 36.86% soluble sugars and 35.38 - 55.55% starch contents were lost as a result of cooking of the legumes. Earlier workers also reported that cooking caused some of the bean cells to separate rather than to break because of which cell contents (proteins, minerals and sugars) were released to the surrounding media and consequently, caused reduction in the nutrients of beans (Kon 1979; Rincon *et al* 1993).

The amount of neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and lignin in these five food legumes was 19.44 - 24.98%, 4.23 - 8.49%, 2.67 - 6.60%, 12.74 - 20.78% and 1.40 - 1.89%, respectively (Table 2). Variable amounts of these dietary fibre components from the legumes were lost as a result of pressure cooking. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents of these legumes reduced to 11.52 - 18.32% and 10.87 - 19.66% because of cooking. Reduction in cellulose by 11.49 - 21.55% and hemicellulose by 17.85 - 27.22% was observed whereas 3.03 - 7.40% lignin contents were reduced during cooking process. These results are consistent with the findings of earlier workers who found reduction in cellulose and hemicellulose contents of legumes during different cooking processes (Vidal-Valverde and Frias 1991).

Phytic acid and tannin contents in the food legumes were found to be 223 - 599 mg /100g and 164 - 371 mg /100g, respec-

Table 2
Nutrients, anti-nutrients and dietary fibre components of raw legumes

Legumes	Nutrients %				Anti-nutrients mg /100g		Dietary fibre components %				
	Protein	Minerals	Soluble sugar	Starch	Phytic acid	Tannins	NDF	ADF	Cellulose	Hemicellu-loses	Lignin
Black grams	19.43	3.07	7.09	41.26	223.30	164.70	21.23	8.49	6.60	12.74	1.89
Chick-peas	22.62	2.73	5.50	42.00	289.00	186.70	21.31	7.22	5.57	16.14	1.65
Lentils	26.00	3.07	5.21	37.62	351.30	315.67	24.48	4.23	2.83	20.25	1.40
Red kidney beans	23.69	5.66	7.32	44.00	599.70	371.69	24.98	5.20	3.66	20.78	1.54
White kidney beans	22.48	3.73	8.46	47.00	388.00	189.00	19.44	4.37	2.67	15.07	1.70

Table 3
Digestibility of protein and starch of raw legumes

Legumes	Digestibility %	
	Protein digestibility %	Starch digestibility %
Black grams	37.00	44.44
Chick-peas	39.68	45.00
Lentils	45.72	42.00
Red kidney beans	33.77	48.00
White kidney beans	35.29	49.37

tively (Table 2). About 53.43 to 66.11% phytic acid and 25.23 - 50.00 % tannin contents were reduced when water soaked legumes were cooked in a pressure cooker for 15 min. Reduction in anti-nutrients have already been observed by earlier workers during cooking of cowpea, winged beans and field beans (Laurena *et al* 1984;Ogun *et al* 1989).

Protein and starch digestibility of the raw legumes varied from 33.77 - 45.72 and 42.00 - 49.37%, respectively (Table 3). The digestibility of protein and starch was improved by 51.07 - 66.09% and 64.31 - 76.19%, respectively on cooking legumes. Improvement in starch digestibility by 15.28 to 25.92% was higher than protein digestibility of these legumes on cooking. Better improvement in starch digestibility could be attributed due to hydrolysis of starch under drastic conditions of heating under pressure. Mbofung *et al* (1999) also reported distinct improvement in starch digestibility of cowpeas after cooking. Partial removal of anti-nutrients (phytic acids, tannins) might be responsible for improving the digestibility of protein and starch of the legumes. However, it is also possible that some structural changes might have occurred which increased the susceptibility to enzymatic attack and ultimately improved the digestibility of these two nutrients after cooking process.

Key words: Legumes, Nutrients, Anti-nutrients, Physical characteristics, Soaking, Cooking.

References

AOAO 1990 *Official Methods of Analysis*. Association of Official Analytical Chemists. 15th ed. Washington, D.C., USA.

Akinyele I O, Onigbinde A C, Hussain M A, Omolou A 1986 Physico-chemical characteristics of 18 cultivars of Nigerian cowpeas *(Vigna unguiculata)* and their cooking properties. *J Food Sci* **51** (6) 1483 - 1485.

Attia R S, El-Tabey Shehate A M, Aman M E, Hamza M A 1994 Effect of cooking and decortication on the physical properties, the chemical composition and the nutrition value of chick-peas. *Food Chem* **50** 125 - 131.

De-Leon L F, Elias L G, Bressani R 1992 Effect of salt solutions on the cooking time, nutritional and sensory characteristics of common beans. *Food Res Int* 131 - 136.

Kon S 1979 Effect of soaking temperature on cooking and nutritional quality of beans. *J Food Sci* **44** (6) 1329 - 1331.

Laurena A G, Van-Den T, Mendoza M A T 1984 Effects of condensed tannins on the *in vitro* protein digestibility of cowpeas *(Vigna unguiculata* A.Walp). *J Agric Food Chem* **32** (5) 1045 - 1048.

Mbofung C M F , Rigby N, Waldon 1999 Use of two varieties and hard to cook beans. *(Phaseolus vulgaris)* in the processing of Koki. *Plant Food Hum Nutr* **54** 131 - 150.

Morrow B 1991 The rebirth of legumes. *Food Tech* **45** 96 - 121.

Ogun P O, Markakis P, Chenowth W 1989 Effect of processing on certain anti-nutrients in cowpeas *(Vigna unguiculata)*. *J Food Sci* **54** (11) 1084 - 1085.

Phirke A V, Charvan J K, Jadhar S J, Salunkhe D K 1982 Physical properties, chemical composition, cookability and solubilization of proteins of dry beans. *Legumes* **5** 91 - 96.

Rincon F, Ros G, Collins J L 1993 Mineral losses in cowpeas by pressure heating in water. *J Food Sci* **58** (9) 856 - 858.

Sefa Dedh S, Stanly D W 1979 The relationship of micro structure of cowpeas to water absorption and dehulling properties. *Cereal Chem* **56** 379 - 381.

Singh U, Subrahmanyam M, Kumar N 1991 Cooking quality

and nutritional attributes of some newly developed cultivars of chick-pea. *J Sci Food Agric* **55** 37 - 46.

Stanley D M 1992 Hard beans, A problem for growers, processors and consumers. *Hort Technol* **2** 370 - 378.

Van der Poel A F B, Gravandoel S, Boer H 1991 Effect of different processing methods on the tannin contents and protein digestibility of faba beans. *Anim Feed Sci Technol* **33** 49 - 58.

Vidal-Valverde C, Frias J 1991 Legume processing effects on dietary fibre components. *J Food Sci* **56** 1350 - 1352.

Short Communication

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ALLELOPATHIC EFFECT OF AQUEOUS EXTRACTS OF *CALOTROPIS PROCERA* ON GERMINATION AND SEEDLING GROWTH OF MAIZE

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The effects of 24 and 48 h leaf extracts of *Calotropis procera* on radicle and plumule growth of four cultivars of maize (Oba Super I, II, III and IV) were examined. Both extracts demonstrated considerable inhibitory effects on the growth of radicle and plumule of the cultivars and the severity of the inhibition was observed to increase with the increase in the duration of the extraction.

The growth of Oba Super 1 tends to be the least inhibited while the growth of Oba Super III appeared to be mostly inhibited by the extracts when the growth and development of their radicle and plumule were compared to those of the control.

The study concluded that allelopathy might have played a major role in the suppressive ability of this weed on the seedling growth of maize.

Calotropis procera, a member of Asclepiadaceae (Olorode 1984), is one of the most prominent weeds in the Savanna zone of Nigeria, where it constitutes a menace to cowpea, guinea corn and maize farms. Field observations have revealed a significant reduction in yields from the farms, where this weed is found.

Previous studies, such as (Evanari 1949; Rice 1984) among others, had revealed that most weeds affect their neighbours by releasing allelochemicals, which inhibit the growth of their neighbours directly or indirectly by altering the physical and chemical characteristics of the soil and the availability of nutrients, pH, total phenolic levels and microbial population (Blum & Shaffer 1988; Inderjit & Dakshini 1992, 1994).

Therefore, there is a growing interest in the study of allelopathy among weed scientists and plant ecologists in the recent time. The study being reported here aimed at determining the inhibitory potentials of the extracts of *C. procera* on the germination and seedling growth of maize, a widely cultivated crop in Nigeria.

Maize seeds from four cultivars, namely Oba Super I, II, III and IV, were obtained from the International Institute for Tropical Agriculture, Ibadan, Nigeria and used in this study.

Leaves of *Calotropis procera* were collected along Ilorin - Jebba road in the Savanna region of Nigeria. 500 g of fresh leaves were soaked in one litre of distilled water for a period of 24 h. Another 500 g were soaked for 48 h. The solutions were filtered and the filtrate was used as aqueous extracts.

Sterilized petri- dishes were double - layered with Whatman No.1 filter papers. Five seeds of the respective cultivars were placed in each petri - dish. The petri - dishes were moistened daily with the 24 and 48 h, aqueous extracts of the leaves of

Table 1

Effect of aqueous extracts of *C. procera* on the radicle and plumule lengths (cm) of maize cultivar Oba Super I

Plant parts	Extract time (h)	Experimental time (h)*						Average % decrease
		24	48	72	96	120	144	
	Control	-	1.30	3.92	5.73	6.95	8.05	-
Radicle	24	-	0.95 (26.90)	1.08 (72.40)	2.95 (48.50)	4.01 (42.30)	5.27 (34.50)	- (44.90)
	48	-	0.56 (56.90)	0.92 (76.50)	1.45 (74.70)	3.24 (53.40)	4.01 (50.20)	- (62.30)
	Control	-	1.20	1.31	2.38	2.98	3.68	-
Plumule	24	-	- (100.00)	0.51 (61.10)	0.97 (59.20)	1.21 (59.40)	1.88 (48.90)	- (65.70)
	48	- -	- (100.00)	0.31 (76.30)	0.53 (77.70)	0.71 (73.50)	1.06 (71.20)	- (79.70)

* Figures in brackets represent the % decrease over control.

Table 2
Effect of aqueous extracts of C. *procera* on the radicle and plumule lengths (cm.) of maize cultivar Oba Super II

Plant parts	Extract time (h)	Experimental time (h)*						Average % decrease
		24	48	72	96	120	144	
	Control	-	1.40	3.98	6.92	9.01	10.89	-
Radicle	24	-	0.72	1.51	2.01	3.96	4.75	-
			(48.60)	(62.10)	(71.00)	(56.00)	(56.40)	(58.80)
	48	-	0.56	0.98	1.75	2.57	3.01	-
			(60.00)	(75.40)	(74.70)	(71.50)	(72.40)	(70.80)
	Control	-	1.36	1.98	2.58	3.30	4.02	-
Plumule	24	-	-	0.52	0.98	1.08	1.73	-
		-	(100.00)	(73.70)	(62.00)	(67.70)	(57.00)	(72.10)
	48	-	-	0.23	0.38	0.91	1.22	-
		-	(100.00)	(88.40)	(85.30)	(72.40)	(69.70)	-

* Figures in brackets represent the % decrease over control.

Table 3
Effect of aqueous extracts of C. *procera* on the radicle and plumule lengths (cm) of maize cultivar Oba Super III

Plant Parts	Extract time (h)	Experimental time (h)*						Average % decrease
		24	48	72	96	120	144	
	Control	-	0.28	1.86	3.27	4.01	4.78	
Radicle	24	-	-	0.21	0.53	0.99	1.32	
		-	(100.00)	(88.70)	(83.30)	(75.90)	(72.40)	(89.10)
	48	-	-	0.14	0.25	0.48	0.96	
		-	(100.00)	(92.50)	(92.40)	(88.00)	(79.90)	(90.60)
	Control	-	0.34	0.78	1.82	2.65	3.12	
Plumule	24	-	-	-	0.25	0.51	1.05	
		-	(100.00)	(100.00)	(86.30)	(80.80)	(66.30)	(86.70)
	48	-	-	-	0.23	0.49	1.03	
		-	(100.00)	(100.00)	(87.40)	(81.50)	(67.00)	(87.20)

* Figures in brackets represent the % decrease over control.

C. procera. Each treatment was replicated five times. A control treatment was also set up and moistened daily with distilled water.

The petri - dishes were kept at room temperature in a growth chamber where germination measurements were recorded at 24 h intervals. The results obtained from the extract - treated seed were compared statistically to those obtained from the control experiment.

The results of the different aqueous extracts of the leaves of *C. procera* on radicle and plumule growth of maize are shown in Table 1 - 4. Both 24 and 48 h extracts demonstrated considerable inhibitory effects on the growth of radicle and plumule of the maize cultivars. The severity of the inhibition was more pronounced at 48 h extract time than those of the 24 h extract time.

Results obtained from all the cultivars tend to follow the same trend. The growth and development of the radicle and plumule decreased with the increase in the duration of the extraction. Statistical analyses revealed that these reductions were significantly different when compared to the control at 5% level.

The radicle of Oba Super I was least affected by the extracts,

Table 4
Effect of aqueous extracts of C. *procera* on the radicle and plumule lengths (cm) of maize cultivar Oba Super IV

Plant Parts	Extract time (h)	Experimental time (h)*						Average % decrease
		24	48	72	96	120	144	
	Control	-	1.29	2.85	5.10	6.25	7.20	
Radicle	24	-	0.36	0.87	1.35	2.10	2.85	
		-	(72.10)	(69.50)	(73.50)	(66.40)	(60.40)	(68.40)
	48	-	0.30	0.76	1.28	1.96	2.30	
		-	(76.70)	(73.30)	(74.90)	(68.60)	(68.10)	(72.30)
	Control	-	1.76	2.99	3.36	3.96	4.26	
Plumule	24	-	-	0.32	0.89	1.28	1.96	
		-	(100.00)	(89.30)	(73.50)	(67.70)	(54.00)	(76.90)
	48	-	-	-	0.30	0.71	1.32	
		-	(100.00)	(100.00)	(91.10)	(82.10)	(69.00)	(88.40)

* Figures in brackets represent the % decrease over control.

as the percentages decrease over the control were 44.90 and 62.30 at 24 h and 48 h extracts, respectively (Table 1). Radicle that mostly inhibited was of Oba Super III with the percentages decrease of 89.10 and 90.60 at 24 h and 48 h extracts, respectively (Table 3). The percentages decrease of the radicle of Oba Super II and Oba Super IV were 58.80 and 70.80, 68.40 and 72.30 at 24 h and 48 h extracts, respectively.

The inhibition of the growth and development of the plumule also followed the trend above. Oba Super I tends to be the least inhibited at both 24 h and 48 h extract (Table 1) while Oba Super III appeared to be the most inhibited at both 24 h and 48 h extract (Table 3). Plumule inhibition was above 70% when compared to the controls at both 24 and 48 h extracts in Oba Super II and IV (Tables 2 and 4).

The role of allelopathy in the spatial distribution of weeds cannot be over - emphasized. The latex exudates some of the members of the genus *Calotropis* which has been found to contain a strong allelochemical called Calotropin (Watt & Breyer - Brandwijk 1962; Bouguent 1972; Daubenmire 1974). This compound might be responsible for the inhibitory effects demonstrated in this study by this weed.

Allelochemicals, though present in all plants parts, have been found to be mostly concentrated in the leaves. For example, studies had revealed that the allelochemicals present in *Chromoleana odorata,* (Ogbe *et al* 1991; Gill *et al* 1993), *Setaria faberii* (Bell & Koeppe 1972), *Cyperus rotandis* (Alams & Azini 1991) and *Euphorbia heterophylla* (Tijani - Eniola & Fawusi 1989; Kayode 1998) were concentrated in their leaves. The leaf extracts from these weeds, irrespective of the duration of extraction, inhibited germination and seedling growths in *Zea mays*, *Vigna unguiculata*, wheat, tomato and cowpea, respectively.

In conclusion, Idu & Omonhinmin 1998 had shown that the degree of inhibition demonstrated by extracts from the different component parts of *C. procera* were similar. Thus, it could be suggested that allelopathy might have played a prominent role in the spatial distribution and suppressive ability that this weed exerts on neighbouring plants in the field.

***Key words*:** Allelopathy, Inhibition, *Calotropis procera*, Radicle, Plumule.

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References

Alams S M, Azini R 1991 Effects of purple nutsedge (*Cyperus rotundus*) leaf extract on germination and seedling growth of wheat (W. Pavon). *Pak J Weed Sci* **4** 56 - 61.

Bell T D, Koeppe D E 1972 Non - competitive effects of giant foxtail on the growth of corn. *Agron J* **64** 321 - 325.

Blum U, Shafer S R 1988 Microbial population and phenolic acid in soil. *Soil Biol and Bioch* **20** 793 - 800.

Bouguent A 1972 *Plantes Medicinales du Congo Brazzaville. Btraveanx orstom.* Paris No.13, France, pp 15.

Daubenmire R F 1974 *Plant and Environment.* 3rd ed. John Wiley and Sons, New York, USA, pp 54 - 70.

Evanari M 1949 Germination inhibitors. *Botanical Review* **15** 159 - 194.

Gill L S, Anoliefo G O, Iduoze U V 1993 Allelopathic effect of

aqueous extract of Siam weed on growth of Cowpea. *Chromoleana Newsletter* **8** 1 - 4.

Idu M, Omonhinmic C A 1998 Allelopathic effect of *Calotropis procera* on the germination of *Helianthus annus* seeds. *Comp Newsletter* **32** 31 - 36.

Inderjit A, Dakshini K M M 1992 Interference potential of *Pluchea lanceolata* (Asteraceae): growth and physiological response of asparagus bean, *Vigna unguiculata* var. *serquipedalis*. *American J Botany* **79** 799 - 804.

Inderjit A, Dakshini K M M 1994 Allelopathic effect of *Pluchea Ianceolata* (Asteraceae) on characteristics of four soils and tomato and mustard growth. *American J Botany* **8** 799 - 804.

Kayode J 1998 Allelopathic effect of aqueous extracts of *Euphorbia heterophylla* L. on radicle and plumule growth of cowpea (*Vigna unguiculata* L.) Walp. *Biosci Res Commu* **10**(1) 23 - 26.

Ogbe F M O, Gill L S, Iserhien E O O 1994 Effect of aqueous extracts of *Chromolaena odorata* L. on radicle and plumule growth and seedling height of maize, *Zea mays* L. *Compositae Newsletter* **25** 31 - 38.

Olorode O 1984 *Taxonomy of West African flowering plants.* Longman Group Limited London, UK and New York, USA, pp 156.

Rice E L 1984 *Allelopathy*. 2nd ed. Academy Press, New York, USA, pp 1 - 6.

Tijani - Eniola H A, Fawusi O A 1989 Allelopathic activities of crude methanol extract of Siam weed and wild poinsettia on seed germination and seedling growth of tomato. *Nigerian Journal of Weed Science* **2** 15 - 20.

Watt J M, Brayer - Brandwijk M G 1962 *Medicinal and Poisonous plants of Southern and Eastern Africa*. E and S Livingstone, Edinburgh and London, UK, pp 65 - 72.

Short Communication

Pak. J. Sci. Ind. Res. 2004 ***47****(1) 73 - 75*

IN VITRO ASSESSMENT OF THE PROBIOTIC PROPERTIES OF *LACTOBACILLUS ACIDOPHILUS* FROM FAECES AND FRESH COW MILK

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Probiotics are viable bacteria used as feed additives, which produce beneficial effects that bring about a balance of the intestinal flora. Strains of *Lactobacillus, Pediococcus, Bacteriodes, Bifido bacterium, Bacillus, Streptococcus and Escherichia coli* have been used as probiotics (Fuller 1986).

Lactobacilli are found in the normal intestinal flora of chickens and other animals from the first few days of their life (Fuller 1986). Their ability to inhibit both Gram- positive and Gram-negative bacteria had been reported (Klaenhammer 1988; DeVuyst and Vandemme 1994; Jin *et al* 1996). Chang and his co-workers (Chang *et al* 2001) recently reported that *Lactobacillus reuteri* BSA131 sourced from pig faeces strongly inhibited pathogenic bacteria used as indicator organism. Moreover, the adhesion of *Lactobacilli* to the epithelial wall of the small intestine of some animals had also been reported (Sarra *et al* 1992; Jin *et al* 1996).

Lactobacillus acidophilus produce lactic acid and small amount of hydrogen peroxide to suppress harmful bacteria (Price and Lee 1970; Gilliland and Speck 1977). Some strains produce bacteriocins such as acidophilin, lactocidin and acidolin (Gilliland 1989). The resistance of *Lactobacillus acidophilus* to bile salt and pH had been reported (Andrez and Leszek 2001).

Studies on the antagonistic effect of wild strain of *Lactobacillus acidophilus* from faeces and fresh milk on some pathogen and their adherence to the IEC (Ileum epithetial cell) of albino rat is lacking. The aim of the present work was to investigate the antagonistic effect and adhesion properties of *Lactobacillus acidophilus* strains to the IEC of albino rat.

Source of Lactobacillus isolates. Fresh cow milk was obtained from the university farm. Faeces of man was collected from a student while faeces of pig and albino rat were obtained from the piggery and experimental animal house of the Federal University of Technology, Akure, Nigeria.

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Isolation and characterisation of isolates. *Lactobacillus* isolates were obtained using MRS agar (LABM). Incubation was at 37˚C under an anaerobic environment generated inside a dessicator. Dry crab – like colonies with projecting outgrowth were subjected to morphological and biochemical test for identification (Parker and Collier 1990).

Source of indicator bacteria. Pure culture of *Bacillus cereus* NCIB 6349, *Escherichia coli* Type 1 NCIB 86, *Pseudomonas aeruginosa* NCIB 950, *Klebsiella pneumoniae* NCIB 14070, and *Staphylococcus aureus* NCIB 8588 were obtained from culture collection of the Department of Microbiology, Obafemi Awolowo University, Ile Ife, Nigeria. They were maintained on nutrient agar (LABM) slant throughout the duration of the study.

In vitro antagonism assay. The agar diffusion assay of Schillinger and Lucke (1989) was used to assay the inhibitory effect of *Lactobacillus acidophilus* strains against the indicator bacteria. This involves seeding Tryptone Soya agar (LABM) plates with the test bacteria and introducing 0.05ml (50 µl) of overnight broth culture *L.acidophilus* into holes bore with 3mm cork borer. The plates were incubated aerobically at 37˚C for 24h after which they were examined for zones of inhibition.

Preparation of intestinal epithelial cell of albino rat. Ileum epithelial cell of 4-weeks-old albino rat was prepared by using the method of Jin *et al* (1996). The epithelial cells of the ileum were scrapped off gently using the edge of a microscope slide and the scrapping suspended in phosphate buffer saline (PBS) of pH 7.3. The suspended scrapping was stored in ice for 15 min to allow the debris to settle. The sedimented debris was removed and the supernatant fluid centrifuged for 10 min at 2 g to remove large tissue cluster. It was centrifuged again at 120 g for 10 min to spin down the cells in suspension. The IEC was then suspended to a concentration of 6×10^5 cell / ml.

In vitro adhesion assay. The method described by Jin *et al* (1996) was adopted. Cells from overnight anaerobic cultures of *Lactobacillus acidophilus* in MRS broth was washed and suspended in PBS to a density of 10^8 cells/ml. One fifth of 1ml (0.2ml) of epithelial cell suspension was added to 0.8ml of the overnight anaerobic broth culture of *L.acidophilus* and incubated for 1h at 37˚C. Adhesion of the *L.acidophilus* strains to IEC was assessed using light microscopy with phase contrast illumination (x100).

Statistical analysis. The differences between the mean of the *L.acidophilus* attached to the IEC was assessed using the one way ANOVA (SPSS version 10.0) with the level of significance set at $P < 0.05$.

Table 1
Antimicrobial activities of faecal strains of *L.acidophilus* strains towards indicator bacteria

Indicator bacteria	Isolates Zone of inhibition (mm)*			
	1H	1P	1A	1C
Bacillus cereus	3.0 ± 1.0	3.5 ± 1.0	3.5 ± 1.0	NI
Escherichia coli	2.5 ± 0.0	1.0 ± 1.0	1.5 ± 0.5	6.0 ± 1.7
Pseudomonas aeruginosa	NI	2.5 ± 0.9	NI	15.0 ± 0.5
Klebsiella pneumoniae	4.0 ± 0.1	3.0 ± 1.0	2.0 ± 1.0	NI
Staphylococcus aureus	3.5 ± 1.3	2.0 ± 0.5	NI	NI

*Values are mean ± SD of three replicates; NI: No inhibition
H: Human isolate; P: Pig isolate; A: Albino rat isolate; C: Cow milk.

Table 2
Attachment of *L.acidophilus* strains to the IEC of albino rat

Isolates	Bacteria / IEC *
1H	15
1P	5
1A	23**
1C	10

*Each value is a mean of three replicates. **Value is higher and significantly different ($P < 0.05$) from other. Contact time (1h) at 37°C.

L. acidophilus strain was isolated from fresh cow milk, human, pig and albino rat faeces, respectively. The strain from pig was able to inhibit all the indicator bacteria (Plate B) while the isolate from the other sources could not inhibit one or two of these bacteria (Table 1). The highest level of inhibition (15 mm) was recorded for isolate from fresh milk against *Pseudomonas aeruginosa* (Plate A). The result obtained contradicts the earlier report of Gilliland and Speck (1977). These workers showed that *Lactobacilli* showed stronger antibacterial properties against Gram - positive than Gram - negative bacteria.

Recently, the antibacterial effect of *Lactobacillus* isolates have been demonstrated against *Salmonella* and *E.coli* by agar diffusion / spot methods (Oyarzabal and Conner 1995; Jim *et al* 1996). The ability of the isolate to antagonise the growth of these indicator bacteria as observed in the present report is a good index that these strains can produce metabolic products that can inhibit the growth of pathogen in the intestine. Juven *et al* (1992) reported that strains of *L. acidophilus*147 from chicken intestine produce lactic acid, hydrogen peroxide and a bacteriocin. All these substances have antibacterial properties. Figure 1, shows the plates (A & B) of the inhibitory effect of *L.acidophilus* strains on some of the indicator bacteria.

The four *L.acidophilus* strains were able to adhere to the IEC of albino rat. The highest level of adhesion of 23 bacteria/cell was recorded for isolate from albino rat while the least was observed for isolate from pig (5 bacteria/cell) (Table 2). Several workers have reported the host specificity of bacteria strain adhesion to epithelial cells of chicken crop, rats and pig

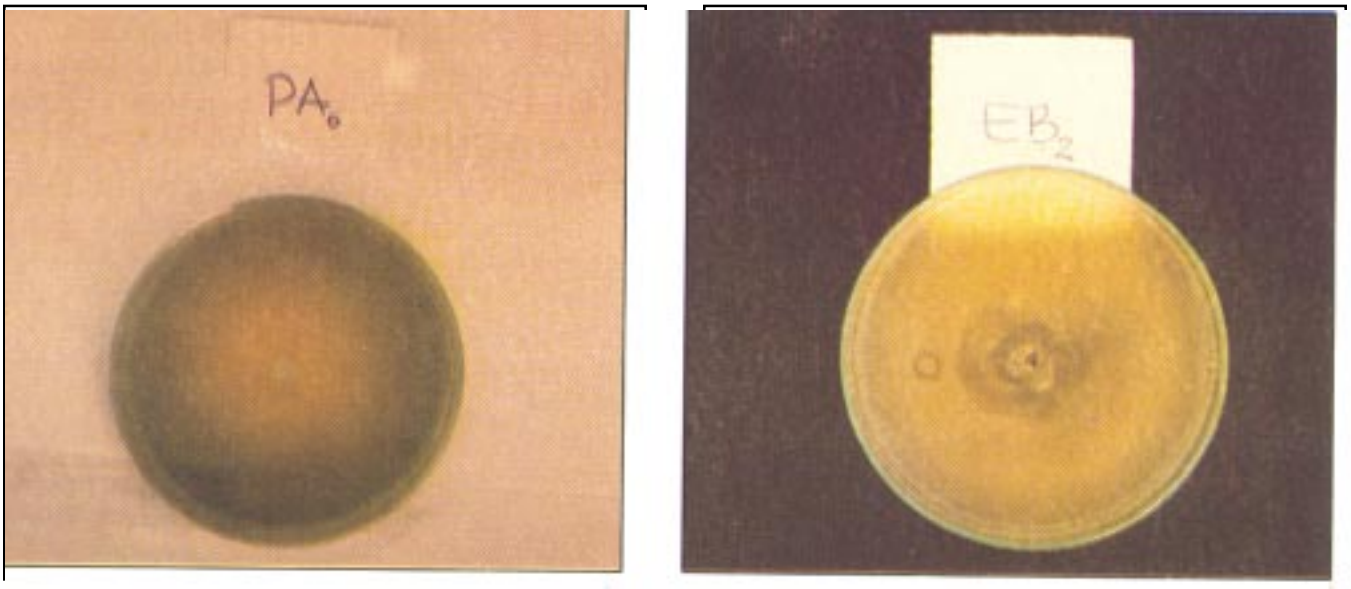

Fig 1. Inhibition of bacteria indicator by *L.acidophilus* strains. The indicator of plate 'A' is *Pseudomonas aeruginosa* NCIB 950 while plate 'B' is *Bacillus cereus* NCIB 6349.

sqamous *in vitro* (Fuller 1975; Suegara *et al* 1975; Barrow 1980). The adhesion ability varies between bacterial species and even different strains of the same species show variations (Fuller 1986). This may account for the high adhesion recorded for strain isolated from albino rat faeces.

A temperature of 37˚C was used to ascertain the adhesion capability of the different strains to the IEC of albino rat. Fuller (1975) had earlier reported that temperature in the range of 4˚C to 47˚C has no effect on the ability of *Lactobacilli* to adhere to the IEC of chicken, hence a temperature of 37˚C was adopted. A contact time of 1h (more than 30 mins) was adopted as recommended by Jin *et al* (1996). There was a significant difference ($P< 0.05$) in the adhesion of the different strains to albino rat IEC.

This study shows that wild strain of *L.acidophilus* from the faeces of human, pig and albino rat can produce antibacterial agent against major pathogenic bacteria. It also reveals that different strains of *L. acidophilus* have varying degree of adhesion to the IEC of albino rat with the isolate from albino rat having the highest adhesion.

***Key words*:** Probiotic, *Lactobacilli,* Antagonism, Adhesion.

References

Andrzej F, Leszek S 2001 Sensitivity of probiotic *Lactobacillus acidophilus* and *L. casei* to bile salt. File: //A:/ paper - 09. htm.

Barrow P A, Brooker B E, Fuller R, Newport M J 1980 The attachment of bacteria to the gastric epithelium of the pig and its importance in the microecology of the intestine. *J Appl Bacteriol* **48** 147 - 154.

Chang Y, Kim J, Kim H, Kim W, Kim Y, Park Y 2001 Selection of a potential probiotic *Lactobacillus* strain and subsequent *in vivo* studies. *Antonie van Leeuwenhoek* **80** 193 - 199.

DeVuyst L, Vandemme E L 1994 Antimicrobial potential of lactic acid bacteria. In: *Bacteriocins of Lactic Acid Bacteria,* eds. DeVuyst L & Vandamme E L, London: Blackie Academic And Professional, UK, pp 91 - 142.

Fuller R 1975 Nature of the determinant responsible for the adhesion of *Lactobacilli* to chicken crop epithelial cells. *J Appl Bacteriol* **33** 245 - 250.

Fuller R 1986 Probiotics. *J Appl Bacteriol* Symposium supplement. 1S - 7S.

Gilliland S E, Speck M L 1977 Antagonistic action of *Lactobacillus acidophilus* towards intestinal and food pathogen in associative culture. *J Food Prot* **40** 820 - 823.

Gilliland S E 1989 *Acidophilus* milk products: A review of poten-tial benefits to consumers. *J Dairy Sci* **72** 2483 - 2494.

Jin L Z, Ho Y W, Abdullah N, Ali M A, Jalaludin S 1996 Antagonistic effects of intestinal *Lactobacillus* isolates on pathogens of chicken. *Lett Appl Microbiol* **21** 137 - 141.

Juven B J, Schved F, Linder P 1992 Antagonistic compounds produced by a chicken intestinal strain of *Lactobacillus acidophilus*. *J Food Prot* **55** 157 - 161.

Klaenhammer T R 1988 Bacteriocins of lactic acid bacteria. *Biochimie* **70** 337 - 349.

Oyarzabal O A, Conner D F 1995 *In vitro* fructo - oligosaccharide utilization and inhibition of *Salmonella* spp. by selected bacteria. *Poultry Sci* **74** 1418 - 1425.

Parker M T, Collier L H 1990 *Streptococcus* and *Lactobacillus*. In: *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*. Edward Arnold, London, UK, 8th ed. pp 148 - 152.

Price R J, Lee J S 1970 Inhibition of *Pseudomonas* species by hydrogen peroxide producing *Lactobacilli*. *J Milk and Food Tech* **33** 13 - 18.

Sarra P G, Morelli L, Bottazi V 1992 The lactic acid microflora of fowl. In: *The Lactic Acid Bacteria in Health and Disease*, ed. Wood B J B, London, New York: Elsevier Appl Science, The Netherlands pp 3 - 21.

Schillinger U, Lucke F 1989 Antibacterial activity of *Lactobacillus sake* isolated from meat. *Appl and Environ Microbiol* **55** 1901 - 1906.

Suegara N, Morotomi M, WatanabeT, Kawai Y, Mutai M 1975 Behaviour of microflora in the rat stomach: adhesion of *Lactobacilli* to the keratonized epithelial cells of the rat stomach *in vitro*. *Infection and Immunity* **12** 173 - 179.

Review Article

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TOXICITY OF DYES AND DYE INTERMEDIATES

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Dyes and pigments are mostly colored substances used for coloration.The chemicals used for their synthesis are hazardous for human life. The metabolism occurs primarily in liver and the metabolites formed are transported in the blood where they can form protein adduct or undergo renal filtration in urinary bladder lumens where at acidic pH, they can react covalently with DNA and the carcinogen DNA adducts formed cause disorders in the whole metabolic reactions. Many carcinogenic/ mutagenic hazards, which occur in the body, have been summarized for public awareness.

Key words**:** Toxicity of dyes, Coloration, Dye intermediates, Pigments.

Introduction

Dyes are intensely colored substances that can be used to produce a significant degree of coloration when dispersed in or reacted with other materials by a process which at least temporarily destroys the crystal structure of the substrate. They are retained in the substrate by adsorption solution and mechanical retention or by ionic or covalent chemical bonds (Anon 1974).

Organic pigments are finely divided crystalline solids. They are insoluble in the systems in which they are used, like inks, surface coatings, plastics and artificial fibres, must be dispersed in them with the expenditure of mechanical energy (Schewaebel and Nordmyer 1971).

Dye Intermediates are the chemicals or substances which are used in the synthesis of dyes and pigments. For example, intermediates used in the production of dyes and pigments are naphthols, naphylaminophenols and many others, having different substituents on aromatic rings and having structure as required according to their use in any class of dyes (Anon 1955).

Purpose of this review is to create awareness about the toxicity and carcinogenicity of these synthetic dyes and intermediates among who are manufacturing these dyes or are using them in their goods. This review covers the literature from 1956 to 2002 with all the hazardous/carcinogenic / mutagenic effects of dyes and dye intermediates on humans and animals. Toxicity of many intermediate chemicals are presented in Table 1 and 2. Various reports are already can be seen in the literature (Clontero *et al* 1990, Anliker 1979) however, these reports lack mechanism of toxicity induced by these synthetic dyes.

Prohibition of certain dyestuffs in developed countries began with the discovery of that handling benzidine-based dyes that could cause cancer of the bladder for over 25 years (Melick *et al* 1955, Huang *et al* 1979). Studies comparing the combined effect of chemical carcinogens on laboratory rats revealed that concurrent, sequential or mixed quantities of compounds targeted at liver tissue resulted in syncarcinogenic effects. Current test of relevance to human tissue involved change in morphology in respect of stiochiometric adducts, haemoglobin or DNA.

Carcinogenic aromatic amine metabolism and DNA adduct detection in humans. Initial metabolism of an aromatic amine occurs primarily in the liver and involves N-hydroxylation by cytochrome P - 450 IA2 and N-acetylation by acetyltransferase which serve as competing activation and detoxification reactions, respectively. Both the enzymes are polymorphic in humans and can be readily assessed using caffeine ingested in coffee followed by urinary metabolite analysis. N-hydroxyarylamines can then be transported in the blood where they can form protein adducts with haemoglobin (Hb) or undergo renal filtration into the urinary bladder lumen where, at acidic pH, they can react covalently with urothelial DNA. Carcinogen-DNA adduct levels in human bladder are smoking-related and the C_8-substituted deoxyguanosine derivatives of 4-aminobiphenyl are the major product formed.

Alternatively, N-hydroxyarylamines can be conjugated in the liver by glucuronyl transferases which provides a mechanism for biliary transport of the colon lumen where α-glucuronidases can regenerate the aglycon. In the colon mucosa, acetyltranferases can further activate the N-hydroxy metabolite by *O*-acetylation, and persons with rapid acetylation are known to be at higher risk for colorectal cancer. Detection of specific arylamine - DNA adducts in human colon should provide direct evidence for the role of these carcinogens in the etiology of this disese (Kadlubar 1991, Pei *et al* 1993).

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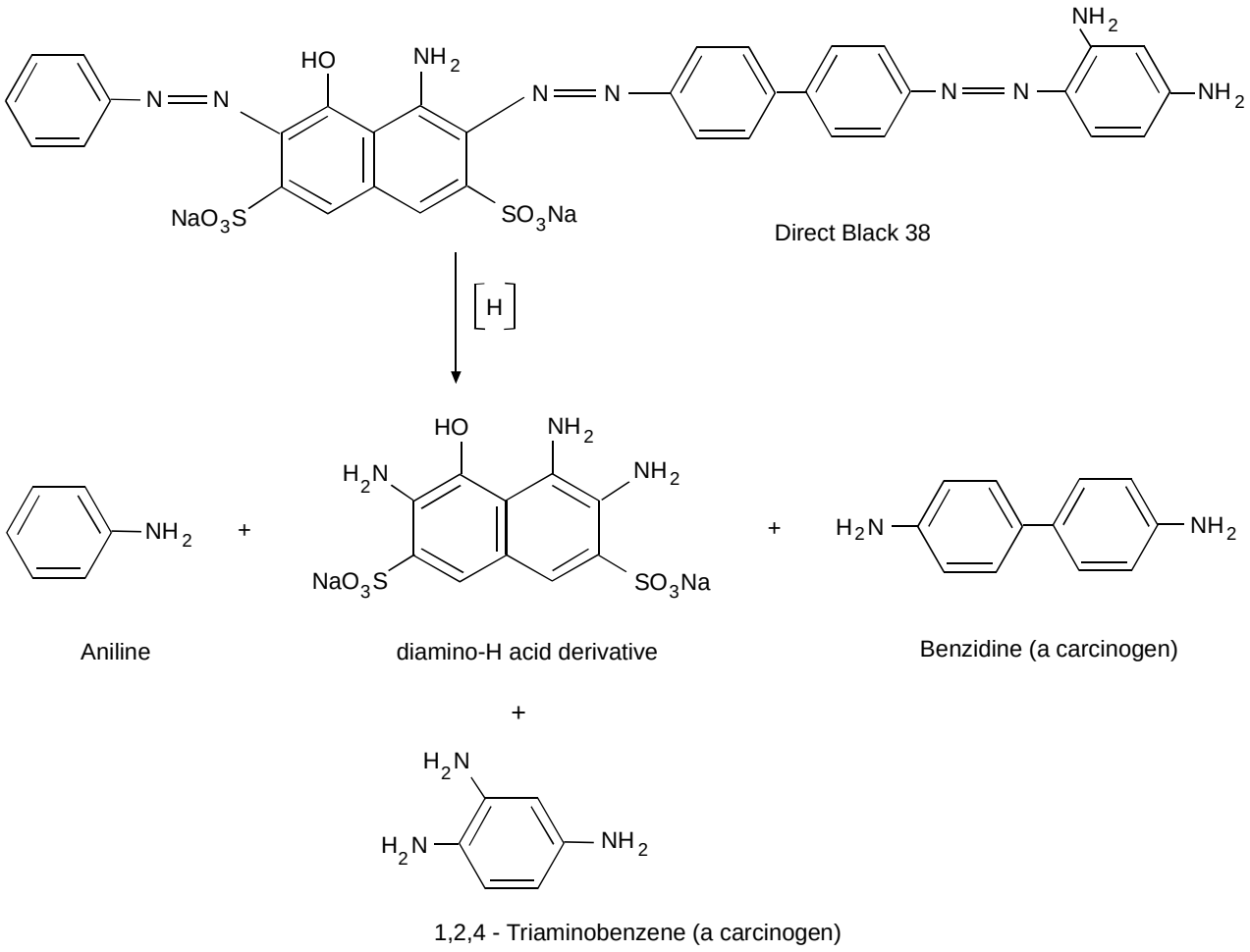

Scheme 1. The metabolism of an azo dye via a reductive- cleavage reaction.

Table 1
Dyes Intermediates

Compound/Class	Area of application
Monochloroanilines	Intermediate for herbicides, pharmaceuticals and azo dyes
Dichloroanilines	Dyes, azo dyes, isocyanates, plant protection agents
Trichloranilines	Pigment intermidiates
Toluidines	Herbicides, Pharmaceuticals, dye Intermediates
Benzidines and substituted benzidines	Intermediates for azo dyes and pigments, cross linking agents for polyurethanes and polyamides

The azo dyes have ability to undergo reductive cleavage of the azo linkage. The metabolism *via* a reductive - cleavage reactions has been shown in Scheme - 1.

Toxicity of Dyes and dye Intermediates. Certain dye intermediates e.g., substituted aromatic amines (anilines and benzidines) are known to possess high toxicological potential. They are classical environmental pollutants by virtue of their high degree of water solubility and the large amounts produced annually in the chemical industry for the synthesis of dyes and other compounds. As a consequence of their common usage, certain members of aromatic amines and benzidines are commonly found not only in waste water but also in surface water. For this reason, their accumulation in free or bound form is also possible in sediments or suspended matter (Scholz *et al* 1988). Some examples given in Table 1.

*Dyes.*Azo and anthraquinone dyes are the major chemical classes of commercial dyes. Among azo dyes, the direct dyes and pigments are the major classes which are derived from mono and disubstituted anilines. Much of the research on the ecological aspects of nitro, azo and anthraquinone dyes has shown the potential carcinogenic risk to humans and animals.

The International Agency for Research on Cancer (IARC) has also reported an evaluation of the Carcinogenic and Mutage-

Table 2
Toxicity of some colorants

C.I. Generic name & No.	CAS No.	CAS Name	Active ingredient in test sample (%)	Toxicological			Symbol
					Irritation		
				LD50 (mg/kg rat)	Skin rabbit	Eye rabbit	
C.I. Acid Orange 156 C.I. 26501	68555-86-2	Benzenesulphonic acid, 4-[(5-methoxy-4-[(methoxyphenyl)azo]-2-methylphenyl]-azo]-, sodium salt	95	120200	-	-	T
C.I. Acid Orange 165 C.I. 28682	85030-26-8	Benzenesulphonic acid, 3-[(4-[(3,5-dimethyl-IH-pyrazol-4-yl)azo]2,5-dimethoxyphenyl)azo]-, sodium salt	100	60	-	+	T
C.I. Basic Blue 3 C.I. 51004 (chlorozinoate)	63589-47-9	Phenoxazin-5-ium, 3,7-bis(diethylamine)-, (T-4)- 95 100 - + T(chlorozincate) tetrachlorozincate (2-1) (2:1)	95	100	-	+	T
C.I. Basic Blue 7 C.I. 42595	2390-60-5	Ethanaminium, N-[4-((4-(diethylamino)-phenyl] [4-(ethylamino)-1-napthalenyl]methylene]-2, 5-cyclohexadien-1-ylidine]-N-ethylchloride	>98	100	-	+	T
C.I. Basic Blue 81 C.I. 42598	73309-46-3	Ethanaminium, N-[4-(diethylamino)-phenyl] [(4-ethoxyphenyl)amino]-1-naphthalenyl]methylene]2,5-cyclohexadien-1-ylidine]-N-ethyl-, chloride	>98	205	-	+	T
C.I. Basic Red 12 C.I. 48070	6320-14-5	3H-Indolium, 2-[3-(dihydro-1,3,3,-trimethyl-2H-indol-2-ylidine)-1-propenyl]-1,3,3,-trimethyl-, chloride	>98	25+ 310	-	+	T
C.I. Basic Violet 16 C.I. 48013	6359-45-1	3-H-Indolium, 2-(2-[4-(diethylamino)phenyl]-ethenyl]-1,3,3-trimethyl-, chloride	94	90	-	+	T
C.I. Basic Yellow 21 C.I. 48060	6359-50-8	3H-Indolium, 2-[2-[2,3-dihydro-2-methyl-1H-indol-1-yl]ethenyl]1-3-trimethyl-, chloride	>98	171	-	+	T
C.I. Direct Orange 62 C.I. not available	70304-37-9	Benzensulphonic acid, 2,2″-(1,2-ethenediyl)-bis(5-[(2-methoxy-5-methyl-4-[(4-sulphophenyl)azo]phenyl]-azoxy]-tetrasodium salt	96	150	-	+	T
C.I. Basic Blue 81 C.I. 42598	2390-60-5	Ethanaminium, N-[(4-diethylamino)-phenyl][(4-ethoxyphenyl)amino)-1-napththalenyl)]methylene]-2,5-cyclohexadien-l-ylidine]-N-ethyl-, chloride	>98	205	-	+	T
C.I. Azoic Diazo Component 20 C.I. 87175	120-00-3	Benzamide, N-(4-amino-2,5-diethoxyphenyl)-	90	49	-	-	T
C.I. Azoic Diazo Component 24 C.l. 37155	6268-05-09	Benzamide, N-(4-amino-2,5-dimethyoxyphenyl)-	90	70	-	-	T
C.I. Azoic Diazo Component 41 C.l. 37165	99-21-8	Benzamide, N-(4-amino-5-methoxy-2-methylphenyl)-	90	115	-	-	T

Symbol T=Toxic

nic risk of some aromatic amines and related nitro compounds and azo dyes to man. It seems clear from these reports that, as a general rule, azo dyes which are based on these aromatic amines and which also lack at least one sodium sulfonate (-SO_3Na) group pose a risk to man and this point is also supported by more recent work. On the other hand, those dyes which contain two or more hydrophilic - SO_3Na groups are non-mutagenic and non-carcinogenic (Anon 1982).

For a number of colorants e.g, the benzidine - based dyes for which the metabolism in man and animals has been shown to involve reductive cleavage of the azo bonds. The use of such dyes in high-exposure applications e.g. hair dyes, children's wear, finger paints, do-it-yourself dyeing or under poor working conditions in processing plants, poses an elevated risk.

Toxicity of dye intermediates and their biochemistry. Health Hazard Information. The ETDA members have completed the project which involved the testing of over 5000 commercial dyes during 5 - 7years and reported the acute oral toxicity on the rats and rabbits. The analysis of short - term health effects showed skin and eye irritation which may occur immediately or shortly after exposure to these aromatic amine (Hunger and Jung 1991, Sewekow 1997).

Chronic Health Effects. At present, the possible chronic effects that attracting most of the attention are carcinogenicity and to a lesser extent sensitization. Technical dyes (excluding food, hair, cosmetics and drug dyes), properly handled and used, are only taken up in small traces, if at all. The available evidence indicates that these trace quantities do not present any unreasonable risk. However, a risk situation could arise due to improper handling or use. This is the main reason for ETDA's continuing efforts to identify any such product. The chronic (long - term) health effects can occur at some time after exposure to these aromatic amines and can last for months or years (Slawomir *et al* 1998).

Cancer Hazard. The aromatic amines are carcinogens in humans and cause liver, breast, bladder intestine and skin cancer not only in humans but in animals also (Helmes *et al* 1986).

Many scientists believe that there is no safe level of exposure to a carcinogen. Such substances may also have the potential for causing reproductive damage in humans. These compounds caused cancer in the offspring of animals exposed during their pregnancy. Other long - term effects may cause a skin allergy. If allergy develops, very low future exposure can cause itching and a skin rash (Rockeville 1984, Hillier and Rome 1986).

Acute (short-term) ecological effects. Acute toxic effects may include the death of animals, birds or fish and death or low growth rate in plants. Acute effects are seen after two or four days when animals or plants come in contact with a toxic chemical substance (Jaskot and Costa 1994).

Chronic (Long-term) Ecological effects. Chronic toxic effects may include shortened lifespan, reproductive problems, lower fertility and changes in appearance or behavior. Chronic effects can be seen long after first exposure to a toxic chemical (Sun *et al* 1998).

Water solubility. The solubilities of these aromatic amines is between 1 to 1,000 mg in a liter of water.

Distribution and persistence in the environment. These compounds are moderately persistant in water, with a half - life of between 20 to 200 days. The half life of a pollutant is the amount of time it takes for one - half of the chemical to be degraded. About 60 - 80% of these aromatic amines will eventually end up in water, the rest will be divided about equally between terrestrial soils and aquatic sediments.

Bioaccumulation in aquatic organisms. Some substances increase in concentration or bioccumulate, in living organisms as they breath contaminated air, drink contaminated water, or eat contaminated food. These chemicals can become concentrated in the tissues and internal organs of animals and humans.

Biochemistry of dyes/dye intermediates and their carcinogenicity. There is sufficient evidence for the carcinogenicity in experimental of these aromatic amines animals in when administered in the diet, these aromatic amines induced transitional cell carcinomas of the urinary bladder in hamsters and female dogs and hepato-cellular carcinomas in female dogs (Lakshmi *et al* 1995).

When administered by transplacental exposure, the compound increased the incidences of lymphoid leukemia in mice (Kennelly *et al* 1982).

The dye intermediates have ability to undergo reductive cleavage of the azo linkage. The metabolism by a reductive cleavage reaction has been shown in Scheme - 1.

Binding of benzidine / derivatives to rat and mouse tissue DNA. Heamoglobin binding of benzidine and some benzidine congeners (Beland *et al* 1997).

Hydroperoxidase I catalyzed peroxidative activation of benzidine / derivatives to a mutagen in *Salmonella tuphimurium* (Jung *et al* 1985).

Comparative activation of benzidines to mutagens by hepatic S9 and microsomes from rat pretreated with different inducers of Cytochrome P - 450 (Parkinson *et al* 1983).

Table 3
Mutagenicity data of some dye intermediates

Test	Mutagencity (Revertants μmol^{-1})	
	Standard Assay S9	Prival Modification
Aniline	Negative	Negative
3-Aminoquinoline	Negative	-
5-Aminoquinoline	42	-
3-Aminopyridine	0.7	-
4-Amino-N,N-bis-(2-hydroxyethyl)aline	1007	689
4-Aminodiphenylamine	Negative	154
4-Amino-N,N-dimethylaniline	Negative (toxic)	-
2-Aminothiazole	4.1	-
3-Aminophenol	1.7	-
Chromatropic acid	Negative	Negative
Broenner's acid	4.9	7.7
Cleve's acid	4.9	12.5
S-acid	13.8	24.4
Gamma acid	15.0	32.6
H-acid	Negative	Negative
J-acid	5.2	41
4-Amino-1-naphthol	1413	Negative
4-Amino-5-hydroxy-8-phenylazo-2,7-naphthalenedisulfonic acid disodium salt	Negative	Negative
4-Amino-8-(4-carboxyphenylazo)-5-hydroxy-2,7-naphthalenedisulfonic acid trisodium salt	Negative	Negative
7-Amino-4-hydroxy-1-phenylazo-2-naphthalenesulfonic acid monosodium salt	16.1	67.8

Benzidine and derivatives induce micronuclei in the bone marrow and the fetal liver of mice after gavage (Przybjewska *et al* 1985).

Mutagenicity of some benzidine congeners and their acetylated and diacetylated derivatives in different strains (Hatcher and Swaminatham 1992).

Induction of hepaticmicrosomal cytochrome P - 448 - mediated oxidases by benzidine derivatives in the rat (Parkinson *et al* 1983). Binding of benzidine derivatives to DNA and polyribonucleotides (Osborne and Monogr 1984).

Mutagenicity of some congeners of benzidine in the *Salmonella typhimurium* assay system (Kranen *et al* 1997).

Covalent interaction of benzidine/derivative with hepatic lipids. Enzymic basis and stability of the adducts (Mule and Lomte 1994).

Activation of benzindine/derivatives in rat liver microsomes to mutagens with the involvement of Cytochrome P - 450 (Manus 1989).

Structure - toxicity relationship. The toxicity and carcinogenic activity also depend on the structure and substitution on biphenyls. For dyes that are carcinogenic and are resistant to chemical attack, such as true azo dyes, the dye itself is likely to be procarcinogen. In contrast, azo dyes which exist in the hydrazone form are more likely to be broken down e.g. reduced. In this case, the procarcinogen is likey to be an amine breakdown product of the dye and the ultimate carcinogenic potential can then be deduced from the availabitlity of a suitable active site on the metabolite. Azo pigments because of their extreme insolubilities are likely to be broken down, even if they exist in the hydrazone form (Gregory 1986).

A relationship also exists between structure and carcinogenic activity of substituted benzidines. Proton accepting substituents which are capable of forming intramolecular hydrogen bonds in ortho - position in relation to amino groups, decrease the carcinogenicity of the title intermediates of dye synthesis. Lipophilicity of the compounds and electronic effects of the substitents are found to be the main parameters of carcinogenicity. Substitution at both *ortho* positions 2, 6-disubstitued aniline and 2,4,5-trimethyl aniline could prevent genotoxicity due to steric hindrance of an enzymic activation to electrophilic intermediates (Belogoro *et al* 1981).

Direct Dyes. It is now well established that the ability of azo dyes to undergo reductive cleavage of the azo linkage could lead to an indirect route of exposure to an established carcinogen. The absorbance potency of these dyes, however was the most potent of the dyes tested and their inducing activity was much greater than that of its azo reduction products (Kornburst and Barfknecht 1984). The literature surveyed contains the following reports which described work published on the toxicity of direct dyes.

A report on the toxicology and carcinogensis properties of C.I. Direct blue 14 and 15 was published under the national toxicology programe. The rats were fed these dyes in drinking water and the experimental results showed a clear evidence of carcinogenic activity, as indicated by benigu and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity and small and large intestine. Increased incidences of mononuclear cell leukemia and neoplasms of the brain may have been related to chemical administration (Anon 1992, 1993).

A thirteen-week subchronic toxicity studies were conducted on direct blue 6 (Anon 1974), direct black 38 and direct brown 95 by administering the test chemicals in feed to rats and mice. Deaths occurred among rats but not among mice during the test period. In rats, neoplastic lesions of the liver, hepatocellular carcinomas and neoplastic nodules, occurred only in the treated groups (Roben *et al* 1980).

In an investigation, the textile dyes, Navy blue M3R(I) and direct brown 2G(2), not only reduced the percentage of seed germination in *Vigna radiata* (L.) but also suppressed various morphological, biochemical and physiological parameters. I affected the various parameters more adversely than 2. The adverse effects of both dyes were higher in black soil than in red soils (Wilczek *et al* 1992). Toxicological and carcinogenesis studies were conducted by adiministering direct blue 218 in feed to groups of male and female rats in *Salmonella typhimurium*, cultured Chinese hamster ovary cells and *Dorsophila melanogaster* and their carcinogenic and toxic effects are reported (Anon 1994).

The azo reductase activity of a cell - free extract of *Fusobacterium* sp.2 was characterized using trypan blue (I) as a substrate. Either chemical reduction of this dye with sodium hydrosulfite or reduction by the cell-free extract produces a mutagenic product, *o* - tolidine. The *o* - tolidine is mutagenic in the Ames *Salmonella* / mammalian - microsome mutagenicity test when activited by a rat liver S9 preparation (Hartman *et al* 1978).

The effects of prenatal administration of the benzidine-based dyes which are Congo red, Diamine blue and Chlorazil black E, whereas, the dimethylbenzidine-based dyes include: Trypan blue, Evans blue and Benzopurpurin 4B and dimethoxybenzidine-based were investigated, whereas, Chicago sky blue, Azoic diazo component 48, a dimethoxybenzidine congener, two diazo dyes , Naphthol blue black and Sudan III were tested for developmental testicular toxicity. In mice and rats, prenatal exposure to the dye, Congo red permanently reduces the number of germ cell in male and female offspring. In this study, the structural component of the dyes responsible for the parental induction of germ cell aplasia was identified. Only benzidine-based dyes altered testicular development and caused hypsopermatogenesis in mice during adulthood. Dimethyl-and dimethoxybenzidine based dyes were without effect (Gray *et al* 1993).

β -Amyloid peptides are neurotoxic when applied to primary cultures of hippocampal neurons from the embryonic rat. This neurotoxic effect can be inhibited completely by certain diazo dyestuffs. The most potent of these are Congo black(I) and Congo rubin, while direct garnet and sodium-4-aminonaphthalene-1- sulfonate are inactive. I also inhibits the neurotoxic effects of the human pancreatic amyloidogenic peptide amylin. It is postulated that these dyes, by interacting with the (- pleated sheet structure of amyloidogenic peptide, prevent aggregation and hence neurotoxicity (Bargevin *et al* 1994). In a similar study the gonadal effects of fetal exposure to the azo dye Congo red (I) in mice was investigated. This study describes the relationship between gonadal agenesis and fertility in male and female mice exposed to the diazo dye (I). Maternal (I) treatment inhibited testicular and ovarian function in the offspring after oral administration. It was found that prenatal exposure to the dye (I) affects the gonads of both male and female offspring, but only the female offspring display reduced fertility (Gray *et al* 1992).

Food colors. The toxic effects of nine natural food dyes on *Paramecium caudatum* showed the inhibitory effects on leucine aminopeptidase acid phosphatase and esterase *in vitro* and were proportional to the toxic effects of the dyes expressed in terms of the survival time of *P. caudatum* (Chughtai *et al* 1993). In a similar studies, the toxic effect of Xanthene dyes containing halogen atoms were found to be more toxic than other types of food dyes. Phloxin and Rose Bengal (containing chlorine) were particularly toxic. The inhibitory effect of food dyes on leucine aminopeptidase were not consistent with their toxic on *A.salina*. On the other hand, the inhibitory effects of food dyes on lactate dehydrogenase *in vitro* were consistent with the toxic effects of the dyes on *A.salina* (Sako *et al* 1979).

The carcinogenic effect of Metanil Yellow on albino mice have developed hemangioendotheliosarcoma in 80% of the female albino mice and none in males after feeding them food colour additive for 1 year at a dose of 3.0 g / kg body weight. Degenerative changes and metastates were also observed in other important vital organs of the body such as stomach, ileum, rectum, liver, spleen, kidney and ovary (Prasad and Rastogi 1982).

A skin painting studies in mice was conducted with 14 Food, C, D and C colours: FD and C blue No. 9, red No.10, red No.19 & red No. 21, red No. 27 and red No. 31, red No. 36, orange No. 5, orange No. 10 and orange No. 17. These 14 cosmetic colour were submitted to dermal toxicity testing. Dosage levels were based on lipstick use determination made in a group of human female volunteers. The groups of lipstick colors were divided into 3 treatment series and painted on twice weekly to an area 6 cm. A total of 1400 mice were used comprising groups of 100 mice (50/sex) plus an additional pos. control group of the same size and a vehicle control group of 300 mice (150/sex). All colours were prepared at 1.0% suspensions in water. The pos. control received benzo[a] pyrene(1) dissolved in acetone.

Survival was approximated equivalent in all experimental groups except the pos. controls who died earlier which was consistent with survival recorded by others for I-treated mice. Extramedullary hematopoiesis was found in all treated groups, equivalent to the findings in the controls. The repeated application of 0.1 ml containing 1.0% dye did not increase the incidence of neoplasia when compared to controls in any of the groups receiving application of the 14 days (Carson *et al* 1984).

The mutagenicity of food colors in the Ames/*Salmonella* of 14 food dyes tested, including Methylene green 203A, Strawberry red 1023, Purple red 0048, Raspberry red 0059 and Black 0051 was determined but none was mutagenic. In another investigation, the effect of food colors on the induction of sex-linked recessive lethal mutation with *Dorsophila melanogaster* (Rapic *et al* 1985) was determined of 13 food dyes tested, inclusive Citronine rose 200A, Methylene green 203A, Black 0051, Purple red 0048, Strawberry red 1023 and Raspberry red 0059, none were mutagenic in a sex-linked recessive lethal mutation test with *D. melanogaster* (Vijosevic *et al* 1985). In a similar report, the results of *in vivo* study on mammalian chromosomes of genotoxicity of 16 food dyes tasted, including Raspberry red 0059, Orange color 963, Strawberry red 1023, Citron rosy 200 A and Chocolate brown 1022, none was genotoxic to bone marrow cell chromosomes when given to mice as a single dose of a 1% aqueous solution (Jankovic *et al* 1985).

The mutagenic evaluation of Orange color 963, Vanilla red 0050, May green 1025, Black 0051 and Methylene green 203A by the micronucleus test (no of polychromatic and normochromatic erythrocytes containing micronuclei) with rats only 1 (Black / 0051) increased the percentage of erythrocytes with micronuclei indicating mutagenic activity by this dye (Savkovic *et al* 1985). In another investigation, the influence of 16 food colors on the induction of dominant lethal mutation and spermatozoid abnormality in mammals was also determined. None of 16 tested food dyes reduced fertility or caused spermatozoid abnormalities. No significant changes in dominant lethal mutations were induced. The dyes tested included Orange color 963, Vanilla red 0050, May green 1025, Black 0051 and Methylene green 203A (Konishi *et al* 1992, Dillion *et al* 1994).

The carcinogenicity testing of Food red No.106 (acid red) (I) in male and female rats were determined. Body and organ weights, hematology, urinalysis and histopathological evaluations do not reveal any evidence of adverse effects associated with the compound relative to the untreated controls. The spectrum, incidence and histology of tumors developing in both treated and control animals were consistent with spontaneous incidences reported in this strain of rat. This study thus indicates that (I) is not carcinogenic to F 344 rats after 2 year of dietary administration at a maximal level of 5.0% in the basal diet by weight (Osman *et al* 1984).

The effect of feeding synthetic food and drug colorants belonging to four different chemical classes i.e. Brilliant black (BK), Brilliant blue (B), Erythrosine (Er) and Indigo carmine (In) were studied. Groups of male and female mice were fed-libitum diets mixed with the synthetic food colorants. The activities of liver and heart tissues were inhibited in male and female mice relative to control and males were more affected than females. The blood hemoglobin (Hb) content was increased under the effect of the 4 synthetic food colorants in the order: Er > Bk > In > Bl as well as the red blood cells while white blood cell count were increased relative to control in male and female mice. Pathalogical and histopathological changes were observed either in female or male organs especially in the liver and stomach. Some changes were found in the liver tissues and cell nucleus (polynucleus) in addition, enlargement of the stomach size was also evident (Nishiuchi 1984).

The toxicity of 25 dyes on freshwater organisms was tested. These synthetic dyes used in food was tested on carp (*Cayprinus cariop*), *Dephnia carinata*, *Indoplanorbis exustus*, larvae of *Sympetrum frequens* and tadpoles of toad (*Bufo bufo*) and frog (*Rana brevipoda porosa*). With the exception of the effect of Food Black No. 105 (Rose Bengal) on carp, all the tested dyes showed very low toxicity to all tested fresh water organisms with median lethal concentration value (LC50) of > 40 ppm (Wagner *et al* 1995).

Basic and Solvent Dyes. The potential for methylene blue genotoxicity was investigated in two mammalian test systems. Different concentrations of Methylene blue were prepared in plasma (heat-treated at 56°C for 1h to reduce cytotoxicity) and used, without illumination, in an *in vitro* mouse lymphoma cell assay designed to detect forward mutations in the gene encoding thymidine kinase. The assay was performed in the presence and absence of rat liver microsomal fraction. Methylene blue did not increase the frequency of micronuclei in polychromatic red cells harvested from bone marrow. It is mutagenic in cultured mammalian cells (Michaels *et al* 1985).

The toxicity and sorption of 5 azo and triphenylmethane dyes named as Basic violet 1(I), Basic violet 2, Basic violet 3, Basic green 4 and Tropaeolin O were established by determining the percent to fresh water microbiota and determined the risks, that these dyes may pose to the aquatic environment. Basic violet 3 was the most toxic, with a mean survival rate of 20.7% at a dye concentration of 5.0 mg/l. Tropaeolin O was the least toxic, with a survival rate of 92.0%. Survival increased with

decreasing dye concentration equilibrium. Partition coefficients were higher for viable cells than for heat-killed cells, suggesting that a metabolic process may be involved in sorption of these dyes or that autoclaving the cells reduces the organisms' cation exchange capacities (Milanova *et al* 1997).

Aquatic toxicity effects of dyes used in the manufacture of news print and telephone directory-grade papers were studied on luminescent bacteria, rainbow trout and activated sludges. The results showed that triphenyl methane dyes, used in making telephone directory-grade papers inhibited respiration of flora in activated sludge at higher concentrations not likely to exist in mill effluents. Data from microtox tests agreed with fish toxicity data (Ahmed *et al* 1985).

The toxicity of Aniline blue, Methylene blue, Xanthene, Eosin and Fuchsin dyes against the cotton leaf worm (*Spodoptera littorialis*) was investigated for their possible use in combination with pyrethroids. Deltamethrin was the most effective against (*S. littoralis*) 4th instar larvae followed by flucythrinate and cypermethrin, whereas, Aniline blue and Methylene blue showed higher toxic index than Xanthine followed by Eosin and Fuchsin. The joint action studies demonstrated strong synergism in mixtures of deltamethrin with Fuchsin, Methylene blue or in the mixture of Flucythrinate with Xanthine or Eosin. The other combinations indicated only additive joint action (Vachalkova 1996).

The reduction of some triphenylmethane dyes and their potential carcinogenic activity was examined in strictly anhydrous solutions in the absence and presence of α-lipoic acid. The highest potential carcinogenicity values determined for Crystal violet and Methyl violet are found to be 0.420 and 0.440, respectively (Selyuzhitskii *et al* 1982).

A study was conducted to find out the blastomogenic properties of Basic blue K (1) on animals. I induced formation of 2 tumors in 2 males of 10 and 10 tumors in 7 females of 13; no tumors were developed in control animals (Zimina and Pavlenko 1991).

The toxic and mutagenic effects of arylmethane dyes Victory blue (C.I. 44040), Methyl violet (C.I. 42535) and Brilliant green (C.I. 42040) and the carcinogenic aminoazo dye Chrysoidin (C.I. 14270) were evaluated. All the dyes exhibited high toxicity (as cell killing and growth inhibition) and increased the frequency of nuclear point mutations and cytoplasmic mutations of respiratory deficiency (Serova *et al* 1992).

The frequency of neoplasms and chromosomal aberrations (CA) in the liver and bone marrow were investigated after administration of carcinogenic *O*-aminoazotoluene (OAT) and non-carcinogenic analog - 4 - aminoazo-toluene (AB) to male and female rats in the early postnatal period. AB increase of CA in the liver was small (although substantial in males), but the increase was high in the bone marrow of both sexes. In contrast, OAT increase of CA was higher in the liver than that in the bone marrow and it was higher in the males. In addition, OAT induced greater number of hepatic neoplasm in the males. Both these effects of OAT in males suggest a relation between its mutagenic and carcinogenic properties (Kaledin *et al* 1994; Ashby *et al* 1994).

The evaluation of Butter yellow (4-dimethylaminoazobenzene) (I) and 12 of its structural analog were made in a cell transformation assay. The *in vitro* results agreed with long-term animal data for 8 compounds, but disagreed in finding I-4 sulfonic acid and sodium salts, 4-trifluoromethyl-I and 4-diethylaminoazobenzene pos. 9-Phenylazotoluidine and N-methyl-5-phenylazoindoline may have carcinogenic potential and 3,5-dimethyl-4-aminozobenzene-4-sulfonic acid may be noncarcinogenic. Addition of azobenzene to the *in vitro* assay medium increased the transforming potency of I 25-fold. This assay cannot be relied upon to predict the *in vivo* potency of a carcinogen (Sandhu and Chipman 1991).

The role of oxidation and azo reduction in the activation of Chrysoidine (I) dyes to genotoxic products. The enzymes involved in metabolic activation of (I) azo dye to genotoxic products were investigated. The mutagenicity of the component 2,4-diamino-3-methylazobenzene in *Salmonella typhimurium* strain TA 100 potentiated > 4-fold. The results indicate a role for cytochrome P - 450 particularly, cytochrome P - 448 in metabolic activation of these dyes and indicate that azo reduction was a detoxification process. Neverthless, the oral and injected dosing of Chrysoidine to rats led to unscheduled DNA synthesis in hepatocytes (Collier *et al* 1993).

The mechanism of azoreduction of *p*-dimethylamino-azobenzene (I) dye carcinogens was conducted by rat liver microsomal cytochrome P - 450. To elucidate the mechanisms involved, the reduction of structurally related azobenzenes by hepatic microsomes was investigated. High substrate reactivity was observed for I, its corresponding secondary (II) and primary (III) amines and *p*-hydroxyazobenzene (IV). In contrast, only negligible rates were obtained for unsubstituted azobenzene (V), hydroazobenzene (VI), *p*-isopropylazobenzene (VII) and (VIII), the benzoylamide derivatives of III. These results clearly indicate that electrons-donating groups, such as hydroxyl or primary, secondary and tertiary amines, are essential for binding of azo dye carcinogens to liver microsomal cytochrome P - 450 and by implication, their enzyme reduction. No inhibition of azoreduction of I and IV was obtained by

addition of VII, V or IV to the reaction mixture. In contrast, very weak binding was observed for the unreactive compounds VII, VIII, V, VI. Thus, there is good correlation between binding and substrate reactivity. The apparent lack of binding may explain the inability of the non-reactive compounds to inhibit azoreduction. The difference in the reduction rate observed for V *vs*. IV suggested that hydroxylation would facilitate the reduction of an otherwise non-reactive azo dyes (Zbaida *et al* 1989, Anon 1992).

Pigment and vat dyes. Under National Toxicology Program (NTP), a technical report on the toxicology and carcinogenesis studies of C.I. Pigments red 3(I) in rats and mice is prepared. Under the condition of 2-year feeding studies, there was some evidence of carcinogenic activity of (I) in male rats as exhibited by increased incidences of benign pheochromocytomas of the adrenal gland. The marginal increase in the incidences of squamous cell papillomas of the skin and Zymbal's gland carcinomas may have been related to (I) administration. There was some evidence of carcinogenic activity of (I) in female rats as indicated by the increased incidence of hepatocellular adenomas. There was some evidence of carcinogenic activity of (I) in male mice as exhibited by the increased incidences of tubuler adenoma of the renal cortex and follicular cell adenomas of the thyroid glands. There was no evidence of carcinogenic activity of (I) in female mice that received 12,500, 25,000 or 50,000 ppm. The incidences of mononuclear cell leukemia and preputial gland tumors in male rats and mononuclear cell leukemia, mammary gland fibroadenoma and clitoral gland tumors in female rats were lower in the exposed groups. The incidences of liver foci were markedly increased in exposed male and female rats. The severity of nephropathy was observed in male and female mice, cytomegaly (karyomegaly) of renal tubule epithelium was observed in male mice. Thyroid follicular cell hyperplasia occurred with an increased incidence in male and female mice receiving (I) (Anon 1994).

In a similar study, toxicology and carcinogenesis of C.I. Pigment Red 23(I) was determined in rats and mice. Under the conditions of the 2-year feed studies, there was equivocal evidence of carcinogenic activity of (I) in male rats as evidenced by a marginally increased incidence of renal tubule cell neoplasms. There was no evidence of carcinogenic activity of (I) in female rats fed diets containing 10,000, 25,000 or 50,000 ppm. The severity of kidney nephropathy was increased in exposed male rats. In mice, (I) caused an increased in hyperkeratosis and epithelial hyperlasia of the forestomach (Hofman and Schmidt 1993).

The possible metabolism of Pigment Yellow 17(I), a 3,3´-dichlorobenzidine based pigment was investigated after inhalation exposure in rats. Rats were exposed by inhalation to the technically highest administrable concentration of 230 mg (I) / m^3 air for 4 h. Inhalability of the dust was guaranteed by a mass - median aerodynamic diameter of 1.0 - 1.1 m. For 14 days after exposure, urine and serum samples were analyzed for 3,3´-dichlorobenzidine, the parent carcinogenic amine of the test compound. No 3,3´-dichlorobenzidine could be detected either in urine of blood, the detection limit being 5 mg/ml for both media. Based on the results of this study there is no evidence for metabolic cleavage of (I) to 3,3´-dichlorobenzidine in the rat (Kurlyanskii *et al* 1988).

The water-soluble phthalocyanine dyes are found to be low toxic and are not very harmful. The copper phthalocyanine derivatives-disulfonic acid, trisulfonic acid reactive turquoise 2 Z and reactive turquoise K affected hepatic mitochondrial electron transport chain. Toxicological properties of dyes are also described (Rannung *et al* 1992).

The presence of genotoxic and bioactive components in indigo-dyed fabric was examined. Extractions of pure cotton and jean fabrics were tested for mutagenicity in *Salmonella typhimurium* strains TA 98 and TA 100. Synthetic indigo, indirubin and isatin were tested in competition expermients *in vitro*. The mutagenicity of the indigo dyed fabric was dependent on type and treatment of the fabric. Extracts of both bleached and nonbleached jeans gave mutagenic effects on TA 98 (S9 and TA 100 S9). The greatest effects were seen in the presence of S9. Indigo can, therefore, still be a potential health risk either by eliciting toxic effects of other compounds or by being a non - genotoxic carcinogen. The world wide use of jeans with a possible exposure of a large population to genotoxic and biological active components emphasizes the need for a more thorough characterization of these effects (Conde *et al* 1984).

The contact dermatitis was determined in 3 patients induced by the use of dyed textiles or by occupational exposure to azo dyes *p*-aminoazobenzene (I), disperse yellow 3, disperse orange 3, diazodiethylaniline and diazodimethylaniline (Kosaka *et al* 1991).

Miscellaneous. The genotoxic components of commercially available synthetic dyes were examined. Dyes which showed toxicity were separated on silica gel coated plates and the genotoxicity of each component was examined. Among the dyes examined, the main component of Disperse red 73 showed genotoxicity. In the case of 5 other dyes, Acid black 26, Acid black 50, Acid brown 2, Disperse red 145 and Disperse red 157, a minor component of Disperse violet 52 was not determined because of the insolubility of its main component (Wedzisz and Grzelka *et al* 1988).

Table 4
Characterization of 1,5-dihydroxy napthalene formaldehyde (1,5-DHNF) Oligomer

1,5-dihydroxy Napthalene Formaldehyde oligomer (1,5-DHNF)	Color	Softning point (°C)	Elemental analysis(%) C Calcd	C Found	H Calcd	H Found	Mn estimated by VPO
	Brown	> 230	77.41	77.30	5.37	5.30	744

Where VPO=Vapour Pressure Osmometry.

LD50 (96 h) values of Mordant black 11, Basic brown 1 and Disperse yellow 1 in guppies (*L. reticulatus*) were found to be 4.02, 3.06 and 3.74 mg / l, respectively; thus the dyes have moderate toxicity to fish. LC50 of Mordant black 15, Acid violet 7, Acid black 26, Acid yellow 44, Acid violet 49, Acid blue 7 and Direct black 32 and 151 were > 400mg/l; these dyes can be considered non-toxic (Kour *et al* 1993).

The mutagenicity testing of textile (azo) dyes with *Salmonella* / microsome assay. Five textile azo dyes-Acid violet 17, Acid green 16, Acid red 85, Acid red 114 and Direct green 6 were tested for bacterial mutagenicity with *S.typhimurium* TA98 and TA100 strains using a plate incorporation assay. After testing over the concentration range 1 - 500 mg with and without metabolic activation these dyes showed no mutagenicity or toxicity (Flammang *et al* 1992).

Dyes intermediate toxicity. The DNA adduct levels were determined in congenic rapid and slow acetylator mouse strains following chronic administration of 4-aminobiphenyl. The levels of the hepatic DNA adducts were 2-fold higher in the liver of the female as compared to the male animals. The DNA adducts also increased with the dose in bladder of the male mice, but in contrast to the liver, the adduct levels were 2-fold lower in the bladder DNA of the female mice (Hughes *et al* 1992).

New thiophene analogs of benzidine and 4-aminobiphenyl have been tested for their carcinogenicity in the *Salmonella* revere-mutation assay Ames and the cell-transformation assay of styles. Their activity profiles *in vitro* were consistent with their known potential carcinogenicity. The possible carcinogenicity of the analogs *in vivo* is discussed (Ashby *et al* 1978).

3,3′-Dimethylbenzidine dihydrochloride (I) is one of five chemicals being evaluated in 2-years carcinogenicity and toxicity studies as part of NTP's Benzidine Dye initiative. Toxicology and carcinogenesis studies were conducted by administering (I) (approximately 99% pure) in drinking water to groups of rats of each sex, whereas, Genetic toxicology studies were conducted in *Salmonella typhimurium*, Chinese hamster ovary (CHO) cells and *Drosophila melanogaster*. Hematology and serum chemical analyses and thyroid hormone determinations were conducted and the results indicated a rapid declining of animal survival (Anon 1991).

It has been reported that the carcinogen 3,3′-dichlorobenzidine is bioactivated in the liver *in vivo* and *in vitro* to mutagenic and lipid - binding metabolites. To characterize the metabolites involved, adult male rats with treated with a single dose of either DBC, the spin trap 1-phenyl-N-tert - butylnitrone (PBN) and both chemicals. It is concluded that in the rat aryl radicals may be the predominant stable radicals from arylamines in the liver, whereas, radicals derived from N-oxygenation may predominate in the blood. The tissue distribution of the two may predominate in the blood. The tissue distribution of the two radical species may reflect the major site of their formation from some arylamines in the rat (Iba *et al* 1991).

The non-genotoxic rodents of the potent bladder carcinogens, *o*-anisidine and *p*-cresidine has been reported which indicated that the genotoxicity of *o*-anisidine was acknowledged and misquoted data cited (Bolcsfoldi *et al* 1992). In another studies, the mutagenicity of *o*-anisidine to the bladder of lacl transgenic mice was also conducted. A single oral administration of the maximum tolerated dose level (750 mg/kg) of *o*-anisidine to mice yielded negative results in 32P - post - labelling assays of bladder and liver DNA (24 h after dosing). The possibility that *o*-anisidine is mutagenic and carcinogenic to the rodent bladder via formation of radical species is suggested (Sasaki *et al* 1994).

The toxicities of chloroanilines to *Photobacterium phosphoreum* was determined and their correlations with effects on other organisms and structural parameters using the Microtox assay was presented. The values obtained correlated well with the toxic effects of these compounds to 4 different species of yeast and with the effects of the octanol / water partition coefficient (Ashby 1992).

The toxicity, DNA binding and adduct formation of chloroaniline and 4,4′-methylene - bis (2 - chloroaniline) has been found and the data showed DNA damage in cultured explants

of human and dog bladder urothelium. The level of DNA damage in human bladder was higher than that in the dog. 33 *P*-postlabeling analyses indicated that Methylene-bis (2-chloroaniline) forms several DNA adducts in both human and dog bladder tissues. These results suggest caution in the occupational exposure of humans to Methylene-bis (2-chloroaniline) (Ribo and Kaiser 1983).

The aromatic amine metabolism catalyzed by prostaglandin H synthase (PHS) was investigated as an enzyme system responsible for the conversion of carcinogens to reactive metabolite(s) in extrahepatic tissues. The bladder carcinogens benzidine 2-aminofluorene, and 2-naphthylamine are oxidized by PHS to free radicals. Metabolic activation of carcinogenic aromatic amines catalyzed by hepatic cytochrome P - 450 proceeds via an N-hydroxylation pathway analogous to other well - studied aromatic amine carcinogens. PHS - catalyzed metabolism of these compounds results in the formation of reactive species which bind covalently to cellular macromols (Stoner *et al* 1988).

The metabolism of naphthylamines in isolated rat hepatocytes has been studied and compared in freshly isolated hepatocytes from 3-methylcholanthrene (MC) - treated and untreated rats. At 10 (M, 2-naphthylamine was mainly N-acetylated and N-glucuronidated. Minor pathways led to C-oxidation and N-oxidation. In hepatocytes, from MC-treated rats total metabolism was slightly affected (1.5-fold increase). Similar experiments were carried out with 1-naphthylamine. Its N-glucuronide was the predominant metabolite (68%) followed by the N-acetylated compound (15%) while C-oxide was low and N-oxidized metabolites could not be detected, even after induction. Thus, MC treatment markedly shifted 2-naphthylamine metabolism from N-acetylation and N-glucuronidation to N-and C-oxidation. In the case of 1-naphthylamine metabolism, extensive N-glucuronidation together with the lack of N-oxidation may prevent carcinogenesis (Eling *et al* 1987). In a similar study, rat peritoneal macrophages were incubated with 2-naphthylamine (I), a well known carcinogen and respiratory burst was studied. (I) induced a time and dose-dependent stimulation of superoxide anion production which was suppressed by superoxide dismutase treatment. Other observations were as follow (i) the simultaneous presence of polymyxin B and stauros-porine inhibitors of protein kinase C, inhibited (I)-dependent O_2 -production; (ii) NADPH-oxidase contained in postnuclear fraction from (I)-incubated macrophages showed a greater activity than control fractions; (iii) the stimulation of O_2 -production elicited by (I) was several-fold enhanced in activated macrophages compared to resident cells. These data suggest that (I) produces the activation of NADPH-oxidase through protein kinase C (Orzechowski *et al* 1992).

The toxicity of toluidine isomers have been determined. It has been reported that *p*-toluidine was 2-fold more toxic than the *ortho* and *meta* isomers according to LD50 in rats, mice and rabbits. The *m*-toluidine had the most damaging effect on blood composition, followed *o*-toluidine and *p*-toluidine. The last also showed some hepatotoxicity. The *meta* isomer somewhat impaired the N excretion by the kidney. Skin resorption followed the order *ortho* > *meta* > *para*. The most local irritating action in rabbit eye was shown by *m*-toluidine (Chiara and Sobrino 1992). In similar work, the author have re-evaluated the carcinogenicity of *o*-toluidine and presented the evidence of carcinogenicity/genotoxicity of *o*-toluidine (Vashenko *et al* 1977). The preventions of occupational urinary bladder tumours in the manufacture of toluidines were investigated. The results of experimental, hygienic and occupational - pathology investigations indicate the carcinogenic nature of *o*-toluidine. A most effective measure for radically improving working conditions is the use of the catalytic method. The maximum perimissible concentration of I should be revised, taking into account its carcinogenic activity.

Among the aromatic amines examined, the mutagenicity was in the order: 3,3′-dichlorobenzidine > toludine > benzidine> 2-naphthylamine. Dichlorobenzidine was extremely mutagenic in the Ames assay with *Salmonella typhimurium* TA 98 with S9 microsomal activation. The last 2 aromatic amines were moderate mutagenicity. Tobias acid and 2,2′, 4,4′-tetra-aminobiphenyl gave negative results with *S. typhimurium* TA 98 and 100 stains and with or without S9 microsomal activation. The carcinogenicity of these compounds was in the same order as their mutagenicity of these compounds. Plus tobias acid and tetra-aminobiphenyl were not carcinogenes.

Conclusion

The primary motivation for the design of novel, marketable dyes and pigments have been the need for colorants having improved technical performance. In more recent years, consumers are getting awareness of the hazards of synthetic dyes and pigments. It has therefore, become clear that the toxicological properties of dyes and their precursors must also be factored into the dye design equation. This means that the development of environmentally friendly colorants must embrace all aspects of the life cycle of synthetic dyes, from manufacture through dye application and handling of residual dye bath color. In this regard, it is also clear that dye chemists must continue to work closely with genetic toxicologists and environmental toxicologists to assure the viability of our industry without compromising human health or the

environment. It is the role of dye chemists to work especially closely with genetic toxicologists to enhance our understanding of the nature of the binding site(s) with which azo dyes interact *en route* to eliciting a genotoxic response. The ability to define these sites and develop working models would shed light on the reason that closely related dye structures can differ significantly in genotoxicity. Many efforts have been made to meet this challenge and synthesize intermediates to develop non-genotoxic dyes. It should also be excersied to handle these intermediates properly to prevent/reduce the health risks.

References

Ahmed Y M, Mustafa A M A, Eleiva M A 1985 Toxicity of certain dyes as insecticides and their joint action with some pyrethroids. *J Environ Sci Health Part-B* **20** 689 - 699.

Anliker R 1979 Ecotoxicology of dyestuffs - a joint effort by industry. *JSDC* **95** 317 - 326.

Anonymous 1955 *The Chemistry of Synthetic Dyes and Pigments*. Edited by H A Lubs, Organic Chemical Department, E I Du Pont de Nemours and Company, p 65.

Anonymous 1971 Schwaebel Nordmeyer, *Text Chem Colorist* **3** 133.

Anonymous 1974 *Riegles Hand Book of Industrial Chemistry, published by Von Nostrand Reinhold Company*, 7th ed. pp 667.

Anonymous 1982 Monographs on the evaluation of the carcinogenic risk of chemicals to man. lyon IARC, Vol. **27**.

Anonymous 1991 *Carcinogenic aromatic amine metabolism and DNA adduct detection in humans*. F F Kadlubar, *Proc Int Symp Princess Takamatsu Cancer Res Fund* **21** 329 - 338.

Anonymous 1992 NTP technical report on the toxicology and carcinogenesis studies of CI direct blue 15 (desalted industrial grade) in F344/N rats (Drinking water studies). *Natl Toxicol Program Tech Res Ser* **397** pp 249.

Anonymous 1993 Toxicology and carcinogenesis studies of CI direct blue 15 (CAS No.2429-74-5) in F 344/N rats (drinking water studies). *National Toxicology Program, Gov Rep Announce Index* **93**.

Anonymous 1994 NTP technical report on the toxicology and carcinogenesis studies of C.I. direct blue 218 (Cas No. 28407-37-(6) in F 344/N rats and B6 C3F1 mice (Feed studies). *Natl Toxicol Program Tech Rep Ser* **430** pp 284.

Anonymous NAT 1991 Toxicology and carcinogenesis studies of 3,3´-dimethylbenzidine dihydrochloride (CAS No.612-82-8) in F 344/N rats (drinking water studies). *National Toxicology Program Report*, NTP-TR-390, NIH/BUB-91-2845; order No.PB 92-103779, pp 237.

Anonymous NTP 1994 NTP technical report on the toxicology and carcinogenesis studies of C.I. Pigment Red 3(CAS No. 2425-85-6) in F344/N rats and B6C3F1 mice (Feed studies). *Natl Toxicol Program Tech Rep Ser* **407** pp 292.

Ashby J 1992 The non-genotoxicity of *ortho* - anisidine. *Mutat Res* **279** 255 - 256.

Ashby J, Short J M 1994 Mutagenicity of *ortho* - anisidine to the bladder of lac I transgenic B6 C3 F1 mice: Absence of 14C or 32P bladder DNA adduction. *Carcinogenesis* **15** 2291 - 2296.

Ashby J, Styles J A, Paton D 1978 Thiophene analogs of the carcinogens benzidine and 4 - aminobiphenyl:evaluation *in vitro*. *Br J Cancer* **38** 34 - 50.

Bargevin M C, Passat M, Daniel N, Capet M, Doble A 1994 Congo Red protects against toxicity of α-amyloid peptides on rat hippocampal neurons. *Neuro Report* **5** 2429 - 2432.

Beland F A, Melchior W B J, Mourato L L G, Santos L G, Amelia M, Marques M M 1997 Arylamine - DNA adduct confirmation in relation to mutagenesis. *Mutat Res* **376** 131 - 219.

Belogoro V V, Muller N R, Ivin B A, Filov V A 1981 Quantitative relation of antitumour activity and toxicity with the structure of some para-substituted N,N-di(2-choloroethyl) aniline. *Eks P Onkol* **3** 59 - 62.

Bolcsfoldi G, Hellmer L 1992 Comments on the paper, The non-genotoxic rodents of the potent bladder carcinogens *ortho*-anisidine and *para*-cresidine. *Mutat Res* **279** 223 - 224.

Carson S 1984 Skin painting studies in mice-11 colors. *J Toxicol Cutaneous Ocul Toxicol* **3** 357 - 370.

Chaira M D, Sobrino F 1992 2-Naphthylamine induces anion superoxide production in rat perioteneal macrophages. *Biochem Pharmacol* **43** 1841 - 1845.

Chughtai S R, Mohammad A S, Siddiqui B A 1993 Genotoxicity testing of some dyes in diploid yeast *Saccharomyces cerevisiae*. *Pak J Sci Ind Res* **35** 283 - 287.

Clonfero E, Venier P, Granella M, Levis A 1990 Azo dyes for leather: mutagenic and carcinogenic hazards *Med Cav* **81** 222 - 229.

Collier S W, Storm J E, Bronaugh R L 1993 Reduction of azo dyes during *in vitro* percutaneous absorption. *Toxicol Appl Pharmacol* **118** 73 - 79.

Conde-Salazt L, Guimeraens A, Romero L, Harto A 1984 Contact dermatitis from azo dyes. *Med Seg Trab* **31** 29 - 33.

Dillion D, Combes R, Zeiger F 1994 Activation by cecal reduction of the azo dye D and C black No.9 to a bacterial mutagen. *Mutagenesis* **9** 295 - 299.

Eling T E, Petry T W, Hughes M F, Krauss R S 1988 Aromatic amine metabolism catalyzed by prostaglandin H synthase. *Carcinogen mutagen responses aromatic amines and nitroarenes Proc Int Conf Carcinog Mutagen.N-substi-*

tuted aryl compd **3** 161 - 172.

Flammang T J, Couch L H, Levy G N, Weber W W, Wise C K 1992 DNA adduct levels in Congenic rapid and slow acetylator mouse strains following chronic administration of 4-aminobiphenyl. *Carcinogenesis* **13** 1887 - 1891.

Gray (Jr.) L E, Ostby J S, Kavlock R J, Marshall R 1992 Gonadal effects of fetal exposure to the azo dye Congo black in mice: infertility in female but not male offspring. *Fundam Appl Toxicol* **19** 411 - 422.

Gray L E, Ostby J S 1993 The effect of prenatal administration of azo dyes on testicular development in the mouse. A structure activity profile of dyes derived from benzidiene, dimethylbenzidine, or dimethoxybenzidine. *Fundam Appl Toxicol* **20** 177 - 183.

Gregory P 1986 Azo dyes: Structure-Carcinogenicity relationship. *Dyes Pigments* **7** 45 - 56.

Hartman C P, Fulk G E, Andrews A W 1978 Azo reduction of Trypan blue to a known carcinogen by a cell-free extract of a human intestinal anaeroba. *Mutat Res* **58** 125 - 132.

Hatcher J F, Swaminathan S M 1992 Microsome-mediated transacetylation and binding of N-hydroxy-4-amino-biphenyl to nucleic acids by hepatic and bladder tissue from dog. *Carcinogenesis* **13** 1705 - 1711.

Helmes C T, Sigma C L, Fung V A 1986 A study of azo and nitro dyes for the selection of candidates for carcinogen bioassay. *J Environ Sci Health* **A19**, Hillier R A *International Congress on Industrial Hygiene*, Rome, Italy.

Hillier R A 1986 Effects of using liver fractions from different mammals, including man, on results of mutagenicity assay in *Salmonella typhimurium. International Congress on Industrial Hygiene*, Rome, Italy.

Hofmann T, Schmidt D 1993 Investigation of possible metabolism of Pigment yellow 17, a 3,3´-dichlorobenzidine-based pigments after inhalation exposure in rats. *Arch Toxicol* **67** 141 - 144.

Huang M T, Miwa G T, Lu A Y H 1979 Rat liver cytosolic azo reductase. Purification and characterization. *J Biol Chem* **254** 3930.

Hughes M F, Smith B J, Eling T E 1992 The oxidation of 4-aminobiphenyl by horseradish. *Chem Res Toxicol* **5** 340 - 355.

Hunger K, Jung R 1991 *Chimia* **45** 297 - 300.

Iba M M, Ghosal A, Poyer J L, Downs P, Massion W H 1991 *Prog Pharmacol Clin Pharmacol* **8** 255 - 266.

Jankovic S V, Jelic V 1985 Mutagenic evaluation of food colors by the micronucleus test. *Hrana Ishrana* **26** 27 - 28.

Jaskot R H, Costa D L 1994 Study on the genotoxic activity of selected dyes by a micronucleus test. *Fundam Appl Toxicol* **22** 103 - 112.

Kaledin V L, Ronishevskaya G M, Zvereva L N 1994 Apparent discrepancy between carcinogenic and mutagenicity effects of aminoazo dyes in suckling mice. *Dokl Akad Nauk* **335** 810 - 812.

Kaur A, Singh R, Grover I S 1993 Mutagenicity testing of textile (azo) dyes with *Salmonella* / microsome assay. *J Environ Biol* **14** 327 - 333.

Kennelly J C, Hertzog P J, Martin C N 1982 The release of 4,4´-diaminobiphenyls from azo dyes in the rat. *Martin Carcinogenesis* **5** 947-951.

Konishi N, Kitahori Y, Nishioka H, Yane K, Hiasa Y 1992 Carcinogenicity testing of food red 106 (acid black) in Fischer 344 rats. *J Toxicol Pathol* **5** 157 - 165.

Kornburst D J, Barfknecht T R 1984 Comparison of 7 azo dyes and their azo reduction products in the rat and hamster hepatocyte primary culture / DNA repair assays. *Mutat Res* **136** 255 - 266.

Kosaka H, Nakamura S 1991 Comments on the paper. The non-genotoxic rodents of the potent bladder carcinogens *ortho*-anisidine. *Chem Express* **6** 257 - 260.

Kranen M, Donk J N M, Laires A, Rueff J, Vermuelen N P E 1997 Characterization of enzyme activities and co-factors involved in bioactivation and bioinactivation of chemical carcinogens in the tester strain *Escherichia coli* K12 M x 100 and *Salmonella typhimurium* LT2 TA100. *Mutagenens* **12** 245 - 254.

Kurlyanskii B A, Torshina N L, Balabanova E L, Nevzorova N I, Kibrik V B E 1988 Some characteristics of the toxic effect of water soluble phthalocyanine dyes. *Gig Tr Prof Zabol* **9** 47 - 48.

Lakshmi V M, Zenser T V, Goldman H, Spencer G G, Gupta R C, Hsu F F, Davis B B 1995 The role of acetylation in benzidine metabolism and DNA adduct formation in dog and rat liver. *Chem Res Toxicol* **8** 711 - 720.

Le J, Jung R, Kramer M 1985 Effects of using liver fractions from different mammals, including man, on results of mutagenicity assays in *Salmonella typhimurium*. *Food Chem Toxicol* **23** 695 - 700.

Mc Manus M E 1989 The role of cytochromes P - 450 and N-acetyl transferase in the carcinogenicity of aromatic amines and amides. *Clin Exp Pharmacol Physiol* **16** 491 - 495.

Melick W F, Escue H M, Naryka J J, Mezera R A, Wheller E P 1955 Bladder tumors due to a carcinogen Xenylamine. *J Urol* **74** 760.

Michaels G B, Lewis D L 1985 Sorption and Toxicity of azo and triphenylmethane dyes to aquatic microbial populations. *Environ Toxicol Chem* **4** 45 - 50.

Milanova F, Wood S, Sithole B B 1997 Toxicity of news prints dyes to rainbow trout and activated sludge. *Tappi J* **80** 113 - 118.

Mule M B, Lomte V S 1994 *Cyperkill* (cypermethrin) toxicity to lipid metabolism in *Thiora tuberculate*. *Proc Acad Environ*

Biol **3** 131 - 134.

Nishiuchi Y 1984 Toxicity of agrochemicals to fresh water organism: 2. Dyes. *Suisan Zoshoku* **32** 173 - 175.

NTP 1992 Toxicology and carcinogenesis studies of CI Pigment Red 23 (CAS No. 6471-49-4)in F 344 rats and B6 C3F1 mice (Feed studies). *Natl Toxicol Program Tech Rep Ser* **411** pp 287.

Orzechowski A, Schrenk D, Bock K W 1992 Metabolism of 1 and 2-naphthylamines in isolated rat hepatocytes. *Carcinogenesis* **13** 2227 - 2232.

Osborne M R 1984 DNA Interactions of reactive intermediates derived from carcinogenes. *ACS Monogr* **182** 485-524.

Osman M A, Hussien R M, Adel S A, Abdel-Halim M, Sherifa H S 1984 Biochemical effect of some synthetic food and drug colorants on liver functions, some hormones and hemotological parameters in male and female mice. *Bull Fac Pharm* **33** 173 - 175.

Parkinson A, Safe S H, Robertson L W, Thomas P E, Ryan O E, Reik L M, Levin W 1983 Immunochemical quantitation of cytochrome P - 450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rats. A study of structure-activity relationships. *J Biol Chem* **258** 5967 - 5976.

Parkinson A, Stephen H S, Robertson L W, Thomas P E, Ryan D E, Reik L M, Levin W 1983 Immunochemical quantitation of cytochrome P - 450 isozymes and epoxide hydrolase in liver microsomes from polychlorinated or polybrominated biphenyl-treated rat. *J Biol Chem* **258** 5967 - 5976.

Pei S, Zhu X, Xiang M, Yang L, Dai O 1993 *Recent Adv Chem Mol Biol Cancer Res Int Symp*, pp 111 - 119.

Prasad O., Rastogi P B 1982 Orange II-induced cytogenetic changes in albino mice. *Natl Acad Sci Lett* **5** 205 - 207.

Przybojewska B, Dziubaltowska E 1985 Study on the genotoxic activity of selected dyes by a micronucleus test. *Bromatol Chem Toksykol* **18** 280 - 283.

Rannung U, Bramstedt H, Nilsson U 1992 The presence of genotoxic and bioactive components in indigo - dyed fabrics - a possible health risk. *Mutat Res* **282** 219 - 225.

Rapic V, Latinovic M 1985 Mutagenicity of food colors in the Ames / *Salmonella* assay. *Hrana Ishrana* **26** 19 - 21.

Ribo J M, Kaiser K L F 1983 Toxicities of chloroaniline to photobacterium phosphorium and their correlations with effects on other organisms and structural parameters. *QSAR Environ Toxicol* 3136.

Roben J F, Dill G S, Ward J M 1980 Thirteen-week subchronic toxicity studies of direct blue 6, direct black 38 and direct brown 95 dyes. *Toxicol Appl Pharmacol* **54** 431 - 442.

Rockeville M D 1984 Process of Seminar, Risk management of existing chemicals (Chemical Manufacturers Association), Government Institute Inc., USA.

Sako F, Kobayashi N, Taniguchi N 1979 Toxicity test for food dyes using *Artemia salina* and its enzyme activity. *Shokuhin Eiseigaku Zasshi* **20** 233 - 238.

Sandhu P, Chipman J K 1991 *Prog Pharmacol Clin Pharmacol* **8** 319 - 325.

Sasaki Yu F, Sakaguchi M, Yamada H 1994 Evidence for nonclastogenicity to mice of possible human carcinogen (IARC) para-cresidine. *Mamm Mutagen Study Group Commun* **2** 83 - 85.

Savkovic, Pecevski J, Alavantic D, Sunjarevic I, Radivojevic D 1985 Influence of some food colors on the induction of dominant lethal mutation and spermatozoid abnormality in mammals. *Hrana Ishrana* **26** 29 - 31.

Scholz B, Palanschek N 1988 The determination of substituted aromatic amines in water and sediment samples. *Fresenius Z Anal Chem* **331** 282 - 289.

Selyuzhitskii G V, Malysheva V V, Zus B A 1982 Experimental study of the blastomogenic properties of basic blue K. *Gig Saint* **4** 30 - 32.

Serova IA, Kaledin V I, Semenova L A 1992 Inheritance of susceptibility to induction of hepatic tumors and stimulations of α-fetoproteins synthesis at early stages of hepatocarcinogenesis induced by *ortho*-amino-azotoluene in DD/He and CC 57 BR/MV mice. *Eksp Onkol* **14** 31 - 35.

Sewekow U 1997 Leather articles under the aspect of the German legislation for consumer protection. *Leder* **48** 187 - 192.

Slawomir K, WlokPrzegl 1998 Dyes, the environment and man, Part V, a. Effect of dyes on mammals and man. *Tech Wolk* **4** 23 - 25.

Specht K, Platzek T 1995 Textile dyes and finishes, Remarks to toxicological and analytical aspects. *Dtsch Lebensm Rundsch* **91** 352 - 358.

Stoner G D, Shivaparker M N, Schut H A J, Lehman T A 1988 DNA binding and adduct formation of 4,4´-methylene-bis (2-chloroaniline) (MOCA) in explant cultures of human and dog bladder. *Carcinogen Mutagen Responses Aromatic Amines and Nitroarenes Proc Int Conf Carcinog Mutagen. N-Substituted Aryl Compd* **3** 237 - 240.

Sun H, Huang G, Wang C, Song W 1998 Toxic effect of azo dyes to *Scenedesmus obliquus* and relationship between structure and activity. *Huanjing Kexue* **19** 22 - 25.

Vachalkova A, Novotny L, Blesova M 1996 Polarographic reduction of some triphenylmethane dyes and their potential carcinogenic activity. *Neoplasma* **43** 113 - 117.

Vashenko N M, Yaremenko V D, Baturina T S, Nedbailo E P 1977 Comparative toxicity of toluidine isomers. *Deposited Doc VINITI* 4035 - 4077.

Vujosevic M, Zivkovic S, Mikalacki J 1985 *In vivo* study on mammalian chromosomes of genotoxicity of food colors. *Hrana Ishrana* **26** 25 - 26.

Wagner S J, Cifone M A, Dodd R Y, Myhr B 1995 Mammalian genotoxicity assessment of methylene blue in plasma: implications for virus inactivation. *Transfusion* **35** 407 - 413.

Wedzisz A, Grzelka M 1988 Determination of some toxicity parameters of dyes produced by dyestuff factory *Boruta* Part II. Toxicity studies in mammals. *Bromatol Chem Toksykol* **21** 61 - 64.

Wilczek S R, Krishnasamy R, Subburam V 1992 Toxicity of selected dyes to *Vigna radiata* (L.). *Geobios* **19** 81 - 87.

Zbaida S, Stoddart A M, Levine W G 1989 Role of electronic factors in binding and reduction of azo dyes by hepatic microsomes. *Chem Biol Interact* **69** 61 - 71.

Zimina T A, Pavlenko V V 1991 *Genetika* **26** 2246 - 2249.

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EXAMPLES

Journal Articles

In Bibliography:

Reid R W, Watson J A 1995 Reaction of lodgepole pine to attack by blue stain fungi. *Can J Bot* **45** (2 Part 1) 45-50.

Weenen H, Nkunya M H H, Bray D H, Mwasumbi L B, Kinabo L S, Kilimali V A E B 1990 Antimalarial activity of Tanzanian plants. *Plant Medic* **56**(4) 368-370.

Solhein H 1992a The early stages of fungal invasion in Norway spruce. *Can J Bot* **70** (4 Ser A) 112-119.

Solhein H 1992b Fungal succession in sapwood. *Eur J Pathol* **22** 143-158.

In Text:

(Reid and Watson 1995)

(Solhein 1992a)

(Solhein 1992a & b)

(Weenen *et al* 1990; Solhein 1992a; Reid and Watson 1995)

Chapters of Books

Demarchi DA, Marsh RD, Harcombe AP, Lea EC 1990 The environment. In: *The Birds of British Columbia*, eds Cambell R W & McNall M C E. Royal British Columbia Museum, Victoria, B C USA, Vol 2, 2nd ed, pp 366-425.

Books

Bennet O C, Myers J E 1974 *Momentum, Heat and Mass Transfer*. McGraw Hill, New York, USA, pp 540-562.

Thesis

Hu C 1989 A comparative study of peanut peroxidase isozyme. Ph.D thesis, Department of Plant Sciences, University of Western Ontario, London, Ontario, Canada.

Papers of Conferences/Symposia/Seminars

Jhonson S R, Knapp A K 1994 The role of *fire in Spartina pectinator* dominated tallgrass prairie wetlands. In: *Proceedings on 19th Tall Timbers Fire Ecology Conference*, Tallahassee, Fl, USA, November 3-6, 1993.

Technical Reports

Tiller FM, Leu WF 1984 *Solid Liquid Separation for Liquefied Coal Industries*. Final Report for RP - 1411-1. EPRI, AP-3599, Electric Power Research Institute, Palo Atto, CA, USA.

Patents

Verschuur E 1978 *Agglomerating Coal Slurry Particles*. US Patent 4126426.

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