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Efficient Method of Choosing Potential Parents and Hybrids: Line × Tester Analysis of Spring Wheat (*Triticum aestivum* L.) Cultivars


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Abstract. The study was conducted to estimate the general combining ability (GCA) and specific combining ability (SCA) of wheat genotypes crossed in a line × tester fashion. The mean squares due to *F*₁ hybrids, female lines, male testers/pollinators and lines × tester interaction were significant for majority of the characters studied. The significance of GCA and SCA variances thus suggested that both additive and non-additive genes were controlling majority of the characters, yet additive genes were more prominent because variances due to GCA by and large were higher than due to SCA. Among the three female lines evaluated, Khirman displayed maximum positive GCA effects for spike length (0.08) and seeds/spike (0.67), while other female lines which showed maximum positive GCA effects were Mehran for plant height (3.05), number of tillers/plant (1.00), spikelets/spike (1.92) and seed index (3.42) and Kiran for seeds/spike (0.67) and yield/plant (1.86). From the male testers, TD-1 exhibited greater GCA effects for number of tillers/plant (2.96), spikelets/spike (0.25), seed index (0.61) and yield/plant (2.22), whereas, Marvi displayed highest positive GCA effects for plant height (2.88), spike length (0.37) and seeds/spike (6.41). The specific combining ability estimates indicated, if hybrid crop development is feasible then, crosses Mehran × TD-1 for spike length; Kiran × TD-1 for plant height and seeds/spike and Khirman × Marvi for number of tillers/plant, spikelets/spike, seed index and yield/plant may be the hybrids of choice.

Keywords: combining ability, line × tester analysis, quantitative traits, *Triticum aestivum* L. additive genes, non-additive genes

Introduction

Over the past three decades, increased agricultural productivity occurred largely due to the evolution of high-yielding cultivars and increased fertilizer use. However, with the introduction of semi-dwarf wheat cultivars, wheat productivity has been augmented in all the major cropping systems representing the diverse and varying agro-ecological conditions (PARC, 2006). Further advancement in the yield of wheat requires certain information regarding heterosis and combining ability of parents which is useful for the exploitation of successful hybridization programme and hybrid crop development. The nature of gene action involved in the expression of quantitative traits of economic importance has got an additional role in relying upon the breeding strategies. Thus, the fundamental issue in hybrid breeding is the choice of parents and identification of superior hybrid combinations. Earlier, it was reported as high as 141.7 and 18.9 % heterosis in tillers/plant and grain yield/plant, respectively (Sedeque *et al*., 1991). Fida *et al*., (2004) measured positive heterotic effects as 11.61, 61.90, 30.67 and 51.89% for plant height, tillers/plant, grains/ spike and grain weight, respectively. Another study by Patwary *et al*. (1986), made on grain yield/plant from seven cultivars and their 42*F*₁ hybrids revealed that 12 crosses showed significant positive heterosis in grain yield varying from 77.15 to 160.43%. As such heterosis expressed as per se hybrids is not as much reliable as the SCA of the parents (Baloch and Bhutto, 2003).

Due to its greater genetic diversification, wheat provides many opportunities for the development of new high yielding genotypes through crossing and recombination of desirable genes. Nevertheless, an understanding of the genetic factors that govern the yield components is the primary step towards any breeding endeavors. For wheat breeders, the search for desirable germplasm is a continuous process and the development of new varieties is an un-ending goal. In this context, knowing the extent of inheritance of desirable traits from parents to the offspring is of utmost importance for further improvement.
For this purpose, the line × tester mating design was used to estimate GCA and SCA variances and their effects. Though, diallel is most commonly used mating design for estimating combining ability, yet it involves fewer and same set of parents being male and females thus gives just only one estimate of GCA whereas, line × tester mating design uses different sets of males and females hence provides two independent estimates of GCA of lines and testers separately. A general combining ability is defined as an average performance of a line in a series of crosses while specific combining ability connotes those instances where certain hybrids are either better or poorer than would be expected on average performance of parents in hybrid combinations (Sprague and Tatum, 1942). Despite the fact that, a lot of research on combining ability has been carried-out by wheat breeders, yet controversy in results always remained a debate which existed either due to material used, or environment in which material was tested or the breeding methodology adopted. Rajara and Maheshwari (1996) and Dhadhal and Dobariya (2006) reported the importance of both additive and non-additive genes yet found preponderance of non-additive gene effects for grain yield/plant, plant height and spikelets/spike. Similarly, Singh and Singh (2003) studied 80 F1s developed from 4 lines crossed with 20 testers evaluated via line × tester analysis. The estimates of variance components due to general combining ability (GCA) and specific combining ability (SCA) indicated the predominance of non-additive genes for plant height, spike length, number of spikelets/spike, grain weight/spike, 100-seed weight and yield/plant. Ribadia et al. (2007) estimated general and specific combining ability and found high proportion of non-additive genes for plant height, length of main spike, spikelets/spike, grain yield/plant and grain weight/spike. Nevertheless, contrary to these findings, Hamada et al. (2002) while working with thirty-five introduced wheat lines crossed with four local wheat cultivars as testers and produced 140 hybrids using line × tester analysis to estimate combining ability effects and gene action for plant height, spike length, number of kernels/spike, 1000-kernel weight and grain yield/plant. They found significant differences for lines, testers and lines × testers’ interaction for all the traits except spike length. Their results further revealed that additive gene effects were larger than those of the non-additive ones. Similarly, Vanpariya et al. (2006) crossed 10 lines with 4 testers and found that both additive and non-additive gene actions were important, yet the ratio of GCA/SCA showed the preponderance of additive genes for plant height, length of main spike and spikelets/spike. While non-additive genes were prevailing for grain yield/plant, grains/spike, 100-grain weight and grain yield/spike. Esmail (2007), crossed ten bread wheat lines with three testers and noted additive as well as dominance genetic components playing a role in the inheritance of plant height. The present study therefore was aimed at estimating the combining ability of bread spring wheat genotypes by line × tester analysis for quantitative traits.

Materials and Methods

The research was conducted during 2007 at the Experimental Field, Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Province of Sindh, Pakistan, so as to identify good general and specific combining parents and also to determine the nature of gene action governing for different characters in spring wheat genotypes by line × tester analysis. The trial comprised of 6 F1 hybrids and their 5 parents (three lines, Khirman, Mehran-89, Kiran-95 and two testers, TD-1, Marvi). The experiment was laid-out in a Randomized Complete Block Design with three replications. The analysis of variance was carried-out according to statistical methods developed by Gomez and Gomez (1984) whereas, the general and specific combining ability variances and effects were estimated according to methods developed by Kempthorn (1957) and adopted by Singh and Choudhry (1979). The data were recorded on plant height (cm), number of tillers/plant, spike length (cm), grains/spike, spikelets/spike, yield/plant (g) and seed index (1000-grain weight in g). All the cultural practices were done as and when required while fertilizer and irrigations were applied according to the recommendations of wheat crop for local conditions.

Results and Discussion

The research was conducted for estimating the general combining ability (GCA) and specific combining ability (SCA) of wheat genotypes for some quantitative traits via line × tester analysis. The mean squares due to F1 hybrids, female lines, male testers and lines × tester interactions were significant for all the characters, except GCA of line and testers for spike length and spikelets/spike, respectively and SCA for spike length only were non-significant (Table 1). The significance of mean squares due to female and male inbreds both designate GCA variances while female × male interaction which designate SCA variances employed that additive as well
as dominant genes were important for most of the characters studied. In respect to per se F₁, hybrids’ performance summarized in Table 2 indicated that cross Mehran × TD-1 produced maximum number of tillers/plant (26.5), gave more spikelets/spike (25.0), recorded highest seed index (40.4g) and produced maximum seed yield/plant (53.6g). However, hybrid Khirman × Marvi measured tallest plants (94.5cm), and gave more seeds/spike (92.5), while Kiran × Marvi, gave longer spikes (14.8cm). By and large, per se hybrid performance was not reflected in general or in specific combining ability of the parents except Mehran × TD-1 which was good as per se F₁ hybrid and average general combiners as well for number of tillers/plant, spikelets/spike, seed index and yield/plant, therefore both the parents can reliably be used in hybridization and selection programme to improve majority of the characters (Table 2, Fig. 1). Similar to our findings, Vanparya et al. (2006) found that parents in cross CPAN 1933 × GW 173 was one most promising parent as it had high SCA effect and simultaneously as best per se F₁ hybrid for length of main spike, spikelets/spike and number of grains/spike. However, the lowest yielder per se F₁ hybrid (Khirman × Marvi) was the specific combiner (Fig. 1). These results suggested that per se F₁ hybrid performance should not always be taken granted for having good SCA also. Mean squares due to GCA for both lines and testers were significant for majority of the characters (Table 1). Similar to our findings, Nazan (2008) had found significant GCA variances for grain yield/spike, plant height, spike length, spikelet number/spike, kernel number/spike,

Table 1. Mean squares from lines × testers analysis for various characters in spring wheat (Triticum aestivum L.)

<table>
<thead>
<tr>
<th>Traits</th>
<th>F₁ hybrids</th>
<th>Lines (GCA)</th>
<th>Testers (GCA)</th>
<th>Lines × Testers (SCA)</th>
<th>Error</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DF = 5</td>
<td>DF = 2</td>
<td>DF = 1</td>
<td>DF = 2</td>
<td></td>
</tr>
<tr>
<td>Plant height</td>
<td>109.56**</td>
<td>69.29**</td>
<td>198.38**</td>
<td>40.88**</td>
<td>7.97</td>
</tr>
<tr>
<td>Tillers/plant</td>
<td>96.78**</td>
<td>6.50**</td>
<td>210.04**</td>
<td>28.67**</td>
<td>1.25</td>
</tr>
<tr>
<td>Spike length</td>
<td>1.78**</td>
<td>1.17</td>
<td>3.38**</td>
<td>0.50</td>
<td>0.33</td>
</tr>
<tr>
<td>Spikelets/spike</td>
<td>10.43**</td>
<td>22.17**</td>
<td>1.50</td>
<td>13.50**</td>
<td>0.72</td>
</tr>
<tr>
<td>Seeds/spike</td>
<td>443.47***</td>
<td>10.67**</td>
<td>988.17**</td>
<td>115.17**</td>
<td>12.53</td>
</tr>
<tr>
<td>Seed index</td>
<td>54.60**</td>
<td>70.21**</td>
<td>8.88**</td>
<td>92.50**</td>
<td>1.85</td>
</tr>
<tr>
<td>Yield/plant</td>
<td>82.05**</td>
<td>75.95**</td>
<td>117.64**</td>
<td>49.49**</td>
<td>1.95</td>
</tr>
</tbody>
</table>

** = Significant at 1% probability level.

Table 2. Specific combining ability estimates and per se average performance of F₁, hybrids (in parenthesis) for various characters in spring wheat (Triticum aestivum L.)

<table>
<thead>
<tr>
<th>F₁ hybrids</th>
<th>Plant height</th>
<th>Tillers/plant</th>
<th>Spike length</th>
<th>Spikelet/spike</th>
<th>Seeds/spike</th>
<th>Seed index</th>
<th>Yield/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kirman × TD-1</td>
<td>-2.83</td>
<td>-1.33</td>
<td>0.01</td>
<td>-1.5</td>
<td>-4.07</td>
<td>3.88</td>
<td>1.15</td>
</tr>
<tr>
<td>(84.0)</td>
<td>(21.3)</td>
<td>(13.5)</td>
<td>(20.0)</td>
<td>(71.5)</td>
<td>(39.5)</td>
<td>(47.8)</td>
<td></td>
</tr>
<tr>
<td>Mehran × TD-1</td>
<td>-5.63</td>
<td>-2.08</td>
<td>0.51</td>
<td>-2.25</td>
<td>5.47</td>
<td>-8.40</td>
<td>-2.70</td>
</tr>
<tr>
<td>(91.8)</td>
<td>(26.5)</td>
<td>(13.3)</td>
<td>(25.0)</td>
<td>(74.3)</td>
<td>(40.4)</td>
<td>(53.6)</td>
<td></td>
</tr>
<tr>
<td>Kiran × TD-1</td>
<td>9.5</td>
<td>-6.83</td>
<td>-0.24</td>
<td>1.75</td>
<td>10.17</td>
<td>4.16</td>
<td>-6.28</td>
</tr>
<tr>
<td>(84.0)</td>
<td>(22.3)</td>
<td>(13.5)</td>
<td>(22.0)</td>
<td>(79.0)</td>
<td>(32.2)</td>
<td>(49.2)</td>
<td></td>
</tr>
<tr>
<td>Khirman × Marvi</td>
<td>-0.38</td>
<td>9.84</td>
<td>-0.99</td>
<td>4.00</td>
<td>-14.16</td>
<td>6.11</td>
<td>11.37</td>
</tr>
<tr>
<td>(94.5)</td>
<td>(18.0)</td>
<td>(14.3)</td>
<td>(22.5)</td>
<td>(92.5)</td>
<td>(30.8)</td>
<td>(41.1)</td>
<td></td>
</tr>
<tr>
<td>Mehran × Marvi</td>
<td>-0.88</td>
<td>-0.41</td>
<td>0.51</td>
<td>-1.25</td>
<td>6.08</td>
<td>-8.65</td>
<td>-6.42</td>
</tr>
<tr>
<td>(93.3)</td>
<td>(16.3)</td>
<td>(13.5)</td>
<td>(23.0)</td>
<td>(85.8)</td>
<td>(39.7)</td>
<td>(45.8)</td>
<td></td>
</tr>
<tr>
<td>Kiran × Marvi</td>
<td>-0.25</td>
<td>0.84</td>
<td>0.26</td>
<td>-0.75</td>
<td>-3.41</td>
<td>3.28</td>
<td>2.85</td>
</tr>
<tr>
<td>(89.3)</td>
<td>(18.0)</td>
<td>(14.8)</td>
<td>(20.0)</td>
<td>(85.0)</td>
<td>(37.6)</td>
<td>(50.5)</td>
<td></td>
</tr>
<tr>
<td>S.E. (si.)</td>
<td>1.993</td>
<td>0.559</td>
<td>0.288</td>
<td>0.424</td>
<td>1.769</td>
<td>0.680</td>
<td>0.699</td>
</tr>
</tbody>
</table>

LSD (5%) for means 4.4 1.7 1.779 2.6 10.9 4.195 4.3

si = Significance level of specific combining effects.
and 1000 kernel weight. The parents Goen and S-46 for KNS and P-311 and SB - 333 for 1000 kernel weight and grain yield/spike showed positive GCA values. So far, GCA effects of lines are concerned (Table 3), Khirman displayed maximum GCA effect for spike length (0.08) and seeds/spike (0.67), while Mehran registered highest GCA effects for plant height (3.05), number of tillers/ plant (1.00), spikelets/spike (1.92) and seed index (3.42) and Kiran for seeds/spike (0.67) and yield/plant (1.86). However, among the testers, TD-1 manifested highest GCA effects for number of tillers/plant (2.96), spikelets/ spike (0.25), seed index (0.61) and yield/plant (2.22) whereas, Marvi displayed maximum positive GCA effects for plant height (2.88), spike length (0.37) and seeds/spikes (6.41). The GCA effects of lines and testers, therefore suggested that parents Mehran and Khirman among the female lines and TD-1 and Marvi among the testers may be preferred for hybridization and selection of desirable plants from segregating population so as to improve majority of the characters.

In consonance to present findings, Esmail (2007) also found three wheat varieties, Jup/Biy, Giza-164 and Sids 4, those exhibited large GCA effects for number of spikes/ plant. The tester cultivar Giza 168 was at the top of GCA effects for yield and its components. Ribadia et al. (2007) from line × tester analysis noted that female line Flamingo's was a good general combiner for as many as six yield characters while CPAN-6153 was good general combiner for main spike length. While among the males, H-6178 (5) 6-4-5 exerted significant positive GCA effects for length of main spike, spikelets/ spike and grain yield/plant, yet JD-98-16 and HI-8498 were good general combiners for plant height.

The results for specific combining ability (SCA) effects (Table 2) indicated that maximum positive SCA effect of 0.51 was displayed by the hybrid Mehran × TD-1 for spike length, whereas, Kiran × TD-1 for plant height (9.50), seeds/spike (10.17) and Khirman × Marvi for tillers/plant (9.84), spikelets/spike (4.00), seed index (6.11) and yield/plant (11.37). These results suggested that various hybrids may be considered for hybrid crop development. Present findings are in agreement with those of Inamullah et al. (2006) who reported high SCA effects for tillers/plant, plant height, spike length, grains/ spike, 1000-grain weight and yield/plant in hybrids FS × Dera, Tat × SQ, Tat × SARC, Tat × SQ, Tkb × SARC and SQ × Dera, respectively.

**Conclusion**

It can be concluded from the present research that the hybrids differed significantly for their mean performance regarding all the traits studied except spike length. The

![Table 3. General combining ability estimates of line and testers for various characters in spring wheat F₁ hybrids](image)

<table>
<thead>
<tr>
<th>Parents/Female parents:</th>
<th>Plant height</th>
<th>Tillers/plant</th>
<th>Spike length</th>
<th>Spikelet/spike</th>
<th>Seeds/spike</th>
<th>Seed index</th>
<th>Yield/plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Khirman</td>
<td>-0.20</td>
<td>-0.75</td>
<td>0.08</td>
<td>-0.83</td>
<td>0.67</td>
<td>-1.71</td>
<td>-3.55</td>
</tr>
<tr>
<td>Mehran</td>
<td>3.05</td>
<td>1.00</td>
<td>-0.40</td>
<td>1.92</td>
<td>-1.33</td>
<td>3.42</td>
<td>1.70</td>
</tr>
<tr>
<td>Kiran</td>
<td>-2.82</td>
<td>-0.24</td>
<td>-0.33</td>
<td>-1.08</td>
<td>0.67</td>
<td>-1.71</td>
<td>1.86</td>
</tr>
<tr>
<td>S.E. (gi.)</td>
<td>0.998</td>
<td>0.395</td>
<td>0.204</td>
<td>0.300</td>
<td>1.251</td>
<td>0.481</td>
<td>0.494</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testers/Male parents:</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TD-1</td>
<td>-2.86</td>
<td>2.96</td>
<td>-0.37</td>
<td>0.25</td>
<td>-6.42</td>
<td>0.61</td>
<td>2.22</td>
</tr>
<tr>
<td>Marvi</td>
<td>2.88</td>
<td>-2.95</td>
<td>0.37</td>
<td>-0.25</td>
<td>6.41</td>
<td>-0.61</td>
<td>-2.21</td>
</tr>
<tr>
<td>S.E. (gi.)</td>
<td>0.815</td>
<td>0.322</td>
<td>0.166</td>
<td>0.245</td>
<td>1.021</td>
<td>0.392</td>
<td>0.404</td>
</tr>
</tbody>
</table>

gi = Significance level of general combining effect.
GCA and SCA variances were significant for all the traits with few exceptions, yet the magnitude of variances due to GCA for both lines and testers were generally higher than SCA (lines × tester interaction) indicating preponderance of additive genes in the control of traits. Among the lines, Mehran and Khirman and from the testers, TD-1 and Marvi were the best general combiners for all the traits studied thus can reliably be used in hybridization programmes so as to select the desirable plants from segregating populations. The specific combining ability effects indicated that for hybrid crop development, crosses Mehran × TD-1 for spike length; Kiran × TD-1 for plant height and seeds/spike and Khirman × Marvi for number of tillers/plant, spikelets/spike, seed index and yield/plant may be the hybrid of choice to exploit heterosis for higher productivity.

References


Sulphur Supply Enhances Wheat Growth and Yield on Saline-Sodic Soil

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(received November 10, 2010; revised June 15, 2011; accepted June 20, 2011)

Abstract. The effect of different S levels (0, 25, 50 and 75 kg S/ha) on growth and ionic concentration of wheat variety Inqalab-91 directly sown on saline-sodic soil (ECe=5.65 dS/m, pH=8.57 and SAR=17.38) was evaluated in a field experiment. Treatments were arranged using randomized complete block design (RCBD) with three replications. The crop was harvested at maturity, data on tillering, plant height, spike length, number of grains/spike, 1000-grain weight and yields (grain and straw) were recorded. Na, K, Ca and S concentrations in grain and straw were estimated using atomic absorption spectroscopy. Tillering, number of grains/spike, 1000-grain weight and grain yield significantly (p ≤ 0.05) increased by enhancing the S application. Maximum wheat yield (4.66 t/ha) was recorded when S was applied at 75 kg/ha, which was 43% more than the control treatment. Maximum number of tillers/plant (161) and number of grains/spike (56) were recorded with sulphur applied at 75 kg/ha. Positive correlation (r = 0.85, r = 0.88) between calcium, potassium and negative correlation (r = -0.84) between grain sodium content and wheat grain yield was recorded. It indicates that presence of significantly higher Ca and K contents of grain receiving S application might possibly help plants to attain more Ca and K and avoided sodium uptake to alleviate salinity/sodicity stress. Economical analysis showed that maximum value cost ratio (5.5:1) was achieved with the application of 25 kg S/ha.

Keywords: wheat growth, gypsum application, salt affected soil, sulphur levels

Introduction

Sulphur is a constituent of three S-containing amino acids (cistern, cystine and methionine), which are the building blocks of protein and a key ingredient in the formation of chlorophyll (Duke and Reisenauer, 1986). Without adequate S, crops cannot reach their full potential in terms of yield or protein content (Zhao et al., 1999). Wheat plants have a lower requirement for S than legumes and oilseed crops (Duke and Reisenauer, 1986). Sulphur is an essential element for growth and physiological functioning of plants (De Kok et al., 2002). Sulphur improves K/Na selectivity and increases the capability of calcium ion to decrease the injurious effect of sodium ions in plants (Badr et al., 2002; Wilson et al., 2000). Wheat requires a relatively high amount of supplemental S due to incompatibility of conditions with its period of most rapid growth during early spring, when the rate of S release from soil organic matter is quite slow (Johnson, 1999). Significant yield increases of winter wheat in response to S additions have been reported elsewhere (McGrath and Zhao, 1995; Randall and Wrigley, 1986). Elemental S and sulphate fertilizers increase 36% wheat grain yield (Riley et al., 2000). Under sulphur deficient conditions, crop growth and yield are declined, and the produce quality is adversely affected (Schonhof et al., 2007). Since the site under investigation is deficient in S, therefore, this study was designed to address the S issue.

Materials and Methods

A field experiment was conducted to study the effect of S on growth and yield of wheat (Var. Inqalab-91) at Malik Farm, Farooqabad, and Sheikhupura during 2009-10. Sulphur treatments were assigned using randomized complete block design (RCBD) during three replications. The S treatments in this study were control, 25 kg S/ha, 50 kg S/ha and 75 kg S/ha. Gypsum as a source of S was selected for application. Different S levels were applied in designated treatments having plot size of 3.5x20 m. The recommended doses of N, P2O5 and K2O at 100, 80 and 50 kg/ha, respectively were applied to all treatments. The crop was irrigated with tube well water throughout the growth period. All necessary plant protection measures were done whenever required. At maturity plants were harvested to record data on tillers/plant, spike length, number of grain/spike, 1000-grain weight and straw and grain yields/plant. Plant samples were oven dried at 60 °C to a constant weight and recorded dry matter yield. Grain and straw
samples were ground using Wiley mill. Plant samples were then digested in perchloric-nitric acid (2:1 IN) mixture (Rhoades, 1982) to estimate Na, K, Ca and Mg by atomic absorption spectroscopy. Available SO₄ –S of soil samples was determined by the method as described by Bardesly and Lancaster (1960). The data thus obtained were analyzed using MSTATC and treatment means were separated using LSD. Tube well water applied to wheat crop had high residual sodium carbonates however, the soluble salts were present in permissible limit. The soil was saline sodic in nature. It was deficient in sulphur i.e less than 10 ppm (Ahmad et al., 1994). The physico-chemical properties of soil (Table 1) and the quality of tube well water applied to plants are given in Table 2, respectively.

**Results and Discussion**

Application of S to wheat crop grown on saline-sodic soil had positive influence on growth and yield of wheat (Table 3). The effect of S application on tillering, plant height, spike length, number of grain/spike, 1000 grain weight, straw and grain yield remained statistically significant. Maximum tillers (161) were recorded on plants received 75 kg S/ha followed by plants received 50 and 25 kg S/ha. Plant height and spike length were the highest in treatment receiving 25 kg S/ha. The highest 1000 grain weight (38 g) was recorded in treatment receiving 25 kg S/ha followed by 75 and 50 kg S/ha. High straw (10.26 tons/ha) and grain (4.66 tons/ha) yields were attained by plants treated with 75 kg S/ha which is 43% higher than control treatment. The S treatment receiving 50 kg S/ha registered second highest grain yield (3.91 tons/ha) which is 20% higher than control treatment followed by treatments receiving 25 kg S/ha producing 17% higher yield as compared to control treatment. Gupta et al. (2004) reported that S application significantly enhances wheat yield and yield components. This was possibly due to increased Ca and K in soil resulted in enhancing the availability of macro and micro-nutrients for healthy plant growth.

**Table 1.** Physico-chemical properties of soil at MK Farm, Farooqabad  

<table>
<thead>
<tr>
<th>Properties</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:1 H₂O)</td>
<td></td>
<td>08.57</td>
</tr>
<tr>
<td>ECe (1:1)</td>
<td>dS/m</td>
<td>05.65</td>
</tr>
<tr>
<td>SAR</td>
<td>(m mole, /L)¹/²</td>
<td>17.38</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>%</td>
<td>07.00</td>
</tr>
<tr>
<td>OM</td>
<td>%</td>
<td>01.33</td>
</tr>
<tr>
<td>Sand</td>
<td>%</td>
<td>33.00</td>
</tr>
<tr>
<td>Silt</td>
<td>%</td>
<td>42.00</td>
</tr>
<tr>
<td>Clay</td>
<td>%</td>
<td>25.00</td>
</tr>
<tr>
<td>SO₄–S</td>
<td>mg/kg</td>
<td>07.26</td>
</tr>
</tbody>
</table>

Textural class (USDA)  
Loam  

<table>
<thead>
<tr>
<th>pH (1:1 H₂O) after S application</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>08.10</td>
</tr>
</tbody>
</table>

**Table 2.** Quality of tubewell water applied to the crop  

<table>
<thead>
<tr>
<th>Quality</th>
<th>Unit</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>--</td>
<td>8.3</td>
</tr>
<tr>
<td>ECw</td>
<td>dS/m</td>
<td>1.6</td>
</tr>
<tr>
<td>RSC</td>
<td>meq/L</td>
<td>14.7</td>
</tr>
<tr>
<td>HCO₃⁻¹</td>
<td>meq/L</td>
<td>16.3</td>
</tr>
</tbody>
</table>

RSC = residual sodium carbonate.

**Fig. 1.** Correlation between Ca content of grain and wheat grain yield.

**Table 3.** Effect of S on wheat growth and yield  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tillers/ plant</th>
<th>Plant height (cm)</th>
<th>Spike length (cm)</th>
<th>Grains/ spike</th>
<th>1000 grain weight (g)</th>
<th>Straw yield (t/ha)</th>
<th>Grain yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64&lt;sup&gt;d&lt;/sup&gt;</td>
<td>71.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.26&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.26&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 kg S/ha</td>
<td>127&lt;sup&gt;c&lt;/sup&gt;</td>
<td>97.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>50 kg S/ha</td>
<td>147&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.91&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>75 kg S/ha</td>
<td>161&lt;sup&lt;a&gt; &lt;/a&gt;c&lt;/sup&gt;</td>
<td>94.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.66&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td>3.86</td>
<td>3.003</td>
<td>0.6892</td>
<td>5.508</td>
<td>2.746</td>
<td>0.8382</td>
<td>0.6125</td>
</tr>
</tbody>
</table>

a, b, c = indicate statistical significant differences among treatments.
Ionic concentration. The data presented in Table 4 indicates that the increasing concentration of S significantly increased K, Ca and Mg and decreased Na contents of grains. The highest content of calcium in grain was found in plants treated with 75 kg S/ha followed by plants treated with 50 kg S/ha. However, compared to the control treatment only, all the other treatments produced grains with significantly higher calcium content. The highest K content of grain was found in plants treated with 75 kg S/ha, followed by plants treated with 50 kg S/ha. Sulphur application ultimately resulted in better nutrient supply to wheat crop.

Figure 1 indicates significant positive correlation (r = 0.85) between calcium contents of grain and wheat grain yield. It indicates presence of significantly higher calcium content of grain, receiving S application help plants to attain more calcium and K to avoid sodium uptake which has been an added advantage to alleviate salinity/sodicity using crop residue incorporation apart

Table 4. Chemical analysis of grains for Ca, Na, K and Mg contents as influenced by S levels

<table>
<thead>
<tr>
<th>S-Treatments</th>
<th>Ca %</th>
<th>Na%</th>
<th>K%</th>
<th>Mg%</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 = Control</td>
<td>0.214</td>
<td>0.217</td>
<td>0.415</td>
<td>0.134</td>
</tr>
<tr>
<td>T2 = (25 kg S/ha)</td>
<td>0.278</td>
<td>0.208</td>
<td>0.434</td>
<td>0.151</td>
</tr>
<tr>
<td>T3 = (50 kg S/ha)</td>
<td>0.280</td>
<td>0.185</td>
<td>0.463</td>
<td>0.142</td>
</tr>
<tr>
<td>T4 = (75 kg S/ha)</td>
<td>0.290</td>
<td>0.183</td>
<td>0.469</td>
<td>0.139</td>
</tr>
<tr>
<td>LSD</td>
<td>0.006318</td>
<td>0.006318</td>
<td>0.006318</td>
<td>NS</td>
</tr>
</tbody>
</table>

a, b, c = indicate statistical significant differences among treatments; NS = non significant difference among treatments.

![Figure 2](image2.png)

**Fig. 2.** Correlation between Na content of grain and wheat grain yield.

![Figure 3](image3.png)

**Fig. 3.** Correlation between K content of grain and wheat grain yield.

Table 5. Economic analysis, partial budget analysis and dominance analysis of S on wheat crop yields

<table>
<thead>
<tr>
<th>Dose</th>
<th>T1 Control</th>
<th>T2 25 kg S/ha</th>
<th>T3 50 kg S/ha</th>
<th>T4 75 kg S/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input cost Rs.</td>
<td>0</td>
<td>2,500</td>
<td>5,000</td>
<td>7,500</td>
</tr>
<tr>
<td>Application Cost</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total cost that vary Rs.</td>
<td>0</td>
<td>2,500</td>
<td>5,000</td>
<td>7,500</td>
</tr>
<tr>
<td>Yield grain kg/ha</td>
<td>3260</td>
<td>3820</td>
<td>3910</td>
<td>4660</td>
</tr>
<tr>
<td>Adjusted yield (10% Low)</td>
<td>2934</td>
<td>3438</td>
<td>3519</td>
<td>4194</td>
</tr>
<tr>
<td>Output price Rs./kg</td>
<td>22</td>
<td>22</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td>Straw yield kg/ha</td>
<td>4260</td>
<td>7160</td>
<td>8700</td>
<td>10260</td>
</tr>
<tr>
<td>Adjusted yield (10% Low)</td>
<td>3834</td>
<td>6444</td>
<td>7830</td>
<td>9234</td>
</tr>
<tr>
<td>Output price Rs./kg</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Gross yield benefits Rs.</td>
<td>72216</td>
<td>88524</td>
<td>93078</td>
<td>110736</td>
</tr>
<tr>
<td>Net benefits Rs.</td>
<td>72216</td>
<td>86024</td>
<td>88078</td>
<td>103236</td>
</tr>
</tbody>
</table>

**Dominance Analysis**

<table>
<thead>
<tr>
<th></th>
<th>TCV</th>
<th>NB</th>
<th>VCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCV</td>
<td>0</td>
<td>2500</td>
<td>5000</td>
</tr>
<tr>
<td>NB</td>
<td>72216</td>
<td>86024</td>
<td>88078</td>
</tr>
<tr>
<td>VCR</td>
<td>-</td>
<td>5.5:1</td>
<td>3.2:1</td>
</tr>
</tbody>
</table>

TCV = total cost that vary; VCR = value cost ratio between values of additional crop produce to the additional money spent on S fertilizer.
from enhancing soil fertility and physical properties. Data in Fig. 2 indicates significant negative correlation \( r = -0.84 \) indicating more sodium uptake where calcium and K uptake was the lowest in control treatment. Data in Fig. 3 shows significant positive correlation \( r = 0.88 \) again indicating more potassium uptake as compared to control treatment. Chemical data indicates that application of sulphur combats salinity/sodicity by enhanced uptake of Ca and K.

**Economical analysis.** Economic viability of any intervention is must for adoption in field and is the basic theme of the research. All the agronomic practices and plant protection measures were same. The input cost in treatments receiving 25 kg S/ha, 50 kg S/ha and 75 kg S/ha was Rs. 2500, Rs.5000 and Rs.7500, respectively. Net benefits attained by treatments receiving 25 kg S/ha, 50 kg S/ha and 75 kg S/ha were Rs. 86024, 88078 and 103236, respectively, which were 19, 22 and 43% higher than control treatment (Table 5). The contribution of S towards wheat yield was investigated. Data in Table 5 indicates that treatments receiving 25 kg S/ha attained the highest value cost ratio (5.5:1) followed by application of 75 kg S/ha (4.1:1).

**Conclusion**

The present study envisages that S application not only enhances growth and yield of wheat on salt affected soil and it may increase the uptake of Ca and K ions and it reduces the uptake of toxic Na ions which helps to improve K/Na ratio.

**References**


Effect of Growth Regulators for *in-vitro* Mass Multiplication of Marigold

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Abstract. This study describes an effective and reproducible protocol for the mass multiplication of marigold (*Tagetes erecta* L.) for commercial purpose. Twenty five different combinations of BAP, IAA, GA3, and AgNO3 were added to the basal MS medium to culture marigold explants. The highest mean number (4.83±0.49) and length (5.28 cm ±1.06) of healthy shoots per explant was observed in media supplemented with 2 mg/L BAP along with 2 mg/L IAA. When these shoots were sub-cultured for root development, the maximum number (17.08±2.44) and length (13.67 cm ±0.98) of roots were produced in media supplemented with 4mg/L BAP and 2 mg/L IAA. Addition of gibberellic acid and AgNO3 did not have any significant effect on shoot proliferation and root development of marigold.

Keywords: tissue culture, marigold, shoot proliferation, root proliferation, *Tagetes erecta* L.

Introduction

*Tagetes erecta* L. (marigold) is a member of the family Asteraceae. This family includes some thirty species of strongly scented annual or perennial herbs that are distributed throughout the world. Marigold is an important ornamental crop having a high market value not only for its flowers but also for its industrial value. This crop is a source of highly desirable components like pigments, lutein, essential oils, thiophene, etc. It possesses nematicidal, fungicidal and insecticidal activities and is also used in poultry feed (Qingxiang et al., 2008; Godoy-Hernandez et al., 2006; Karadas et al., 2006; Pudasaini et al., 2006; Barzana et al., 2002; Vanegas et al., 2002).

The development of an effective protocol for *in-vitro* propagation and mass multiplication using tissue culture technique is highly desirable for elite or exotic varieties of marigold (Choi and Chung, 2007; Miranda-Ham et al., 2006; Hayashi et al., 2005; Vanegas et al., 2002; Delgado-Vargas et al., 2000). Gibberellic acid was used for tissue culture of immature unpollinated disc florets (Kothari and Chandra, 1984). Benzyl adenine, Gibberellic acid and Indole acetic acid were used for leaf-callus and suspension cultures (Kothari and Chandra, 1986). Benzyl adenine and Indole acetic acid were found effective for regeneration from hypocotyls and shoot-tip proliferation from adult plants (Godoy-Harnandez et al., 2006; Misra and Datta 1999). Regeneration through leaf segments using l-glutamine, l-arginine, adenine sulphate and 6-benzyladenine was also observed (Ault, 2002; Venegas et al., 2002; Misra and Datta, 2001). Reports on the genetic transformation of marigold with *Agrobacterium rhizogenes* (Giri and Narasu, 2000) and *A. tumefaciens* (Godoy-Hernandez et al., 2006) are also available. The present study was designed to optimize protocol for *in-vitro* mass multiplication and healthy rooting of marigold.

Materials and Methods

Marigold (*Tagetes erecta*) seeds were surface sterilized in a solution of 50% sodium hypochlorite with a few drops of Tween 20 for 40 min followed by three rinses with autoclaved double distilled deionized water. The disinfected seeds were germinated on Murashige and Skoog (1962) basal medium (MS) supplemented with 30 g/L sucrose and 10 g/L agar at pH 5.74. Fifteen seeds were placed in each glass jar for germination (10.5 cm × 5 cm), with 20 mL of medium and incubated at 25±2 °C under 16 h light (40-50 μmol) in the growth room. Germinated seedlings were transferred to fresh medium with the same composition and were allowed to grow for two weeks. After two weeks, explants of 2 mm size were excised from these seedlings and placed on MS medium supplemented with different concentrations
combinations of 6-benzylaminopurine 2, 3, 4 mg/L (BAP), indole-3-acetic acid 1, 2 mg/L (IAA), Gibberellic acid 0.5 mg/L (GA₃) and silver nitrate (AgNO₃) 3 mg/L (Table 1). All jars were labelled and kept in controlled environment at 25 ± 2 °C under 16 h light (40-50 μmol) in the growth room.

Ten explants were used for each treatment. The experiments were repeated independently at least three times with reproducible results. The data on average number and length of shoot and root were collected and analyzed.

**Table 1.** MS medium supplemented with various combinations of 6-benzylaminopurine (BAP), indole-3-acetic acid (IAA), gibberellic acid (GA₃) and silver nitrate (AgNO₃)

<table>
<thead>
<tr>
<th>Media Code</th>
<th>BAP mg/g</th>
<th>IAA mg/g</th>
<th>GA₃ mg/g</th>
<th>AgNO₃ mg/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G2</td>
<td>2</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>G3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G4</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G5</td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>G6</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G7</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>G8</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>G9</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>G10</td>
<td>3</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>G11</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>3</td>
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<tr>
<td>G12</td>
<td>3</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>G13</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
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<td>G14</td>
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<td>3</td>
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<td>0.5</td>
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<td>G17</td>
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<td>2</td>
<td>0</td>
<td>3</td>
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<td>G18</td>
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<td>0</td>
<td>0.5</td>
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</tr>
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<td>G21</td>
<td>4</td>
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<td>0.5</td>
<td>0</td>
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<td>G22</td>
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<td>1</td>
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<td>3</td>
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<td>G23</td>
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</tr>
<tr>
<td>G24</td>
<td>4</td>
<td>2</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>G25</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

**Results and Discussion**

A consistent and reproducible protocol is presented for the regeneration and mass multiplication of marigold through tissue culture technique. Out of the tested twenty five different Murashige and Skoog (1962) basal media containing 6-benzylaminopurine (BAP 2, 3, 4 mg/L), indole acetic acid (IAA 1, 2 mg/L), gibberellic acid (GA₃ 0.5 mg/L) and silver nitrate (AgNO₃ 3 mg/L), only four combinations were found to be effective for in-vitro mass multiplication of shoots (Table 2).

It was noted that media supplemented with BAP 2 mg/L and IAA 2 mg/L gave the best results. This medium not only supported the highest mean number (4.83±0.49), but also allowed vigorous growth of shoots (Fig. 1).

**Fig 1.** Different stages of in vitro shoot proliferation of marigold.
Table 3. Effective media for in-vitro production of roots and their length (in cm)

<table>
<thead>
<tr>
<th>Media Growth regulators code</th>
<th>Roots/explant (Mean ±SE)</th>
<th>Root length (cm) (Mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G23 (4, 2, 0, 0)</td>
<td>17.08±2.44</td>
<td>13.67±0.98</td>
</tr>
<tr>
<td>G12 (3, 1, 0, 0)</td>
<td>16.50±1.23</td>
<td>5.42±1.49</td>
</tr>
</tbody>
</table>

The shoots grown on this medium were healthy and longer in size (5.28±1.06 cm) as compared to the other media tested (Fig. 2). Other effective concentration of growth regulators for shoot proliferation was BAP 2 mg/L and IAA 1mg/L but the shoots produced were weak and smaller in size. Although media supplemented with BAP (3 mg/L), IAA (1 mg/L), GA3 (0.5 mg/L), BAP (4 mg/L), IAA (2 mg/L) and AgNO3 (3 mg/L) also produced good number of shoots per explant but the shoots were smaller with slower growth (Fig. 2).

Previous studies on optimization of in-vitro propagation of marigold showed that effective concentration of BAP was 0.1 mg/L to 5.0 mg/L, IAA was 0.2 to 3.0 mg/L and GA3 was 0.5 to 20 mg/L (Godoy-Hernandez et al., 2006; Vanegas et al., 2002; Chakrabarty et al. 2000; Misra and Datta, 1999). The present results showed that 2 mg/L concentration of both the BAP and the IAA was enough for producing good number and length of shoots.

The media effective for shoot proliferation did not help in formation of healthy roots for the establishment of plants in soil (Misra and Datta, 1999). When tissue culture-derived shoots were sub-cultured on twenty five different media (Fig.3), only two were found to be effective for in-vitro rooting. Sub-cultured shoots on media supplemented with BAP (4 mg/L) and IAA (2 mg/L) produced the most extensive root system and the highest mean number and size of roots were observed (Table 3).

Table 2. Effective media for in-vitro production of shoots and their length

<table>
<thead>
<tr>
<th>Media Growth regulators code</th>
<th>Shoot/explants (Mean±SE)</th>
<th>Shoot length (cm) (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G7 (2, 2, 0, 0)</td>
<td>4.83±0.49</td>
<td>5.28±1.06</td>
</tr>
<tr>
<td>G13 (3, 1, 0.5, 0)</td>
<td>4.50±0.71</td>
<td>3.34±0.53</td>
</tr>
<tr>
<td>G25 (4, 2, 0, 3)</td>
<td>4.33±0.64</td>
<td>3.96±0.72</td>
</tr>
<tr>
<td>G4 (2, 1, 0, 0)</td>
<td>4.00±0.71</td>
<td>4.54±1.11</td>
</tr>
</tbody>
</table>

Misra and Datta (1999) reported 100% rooting on 0.05 mg/L NAA within 7 days of incubation; they also observed 100% rooting in media supplemented with IAA and IBA but associated with some callusing and vitrification of shoots. Other medium effective for root formation was BAP (3 mg/L) and IAA (1 mg/L) but growth of roots was stunted and their size was short (Fig. 3). However, addition of gibberellic acid (GA3) and AgNO3 in media was not found effective for root formation in marigold.

The results suggested that root initiation requires BAP (4 mg/L), IAA (2 mg/L) and the same media was good for healthy growth of roots (Fig. 4).

Present study also reveals that addition of GA3 and AgNO3 did not have any significant effect on direct shoot or root proliferation from explant (Fig. 2). In contrast, Godoy-Hernandez et al. (2006) found a
significant role of gibberellic acid for the induction of shoot proliferation of marigold. AgNO₃ along with BAP has been used successfully for shoot proliferation of marigold by Misra and Datta (2001) and Fuentes et al. (2000) but in the present study, results were not successful.

This study was conducted to select the most effective concentration and combination of growth regulators for mass multiplication and for healthy rooting, through tissue culture and it was found that use of BAP and IAA in the concentrations mentioned above were enough for both shoot and root formation of marigold. Addition of other growth regulators and changing the concentration either reduced the number of shoots per explant or reduced the size of shoots.

References


High Density Culture of a Cladoceran *Moina micrura* (Kruz, 1874) by Using Poultry Manure

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**Abstract.** The branchipod *Moina micrura* was mass cultured using three different concentrations (250 ppm, 500 ppm, and 1000 ppm) of chicken manure in the medium for 21 days. In the first concentration (250 ppm), the maximum density (1694.44 ± 9.68) of *M. micrura* was obtained on the 9th day of inoculation and the number of plankton declined gradually afterwards. In the second concentration (500 ppm), the maximum density (5659.88 ± 54.35) appeared on the 11th day while in the third concentration (1000 ppm), the maximum density (1510 ± 15.27) was obtained on the 14th day of inoculation. Temperature range of 27-31°C and pH of 6-7 was conducive for optimal growth of *M. micrura*.

**Keywords:** live feed, *Moina micrura*, mass culture, poultry manure

**Introduction**

Zooplankton is important as food for both young and adult fish (Altaff and Mehraj, 2010; Srivastava *et al.*, 2006; Pangano *et al.*, 2000). Zooplankton collected from natural resources are used as diet for fish and prawn larval rearing in many hatcheries although this is an unreliable source for commercial use according to quality. Wild zooplankton may introduce harmful organisms to the hatcheries and hence mass culture under controlled conditions is desirable (Altaff *et al.*, 2002).

*M. micrura* is a cosmopolitan, cyclic parthenogenetic cladoceran with ample morphological and ecological plasticity, inhabiting temperate, tropical and subtropical regions worldwide (Martinez-juerinimo *et al.*, 2007). Organic fertilizers are usually preferred on mineral fertilizers because organic fertilizers provide bacterial and fungal cells, detritus as well as phytoplankton as food for *Moina* (Rottmann *et al.*, 2003). Earlier Jana and Chakraborti (1993a) reported the presence of detritus in the gut of *M. micrura*. Since then different culture techniques have been developed to increase the yield of cladocerans by employing different waste organic products as food sources (Mehraj and Altaff, 2010; Shrivastava *et al.*, 2006; Sivakumar, 2005; Pagano *et al.*, 2000). Among various species of zooplankton, the genus *Moina* of the Cladocera is known to be suitable as initial feed for larval stages of many fishes. The cost of *Artemia* cyst is very high and its use in developing countries may not be appropriate as its production requires some specialized facilities (Hung *et al.*, 1999).

Young *Moina* are less than 400 µm in size, approximately of the same size as that of the adult rotifers and smaller than newly hatched brine shrimps, which die comparatively earlier in freshwater when fed to the fishes. *Moina* can be stored for longer periods by freezing in low salinity water and can be kept alive for several days in the refrigerator for use as live feed of fish larvae. High temperature tolerance of *Moina* is also of great advantage for both commercial fish farmers and hobbyists cultivating live feed at home. *Moina* that are cultured in water enriched with organic manures are fed to bigger fish or brooders (Rottmann *et al.*, 2003). Unfortunately, there is little information available concerning practical mass culture methods of *M. micrura* and the literature on tropical and subtropical zooplankton feeding is very scarce (Pangano, 2008). Animal manures like, cattle manure, poultry droppings and agricultural by-products are wastes worldwide and can be used as potential organic manures to increase the productivity of water bodies.

Poultry manure has many advantages for fertilizing culture media when compared to other manures and algae (Altaff and Mehraj, 2010; Shrivastava *et al.*, 2006). Furthermore, the high price of *Artemia* cysts has increased the fish production cost, therefore cheaper alternative diets with comparable nutritional quality are needed to maintain the competitiveness of ornamental fish on global market (Altaff and Mehraj, 2010; Kumar *et al.*, 2005).
In this context experiments were carried out on mass culture of *M. micrura* using poultry excreta, a waste which will make it a cheap and easily available food source to the fish culturists.

**Materials and Methods**

Chicken manure was collected from a local broiler chicken shop and was dried for 2 days to remove the moisture and stored in plastic jars for further use. Manure was micronized by grinding and the required quantity was dissolved in distilled water to get suspensions of 250, 500 and 1000 ppm for fertilizing culture medium. Microinisation of poultry waste is necessary for efficient filtration of the suspended particles. Zooplankton samples were collected from Chetpet freshwater pond, Chennai, India and were brought to the laboratory with the least disturbance. The adult *M. micrura* were identified and separated using binocular dissection microscope based on the key characters outlined by Altaff (2004).

Experimental aquarium tanks of 50 L capacity arranged in triplicate were filled with 40 L of filtered water and fertilized with chicken manure at the concentrations of 250, 500 and 1000 ppm. After 3 days, *M. micrura* were inoculated in each experimental tank at the density of 50 ± 5 Ind/L containing both adults and neonates. The culture experiment was conducted for 21 days. Water change was carried out at 3 day interval by removing 50% of the water throughout the culture period. Food was administered as a function of population density every 3rd day using the formula of Altaff and Mehraj (2010):

\[ Y = [(\log 10^N/10) \times 0.2] \times V \times d. \]

where:

- \( Y \) = quantity of chicken manure
- \( N \) = population density (Ind/L)
- \( V \) = volume of culture (L)
- \( d \) = no. of days for which the food was administered.

The culture water used in all experiments was filtered tap water, previously aerated for 24 h to dechlorinate the water. To avoid anaerobic conditions in the medium, the sediments (unconsumed food, faeces, and pseudo-faeces) were siphoned out from the bottom three times a week. Excessive fouling was also removed from the walls of the tanks. Wet weight of the animals was determined after draining 10 L of the culture medium over a nylon gauze of 200 μm mesh size and washed several times to remove other debris. The remaining water was absorbed using tissue paper and the animals were weighed on a digital balance with 1 μg sensitivity. Population density was estimated by counting samples, taken at random with a 1 L beaker, after mixing thoroughly the culture volume. Sub-samples of 100 mL and then 10 mL were drawn from these samples. Samples were immobilized using alcohol and counting was carried out using Sedgwick Rafter cell under binocular dissection microscope. Results were expressed as number of individuals per litre (Ind/L).

**Results and Discussion**

In the first concentration (250 ppm), the peak density (1694.44 ± 9.68) of *M. micrura* was obtained on the 9th day of inoculation and the number of plankton declined gradually afterwards. In the second concentration (500 ppm), the peak density (7296.88 ± 54.35) appeared on the 11th day while in the third concentration (1000 ppm) peak density (1510 ± 15.27) was obtained on the 14th day of inoculation. The lowest density was observed in the tanks fertilized with 1000 ppm concentration (1510 ± 15.27). Thus the concentration of 500 ppm chicken manure was found to be more suitable for obtaining high density culture of *M. micrura* than the other concentrations used (Fig. 1). The density (Ind./mL) of protozoan population during different days in the culture medium fertilized with different concentrations of poultry manure is shown in Fig. 2. Higher wet weight of *M. micrura* biomass (mg) was obtained on the 11th day of the culture (Fig. 3).

![Fig. 1. Population density of *M. micrura* (Ind/mL) during different days in the culture medium fertilized with different concentrations of poultry excreta (Mean ± SE).](image-url)
In the present experiment, higher production of *M. micrura* was obtained compared to 1050-2600 Ind/L reported by Punia (1988) and 2500-3500 Ind/L, by Majumdar and Nandy (1989). The dynamic characteristics of zooplankton have led to the use of particular fertilization techniques and species-specific inoculation in culture ponds (Mehraj and Altaff, 2010; Shrivastava et al., 2006). The food supply could be a limiting factor in this type of culture due to high filtering rate. Organic fertilization introduced a complex food web in the fish pond with higher intensity of bacterial activity through the detrital food chain (Safullah and Altaff, 2002). The nutrients beyond the optimum level reduced population of *Moina*, which may be due to the rapid degradation of high nutrient content in the medium, resulting in increased production of ammonia and growth of pathogenic microbes resulting in unfavorable conditions for the growth of population (Adeyemo et al., 1994). Boyd and Doyle (1984) Anuta (1992) and reported that high input of chicken manure and commercial feed depressed dissolved oxygen for a prolonged time and water quality deteriorated in the culture resulting in suppressed growth and mortality of the cultured organisms. Similarly, concentration of nutrients below the optimum level also resulted in reduced populations of *M. micrura* which may be due to insufficient microbial and protozoan populations and lack of sufficient organic compounds, required for the growth and survival of live food organisms. Safullah and Altaff (2002) also reported higher density of *M. micrura* at 500 ppm concentration using different nutrient media. Ray and David (1969) opined that chicken manure-fertilized medium produced a large population of cladocerans quicker than cattle manure dosages. The influence of food on the population density of Cladocera has been reported (Srivastava et al., 2006; Jana and Chakrabarti, 1997; Boersma and Vijverberg, 1996). Usually when environmental conditions are favourable, the progeny is constituted of parthenogenetic females which are clones of mother. When the environmental conditions become unfavorable, sexual reproduction becomes effective, leading to the production of males and females, eventually leading to the formation of resting eggs that enter into diapause, until the conditions become favourable again (Martínez-gerónimo et al., 2007). Gradual decrease after attaining a maximum density may be due to the indirect effects caused by high population densities such as modifying feeding conditions by releasing and accumulating chemical substances or through behavioral indications.

Dissolved oxygen content of the culture medium is another important factor for population growth. In the present study in all culture media, dissolved oxygen level of 4 ± 1 mg/L was maintained through aeration and fertilization of the medium. Oxygenation of the medium was assured by air bubbling from the bottom of the culture; aeration also ensured a more regular distribution of the population over the whole volume and prevented stratification (Rottmann et al., 2003). Several authors have stressed that aeration is an important culturing parameter and some studies showed that dissolved oxygen decreased with organic loads of the media (Sivakumar, 2005; Tay et al., 1991). An absolute prerequisite to maintain cultures is to renew parts of the culture water at regular intervals, to ensure permanent good water quality, thereby providing a favourable environment for population growth in higher densities.
The culture results of Punia (1988) showed a higher density of 1050-2600 Ind/L using ten different raw materials. In the present study a higher density of *M. micrura* population was recorded compared to previous reports (7296.88 ± 54.35) on 11th day of culture in tanks fertilized with 500 ppm of chicken manure. Punia (1988) reported that *D. lumholtzi* in outdoor culture using small containers, produced about 6000 Ind/L. It appears that with proper standardization it is quite possible to culture high density of Cladocera with cheaper organic materials such as agro-industrial waste and manure. Bonou and Saint-Jean (1998) indicated significant correlation in biomass of *M. micrura* and the initial chlorophyll concentration, which suggests that *M. micrura* population cannot be predicted in the availability of algal food alone.

The physico-chemical parameters appear to play an important role in the successful culture of Cladocera. Tay *et al.* (1991) did not report any relationship between physico-chemical parameters and physiological processes in zooplankton. Shrigur and Indulkar (1987) had proposed a range of water temperature between 27-31 °C for optimum growth of *M. micrura*. In the present study, a temperature range of 27-31 °C produced optimum density of cladocerans. Tay *et al.* (1991) reported the upper lethal limit close to 40 °C where 50% mortality of cladocerans occurred.

*Moïna micrura* can be grown in high densities on optimal quantities of chicken manure. Overfeeding causes high mortality due to unfavorable conditions for culture. This implies that zooplankton with similar feeding modes, can be limited by different factors of their food, which partly explains why so many different organisms are found in the plankton (Hutchinson, 1961). The feeding experiments conducted on *M. micrura* revealed that the feed concentration plays a decisive role in obtaining the maximum production and this is in accordance with the studies conducted by Safiullah and Altaf (2002); Okauchi (1991) and Yufera *et al.* (1983). Chicken manure is an excellent food and has many advantages in comparison to other foods, it is available in large quantities; it can be purchased easily at low price; can be used directly after drying; it can be stored for longer periods of time; and it has none of the problems involved in maintenance of algal stocks and cultures. Dried algae are in some cases also an excellent food but they are too expensive to be used at large scale (Altave and Mehraj, 2010). In the present study high density of *M. micrura* was obtained, which is commercially viable, especially in the present scenario of organic farming where production of live feed organisms using manures is of considerable importance in order to reduce the cost of seed production and to produce large number of quality seeds. Moreover, the densities obtained at different days at different concentrations are of aquacultural importance, the doses can be manipulated, depending upon the requirement of the aquaculturists.

**Acknowledgement**

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**References**


The Composition and Relative Abundance of Fish Species in a Mangrove Creek in the Niger Delta, Nigeria, Based on Different Types of Gear

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Abstract. The composition and relative abundance of fish species assemblage of a mangrove creek in the Niger Delta, Nigeria based on different types of gear was assessed at four stations between November 2004 and June 2006. The overall community structure was made up of 25 species of 16 families. Visual observation showed that tilapia species comprising of Sarotherodon melanotheron and Tilapia guineensis were the most dominant species. Samples from baited entrance traps indicated that S. melanotheron dominated T. guineensis. Samples from baited hook and line, and cast net showed dominance of P. elongatus followed by P. quadrifilis. (The baited hook and line is commonly used by the fishers because it is effective in catching diversity of species, except that it is unable to catch bottom feeders such as mullets and tilapia species). Funnel entrance trap had the highest species selectivity, catching only tilapia species. Cast net was more selective in species catch than baited hook and line, but it was effective in catching both pelagic and benthic species. Among the fish species, members of the families: Sciaenidae, Polynemidae, Ariidae, Monodactylidae and Cichlidae were permanent residents; Carangidae, Lutjanidae and Serranidae were temporary residents; while Elopidae, Gobiidae, Dasyatidae, Cynoglossidae, Sphyraenidae and Trichiuridae were rare species.

Keywords: composition, fish species, mangrove creek, Niger delta

Introduction

Knowledge of fish biology and species composition of different water bodies is necessary to enhance the management of water resources (Okereke, 1990). Niger Delta is located in the Southern part of Nigeria bordering the Atlantic Ocean in the Gulf of Guinea ecoregion. It is a flood plain of over 70,000 sq km. The environment has been reported to be highly diverse and sensitive being the home of the largest stands of mangrove in Africa (over 1 million ha) and the fourth largest in the world (Spalding et al., 1997). The relative contribution of mangrove-related species to total fisheries catch is significant (Islam and Ikejima, 2010). Fisheries in and around mangroves provide direct employment to about 0.5 million fishermen worldwide (Matthes and Kapetsky, 1988). However, mangroves have declined due to increasing pressures from human activities including over-harvesting, aquaculture and coastal development (Alongi, 2002). The significant threat of oil pollution, over-exploitation of fisheries and mangrove resources among other environmental pressures coupled with poor interest and recognition for brackish water bodies and aquaculture pose a great danger to the sustainability of Nigeria’s brackish waters and diverse fish resources, and consequently the coastal communities of the nation (Oribhabor et al., 2005).

The importance of mangroves as nurseries has been one of the reasons advanced to support its conservation and management (Sheridan and Hays, 2003). There have been warnings, that supportive data have not been collected and that fish and decapods use of mangroves may not be the same in all areas of the globe (Hoss and Thayer, 1993; Chong et al., 1990). For effective conservation and management of mangroves, it is necessary to synthesize biological and socio-economic information on mangrove processes and values, and bring this to the attention of policy-makers for community development (Walters et al., 2008). The earlier records of mangrove fisheries were those of Blabber and Blabber (1980); Blabber (1980), Day (1974), Austin (1971), Boeseman (1963) and Inger (1955). Fish studies
on Nigerian coastal waters include those of Chindah and Tawari (2001); King (1996a; 1996b); Chinda and Osuamkpe (1994); Wright (1986); Fagade and Olaniyi (1974; 1972). The Buguma Creek has been used by the Federal Government of Nigeria in collaboration with FAO in 1963 for pilot investigation of the possibility of converting mangrove swamps into brackish water fish farms. Oribhabor and Ogbeibu (2010) have assessed the impact of anthropogenic activities on the predatory fishes of the creek. This paper is the first to document the composition and relative abundance of fish species in Buguma Creek, based on different types of gear.

**Study area.** The Buguma Creek is located Southeast of the Niger Delta between longitude 6° 47'E and 6° 59'E, and latitude 4° 31'N and 4° 59'N (Fig. 1) in Asari-Toru Local Government Area of Rivers State. The Buguma creek system consists of the main creek channel and associated interconnecting creeks, which inter-connect and surround Buguma and Ido communities. The Buguma Creek serves as a source of tidal water for the Nigerian Institute for Oceanography and Marine Research/Buguma Brackish Water Experimental Fish Farm, which was constructed between 1963 and 1966 under the auspices of the FAO. The New Calabar River brings the salty ocean water as tidal flows diurnally to the fish ponds (Dublin-Green and Ojanuga, 1999). A more detailed description of the study area and water quality has been given by Oribhabor and Ogbeibu (2009a; 2009b); Ogbeibu and Oribhabor (2008).

**Materials and Methods**

The fish species composition and relative abundance of Buguma Creek fishes in Niger Delta, Nigeria were conducted based on different types of gear. Fish samples were collected monthly at four stations, at flood tides during the period November 2004 to June 2006. Sampling was conducted between 0800 and 1700 h on each sampling day for 6 h, depending on the flow and ebb of tide.

Baited hook and line were used throughout the sampling duration at stations 1, 3 and 4; cast-net was used at station 1 from January to October, 2005; while baited funnel entrance traps were fortnightly used at stations 1 and 2 specifically for tilapia species from August to December, 2005. Fish samples were pooled based on gear, ice-packed after capture, kept chilled under

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**Fig. 1.** Map of the study area: A: Nigeria showing Niger delta, B - Rivers state showing Buguma, C. The study creek showing study station.
ice-blocks in a plastic cooler and immediately transported to the laboratory. In the laboratory, fish specimens were sorted and identified to the species level using the keys and descriptions of Olaosebikan and Raji (2004); Teugels et al. (1992); Schneider (1990) and enumerated. Visual observation of extraneous fish species periodically harvested from two associated recruitment ponds of Nigerian Institute for Oceanography and Marine Research/Buguma Brackish Water Experimental Fish Farm was also carried out during the study period.

Results and Discussion
The overall community structure was made up of 25 species in 16 families (Table 1). There is no ‘best’ method for sampling fishes within mangrove habitats (Faunce and Serafy, 2006). Three sampling gears: baited hook and line, castnet, baited funnel entrance trap coupled with visual observation were used in this study because samples from the most environments are drawn blind with little or no study of the response of the fishes to sampling gear (Bagenal, 1978).

Among the fishing gears, funnel entrance trap was found to have high species selectivity, catching only tilapia species (Fig. 2). A total of 20 species in 14 families, and 16 species in 12 families were recorded for baited hook and line and castnet, respectively (Fig. 3 and 4). Although cast-net was more selective in species catch than baited hook and line it is effective in catching both pelagic and benthic species, unlike baited hook and line, which is specialized in catching only predatory species. Although catch composition varied with geographical habitat and the type of fishing gear and effort, the relatively low diversity found in the present study may in part indicate the over-exploitation of resources, resulting in destruction of habitat and consequent loss of species diversity (Islam and Ikejima, 2010).

Visual observation showed that tilapia comprising of S. melanotheron and T. guineensis were the most dominant species. Samples, from baited entrance traps (Fig. 2) indicated that S. melanotheron (306 individuals) dominated T. guineensis (136 individuals). The dominance of tilapia could be attributed to their prolific precocious breeding habit, ready availability of mud from which they derive their food, and euryhaline nature. Samples from baited hook and line (Fig.3), and cast-net (Fig. 4) showed dominance of P. elongatus followed by P. quadrispinis. Differences in dominant species observed among the fishing gears are in conformity with the finding of Boromthanarath et al. (1991), who noted that dominant species differed by fishing gear types and methods. The baited hook and line fishing is commonly used by fishermen in Buguma because it is effective in catching diverse species, except that it is unable to catch bottom feeders such as mullets and tilapia species. Though quite selective by species, and to a considerable extent by size, in some situations, line fishing may be the most productive (Bagenal, 1978). Also in many of its forms, it can be conducted successfully by a single fisherman.

The low habitat diversity limits the number of fish species inhabiting tidal mangrove creeks (Krumme et al., 2004). Although low number of species was

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ariidae</td>
<td>Arius gigas (Boulenger, 1911)</td>
</tr>
<tr>
<td>Carangidae</td>
<td>Caranx latus (Agassiz in Spix &amp; Agassiz, 1831)</td>
</tr>
<tr>
<td>Cichlidae</td>
<td>Sarotherodon melanotheron (Ruppell, 1852), Tilapia guineensis (Günther, 1862)</td>
</tr>
<tr>
<td>Cynoglossidae</td>
<td>Cynoglossus senegalensis (Kamp, 1858)</td>
</tr>
<tr>
<td>Dasyatidae</td>
<td>Dasyatis margarita (Günther, 1870)</td>
</tr>
<tr>
<td>Elopidae</td>
<td>Elops lacerta (Valenciennes, 1846)</td>
</tr>
<tr>
<td>Gobiidae</td>
<td>Gobius sp. (Peters, 1876)</td>
</tr>
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<td>Haemulidae</td>
<td>Plectrhus macrolepis (Boulenger, 1899), Pomadasys jabertii (Cuvier, 1831)</td>
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<tr>
<td>Lutjanidae</td>
<td>Lutjanus agonus (Bleeker, 1863), Lutjanus goreensis (Valenciennes, 1830)</td>
</tr>
<tr>
<td>Monodactylidae</td>
<td>Psettus sebae (Cuvier, 1831)</td>
</tr>
<tr>
<td>Mugilidae</td>
<td>Liza falcipinnis (Valenciennes, 1836), Mugil cephalus (Linnaeus, 1758), Mugil curema (Valenciennes, 1836)</td>
</tr>
<tr>
<td>Polynemidae</td>
<td>Polydactylus quadrifilis (Cuvier, 1829)</td>
</tr>
<tr>
<td>Sciaenidae</td>
<td>Argyrosmus regius (Asso, 1801), Pseudotolithus (Fonticulus) elongatus (Bowdich, 1825), Pseudotolithus (Pseudotolithus) senegalensis (Bleeker, 1863)</td>
</tr>
<tr>
<td>Serranidae</td>
<td>Epinephalus aeneus (Geoffray St Hilaire, 1809)</td>
</tr>
<tr>
<td>Sphyraenidae</td>
<td>Sphyraena atra (Peters, 1844), Sphyraena guachancho (Cuvier, 1829), Sphyraena sphyraena (Linnaeus, 1758)</td>
</tr>
<tr>
<td>Trichiuridae</td>
<td>Trichiurus lepturus (Linnaeus, 1758)</td>
</tr>
</tbody>
</table>
recorded for Buguma Creek, all mangrove and estuarine systems are similar in that, they have relatively few species that are clearly dominant in abundance (Tongnumui et al., 2002).

Among all the fish species, members of the families; Sciaenidae, Polynemidae, Ariidae, Monodactyliidae, Mugilidae and Cichlidae were permanent residents. Members of the families; Carangidae, Lutjanidae and Serranidae were temporary residents, while those of the families; Elopidae, Gobiidae, Dasyatidae, Cynoglossidae, Sphyraenidae and Trichiuridae were rare species. Most fishes encountered in this study were sub-adults.

The number of species (25) recorded is high when compared to the 9 species of Wright (1986) for shallow water creeks of a Nigerian mangrove. About 30 species were reported in the Kaper mangrove in Malaysia (IPIECA, 1993). Davis (1988) caught 38 species in Austria, Krumme et al. (2004) caught 40 species in small intertidal mangrove creeks in Northern Brazil. Compared with other mangrove and estuarine systems, these records are low. A pool of all the species recorded in Lagos Lagoon, Nigeria over time revealed 115 species (Oribhabor and Ezenwa, 2005). Other high species record of mangrove and estuarine systems are 55 species (Beumer, 1978), 83 species (Little et al., 1988), 128 species (Kimani et al., 1996), 66 species (Laroche et al., 1997) and 135 species (Tongnumui et al., 2002).

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Freshwater Fisheries Technology, New Bussa, Nigeria.


Effect of Different Temperatures and Host Plants on the Biology of the Long-Tailed Mealy Bug 

*Pseudococcus longispinus* (Targioni and Tozzetti) 
(Homoptera: Pseudococcidae)

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**Abstract.** Experiments were done to determine the effects of different temperature levels and three host plants namely, *Cucurbita moschata* (butternut squashes), *Solanum tuberosum* var. Desi (potato), and *Solanum tuberosum* var. Cara (white potato) on the biology of mealy bug *Pseudococcus longispinus* (Targioni and Tozzetti). It was found that the temperature had a significant effect on the development period of both male and female mealy bugs. For males the period decreased with increasing temperatures up to 28 °C, but increased thereafter and host plant had no effect, except at 24 °C. All the aspects of females including total pre-adult, prelarviposition, larviposition period, life span, fecundity and sex ratio were affected by the fluctuating temperatures, but host plant produced no effect on fecundity and sex ratio.

**Keywords:** mealy bug, temperature, host plants, life span, sex ratio

**Introduction**

Long-tailed mealy bug *Pseudococcus longispinus* (Targioni and Tozzetti) is mainly of tropical and subtropical origin and in glasshouses in temperate zone and many of them have become established as pests. It is found in the Mediterranean Basin, Africa, Southern Asia, Far East, Australia, New Zealand, Pacific Islands, USA, Central and South America (CIE, 1958). They attack a wide range of plants including fruits, vegetables and ornamentals and can cause severe damage to leaves, bark, branches, fruit and roots. They may occur under bark and cause some kinds of galls (Copland *et al.*, 1985; Miller and Kosztarab, 1979). The very broad host range of mealy bugs in part explains their success. As sap feeders, they have the potential to be vectors of various viral diseases (Campbell, 1983; Harris, 1981). Some species are known to inject potent phytotoxins during feeding (Lema and Herren, 1985). Their direct damage takes the form of distortion, stunting and yellowing of foliage, early dropping of the flowers and fruits, sometimes followed by defoliation. Indirectly, their copious secretion of honeydew promotes the growth of sooty moulds which can detract from the aesthetic and economic value of these plants (Hattingh, 1993; Copland *et al.*, 1985; Pritchard, 1949).

Clausen (1915) studied the life history of *P. longispinus*, and was experienced considerable difficulty in measuring the rate of larviposition due to the disturbing effect it had upon the female. He observed that the young remain clustered under the body of the parent for one or more days after birth. The periods of time required for different stages were variable. Mating took place largely during the third instar and larviposition began within 10 to 15 days after the third moult. James (1937) studied the pre-adult stages of *P. longispinus* including the egg. He found three nymphal instars in the female and four in the male. The sexes were indistinguishable externally in the first instar but sexual dimorphism was apparent in the second instar. Panis (1969), who described *P. longispinus* as a viviparous species, showed that light, gravity and host plant quality had a great effect on adult orientation and distribution on plant leaves as well as on its sex ratio; mating was obligatory for the production of eggs and the development of the ovaries. Males were capable of several matings. El-Minshawy *et al.* (1974) studied the biology of *P. longispinus* at different stages. They found that the duration of all pre-adult stages was greatly affected by temperature.

Mealy bug is posing a serious threat to the plants, fruits, vegetables and ornamentals in the field and in glasshouses. The long tailed mealy bug has a thick layered
wax on its body which makes hard to control it chemically. Very little information is available regarding long-tailed mealy bugs bionomics. El-Minshawy (1974) found that the duration of all pre adult stages were greatly effected by temperature therefore different temperature regimes were included in the studies. 

The extended development period at 32 °C was a strange response of long-tailed mealy bug to the temperature. Dr. Panis (1969) also observed the same phenomenon in some other mealy bug species as well. The reason for this behaviour in this mealy bug is yet not clear.

The host plants and temperatures used in this study are normally encountered by long-tailed mealy bug in the fields and in the glasshouses. The number of its host plants is increasing gradually. The temperature has some effect on its life cycle but the host plants have a little role. This means this mealy bug can adapt to any host plant easily. This type of information was not available previously especially when similar types of studies were being conducted on its potential host i.e. brown lacewing. In this study, the effect of temperature and three different host plants on the biology of long-tailed mealy bug has been investigated.

Materials and Methods

The experiments were done in the Department of Environment, Wye College, University of London. The effect of four different temperatures, i.e. 20 °C, 24 °C, 28 °C and 32°C and three different host plants, i.e. Cucurbita moschata (butternut squashes), Solanum tuberosum var. Desiree (red potato), and Solanum tuberosum var. Cara (white potato), on the biology of long-tailed mealy bugs was studied. The temperatures for the studies were achieved in controlled temperature incubators. Five reproducing females were released on each host plant for 24 h. The crawlers laid by these females (F₁ generation) were allowed to develop and complete their life cycle on the host plants. For biological studies, five newly emerged 4th-instar females (F₁) from each of these host plants were then isolated and confined with males for fertilisation. These fertilised females were then released singly on each respective host plant. Each female represented one replicate (five replicates). The crawlers laid by the females (F₂ generation) were counted and their positions were noted after settling down. Studies were conducted for development of different stages, pre-larviposition period, larviposition period, fecundity, female longevity and life-span. The observations were taken after every 24 h. For sex ratio determination, another ten such females were released on each host. Their progeny was raised until the determination of sexes.

Data obtained on the development of different stages, fecundity, survival and total life-span, were statistically analysed by using one-way ANOVA and means were compared using Fisher’s test at 5% level of significance. The data for sex ratio were pooled separately into males and females on emergence at each temperature and on each host for Chi squared test. The LSD values were also calculated.

Results and Discussion

Effect of temperature on the development of the male P. longispinus. Temperature had a pronounced effect on the development of males. The first-instar periods were significantly different from each other at all four temperatures (Table 1) with greatest duration at 20 °C and least at 32 °C. Host plants have no significant effect on the duration at any of the temperatures although development appeared shorter on C. moschata at all temperatures (Table 1).

Temperature had a significant effect on the second-instar male. A decrease in the duration of this instar was observed with increasing temperature from 20 °C to 28 °C except on butternut squash where the minimum period was observed at 24 °C (P < 0.05, Table 2). The longest duration was at 32 °C. The effect of host is also evident in this instar. On butternut squash the stadia were significantly shorter than on the other two hosts at 20 °C and 24 °C (Table 2). No significant host effect was found at 32 °C (Table 2).

The third-instar males were also affected by the temperature. The development periods decreased on all the hosts with increase in temperature up to 28 °C (Table 3), but increased again at 32 °C. The host plants had no effect on third-instar males except on butternuts at 28 °C where their development period was significantly shorter than on the other two hosts (P < 0.05, Table 3).

The development period of fourth-instar males was longest at 20 °C and shortest at 28 °C (P < 0.05, Table 4). It increased again at 32 °C (P < 0.05). The host plants had no effect on this stage at any of the temperature levels, except at 24 °C where the fourth-instar on butternuts was significantly shorter than that on the white potato (Table 4).
Overall temperature had a significant effect on the total pre-adult period of the male. The total pre-adult periods were significantly different from each other at all temperatures (P < 0.05, Table 5). The period decreased with increasing temperatures up to 28 °C, but increased thereafter. The host effect was non significant (P > 0.05, Table 5) except at 24 °C (P < 0.05, Table 5).

**Effect of temperature on the development of the female *P. longispinus*.** Temperature had a pronounced effect on the first-instar female. There was a significant decrease in the first-instar periods with increasing temperature which was longest at 20 °C (Table 6). The effect of host plant was also quite evident at 20 °C, 28 °C, and 32 °C. The stadia were significantly shorter on butternut squashes as compared to either potato cv at these temperatures (P < 0.05, Table 6). The effect of host was also noted at 28 °C on *S. tuberosum* var. ‘Desiree’ where the developmental period was significantly longer than the other two hosts (P < 0.05, Table 6).

The second-instar periods gradually decreased with the rising temperature up to 28 °C. There was no significant

### Table 1. Effect of four different temperatures and three host plants on the development of first-instar of male *P. longispinus* (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>14.92 ± 0.16</td>
<td>13.42 ± 0.15</td>
<td>11.35 ± 0.20</td>
<td>10.11 ± 0.14</td>
<td>0.496</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>a A</td>
<td>n = 170</td>
<td>n = 255</td>
<td>n = 230</td>
<td>n = 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>15.45 ± 0.22</td>
<td>13.45 ± 0.17</td>
<td>11.56 ± 0.17</td>
<td>10.38 ± 0.21</td>
<td>0.575</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>a A</td>
<td>n = 142</td>
<td>n = 246</td>
<td>n = 204</td>
<td>n = 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>15.41 ± 0.21</td>
<td>13.62 ± 0.13</td>
<td>11.63 ± 0.13</td>
<td>10.30 ± 0.14</td>
<td>0.468</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>a A</td>
<td>n = 137</td>
<td>n = 244</td>
<td>n = 202</td>
<td>n = 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD5% value</td>
<td>0.612</td>
<td>0.471</td>
<td>0.521</td>
<td>0.506</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

### Table 2. Effect of four different temperatures and three host plants on the development of second-instar of male *P. longispinus* (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>13.09 ± 0.22</td>
<td>8.21 ± 0.21</td>
<td>9.43 ± 0.15</td>
<td>15.27 ± 0.17</td>
<td>0.572</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>b B</td>
<td>n = 170</td>
<td>n = 255</td>
<td>n = 230</td>
<td>n = 31</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>14.33 ± 0.19</td>
<td>9.10 ± 0.16</td>
<td>8.90 ± 0.12</td>
<td>15.19 ± 0.23</td>
<td>0.533</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>a B</td>
<td>n = 142</td>
<td>n = 246</td>
<td>n = 204</td>
<td>n = 30</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>14.27 ± 0.18</td>
<td>9.31 ± 0.17</td>
<td>8.37 ± 0.16</td>
<td>15.38 ± 0.18</td>
<td>0.507</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>a B</td>
<td>n = 137</td>
<td>n = 244</td>
<td>n = 202</td>
<td>n = 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.699</td>
<td>0.566</td>
<td>0.428</td>
<td>0.602</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.
difference in stadia on any host at 28 °C and 32 °C (P > 0.05, Table 7). The development period of second-instar was significantly shorter on butternut squashes than the other two hosts at 20 °C. The period observed on S. tuberosum var. ‘Cara’ was significantly longer than that of C. moschata at 24 °C (P < 0.05, Table 7).

The development period of third-instar females decreased with temperature rising from 20 °C to 24 °C but increased at 28 °C and 32 °C (Table 8). No effect of hosts was found at 20 °C and 24 °C (P > 0.05, Table 8). On butternut the duration of third-instar was significantly shorter than on the other two hosts at 28 °C (P < 0.05, Table 8).

Total pre-adult development period of females was significantly reduced with increasing temperatures from 20 °C to 28 °C on all hosts. The total development period started increasing after 28 °C on all the host plants (P < 0.05, Table 9). The host effect was pronounced at 20 °C and 28 °C. At 20 °C and 28 °C, the total development period was significantly shorter on C. moschata as compared with the other two hosts (P < 0.05, Table 9).

### Table 3. Effect of four different temperatures and three host plants on the development of third-instar of male P. longispinus (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. moschata Butternut</td>
<td>10.35 ± 0.18 aA n = 170</td>
<td>6.32 ± 0.23 aB n = 255</td>
<td>4.39 ± 0.14 bD n = 230</td>
<td>5.12 ± 0.18 aC n = 31</td>
<td>0.554</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>S. tuberosum var. Desiree</td>
<td>10.49 ± 0.13 aA n = 142</td>
<td>6.55 ± 0.15 aB n = 246</td>
<td>4.98 ± 0.10 aC n = 204</td>
<td>5.32 ± 0.15 aC n = 30</td>
<td>0.408</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>S. tuberosum var. Cara</td>
<td>10.82 ± 0.24 aA n = 137</td>
<td>6.83 ± 0.23 aB n = 244</td>
<td>5.00 ± 0.05 aC n = 202</td>
<td>5.26 ± 0.12 aC n = 26</td>
<td>0.044</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.580</td>
<td>0.630</td>
<td>0.326</td>
<td>0.473</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

### Table 4. Effect of four different temperatures and three host plants on the development of fourth-instar of male P. longispinus (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. moschata Butternut</td>
<td>5.38 ± 0.15 aA n = 170</td>
<td>4.49 ± 0.13 bB n = 255</td>
<td>3.49 ± 0.17 aC n = 230</td>
<td>5.18 ± 0.14 aA n = 31</td>
<td>0.441</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>S. tuberosum var. Desiree</td>
<td>5.32 ± 0.14 aA n = 142</td>
<td>4.83 ± 0.11 bB n = 246</td>
<td>3.42 ± 0.13 aC n = 204</td>
<td>5.30 ± 0.08 aA n = 30</td>
<td>0.340</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>S. tuberosum var. Cara</td>
<td>5.60 ± 0.11 aA n = 137</td>
<td>5.08 ± 0.13 bB n = 244</td>
<td>3.45 ± 0.06 aC n = 202</td>
<td>5.20 ± 0.05 aB n = 26</td>
<td>0.278</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.406</td>
<td>0.360</td>
<td>0.397</td>
<td>0.306</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.
Effect of temperature on the prelarviposition period of *P. longispinus*. Temperature had a significant effect on the prelarviposition period. Reductions in periods were observed with increasing temperature, with the maximum at 20 °C and minimum at 32 °C (P < 0.05, Table 10). No host effect was found at 20 °C and 32 °C. The period was significantly longer on *S. tuberosum* var. Desiree at 24 °C (P < 0.05) and shorter on *C. moschata* at 28 °C (P < 0.05, Table 10).

Effect of temperature on the larviposition period of *P. longispinus*. The periods were maximum at 20 °C on all hosts (Table 11) and were reduced until 28 °C with a drastic reduction at 32 °C (P < 0.05, Table 11). The effect of hosts was pronounced at all temperatures except 24 °C. The larviposition period was significantly shorter on *C. moschata* as compared to the other two hosts at 20 °C but was the longest at 32 °C (P < 0.05, Table 11).

### Table 5. Effect of four different temperatures and three host plants on the total pre-adult period of male *P. longispinus* (Mean pre-adult periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>43.74 ± 0.68</td>
<td>32.44 ± 0.57</td>
<td>28.66 ± 0.60</td>
<td>35.68 ± 0.31</td>
<td>1.558</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>n = 170</td>
<td></td>
<td>bC</td>
<td></td>
<td>aD</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>45.59 ± 0.67</td>
<td>33.93 ± 0.4</td>
<td>28.86 ± 0.15</td>
<td>36.19 ± 0.31</td>
<td>1.305</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>n = 142</td>
<td></td>
<td>aC</td>
<td></td>
<td>aD</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>46.10 ± 0.67</td>
<td>34.84 ± 0.60</td>
<td>28.45 ± 0.30</td>
<td>36.14 ± 0.48</td>
<td>1.609</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>n = 137</td>
<td></td>
<td>aC</td>
<td></td>
<td>aD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>2.085</td>
<td>1.665</td>
<td>0.988</td>
<td>1.163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

### Table 6. Effect of four different temperatures and three host plants on the development of first-instar of female *P. longispinus* (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>18.38 ± 0.05</td>
<td>14.30 ± 0.21</td>
<td>13.33 ± 0.17</td>
<td>11.28 ± 0.17</td>
<td>0.483</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>n = 305</td>
<td></td>
<td>aB</td>
<td></td>
<td>bC</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>22.26 ± 0.16</td>
<td>14.36 ± 0.27</td>
<td>13.95 ± 0.08</td>
<td>11.93 ± 0.19</td>
<td>0.558</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>n = 261</td>
<td></td>
<td>aB</td>
<td></td>
<td>aB</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>22.32 ± 0.21</td>
<td>14.59 ± 0.12</td>
<td>13.55 ± 0.11</td>
<td>11.96 ± 0.06</td>
<td>0.412</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>n = 260</td>
<td></td>
<td>aB</td>
<td></td>
<td>bC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.481</td>
<td>0.639</td>
<td>0.392</td>
<td>0.461</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.
C. moschata as compared to other hosts at all the temperatures except 32 °C, where the reverse was the case (Table 12).

**Effect of temperature on fecundity.** No significant effect of temperature on the number of crawlers produced was found at 20, 24 and 28 °C. However, the number of crawlers was very significantly reduced at 32 °C on all the hosts (P < 0.05, Table 13). There was no host effect on the fecundity at any temperature (P < 0.05, Table 13).

**Effect of temperature on the sex ratio.** An effect of temperature on the sex ratio was observed, with the proportion of females greatest at 20 °C and least at 28 °C, increasing again at 32 °C on all hosts (P < 0.05, Table 14). The observed sex ratios were significantly different from the expected at 20 and 24 °C on all the hosts (P < 0.05, Table 14). No significant difference between the observed and expected sex ratio was observed at 28 °C and 32 °C. There was no apparent host plant effect on the sex ratio at any temperature.

A complete biological knowledge of a pest is the pre-requisite for its successful biological control. Very little information is available regarding long-tailed mealy bug’s bionomics. Flanders (1940) concluded

**Table 7.** Effect of four different temperatures and three host plants on the development of second-instar of female P. longispinus (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. moschata Butternut</td>
<td>13.98 ± 0.10</td>
<td>10.19 ± 0.14</td>
<td>9.55 ± 0.14</td>
<td>10.03 ± 0.17</td>
<td>0.472</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>bA</td>
<td>n = 305</td>
<td>n = 365</td>
<td>aC</td>
<td>aB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. tuberosum var. Desiree</td>
<td>16.28 ± 0.19</td>
<td>10.53 ± 0.35</td>
<td>9.66 ± 0.06</td>
<td>9.55 ± 0.19</td>
<td>0.610</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 261</td>
<td>n = 305</td>
<td>B</td>
<td>aC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. tuberosum var. Cara</td>
<td>16.71 ± 0.48</td>
<td>11.35 ± 0.29</td>
<td>9.78 ± 0.13</td>
<td>9.65 ± 0.06</td>
<td>0.854</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 260</td>
<td>n = 301</td>
<td>aC</td>
<td>aC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.931</td>
<td>0.827</td>
<td>0.357</td>
<td>0.432</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

**Table 8.** Effect of four different temperatures and three host plants on the development of third-instar of female P. longispinus (Mean development periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. moschata Butternut</td>
<td>5.19 ± 0.10</td>
<td>4.31 ± 0.14</td>
<td>4.51 ± 0.12</td>
<td>11.91 ± 0.18</td>
<td>0.409</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aB</td>
<td>n = 305</td>
<td>n = 365</td>
<td>bC</td>
<td>aA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. tuberosum var. Desiree</td>
<td>5.43 ± 0.22</td>
<td>4.20 ± 0.15</td>
<td>5.66 ± 0.10</td>
<td>11.22 ± 0.16</td>
<td>0.489</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aB</td>
<td>n = 261</td>
<td>n = 305</td>
<td>aB</td>
<td>bA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. tuberosum var. Cara</td>
<td>5.43 ± 0.23</td>
<td>4.95 ± 0.27</td>
<td>5.54 ± 0.08</td>
<td>11.30 ± 0.10</td>
<td>0.569</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aB</td>
<td>n = 260</td>
<td>n = 301</td>
<td>aB</td>
<td>bA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.591</td>
<td>0.793</td>
<td>0.310</td>
<td>0.471</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.
that long-tailed mealy bug is native to Australia. El-Minshawy et al. (1974) and James (1937), had studied the biology of its different stages. In Auckland, the long-tailed mealy bug has three discrete generations on grapevines in a year.

Studies carried out in Russia by Oganesyan and Babayan (1979) showed that, the host, temperature and humidity had a marked effect on egg viability and duration of embryonic development of *Pseudococcus comstocki* (Kuw.). Considering the duration of the pre-adult stages, the data obtained revealed that all the developmental stages were significantly affected by temperature. A similar relationship of temperature and the pre-adult development periods of *P. longispinus* in different instars were also observed by Clausen (1915) and El-Minshawy et al. (1974).

The pre-adult development duration decreased with the increase in temperature up to 28 °C but started increasing except in crawlers after this temperature. Our findings are in accordance with El-Minshawy et al. (1974), who observed that the development of *P. longispinus* was extended at higher temperatures. The fecundity was

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**Table 9.** Effect of four different temperatures and three host plants on the total pre-adult period of female *P. longispinus* (Mean pre-adult periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>37.55 ± 0.21</td>
<td>28.80 ± 0.42</td>
<td>27.41 ± 0.43</td>
<td>33.22 ± 0.26</td>
<td>1.036</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>bA</td>
<td>n = 305</td>
<td>n = 365</td>
<td>n = 240</td>
<td>n = 37</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>43.97 ± 0.56</td>
<td>29.09 ± 0.8</td>
<td>29.29 ± 0.07</td>
<td>30.70 ± 0.39</td>
<td>1.535</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 261</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>44.46 ± 0.85</td>
<td>30.89 ± 0.65</td>
<td>28.87 ± 0.29</td>
<td>32.91 ± 0.17</td>
<td>1.685</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 260</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>1.854</td>
<td>1.97</td>
<td>0.935</td>
<td>0.880</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

---

**Table 10.** Effect of four different temperatures and three host plants on the prelarviposition period of *P. longispinus* (Mean prelarviposition periods in day’s ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>26.16 ± 0.27</td>
<td>23.26 ± 0.68</td>
<td>22.11 ± 0.05</td>
<td>10.26 ± 0.11</td>
<td>1.116</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>26.30 ± 0.29</td>
<td>24.98 ± 0.29</td>
<td>22.93 ± 0.26</td>
<td>09.94 ± 0.22</td>
<td>0.803</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>26.38 ± 0.31</td>
<td>24.30 ± 0.3</td>
<td>23.98 ± 0.3</td>
<td>10.44 ± 0.12</td>
<td>0.767</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.890</td>
<td>1.412</td>
<td>0.686</td>
<td>0.487</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.
also greatly reduced at higher temperatures. The pre-
larviposition, larviposition periods and adult longevity
of the female were also significantly affected by
temperature variations and were longer at lower
and adult longevity
temperatures and decreased with the increasing
temperature. Wang et al. (1997) and Asante et al. (1991)
observed similar phenomena in *Eriosoma lanigerum*
(Hausmann) (Homoptera: Aphididae) and *Aphis nasturtii*
Kaltenbach, (Homoptera: Aphididae), with the duration
of the life-span of the female being extended at low
temperatures. Oganesy and Babayan (1979) observed
a similar effect of low winter temperature on the
development and population density of the first
generation of *P. comstocki*.

The progeny reared at different temperatures showed
that, temperature had a significant effect on the sex
ratio, with a bias towards females at all tested tempera-
tures except at 28 °C where the reverse occurred as was
also observed by El-Minshawy et al. (1974). The reason
for this deviation is unknown. In contrast to observations
by Panis (1969), there was no significant effect of the
host plants on sex ratio; however temperature does
have an effect on the sex ratio.

### Table 11. Effect of four different temperatures and three host plants on the larviposition period of *P. longispinus*

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>33.92 ± 0.22</td>
<td>26.03 ± 0.94</td>
<td>20.89 ± 0.13</td>
<td>12.05 ± 0.16</td>
<td>1.475</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>bA</td>
<td>n = 10</td>
<td>aB</td>
<td>n = 10</td>
<td>aC</td>
<td>n = 10</td>
<td>aD</td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>35.78 ± 0.33</td>
<td>26.93 ± 0.20</td>
<td>20.11 ± 0.16</td>
<td>10.52 ± 0.15</td>
<td>0.208</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td>aB</td>
<td>n = 10</td>
<td>bC</td>
<td>n = 10</td>
<td>bD</td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>35.55 ± 0.40</td>
<td>26.91 ± 0.44</td>
<td>19.83 ± 0.22</td>
<td>10.05 ± 0.14</td>
<td>0.970</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td>aB</td>
<td>n = 10</td>
<td>bC</td>
<td>n = 10</td>
<td>cD</td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>0.998</td>
<td>1.874</td>
<td>0.536</td>
<td>0.459</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

### Table 12. Effect of four different temperatures and three host plants on the life-span of female *P. longispinus*

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>97.63 ± 0.42</td>
<td>78.09 ± 0.86</td>
<td>70.41 ± 0.43</td>
<td>55.53 ± 0.26</td>
<td>1.603</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>bA</td>
<td>n = 10</td>
<td>bB</td>
<td>n = 10</td>
<td>bC</td>
<td>n = 10</td>
<td>aD</td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>106.05 ± 0.60</td>
<td>81.00 ± 0.79</td>
<td>72.33 ± 0.04</td>
<td>51.20 ± 0.50</td>
<td>1.642</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td>aB</td>
<td>n = 10</td>
<td>aC</td>
<td>n = 10</td>
<td>cD</td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>106.39 ± 0.89</td>
<td>82.10 ± 0.55</td>
<td>72.08 ± 0.35</td>
<td>53.40 ± 0.16</td>
<td>1.674</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>n = 10</td>
<td>aB</td>
<td>n = 10</td>
<td>aC</td>
<td>n = 10</td>
<td>bD</td>
</tr>
<tr>
<td>LSD 5% value</td>
<td>2.044</td>
<td>2.302</td>
<td>1.033</td>
<td>0.994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P 5% value</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in columns followed by same lowercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P ≤ 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.
Table 13. Effect of four different temperatures and three host plants on the fecundity of *P. longispinus* (Mean fecundity ± S.E.)

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
<th>LSD 5% value</th>
<th>P 5% value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>154.48 ± 10.1</td>
<td>181.82 ± 26.7</td>
<td>188.22 ± 34.0</td>
<td>29.46 ± 4.2</td>
<td>66.73</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>aA</td>
<td>aA</td>
<td>aA</td>
<td>aB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>133.30 ± 13.8</td>
<td>158.42 ± 32.5</td>
<td>182.99 ± 12.9</td>
<td>32.40 ± 5.3</td>
<td>56.95</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>aA</td>
<td>aA</td>
<td>aB</td>
<td>cB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>133.30 ± 12.6</td>
<td>158.46 ± 29.8</td>
<td>180.54 ± 22.6</td>
<td>28.20 ± 4.4</td>
<td>59.46</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>aA</td>
<td>aA</td>
<td>aA</td>
<td>bB</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD 5% value | 37.86 | 91.69 | 75.99 | 14.38 |

P 5% value | > 0.05 | > 0.05 | > 0.05 | > 0.05 |

Means in columns followed by same lowercase letters are not significantly different at P = 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); Means in rows followed by same uppercase letters are not significantly different at P = 0.05 (ANOVA one-way, LSD 5% value, Fisher’s test); n represents the total number of individuals.

---

Table 14. Effect of four different temperatures and three host plants on the sex of ratio of *P. longispinus*

<table>
<thead>
<tr>
<th>Host plants</th>
<th>20 °C</th>
<th>24 °C</th>
<th>28 °C</th>
<th>32 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. moschata</em> Butternut</td>
<td>1 : 1.87</td>
<td>1 : 1.57</td>
<td>1 : 0.94</td>
<td>1 : 1.17</td>
</tr>
<tr>
<td>m = 330 f = 620</td>
<td>m = 460 f = 720</td>
<td>m = 510 f = 480</td>
<td>m = 60 f = 70</td>
<td></td>
</tr>
<tr>
<td>χ² = 88.53</td>
<td>χ² = 60.21</td>
<td>χ² = 0.91</td>
<td>χ² = 0.77</td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.340</td>
<td>P = 0.380</td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Desiree</td>
<td>1 : 1.89</td>
<td>1 : 1.52</td>
<td>1 : 0.92</td>
<td>1 : 1.17</td>
</tr>
<tr>
<td>m = 280 f = 530</td>
<td>m = 400 f = 610</td>
<td>m = 490 f = 450</td>
<td>m = 60 f = 70</td>
<td></td>
</tr>
<tr>
<td>χ² = 77.16</td>
<td>χ² = 43.66</td>
<td>χ² = 1.702</td>
<td>χ² = 0.77</td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.192</td>
<td>P = 0.380</td>
<td></td>
</tr>
<tr>
<td><em>S. tuberosum</em> var. Cara</td>
<td>1 : 1.93</td>
<td>1 : 1.46</td>
<td>1 : 0.92</td>
<td>1 : 1.20</td>
</tr>
<tr>
<td>m = 270 f = 520</td>
<td>m = 410 f = 600</td>
<td>m = 490 f = 450</td>
<td>m = 50 f = 60</td>
<td></td>
</tr>
<tr>
<td>χ² = 95.61</td>
<td>χ² = 35.70</td>
<td>χ² = 1.702</td>
<td>χ² = 0.910</td>
<td></td>
</tr>
<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P = 0.192</td>
<td>P = 0.340</td>
<td></td>
</tr>
</tbody>
</table>

χ² test used for the analysis of female sex ratio at 5% level of significance from 1:1 ratio; m represents total number of males and ‘f’ represents total number of females.

Johansson (1964) had stated that the quality of the food influences egg production in many phytophagous insects and thus the egg output may vary with plant species even if they belong to same genus. In the course of the present studies, no significant effect of host plant was observed on fecundity or sex ratio.

During the course of the present studies, the host plants seemed to have no effect on the lower threshold and thermal constants of the male *P. longispinus*, whereas they did have an effect on the lower threshold and thermal constant of the female. The female’s lower threshold temperatures were lower than that of the male on all hosts, suggesting the female’s greater tolerance and survival at low temperatures. It also suggested an early start of development in females. The host effect on *T₁* was most pronounced in females on butternut squash. Lower threshold temperatures of both male and female were lowest on *C. moschata* as compared to the two *S. tuberosum* varieties.

Based upon these data, *P. longispinus* can best be produced in the laboratory on *C. moschata*, at between 20 °C to 28 °C.

References

Bionomics and population growth statistics of...


Laboratory Evaluation of Toxic and Repellent Properties of Dracaena arborea Against Sitophilus zeamais and Callosobruchus maculatus

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(received August 11, 2010; revised September 5, 2011; accepted September 17, 2011)

Abstract. Laboratory evaluation of ethanolic extract of Dracaena arborea leaves partitioned between equal volumes of chloroform, ethyl acetate, n-hexane and n-butanol to obtain various fractions was carried out to assess contact toxicity on filter paper and by topical application, toxicity of extracts applied on grains and repellent action against Sitophilus zeamais (Mots.) and Callosobruchus maculatus Fabricius on stored grains. Insects were exposed to both treated and untreated surfaces and mortality was scored at different times after treatment. Results obtained from the study showed the extract fractions causing significant mortality of both insects exposed to treatments. A contact toxicity of over 80% was recorded against S. zeamais from ethyl acetate fraction. Similarly, 100% mortality was recorded against C. maculatus from ethyl acetate fraction after 96 h of treatment. Results obtained from grain treatment produced a significant mortality of over 60% against C. maculatus from n-hexane fraction while the aqueous fraction significantly killed S. zeamais by over 15% compared with the control treatment. A strong repellent action was evoked against S. zeamais while moderate action was recorded against C. maculatus. An overall repellency of 40% and 24% was recorded from various extract fractions against S. zeamais and C. maculatus, respectively. The results obtained suggest a promising alternative to synthetic insecticides and the incorporation of D. arborea into storage pest management system is advised.

Keywords: toxicity, Sitophilus zeamais, Dracaena arborea, stored grains, extract fractions maculatus, Callosobruchus, repellency

Introduction

Grains in storage are subject to insect pest infestation if left without protectants and the consequence is serious threat to food security. There are records of enormous losses of up to 20 – 30% of stored products arising from insect pest attacks (Obeng-Ofori, 1995). Insect pests associated with stored products are beetles and moths with the common ones being Sitophilus zeamais and Callosobruchus maculatus attacking stored maize and cowpea, respectively. Over the years farmers relied upon the use of synthetic chemicals for the control of storage pests. The attendant consequences of the use of synthetic chemicals have been widely reported (Udo et al., 2004; Obeng-Ofori, et al., 1997; White, 1995; Shaaya et al., 1991; Zettler and Cuperus, 1990). Alternative control measures are still being sought out and one of them is the use of plant materials and plant products. Botanical treatments have been found to be safer to the environment, broad spectrum in action with little or no hazards to man and other animals. One such plant is Dracaena arborea which is a tropical plant growing up to 15 m high and 2.5 m girth with long, broad and blade-like leaves, and commonly utilized as boundary plants for demarcation of plots of land in the Southeastern parts of Nigeria. Okunji et al. (1996) has reported that anti-fungal and anti-parasitic compounds are present in D. arborea.

In this study, ethanolic extract fractions of D. arborea were screened for insecticidal activities against S. zeamais and C. maculatus. Extract fractions have the advantage of economic use of the plant material especially where the plant is not readily available and could also be used for such tests like repellency and tropical bioassay.

Materials and Methods

Insects. S. zeamais and C. maculatus were collected from infested stock of grains at Etaha Iam market, Uyo, Nigeria and reared on sterilized maize and cowpea grains, respectively. After two weeks of oviposition, the parent adults were removed by sieving while emerging progeny were recultured and used for various bioassays.

Preparation of extract fractions. Leaves of D. arborea with voucher number UUH (84a), Faculty of Pharmacy, University of Uyo, were collected from the wild in Uyo,
Nigeria and air dried in the laboratory for five days. Dried leaves were ground and 1 kg soaked in 90% ethanol in glass jars and left to stand in the dark for 72 h to prevent possible volatilization of active principles. Filtration was carried out and ethanol was evaporated using rotary evaporator. The residue obtained was dissolved in 60 mL of distilled water and subjected to partitioning using equal volumes of n-hexane, chloroform, ethyl acetate and n-butanol to obtain different extract fractions used for the various bioassays.

**Contact toxicity on filter paper.** Whatman No. 1 filter paper (10.9 cm diameter) placed in glass petri dishes was impregnated with 200 µL/mL of each extract fraction and 10 adults each of *S. zeamais* and *C. maculatus* were introduced into each dish, respectively. Control treatments had the filter paper impregnated with distilled water and mortality was recorded after 24 h and up to 96 h. Insects were assumed dead on failure to respond to three probes with a blunt dissecting probe after a 5-min recovery time.

**Contact toxicity by topical application.** Forty adult insects, each of *S. zeamais* and *C. maculatus* were chilled in the deep freezer for three minutes to reduce their activity (mobility) and transferred into petri dishes. One micro litre of each extract fraction was applied using a micro pipette to the dorsal surface of the thorax of each insect taken individually. Distilled water was applied to control insects and each treatment was replicated four times. Mortality was recorded after one hour for 48 h.

**Toxicity of extracts applied on grains.** Toxicity of the extract fractions applied on maize and cowpea grains, respectively, against *S. zeamais* and *C. maculatus* was tested by applying 200 mg/kg to 50 g of grains in a 200 mL plastic cup. Ten pairs of each insect species were introduced into treated and control grains. Mortality was recorded after 24 h and up to 96 h after treatment with insects presumed dead on failure to respond to three probes with a blunt dissecting probe.

**Repellency test.** Repellent effect of the extract fractions was tested using the method described by Obeng-Ofori *et al.* (1997). Whatman No.1 filter paper were cut into halves with one half being treated with the extract fractions while the other half was treated with distilled water to serve as the control. After being air dried for one hour, the full discs were re-made by attaching treated and control halves with paper tapes. Each filter paper was placed in a petri dish and ten weevils introduced into the center of the paper. Number of weevils present on control (Nc) and treated (Nt) strips were recorded after 30 min and up to 48 h. Percent, repellency was computed as:

\[
PR = \frac{N_c - N_t}{N_c + N_t} \times 100\%
\]

Where:

- \(PR\) = percent repellency
- \(N_c\) = number of insects present on control strip
- \(N_t\) = number of insects present on treated strip.

### Table 1. Contact toxicity of extract fractions of *D. arborea* applied on filter paper against *S. zeamais* and *C. maculatus*

<table>
<thead>
<tr>
<th>Extract fractions (200 µL/mL)</th>
<th>Percent mortality after treatment</th>
<th>Control</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>7 ± 0.50</td>
<td>17 ± 0.95</td>
<td>28 ± 1.25</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0 ± 0.00</td>
<td>17 ± 0.95</td>
<td>33 ± 1.73</td>
</tr>
<tr>
<td>n-hexane</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>8 ± 0.50</td>
</tr>
<tr>
<td>n-butanol</td>
<td>0 ± 0.00</td>
<td>7 ± 0.50</td>
<td>8 ± 0.50</td>
</tr>
<tr>
<td>Aqueous</td>
<td>7 ± 0.50</td>
<td>7 ± 0.50</td>
<td>23 ± 0.81</td>
</tr>
<tr>
<td><em>C. maculatus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>13 ± 0.81</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>n-hexane</td>
<td>7 ± 0.50</td>
<td>7 ± 0.50</td>
<td>23 ± 0.81</td>
</tr>
<tr>
<td>n-butanol</td>
<td>0 ± 0.00</td>
<td>7 ± 0.50</td>
<td>18 ± 0.95</td>
</tr>
<tr>
<td>Aqueous</td>
<td>7 ± 0.50</td>
<td>7 ± 0.50</td>
<td>13 ± 0.81</td>
</tr>
</tbody>
</table>

Means = (SEM) of four replicates of 20 insects each; LSD test = (P < 0.05); NS = Non significant.
Table 2. Toxicity of extract fractions of *D. arborea* applied topically against *S. zeamais* and *C. maculatus*

<table>
<thead>
<tr>
<th>Extract fractions</th>
<th>Percent mortality after treatment</th>
<th>Control</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td>20 μg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-hexane</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>5 ± 0.50</td>
</tr>
<tr>
<td>n-butanol</td>
<td>70 ± 1.73</td>
<td>75 ± 1.89</td>
<td>75 ± 1.89</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>10 ± 0.57</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>15 ± 0.95</td>
<td>35 ± 0.95</td>
<td>40 ± 0.82</td>
</tr>
<tr>
<td>Aqueous</td>
<td>95 ± 0.50</td>
<td>100 ± 0.00</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td><em>C. maculatus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-hexane</td>
<td>0 ± 0.00</td>
<td>10 ± 1.00</td>
<td>15 ± 1.38</td>
</tr>
<tr>
<td>n-butanol</td>
<td>95 ± 0.50</td>
<td>95 ± 0.50</td>
<td>95 ± 0.50</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
<td>0 ± 0.00</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>95 ± 0.50</td>
<td>100 ± 0.00</td>
<td>100 ± 0.00</td>
</tr>
<tr>
<td>Aqueous</td>
<td>95 ± 0.50</td>
<td>100 ± 0.00</td>
<td>100 ± 0.00</td>
</tr>
</tbody>
</table>

Means = (SEM) of four replicates of 10 insects each; LSD test = (P < 0.05); NS = Non significant.

Table 3. Percent mortality of *S. zeamais* and *C. maculatus* exposed to grains treated with extract fractions of *D. arborea*

<table>
<thead>
<tr>
<th>Extract fractions (200 mg/kg)</th>
<th>Percent mortality after treatment</th>
<th>Control</th>
<th>LSD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
<td>72 h</td>
</tr>
<tr>
<td><em>S. zeamais</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-hexane</td>
<td>0 ± 0.00</td>
<td>5 ± 0.81</td>
<td>5 ± 0.50</td>
</tr>
<tr>
<td>Chloroform</td>
<td>0 ± 0.00</td>
<td>5 ± 0.57</td>
<td>5 ± 0.50</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>0 ± 0.00</td>
<td>5 ± 0.57</td>
<td>5 ± 0.81</td>
</tr>
<tr>
<td>n-butanol</td>
<td>0 ± 0.00</td>
<td>5 ± 1.00</td>
<td>5 ± 1.00</td>
</tr>
<tr>
<td>Aqueous</td>
<td>5 ± 1.15</td>
<td>5 ± 1.15</td>
<td>15 ± 1.29</td>
</tr>
<tr>
<td><em>C. maculatus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-hexane</td>
<td>10 ± 2.16</td>
<td>35 ± 2.71</td>
<td>40 ± 2.60</td>
</tr>
<tr>
<td>Chloroform</td>
<td>10 ± 2.87</td>
<td>30 ± 3.77</td>
<td>35 ± 4.69</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>5 ± 1.41</td>
<td>25 ± 2.44</td>
<td>30 ± 2.98</td>
</tr>
<tr>
<td>n-butanol</td>
<td>10 ± 2.06</td>
<td>30 ± 1.25</td>
<td>35 ± 1.82</td>
</tr>
<tr>
<td>Aqueous</td>
<td>15 ± 1.91</td>
<td>20 ± 2.50</td>
<td>20 ± 2.50</td>
</tr>
</tbody>
</table>

Means = (SEM) of four replicates of 20 insects each; LSD test = (P < 0.05); NS = Non significant.

Results and Discussion

Contact toxicity on filter paper. Results of the toxic effect of extract fractions of *D. arborea* applied on filter paper against *S. zeamais* and *C. maculatus* is shown in Table 1. There was a contact mortality of 80% from the ethyl acetate fraction while *n*-hexane and chloroform fractions recorded a mortality of 45% and 15%, respectively after 96 h of treatment against *S. zeamais*. The aqueous fraction also significantly induced a mortality of 60% in *S. zeamais* after 96 h of insect exposure to treated filter papers.

Contact toxicity by topical application. Significant insect mortality was recorded from topical application of the different extract fractions against the two insect species (Table 2). Ethyl acetate and the aqueous fractions were observed to induce 100% mortality of the insects after 96 h treatment.

Toxicity of the extract fractions applied on grains. Different levels of toxicity of the extract fractions were observed against *S. zeamais* and *C. maculatus* after 96 h of exposure to treated grains (Table 3). The *n*-hexane fraction recorded a mortality of 60% against *C. maculatus* while the aqueous fraction produced 15% mortality in *S. zeamais*. 
Table 4. Percent repellency (PR) values for extract fractions of *D. arborea* tested against *S. zeamais* and *C. maculatus*

<table>
<thead>
<tr>
<th>Extract fraction</th>
<th>Mean percent repellency</th>
<th><em>S. zeamais</em></th>
<th><em>C. maculatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>n</em>-hexane</td>
<td>38</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Chloroform</td>
<td>44</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>44</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td><em>n</em>-butanol</td>
<td>25</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Overall PR</td>
<td>40</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>11.04</td>
<td>17.46</td>
<td></td>
</tr>
</tbody>
</table>

Mean = (SEM) of four replicates of 10 insects each; LSD test = (P <0.01).

**Repellency test.** Table 4 shows the repellent effect of the various extract fractions against the two insect species with *S. zeamais* being significantly repelled by about 40% while *C. maculatus* had an overall repellency of 24%. The individual extract fractions showed the aqueous fraction repelling *S. zeamais* and *C. maculatus* by 50% and 33%, respectively. Ethyl acetate fraction also repelled *S. zeamais* and *C. maculatus* by about 44% and 25%, respectively.

The significant mortality of *S. zeamais* in filter paper impregnated with the extract fractions indicated the presence of residual toxic principle in *D. arborea*. Some secondary metabolites identified as manniSpirostan A and spiroinazol A have been identified in *D. arborea* (Okunji et al., 1996). *S. zeamais* was more susceptible to filter paper treatment of the extract fractions probably because of their close contact with treated surfaces, compared to *C. maculatus* which were observed to be very mobile and hanging on the underside of perforated petri dish covers. This observation has also been reported against these insect pests when tested against methanol extract of *Zanthoxylum xanthoxyloides* Lam. (Udo et al., 2004, Ofuya and Okoku, 1994).

The extract fractions applied topically against *S. zeamais* and *C. maculatus* induced significant insect mortality of the two insect species and dead beetles were found having their metathoracic wings stretched outside the elytra thus suggesting contact toxicity and not due to ingestion of treated grains (Obeng-Ofori et al., 1997). Topical application therefore, facilitated the direct contact of toxicants or active ingredients in *D. arborea* with insects’ bodies (Adedire and Ajayi, 1996). Udo and Eipidi (2009) reported similar results on the efficacy of ethanolic extract fractions of *Ricinodendron heudelotii* (Baill) Pierre ex Pax against the two insect pests.

*C. maculatus* was more susceptible to contact action of the extract fractions applied on grains than *S. zeamais* probably because of the absence of hard and highly sclerotized thoracic cuticle as in their *Sitophilus* counterpart. Similar results were obtained by other researchers (Epidi et al., 2008; Owusu et al., 2007), who reported on the efficacy of the leaf powder of *D. arborea* against the two insect species.

The significant repellent action observed against *S. zeamais* and *C. maculatus* is noteworthy as this could prevent the insects from settling, feeding and laying eggs into grains protected with extracts of *D. arborea*. The result obtained from this study recommends the incorporation of *D. arborea* in storage pest management systems particularly in Africa. It could become an important supplement or alternative to synthetic insecticides.

**Acknowledgement**

The author is grateful to Dr. (Mrs.) Uduak Essiet, a Botanist in the University of Uyo for identifying the plant specimen.

**References**


Evaluation of Neutralizing Efficacy of Acorus calamus and Withania somnifera Root Extracts Against Bangarus caeruleus Venom

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†Department of Microbiology, Ayya Nadar Janaki Ammal College (Autonomous), Sivakasi, India

(received February 14, 2011; revised May 19, 2011; accepted June 18, 2011)

Abstract. Acorus calamus and Withania somnifera root extracts when tested against Bangarus caeruleus venom, both the plant extracts neutralized various pharmacological activities induced by B. caeruleus venom. About 0.12 mg of A. calamus and 0.15 mg of W. somnifera root extracts completely neutralized the lethal activity of 2LD₅₀ of B. caeruleus venom. Various pharmacological activities like haemorrhagic, coagulance, edematous, fibrinolytic and phospholipase activities were effectively neutralized by both the extracts.

Keywords: venoms, plant extracts, lethality, Acorus calamus, Withania somnifera, Bangarus caeruleus

Introduction

Snake bite constitutes major health problem in India. It is estimated that in the annual global burden of 4,21,000 envenomations and 20,000 deaths, India alone contributes 81,000 envenomations and 11,000 deaths. Snake bites are seen often among agricultural workers and among those going to the forests. Based on the above statistics, it appears that every 10 sec, one individual is envenomed and one out of four dies due to snake bite (TNHSP, 2008). The male/female ratio among the victims is approximately 3:2. Many deaths occur before the victim reaches the hospital. Common poisonous snakes found in India are Cobra (Naja naja), Krait (Bangarus caeruleus), Russell’s viper (Daboia russelli) and saw scaled viper (Echis carinatus). Anti-venom immunotherapy is the only specific treatment against snake venom envenomation (Bawaskar, 2004). Antiserum development in animals is time consuming, expensive and requires ideal storage condition. There are various side effects of antivenom drugs such as anaphylactic shock, pyrogen reaction and serum sickness. Most of these symptoms may be due to the action of high concentrations of non-immunoglobulin proteins present in the commercially available horse polyvalent antivenom. Over the years many attempts have been made for the development of snake venom antagonists especially from plants sources. Extracts from plants have been used by traditional healers, especially in tropical areas where there are plentiful plant resources, in therapy for snake bite for a long time (Gomes et al., 2010; Daduang et al., 2005). There have been many attempts to study these plants to assess their effectiveness (Otero et al., 2000; Houghton and Osibogun, 1993). India has a rich tradition of the usage of medicinal plants. Several snakebite antidotes of plant origin were recommended in old drug recipes for the treatment of snakebite (Alam and Gomes, 2003). The plants Acorus calamus or sweet flag (common name) is a valuable medicinal plant found almost through out India. It possesses anti-inflammatory activity, which is evident from a number of studies. Withania somnifera Dunal (Ashwagandha) belonging to the family Solanaceae has been used in several indigenous Ayurvedic drug preparations for maintaining health as well as for several disease conditions. In herbal medicine, ashwagandha has been traditionally used as alternative, aphrodisiac, nerve tonic, rejuvenator and in inflammation, arthritis and a number of other disorders. Methanolic extracts of Andrographis paniculata and Aristolochia indica possess potent snake venom neutralizing capacity and could potentially be used for therapeutic purposes in case of snakebite envenomation (Meenatchisundaram et al., 2009a). Aqueous extract of Mimosa pudica root possesses compounds, which inhibit the activity of Naja naja and B. caeruleus venoms (Meenatchisundaram et al., 2009b). The aqueous extract of Mucuna pruriens inhibits the activity of N. naja and B. caeruleus venom (Meenatchisundaram et al., 2010). The present investigation explored the extraction and neutralizing potential of A. calamus and W. somnifera root extracts which were tested against B. caeruleus venom in vivo and in vitro.

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Materials and Methods

Venom and experimental animals. Freeze-dried snake venom powder of *B. caeruleus* was obtained from Irula’s Snake Catchers Industrial Co-operative Society Limited, Chennai and was stored at 4 °C. Male inbred Swiss albino mice 18-20 g were used for the studies. The Institutional Animal Ethics Committee clearance at the Institute of Vector Control and Zoonoses, Hosur, was obtained to conduct the experiment.

Medicinal plants and preparation of extracts. *A. calamus* and *W. somnifera* plants were obtained from Nehru Herbal Gardens, Coimbatore and the extracts were prepared as stated by Uhegbu et al. (2005) using distilled water as the solvent. 20 g of powdered sample of the herb was extracted by soaking in 180 mL of distilled water in a beaker, stirred for about 6 min and left overnight. Thereafter, the solution was filtered using filter paper Whatman No. 1 and the extract was evaporated to dryness under reduced pressure below 40 °C. The plant extract was expressed in terms of dry weight.

Plant lethality test. The lethal effect of plant extracts was determined by the method of Hung et al. (2004). Five groups of six mice each were used; each group was treated with increasing concentration of plant extract at a final volume of 0.5 mL by intravenous administration through the tail vein. LD₅₀ was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 h of injection.

In vivo assessment of venom toxicity and anti-venom effect of plant extracts. Lethal toxicity of *B. caeruleus* venom. The median lethal dose (LD₅₀) of *B. caeruleus* venom was determined according to the method developed by Theakston and Reid (1983). Various concentrations of venom in 0.2 mL of physiological saline was injected into the tail vein of mice (18-20 g), using groups of 3-5 mice for each venom dose. The LD₅₀ was calculated with the confidence limit at 50% probability by the analysis of deaths occurring within 24 h of venom injection. The anti-lethal potentials of *A. calamus* and *W. somnifera* root extracts were determined against 2LD₅₀ of *B. caeruleus* venom. Different amounts of plant extracts (µL) were mixed with 2LD₅₀ of venom sample and incubated at 37 °C for 30 min and then injected intravenously. 3-5 mice were used for each antivenom dose. Control mice received the same amount of venom without antivenom (plant extracts). The median effective dose (ED₅₀) was calculated from the number of deaths within 24 h of injection of the venom/antivenom mixture. The ED₅₀ was expressed as µL antivenom/mouse and calculated by probit analysis.

Edema-forming activity of *B. caeruleus* venom. Minimum edema-forming dose (MED) of *B. caeruleus* venom was determined by the method of Lomonte et al. (1993). Group of four mice were injected subcutaneously in the right footpad with various amounts of venom (0.25 µg-10 µg) dissolved in 50 µL of phosphate-buffered saline (PBS), pH 7.2. The left footpad received 50 µL of PBS alone (control). Edema was calculated as percentage increase in the thickness of the right foot injected with venom compared to the left foot. The thickness of each footpad was measured every 30 min after venom injection with a low-pressure spring caliper (Rojas et al., 2005). Minimum edema-forming dose (MED) was the venom dose that induced 30% edema within 6 h of venom injection when compared to control. The ability of *A. calamus* and *W. somnifera* root extracts in neutralizing the edema-forming activity was determined by pre-incubating constant amount of venom and various dilutions of *A. calamus* and *W. somnifera* root extracts and incubating for 30 min at 37 °C. Then, groups of four mice (18-20 g) were injected subcutaneously in the right footpad with 50 µL of the mixtures, containing venom/plant extracts, whereas the left footpad received 50 µL of PBS alone. Control mice were injected with venom in the right footpad and 50 µL of PBS in the left footpad. Edema evaluated one hour after injection as described by Yamakawa et al. (1976) and was expressed as the percentage increase in thickness of the right footpad compared to the right footpad of the control mice.

Haemorrhagic activity. Minimum haemorrhagic dose (MHD) of *B. caeruleus* venom was determined by the described method of Theakston and Reid (1983). Minimum haemorrhagic dose was defined as the least amount of venom which when injected intradermally (i.d.) into mice results in a haemorrhagic lesion of 10 mm diameter in 24 h. Neutralization of the haemorrhagic activity was estimated by mixing a fixed amount of venom with different amounts of *A. calamus* and *W. somnifera* root extracts. The plant extract-venom mixture was incubated at 37 °C for 1 h and 0.1 mL of the mixture was injected intradermally into mice. The haemorrhagic lesion was estimated after 24 h.

In vitro assessment of venom toxicity and anti-venom efficacy. Phospholipase activity. Phospholipase A2 activity was measured using an indirect haemolytic
assay on agarose-erythrocyte-egg yolk gel plate by the method described by Gutierrez et al. (1988). Increasing concentrations of B. caeruleus venom (μg) was added to 3 mm wells in agarose gels (0.8% in PBS, pH 8.1) containing 1.2% sheep erythrocytes, 1.2% egg yolk as a source of lecithin and 10 mM CaCl₂. Slides were incubated at 37 °C overnight and the diameters of the haemolytic halos were measured. Control wells contained 15 μL of saline. The minimum indirect haemolytic dose (MIHD) corresponds to a concentration of venom, which produces a haemolytic halo of 11 mm diameter. The efficacy of A. calamus and W. somnifera root extracts in neutralizing the phospholipase activity was assessed by mixing the same amount of venom (μg) with different amounts of plant extracts (μL) and incubated for 30 min at 37 °C. Then, aliquots of 10 μL of the mixtures were added to wells in agarose-egg yolk-sheep erythrocyte gels. Control samples contained venom without plant extract. Plates were incubated at 37 °C for 20 h. Neutralization was expressed as the ratio mg antibodies/mg venom able to reduce by 50% the diameter of the haemolytic halo as compared to the effect induced by venom alone.

**Procoagulant activity.** The procoagulant activity was assessed according to the method described by Theakston and Reid (1983) modified by Laing et al. (1992). Various amounts of venom dissolved in 100 μL PBS (pH 7.2) was added to human citrated plasma at 37 °C. Coagulation time was recorded and the minimum coagulant dose (MCD) was determined as the venom concentration, which induced clotting of plasma within 60 sec. Plasma incubated with PBS alone served as control. In neutralization assays, constant amount of venom was mixed with various dilutions of plant extracts. The mixtures were incubated for 30 min at 37 °C. Then 0.1 mL of the mixture was added to 0.3 mL of citrated plasma and the clotting time recorded. In control tubes, plasma was incubated with either venom alone or plant extract alone. Neutralization was expressed as effective dose (ED), defined as the ratio μL antivenom (plant extracts)/mg venom at which the clotting time increased three times as compared to the clotting time of plasma incubated with two MCD of venom alone.

**Fibrinolytic activity:** A modified plaque assay was used (Laing et al., 1992). The minimum fibrinolytic concentration was defined as the concentration of venom that induced a fibrinolytic halo of 10 mm diameter. Neutralization experiments were performed by incubating constant amount of venom with varying amounts of A. calamus and Withania somnifera plant extracts at 37 °C for 1 h. After incubation, the mixture was applied to the wells in the plaque. After 18 h of incubation at 37 °C, fibrinolytic halos were measured.

**Double immunodiffusion.** The interaction of A. calamus and W. somnifera root extracts against B. caeruleus venom was evaluated using double immunodiffusion technique (Ouchterlony, 1953). Agar (1 g) was boiled in 100 mL water in microwave oven. The agar solution was then allowed to cool to 45 °C and then 3.5 mL of it was pipetted onto a precoated slide. The slide was left to solidify at room temperature after which four holes were punched out using a gel puncher. The plugs of agar were removed from each well with a Pasteur pipette attached to a vacuum line. The test was conducted by pipetting 20 μL of B. caeruleus venom (10 mg/mL) and 20 μL of crude plant extract (100 mg/mL) into wells 1 and 2, respectively, followed by incubation overnight at room temperature in a humid chamber. The slide was examined for precipitation lines.

**Statistical analysis.** Statistical evaluation was performed using XL stat 2008 and SPSS 10 Softwares. P < 0.005 was considered statistically significant.

**Results and Discussion**

The lethal toxicity (LD₅₀) of the venom and of the two root extracts was assessed using 18 g, Balb/c strain mice. About 3 μg of the venom was found to be LD₅₀ for 18 g mice. About 1.2 mg and 1.4 mg was found to be the lethal toxicity (LD₅₀) for A. calamus and W. somnifera root extracts, respectively. Neutralization of lethality was carried out by preincubating constant amount of venom with various dilutions of the two root extracts prior to injection. It was found that 0.12 mg of A. calamus and 0.15 mg of W. somnifera root extracts were able to completely neutralize the lethal activity of 2LD₅₀ of the B. caeruleus venom (Table 1, Fig. 1). In edema forming activity, 2 μg of the venom induced edema formation within 3 h which is considered as 100% activity. The edema was reduced down to 20% when 4000 μL of plant extracts/mg venom was given. There was no further reduction in the percentage of edema even when there was an increase in antivenom dose (Fig. 2). In the case of haemorrhagic activity, 2 μg of venom produced a haemorrhagic spot of 10 mm diameter (MHD). Both plant extracts were able to neutralize the haemorrhage induced by the venom. In phospholipase activity (PLA₂), 10 μg of B. caeruleus venom was able to produce 11 mm diameter haemolytic
halo, which is considered to be 1U (U/10 μg). Both root extracts were capable of inhibiting PLA2 dependent haemolysis of sheep RBC’s induced by the venom in a dose dependent manner (Table 2). Minimum coagulant dose (MCD) was determined and it was found that 40 μg of the viper venom clotted 0.3 mL human citrated plasma within 60 sec. In the neutralization assay, the absence of clot formation shows the neutralizing ability of both the plant extracts. High concentration of venom caused rapid clotting that required very high concentration of anti-venom to neutralize. The fibrinolytic effect was effectively antagonized by both the plant extract. The ED50 of *A. calamus* and *W. somnifera* root extracts against the venom were found to be 0.4 and 0.7 mg, respectively. In Double Immunodiffusion test, a visible precipitin line was form between the venom and the plant extracts showing that plant extracts possess potent snake venom neutralizing compounds.

Snakebites are encountered all over the country with a rural/urban ratio of 9:1. They are more common during monsoon and post monsoon seasons. Many of the susceptible populations are poor living below poverty line, living in rural areas with less access to health care. About 10% of snakebite deaths are among the victims who come to the hospital and about 90% die outside, having under-gone other remedies like mantra, magic, and so on. The hospital stay varies from 2 to 30 days, the median being 4 days. The in-hospital mortality varies from 5 to 10%, and the causes are acute renal failure, respiratory failure, sepsis, bleeding and others (TNHSP, 2008). Antivenom against snakes bites are lacking in the rural areas of coastal region. The most common and effective method of treating snake bite victims is through administration of antivenom, a serum made from the venom of the snake (Gomes et al., 2010). Although, use of plants against the effects of snakes bite has been long recognized, more scientific attention has been given during the last 20 years. In our present study we assessed the antivenom potential of *A. calamus* and *W. somnifera* root extracts against *B. caerules* venom. The neutralization ability of snake antivenoms is still assessed by the traditional in vivo lethality assay (minimum effective dose ED50), comparable to those used for bacterial antitoxins, usually performed in mice

### Table 1. Neutralization of *B. caerules* venom induced lethality by *A. calamus* and *W. somnifera* root extracts

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Concentration of <em>B. caerules</em> venom (μg)</th>
<th>Neutrization of venom by plant extracts (ED50 in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acorus calamus</em></td>
<td>6 (2LD50)</td>
<td>0.12 mg</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>6 (2LD50)</td>
<td>0.15 mg</td>
</tr>
</tbody>
</table>

### Table 2. Phospholipase activity of *B. caerules* venom and its neutralization by *A. calamus* and *W. somnifera* root extracts

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Dose of <em>B. caerules</em> venom (μg)</th>
<th>Neutralization of venom by plant extracts (ED50 in mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. calamus</em></td>
<td>10 (1 Unit)</td>
<td>0.11 mg</td>
</tr>
<tr>
<td><em>W. somnifera</em></td>
<td>10 (1 Unit)</td>
<td>0.14 mg</td>
</tr>
</tbody>
</table>

![Logistic regression of Survive by Log(Dose (μL))](image1.png)

**Table 1.** Dose response curve for neutralization of lethality by *A. calamus* and *W. somnifera* root extracts against *B. caerules* venom.
Plant Neutralizing Efficacy for Snake Venom

![Graphs showing thickness and percentage with IgY and IgY concentration levels]  

**Fig. 2.** Neutralization of edema induced by *B. caeruleus* venom by (A) *A. calamus* and (B) *W. somnifera* root extracts in experiments with pre-incubation. Results presented as mean ± SE (N=3). P<0.005 at all antivenoms/venom ratios.

(WHO, 2004). Herbal compounds that possess anti-snake venom activity are tested in experimental animal models through administering in different ways: (A) venom-herbal compounds mixed together, (B) herbal compounds followed by venom and (C) venom followed by herbal compounds (Gomes et al., 2010). In the present preliminary work the anti venom properties of plant extracts were tested by pre-incubation method venom-herbal compounds mixed together and tests for various pharmacological activities like lethality, edema forming activity, hemorrhagic activity, phospholipase activity (PLA2), procoagulant activity caused by *B. caeruleus* venom were undertaken. Neutralization studies can be performed by incubating of venom and plant extracts prior to testing (pre-incubation method). The results showed that both the plant extracts were capable of neutralizing the lethality induced by the venom. The venom showed the presence of PLA2 enzymes through producing haemolytic haloes in indirect haemolytic assays. Both the plant extracts were capable of inhibiting PLA2 dependent haemolysis of sheep RBCs in a dose dependent manner. The medicinal plants, *Camellia sinensis* L. and *Cordia verbenacea* effectively neutralized the phospholipase A2 activity induced by snake venoms (Ticli et al., 2005; Fattepur and Gawad, 2004). Edema-forming activity was assessed for *B. caeruleus* venom and both plant extracts were found to be effective in neutralization of edema induced by venom. There was a significant decrease in the edema (footpad thickness) when there was an increase in the antivenom (plant extract) concentration. Procoagulant activity induced by the venom was studied using human citrated plasma and both the root extracts were found to be effective in the neutralization of procoagulant activity. The present experimental results indicate that *A. calamus* and *W. somnifera* root extracts were effective in neutralizing the main toxic and enzymatic effects of *B. caeruleus* venom. The anti-venom properties of both the plant extracts were potent enough to neutralize the lethality and various pharmacological activities of venom. The result from this preliminary study indicates that both the plant extracts have the potential to be used for therapy in patients with snakebite envenomation. Further investigations are needed for identification and purification of the active components involved in the neutralization of the snake venom.

**References**


A. Ohsaka, K. Hayashi and Y. Sawai (eds.), New York, USA.
Fungi Isolated from Produced Water and Water-Soluble Fraction of Crude Oil

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Abstract. This study was sought to determine the fungi present in the produced water (PW) and water-soluble fraction (WSF) of crude oil as a preliminary approach to determining that fungi can survive in crude oil polluted water and their possible use in bioremediation. Different concentrations of PW and WSF of crude oil samples from Ughelli East Flow Station in Delta State of Nigeria were exposed to onion (Allium cepa) primordial cells at different concentrations for twelve days. Thereafter, samples of the PW and WSF were cultured on Potato Dextrose Agar. Isolates of Thamnidium sp, Gelasinospora sp, Zygorhynchus sp and Colletotrichum sp were found. Zygorhynchus and Colletotrichum were associated with PW while Thamnidium and Gelasinospora associated with the WSF. There were changes in the pH and turbidity of the PW and WSF before and after exposure to Allium cepa cells. At 25% level of treatments there were significant differences in pH and turbidity values of the PW and WSF at P < 0.05 and P > 0.01 before and after exposure to the plant.

Keywords: bioremediation, crude oil, fungi, water soluble fraction, produced water, pollution

Introduction

Over 90% of Nigeria’s income comes from crude oil (Edema and Okoloko, 2008). Oil and gas reservoirs have a natural water layer (called formation water) that, being denser, lies under the hydrocarbons. As a result, oil reservoirs frequently contain some water. However, to achieve maximum oil recovery, additional water is usually injected into the oil surface. Both formation and injected water are eventually produced along with the hydrocarbons. This is called Produced Water (PW).

Neff and Anderson (1981) described PW for ocean discharge as containing up to 48 ppm of petroleum because it has usually been in contact with crude oil in the reservoir rock. Due to rapid mixing with seawater, most physico-chemical features of PW (low dissolved oxygen and pH, elevated salinity and metals) do not pose any hazard to organisms in water. Elevated concentrations of hydrocarbons may be detected in surface sediments that contain aromatic hydrocarbons and metals, up to about 1000 m from the discharge. These aromatic hydrocarbons and metals in PW were reported by Neff and Anderson (1981) to be toxic to organisms.

Crude oil contains a small soluble fraction called water soluble fraction (WSF) (Kavanu, 1964) and it is produced after a long period of oil-water contact (Baker, 1970). As long as incidences of oil spills and hydrocarbon pollution due to oil exploration continue, their effects on the living environment will remain of interest. Organisms exposed to WSF of crude oil take up the dissolved hydrocarbons and react to their effects. Severity of the effects depends on the organisms exposed, the concentration of the compounds and the mode of exposure (Overton et al., 2001). Therefore, the study of the effects of WSF of crude oil is important in order to understand and possibly prevent their undesirable effects. The effects of WSF on the growth and development of different organisms have been studied (Edema, 2010; Edema and Okoloko, 2008). Consequently, there is increasing interest in the use of living organisms in cleaning up oil polluted areas (a process known as bioremediation) instead of the use of chemicals because of their harmful effects and high costs of the latter. Fungi are diverse in their ecological adaptations. The potentials of certain fungi in bioremediation have been reported earlier (Kacprzak et al., 2005; Chigusa et al., 1996). In this study, fungi consistently associated with PW and WSF exposed to onion (A. cepa) root primordial cells in a separate experiment to determine the effect of this exposure on the physico-chemical properties of (PW and WSF) were isolated and identified as an approach to determining whether fungi can survive in crude oil polluted waters (PW and WSF) and any indication of their possible use in bioremediation.
Materials and Methods

Collection of crude oil and preparation of PW and WSF. Crude oil was collected from UgHELLi East Flow Station in the Delta State of Nigeria. The PW is the water that comes with the crude oil. The crude oil used had been standing in the Laboratory for 5 years. To separate the PW from crude oil, the mixture of crude oil and PW was allowed to stand in a separating funnel for 24 h, after which the lower phase was collected and used as stock or 100% PW.

The WSF was prepared according to the method of Anderson et al. (1974). A sample of crude oil (500 mL) was slowly mixed in 500 mL deionized water in a 2 L conical flask using a Gallenkamp table top magnetic bar. Stirring was done for 16 h at room temperature (28 ± 2 °C). The oil-water mixture was allowed to stand for 24 h in a separating funnel. The lower phase was collected and used as stock or 100% WSF and diluted serially with deionized water to give 50% and 25% strength WSF which were stored in screw-cap bottles prior to use. Deionized water was used as control and the three levels of WSF concentrations (25, 50,100%) were used for further experiments, all in triplicates.

Exposure of WSF samples to *A. cepa*. The outer scales of the onion (*A. cepa*) bulbs were removed and the primordial cells were exposed using a razor blade. Some quantities of PW and WSF samples prepared as above were exposed to the onion root primordial cells for twelve days while the remaining was left for determination of fungi present before exposure. The exposure to onion cells was done in order to mimic the natural environment where plants are exposed to the effects of PW and WSF. Thereafter, samples of the PW and WSF were cultured on potato dextrose agar (PDA) (Oxoid, England).

Determination of fungi present in PW and WSF before and after exposure to *A. cepa*. PDA was prepared according to manufacturer’s instructions. Before exposure to onion cells, 1 mL of each WSF concentration was transferred into a petri dish and 20 mL of PDA (containing 50 μg/mL chloramphenicol, to inhibit bacterial growth) was poured into the plate. The plate was swirled round for even mixing and allowed to solidify. The same procedure used for PW and WSF before exposure as described above was also followed after exposure. Control plates contained 1mL deionized water instead of WSF. Also, PW and WSF not exposed to *A. cepa* were plated on PDA to determine presence of fungi. All culture plates were incubated at room temperature (28 ± 2 °C) for 7 days. Culture samples were examined under low (x40) and high (x100) powers of an optical microscope for fungal growth. Fungal identification was done based on colony morphology and microscopic characteristics according to Barnet and Hunter (1998).

Determination of pH. The pH of WSF was taken before and after exposure to *A. cepa*, using a pH meter model PHS-25.

Turbidity. The turbidity of PW and WSF before and after exposure to *A. cepa* was determined with a portable turbidity meter (Hanna I 93 102) at 500 nm.

Statistical analysis. Statistical analysis was carried out using the t-test for paired sample for means.

Results and Discussion

Four genera of fungi were isolated and identified as *Thamnidium*, *Gelasinospora*, *Zygorhynchus*, and *Colletotrichum* (Table 1). While *Zygorhynchus* and *Colletotrichum* were associated with PW of the crude oil, *Thamnidium* and *Gelasinospora* were associated with the WSF. Fungal growth was evident in the PW and WSF both before (data not shown) and after exposure to *A. cepa*. The PW and WSF that were not exposed to onion also had the same fungi growing in them, which indicated that fungi were associated with PW and WSF of the crude oil. The mean pH values before and after exposure to *A. cepa* are shown in Table 2. The pH values after exposure were constantly higher though not significantly different from pH values before exposure to *A. cepa*. The pH values changed to slightly alkaline. Statistical analysis carried out using t-test for paired sample for means showed that the mean values of pH obtained before and after exposure of *A. cepa* to PW and WSF were significantly different (P<0.05). It has been reported that pH influences the oxidation-reduction equilibrium and solubility of ionic forms of several elements (Wild, 1996). Increased pH of the PW and WSF indicates that there was more cation removal from the PW and WSF than anions, as the presence of excess anion raises pH (Wild, 1996). All the pH values were within the maximum permissible level (pH 6.5-9.8) for safe water by World Health Organisation (WHO, 1995). The pH 7.7 of 100% PW has been reported to support catalase activity in plants (Taylor et al., 1998). Edema and Okoloko (2008) reported a decrease in pH of WSF after exposure. The difference between their and our
findings could have arisen as a result of the different plants used as well as a possible role of the fungi present. However, Edema (2010) reported an increase in pH after exposure, which agrees with our findings here.

Turbidity decreased after exposure for both PW and WSF (Table 3). The 25% WSF concentration had the lowest change in turbidity (1.2) and 100% PW had the highest (8.3). The turbidity of PW and WSF decreased as a result of decrease in the concentration of PW and WSF as in the opinion of Michaud (1991). Decrease in turbidity implies that there was increased uptake of ionic particles (Edema and Okoloko, 2008). Also, the effect of pH change on solubility of ions might have contributed to the decrease in turbidity. Fungal density was high after exposure (data not shown). This uptake may be due to the additional uptake by the fungi.

**Zygorhynchus** is a soil-inhabiting fungus belonging to the Class Zygomycetes and Family Mucoraceae and can tolerate high salinity and certain degree of pollution (Ru-Yong, 2002). It is reported to have high resistance to lysis by other microorganisms due to the presence of uronic acid and fucose and phosphate polymers in its cell wall which confer resistance to the cell wall (Ballesta and Alexander, 1971). The ability of this fungus to thrive in PW of crude oil might have been as a result of this attribute. **Zygorhynchus** was isolated from waste-water and sewage sludges by Kacprzak et al. (2005). The occurrence of **Zygorhynchus** in heavily polluted areas such as sewage and crude oil may be due to its more adaptation to polluted environment. It is contrasting to its earlier reported slight tolerance to pollution. It could also be as a result of difference in species reported before and the one found in this work.

**Colletotrichum** was present in PW. It belongs to the Class Ascomycetes (Alexopoulos and Mims, 1979). Species of this fungus are known pathogens causing fruit and leaf rot of plants (Everett, 2003; Saha et al., 2002). A few species cause disease in man and other animals (Marcelino et al., 2008). This is the first documented report of the isolation of **Colletotrichum** from PW of crude oil.

**Thamnidium** was present in WSF. It is a Zygomycete and is commonly isolated from desert and cultivated soils (Arduin and Palma, 2007; Stejskal et al., 2005).

**Gelasinospora**, a soil fungus was detected in WSF (Domsch and Gams, 1972). It is an Ascomycete of the family Sordariaceae (Dettman et al., 2001). Although adapted to aerobic life, it has been reported to also tolerate anaerobic conditions (Ulacio et al., 1998) a property which probably enabled it to survive in WSF.

Kacprzak et al. (2005) opined that ability of fungi to survive in nutrient-limited environments such as polluted water is an indication of their possible use in bioremediation. These results indicate potentials of the fungi isolated for bioremediation of petroleum-polluted water.

### Table 1. Fungal genera isolated from PW and WSF before and after exposure to *A. cepa*

<table>
<thead>
<tr>
<th>Fungi</th>
<th>PW</th>
<th>WSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thamnidium</em> sp</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Zygorhynchus</em> sp</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Gelasinospora</em> sp</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>Colletotrichum</em> sp</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table 2. Mean pH values of PW and WSF before and after exposure to *A. cepa*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Before (Mean and SE)</th>
<th>After (Mean and SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% PW</td>
<td>6.66 ± 0.000</td>
<td>*6.4 ± 0.060</td>
</tr>
<tr>
<td>50% PW</td>
<td>6.70 ± 0.003</td>
<td>*7.90 ± 0.030</td>
</tr>
<tr>
<td>25% PW</td>
<td>6.89 ± 0.003</td>
<td>7.50 ± 0.050</td>
</tr>
<tr>
<td>100% WSF</td>
<td>6.67 ± 0.006</td>
<td>*7.70 ± 0.000</td>
</tr>
<tr>
<td>50% WSF</td>
<td>6.80 ± 0.003</td>
<td>*6.81 ± 0.006</td>
</tr>
<tr>
<td>25% WSF</td>
<td>6.86 ± 0.003</td>
<td>7.30 ± 0.000</td>
</tr>
</tbody>
</table>

*significant difference (<0.05) between the two experimental conditions (before and after exposure); SE = Standard error of mean.

### Table 3. Mean turbidity values of PW and WSF before and after exposure to *A. cepa* (NTU)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Before (Mean and SE)</th>
<th>After (Mean and SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% PW</td>
<td>95.7 ± 0.03a</td>
<td>87.4 ± 0.06b</td>
</tr>
<tr>
<td>50% PW</td>
<td>47.6 ± 0.06</td>
<td>45.5 ± 0.09</td>
</tr>
<tr>
<td>25% PW</td>
<td>21.7 ± 0.06</td>
<td>20.4 ± 0.06</td>
</tr>
<tr>
<td>100% WSF</td>
<td>47.3 ± 0.05</td>
<td>42.6 ± 0.06</td>
</tr>
<tr>
<td>50% WSF</td>
<td>28.5 ± 0.03</td>
<td>24.5 ± 0.12</td>
</tr>
<tr>
<td>25% WSF</td>
<td>18.9 ± 0.00</td>
<td>17.7 ± 0.09</td>
</tr>
</tbody>
</table>

NTU = neph turbidity unit measured at 500 nm; values with different letters in superscripts along the same row are significantly different (<0.01).
References
The Effect of Preservation Methods on the Nutritional Quality of Fluted Pumpkin (Telfaria occidentalis) Leaves

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Abstract. The present research was undertaken to investigate the effect of various blanching and drying treatments on the vitamin C, β-carotene, iron, protein content and drying time of fluted pumpkin (Telfaria occidentalis Hook) leaves. The leaves were subjected to sun or oven drying alone or after blanching in boiling water, dilute citric acid (0.3%) or dilute potassium meta-bisulphite (0.5%). In the absence of blanching pre-treatment, oven drying resulted in shorter drying time for the leaves and a higher vitamin C content of the dried product than sun drying and there was no significant difference (p>0.01) between the protein, β-carotene and iron content of the oven and sun dried products. Leaves blanched in boiling water, dilute citric acid, or dilute potassium meta-bisulphite solution, followed by sun or oven drying had less vitamin C, iron, protein and β-carotene than leaves dried without blanching. Compared with the use of boiling water, blanching in boiling potassium meta-bisulphite reduced the loss of β-carotene, protein and iron; blanching in dilute citric acid reduced the loss of iron. Except for citric acid pre-treatment, blanching prior to drying shortened drying time.

Keywords: fluted pumpkin leaves, blanching, citric acid, potassium metabisulphite, drying, nutrients

Introduction

Green leafy vegetables are a good source of dietary fibre, carotenoids, vitamin C, folate, phytochemicals and certain minerals, but they have low lipid, carbohydrate and protein concentration (Wills et al., 1998). They present an inexpensive and readily available approach to combating certain micronutrient deficiencies. Due to their high moisture and adequate fibre content they provide bulk in the diet and give a feeling of satiety, in the absence of energy dense dietary components. They are helpful for the prevention and management of some chronic conditions that arise partly as a result of excessive energy and inadequate fibre intake. Their phytochemicals also offer protection against disease (Wardlaw and Kessel, 2002).

The plant Telfaria occidentalis Hook, commonly known as fluted pumpkin, oyster nut or ridge gourd belongs to the Cucurbitaceae family. It is indigenous to West Africa, but is found wild or cultivated in West, Central, East and Southern Africa (Bosch et al., 2005). Its primary use is as a source of edible leafy vegetable. Secondary uses of the plant are for forage (leaves), as medicine (leaves and roots), as a source of vegetable oil and condiment (seeds), and for fibre (vine) (Bosch et al., 2005).

Leafy vegetables are highly susceptible to deterioration after harvesting, resulting in heavy post harvest losses. Drying of foodstuffs is an old method for food preservation. Although widespread, cheap and reliable, drying also changes product quality, texture and nutritive value. These changes can be mitigated, however by adequate preparation and pre-treatment before drying. Blanching or scalding in hot water or steam makes the product tender, limits discolouration, eliminates intracellular gases responsible for oxidation reactions, kills harmful bacteria and deactivates enzymes which disintegrate the tissues. Blanching in boiling water makes it possible to incorporate, simultaneously, citric acid and/or sulphur as potassium meta-bisulphite (Rozis, 1997). Blanching and other thermal treatment of green leaves can also enhance the bioavailability of micronutrients by destroying antinutrients and by releasing micronutrients from entrapment in the plant matrix (Hotz and Gibson, 2007; Yang and Tson, 2006; Yadev and Sehgel, 2002; Yeum and Russel, 2002; Henry and Massey, 2001; Rodriguez-Amaya, 1997). Thin-slicing results in better heat and chemical penetration, and facilitates water loss during drying, resulting in shorter drying times and better preservation of heat-labile nutrients.

In this study, fluted pumpkin leaves were thin-sliced, and dried with or without blanching, in sun light or in an oven (an electrically heated ventilated cabinet tray...
dryer) in order to determine the effect of pre-treatment and drying on their nutritive value.

**Materials and Methods**

A batch of fluted pumpkin freshly cut, dark green in colour leaves (about 2.0 kg) was purchased.

All reagents used were analytical grade.

**Preparation of leaves.** Leaves were thin-sliced with a sharp knife and some of the sliced vegetable was blanched.

**Blanching of leaves.** For blanching, each sliced vegetable sample (50.0 g) was kept in a clean cheese cloth and dipped in boiling water, boiling dilute citric acid solution (0.3%) or boiling potassium meta-bisulphite solution (0.5%) for 10 min. The cloth containing the vegetable was removed and gently squeezed to remove water. A total of twenty-four (50.0 g) samples were blanched (eight per pre-treatment). Blanched vegetable was then sun or oven dried (Rozis, 1997).

**Drying of leaves.** Each vegetable sample, blanched or without any pre-treatment, was spread in a thin layer on a net in a ventilated oven maintained at 60°C as well as in a shallow tray in the sun light, at an ambient temperature of 30°C. Material was tested at intervals according to appearance and texture, with the intervals getting shorter as drying progressed. Testing interval was 5 min in the last 30 min of sun or oven drying. The time interval (to the nearest minute) from the beginning of drying to when crumbliness observed was taken as drying time (James and Kuipers, 2003). Material from each dried sample (16 subsamples from each drying condition, 4 untreated and 12 blanched; a total of 32 subsamples) was taken for determination of vitamin C, β-carotene, protein and iron content.

**Analytical methods.** Moisture and protein content were determined by official methods of analysis (AOAC, 1984). Crude protein was determined by the micro-Kjeldahl method and protein content calculated as N × 6.25.

For the determination of vitamin C content, dried and pulverised sample was extracted with 0.5% oxalic acid. Vitamin C in the extract was determined by coupling with 2, 4-dinitrophenyl hydrazine. Absorbance was read at 520 nm (Fafunso and Bassir, 1976).

For the determination of β-carotene content, leaves were extracted using chloroform/methanol (2:1). β-carotene was determined spectrophotometrically at 440 nm after ethanolic NaOH saponification and extraction with chloroform (Bassir, 1963). Iron content was determined by atomic absorption spectrophotometry (Novosamsky, 1983).

**Statistical analysis.** All analyses were done in quadruplicate and all values were expressed as mean ± SEM. The statistical analysis was carried out using unpaired t-test and ANOVA to detect significant differences according to Ogbeibu (2005).

**Results and Discussion**

Three blanching treatments were employed (i) blanching of the vegetable in boiling water (which is the common practice), (ii) blanching in boiling dilute citric acid and (iii) blanching in boiling dilute potassium meta-bisulphite. Beneficial effect of citric acid and meta-bisulphite blanching on the colour of the dried product and also on the flavour and nutrient retention (in the case of citric acid), have been reported (Kendal et al., 2009; Rozis, 1997) and emphasis of this study was to observe the affect of these pre-treatments on the nutrient composition of the dried product.

Table 1 shows the effect of pre-treatment and drying conditions on the nutritional quality of fluted pumpkin leaves.

For the untreated sample, sun dried and oven dried leaves had vitamin C content of 0.967% and 1.190%, respectively. Compared with the oven dried product, sun drying resulted in a loss of about a fifth (18.74%) of this nutrient. Thus oven drying protected vitamin C better than sun drying. The lower value for the sundried material may have resulted from destruction of this material by ultra-violet radiation from the sun and longer drying time, which were not encountered in the oven. Leaves blanched in boiling water and sun dried had vitamin C content of 0.201%; water blanched and oven dried leaves had vitamin C content of 0.177%. Vitamin C content of leaves blanched in citric acid and sun dried was 0.161%. Citric acid blanched and oven dried leaves had vitamin C content of 0.167%. Leaves sun or oven dried, after blanching in dilute potassium meta-bisulphite, had vitamin C content of 0.232% and 0.169 %, respectively.

In all cases, leaves blanched prior to drying had lower vitamin C content than leaves dried without pre-treatment, due to leaching of nutrients, including vitamin C, into the blanching medium. This loss may in addition have resulted from heat denaturation of this vitamin at the blanching temperature. Compared with the untreated
leaves, loss of vitamin C for the blanched and sundried leaves was 76.01-83.35% and about 85% for the oven dried leaves.

The effect of blanching and drying on the β-carotene content of fluted pumpkin leaves is shown in Table 1.

Sun and oven dried leaves had similar β-carotene content (9.92 and 9.63 mg/g), respectively. Leaves blanched in boiling water followed by sun or oven drying had β-carotene content of 4.09 and 4.55 mg/g, respectively. Similar values (4.44 and 4.58 mg/g, respectively) obtained for leaves that were sun or oven dried after blanching in citric acid. However, leaves blanched in potassium meta-bisulphite followed by sun or oven drying had higher content (7.11 and 6.98 mg/g, respectively) of this provitamin. Thus, it appears that blanching in potassium meta-bisulphite might offer better protection to the β-carotene content of the leaves than blanching in boiling water or citric acid.

Table 1. Effect of pre-treatment and drying conditions on the nutritional contents of fluted pumpkin leaves

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Sun dried</th>
<th>% loss</th>
<th>Oven dried</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.967±0.045</td>
<td>-</td>
<td>1.19±0.036</td>
<td>-</td>
</tr>
<tr>
<td>Blanching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiling water</td>
<td>0.20±0.007</td>
<td>79.21</td>
<td>0.17±0.012</td>
<td>85.13</td>
</tr>
<tr>
<td>Boiling in dil. citric acid</td>
<td>0.16±0.003</td>
<td>83.35</td>
<td>0.16±0.004</td>
<td>85.97</td>
</tr>
<tr>
<td>Boiling in dil. K₂S₂O₃</td>
<td>0.23±0.008</td>
<td>76.01</td>
<td>0.16±0.006</td>
<td>85.80</td>
</tr>
<tr>
<td>Control</td>
<td>9.92±0.08</td>
<td>-</td>
<td>9.63±0.12</td>
<td>-</td>
</tr>
<tr>
<td>Blanching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiling water</td>
<td>4.09±0.59</td>
<td>58.77</td>
<td>4.55±0.12</td>
<td>52.75</td>
</tr>
<tr>
<td>Boiling in dil. citric acid</td>
<td>4.44±0.65</td>
<td>55.24</td>
<td>4.58±0.70</td>
<td>52.44</td>
</tr>
<tr>
<td>Boiling in dil. K₂S₂O₃</td>
<td>7.11±0.82</td>
<td>28.33</td>
<td>6.98±0.12</td>
<td>30.63</td>
</tr>
<tr>
<td>Control</td>
<td>10.47±0.28</td>
<td>-</td>
<td>10.39±0.33</td>
<td>-</td>
</tr>
<tr>
<td>Blanching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiling water</td>
<td>6.07±0.24</td>
<td>42.02</td>
<td>8.77±0.44</td>
<td>15.59</td>
</tr>
<tr>
<td>Boiling in dil. citric acid</td>
<td>1.91±0.01</td>
<td>81.80</td>
<td>1.59±0.11</td>
<td>84.65</td>
</tr>
<tr>
<td>Boiling in dil. K₂S₂O₃</td>
<td>7.48±0.12</td>
<td>28.60</td>
<td>9.20±0.13</td>
<td>11.40</td>
</tr>
</tbody>
</table>

β-carotene (mg/g)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Sun dried</th>
<th>% loss</th>
<th>Oven dried</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.92±0.08</td>
<td>-</td>
<td>9.63±0.12</td>
<td>-</td>
</tr>
<tr>
<td>Blanching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Control</td>
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<td>10.39±0.33</td>
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</tr>
<tr>
<td>Blanching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>15.59</td>
</tr>
<tr>
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<td>81.80</td>
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<td>84.65</td>
</tr>
<tr>
<td>Boiling in dil. K₂S₂O₃</td>
<td>7.48±0.12</td>
<td>28.60</td>
<td>9.20±0.13</td>
<td>11.40</td>
</tr>
</tbody>
</table>

Protein content (% dry basis)

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Sun dried</th>
<th>% loss</th>
<th>Oven dried</th>
<th>% loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.728±0.395</td>
<td>-</td>
<td>2.56±0.033</td>
<td>-</td>
</tr>
<tr>
<td>Blanching</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boiling water</td>
<td>0.57±0.062</td>
<td>78.85</td>
<td>0.68±0.372</td>
<td>73.13</td>
</tr>
<tr>
<td>Boiling in dil. citric acid</td>
<td>0.89±0.075</td>
<td>67.23</td>
<td>1.01±0.446</td>
<td>60.26</td>
</tr>
<tr>
<td>Boiling in dil. K₂S₂O₃</td>
<td>0.73±0.033</td>
<td>73.17</td>
<td>0.53±0.028</td>
<td>79.10</td>
</tr>
</tbody>
</table>

Iron content (µg/g)

a = values are recorded as mean±standard deviation of four determinations; b = shows significant (p<0.01) difference when values within the same drying method (sun or oven) but subjected to different pre-treatment methods are compared with the control (no pre-treatment); c = these values showed significant (p<0.01) difference between the drying methods using the same pre-treatment; sun drying was taken as the control; d = using ANOVA, these values show no statistical (p>0.01) difference within the same drying method using different pre-treatment methods.
blanching in potassium meta-bisulphite had protein content of 7.48% and 9.20%, respectively.

Leaves blanched prior to drying had lower protein content than those dried without pre-treatment. Leaves dried after blanching in boiling dilute potassium meta-bisulphite had higher protein content than leaves blanched in boiling water prior to drying. Of all the treatments, blanching in dilute citric acid prior to drying resulted in the highest protein loss. This was probably due to acid hydrolysis of protein to amino acids during blanching, and solubilisation of the latter in the blanching medium. Loss of protein after blanching and drying, ranged from 28.60%-81.80% for the sun dried and 11.40%-84.65% for the oven dried leaves indicating the presence of soluble non-protein nitrogen compounds in fluted pumpkin leaves.

The iron content of fluted pumpkin leaves from blanching and drying treatments is shown in Table 1. Untreated sundried and oven dried leaves had iron content of 2.728 µg/g and 2.564 µg/g, respectively. Water blanched, sun or oven dried leaves had iron content of 0.577 µg/g and 0.689 µg/g, respectively. Leaves blanched in boiling dilute citric acid prior to sun or oven drying had iron content of 0.894 µg/g and 1.019 µg/g, respectively. Potassium meta-bisulphite blanched and sun dried leaves had iron content of 0.732 µg/g; oven dried leaves had iron content of 0.536 µg/g.

Blanched and dried leaves had lower iron content than leaves dried without pre-treatment, due to leaching of the mineral into the blanching medium. Blanching in dilute citric acid or potassium meta-bisulphite appears to have protected iron content better than blanching in boiling water. Compared with the untreated leaves, loss of iron was 67.23%-78.85% for the blanched and sun dried and 60.26-79.10% for blanched and oven dried leaves.

Losses in the activity of heat labile and water soluble nutrients, resulting from thermal treatment of food have been reported previously (Hotz and Gibson, 2007). However, thermal treatment, for example blanching of vegetables, can improve the bioavailability of nutrients, by destruction of antinutrients (Hotz and Gibson, 2007; Yang and Tson, 2006; Yeum and Russel, 2002; Yadv and Sehgal, 2002; Henry and Massey, 2001; Rodrigez-Amaya, 1997) and facilitating the release of nutrients after partial degradation of cell walls combined with protein denaturation and mineral solubilisation from fibre (Kapanidis and Lee, 1995). The effect of different blanching and drying conditions on drying time of leaves is shown in Table 2.

Untreated sun-dried and oven dried leaves had drying times of 235.0 and 123.0 min, respectively. Compared to the untreated leaves, blanching in boiling water decreased drying time to 168.0±5.0 min for the sun dried and 80.0±4.0 min for oven dried leaves. Blanching in boiling dilute potassium meta-bisulphite reduced drying time to 114.0 min and 117.0 min, respectively for sun dried and oven dried leaves. Blanching in citric acid decreased (for the sun dried) or increased (for the oven dried leaves) the drying time to 163.0 min. Thus for leaves blanched in boiling citric acid or boiling potassium meta-bisulphite, drying times were similar for the sun and oven dried leaves. For leaves blanched in boiling water however, oven drying was much faster than sun drying and gave the best (i.e. shortest) drying time.

Blanching in boiling potassium meta-bisulphite gave the shortest sun drying time. Compared with the untreated leaves it shortened drying time by 51.5%, but reduced oven drying time by only 4.9%. Blanching in boiling citric acid shortened sun drying time by 30.6%, and increased oven drying time by 32.5%. Blanching in boiling water reduced sun drying time by 28.5% and reduced oven drying time by 35.0%.

| Table 2. Effect of pre-treatment and drying method on the drying time (min) of fluted pumpkin leaves<sup>a</sup> |
|--------------------------|-----------------|-------|
| Pre-treatment           | Sun             | % reduction | Oven          | % reduction |
| Control                 | 235.0±5.0       | 123.0±4.0<sup>c</sup> |
| **Blanching boiling water** | 168.0±5.0<sup>b,d</sup> | 28.5 | 80.0±4.0<sup>c</sup> | 35.0 |
| Boiling in dil. citric acid | 163.0±1.0<sup>b,d</sup> | 30.6 | 163.0±4.0<sup>b</sup> | - |
| Boiling in dil. K<sub>2</sub>S<sub>2</sub>O<sub>3</sub> | 114.0±3.0<sup>b</sup> | 51.5 | 117.0±1.0<sup>b</sup> | 4.9 |

<sup>a</sup> = values are recorded as the mean of four determinations; <sup>b</sup> = shows significant (p<0.01) difference when values within the same drying method (sun or oven) exposed to different pre-treatment methods are compared with the control (no pre-treatment); <sup>c</sup> these values were statistically different (P<0.01) on comparison between the drying methods after the same pre-treatment; <sup>d</sup> using ANOVA, these values show no statistical (P>0.01) difference within the same drying method after different pre-treatments.
The most common culinary application of fresh or dried fluted pumpkin leaves is as an ingredient in sauces, soups and stews. This application (which involves cooking of the mixture of which the vegetable is an ingredient) would result in the destruction of antinutrients and microorganisms, and improved bioavailability without any loss of nutrients, since whatever is leached from the vegetable stays in the mixture, which is then consumed. Thus, for such application, blanching prior to drying, which results in the loss of nutrients is disadvantageous and unnecessary. If however, the dried vegetable is to be used after rehydration as an ingredient in uncooked food, for example salads, blanching is indeed necessary and confers several benefits. Blanching helps to slow enzyme activity that can cause undesirable changes in flavour and texture. In addition blanching in water or citric acid solution improves the destruction of potentially harmful bacteria during drying, including Escherichia coli 0157:H7, Salmonella species and Listeria monocytogenes (Kendal et al., 2009).

Conclusion
The effect of preservation treatments on the nutritional quality of fluted pumpkin leaves was studied. Regarding the nutrients studied, oven drying (which is more expensive) appears to confer no advantage over sun drying, except for its better preservation of vitamin C content. It however has the advantages of shorter drying time and non-dependence on the weather. Compared with the use of boiling water, blanching in boiling dilute potassium meta-bisulphite offers better protection for vitamin C, β-carotene, protein and iron and shortens the sun drying time. However, citric acid blanching offers better protection than potassium meta-bisulphite for iron but results in serious loss of soluble nitrogen compounds.

References
Short Communication

Biochemical and Molecular Genetic Studies on Some Cyanobacterial Isolates

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and Engy Mohamed Rabea El-Shafê

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Department of Cytogenetic, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
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Abstract. In the present study, the isolation and purification of a set of Cyanobacteria strains belonging to genus Oscillatoria was undertaken, followed by the analyses of phylogenetic relationships using different biochemical and molecular genetic techniques (SDS-PAGE and RAPD-PCR). A total of 45 protein bands were observed within the studied Oscillatoria isolates by SDS-PAGE (only three unique bands, eight monomorphic bands and 37 polymorphic bands). On the other hand, extracted DNA from isolates was used to identify the molecular fingerprints. A sum of 94 polymorphic bands was generated by these primers in the Oscillatoria genotypes under study. A total of 20 unique bands were identified out of the polymorphic ones. These unique bands were used to discriminate among the studied Oscillatoria isolates. Most isolates of Oscillatoria genotypes were discriminated by one or more unique bands. Numerical taxonomic using 45 protein attributes of 19 isolates and RAPD markers on five isolates. Two methods - Clustering (UPGMA) and Principal Component Analysis (PCA) were used for these analyses. The similarities and clusters produced between the studied isolates were discussed.

Keywords: cyanobacteria strains, protein banding pattern (SDS-PAGE), RAPD-PCR, numerical analysis, phylogenetic relationships, Oscillatoria

Cyanobacteria (blue-green algae) produce a large number and variety of bioactive allelochemical substances, with a diverse range of biological activities and chemical structures. Such chemicals are likely to be involved in regulating natural populations and are potentially useful as biochemical tools and as biological control agents (Wu et al., 2005; Biondi et al., 2004; Hirahashi et al., 2001). Microbial biotechnology, including DNA manipulation, facilitates the construction of new bacterial genomes that possess different potentialities.

Cyanobacteria were subjected to molecular genetic studies by Kumari and Parvathi (2009); Havemann and Foster (2008); Guan et al. (2007); Gugger et al. (2002); Itman et al. (2002); Wilson et al. (1999).

The use of phenotypic and genotypic characteristics in cyanobacteria taxonomy was pioneered by Rippka et al. (1979). Ezihlarasi and Anand (2009) used the sequences of 16S rRNA gene in a phylogenetic analysis of Anabaena spp.

Axenic cultures of cyanobacteria isolates were prepared and purified by different methods, then molecular genetic fingerprint were determined by using electrophoresis of proteins banding patterns. Molecular genetic fingerprint to biological active isolates were determined by using RAPD-PCR. Numerical taxonomic study was carried out by using 45 protein attributes on 19 isolates and RAPD markers on five isolates. Two methods used for numerical analysis; Clustering (UPGMA) and Principal Component Analysis (PCA). NTSYSpc version 2.02i (Rohlf, 1998) was used for these analyses.

Sample preparation. In the present study, biological material was nineteen isolates of Oscillatoria species. These isolates were collected from different locations in the eastern part of Qaroun Lake, Egypt. Isolation of cyanobacteria samples was carried out using modified procedure of Brand (1991) and purification was done according to Stein (1979). The extracted samples were centrifuged for 15 min at 4000 rpm at room temperature. The supernatants were taken and filtered through sterile cellulose nitrate membrane filters (0.45μm) for obtaining sterile aqueous cyanobacteria extracts. Whole cell cyanobacteria protein extraction was carried out according to Livne et al. (1992) with a lot of modification.

When preparation of samples were ready for electrophoresis, the frozen cyanobacteria extracts re-suspended
in sample buffer and total protein concentration was determined by Bradford method (Bradford, 1976).

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). Gels were photographed using a Bio-Rad gel documentation system. Data analysis was obtained by Bio-Rad Quantity one software ver: 4.0.3.

**Molecular studies.** DNA was extracted according to Neilan (1995), with some modification. Also, PCR-RAPD reactions applied were based on the protocol of Neilan (1995). PCR-RAPD reactions were conducted using 10 arbitrary 10-mer primers (Operon Technologies, Inc). The successful primers during PCR- are listed in Table 1. Bands were detected on UV-transilluminator and photographed by Gel documentation system (Biometra Bio Doc Analyze - 2000).

**Numerical analysis.** Among nineteen cyanobacteria isolates based on SDS-PAGE and RAPD-PCR markers polymorphism, similarity matrix was developed by SPSS computer package system ver.16. Numerical taxonomic study was carried out by using 45 protein attributes on 19 isolates and RAPD markers on five isolates. Two methods used for numerical analysis; Clustering (UPGMA) and Principal Component Analysis (PCA). NTSYSpc version 2.02i (Rohlf, 1998) was used for these analyses.

**Biochemical analysis.** The fingerprint of nineteen isolates of cyanobacteria is shown in Fig. 1.

**RAPD-PCR amplification analysis.** The randomly amplified polymorphic DNA (RAPD) technique, in conjunction with PCR, has been employed to identify many organisms up to strain level of classification (Williams et al., 1990). The resulting bands may then be used for distinguishing subspecies (Gow and Gadd, 1995).

**Table 1.** List of RAPD primers and their nucleotide sequence

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-02</td>
<td>5′- GTG AGG CGT C-3′</td>
</tr>
<tr>
<td>L-13</td>
<td>5′- ACC GCC TGC T -3′</td>
</tr>
<tr>
<td>Z-09</td>
<td>5′- CAC CCC AGT C-3′</td>
</tr>
<tr>
<td>E-06</td>
<td>5′- AAG ACC CCT C-3′</td>
</tr>
<tr>
<td>F-04</td>
<td>5′- GGT GAT CAG G-3′</td>
</tr>
<tr>
<td>G-05</td>
<td>5′- CTG AGA CGG A -3′</td>
</tr>
<tr>
<td>M-20</td>
<td>5′- AGG TCT TGG G-3′</td>
</tr>
<tr>
<td>O-04</td>
<td>5′- AAG TCC GCT C-3′</td>
</tr>
</tbody>
</table>

**Fig. 1.** SDS-PAGE of total soluble protein banding pattern of the nineteen cyanobacterial isolates.

In the present study, the using of RAPD-PCR technology in the detection of genetic heterogeneity among only five sample of the nineteen axenic cultures of cyanobacterial isolates have been discussed. Table 2 shows the comparison among the total number of amplified bands.

**Fig. 2.** Dendrogram using average linkage (between groups) of nineteen cyanobacterial isolates based on RAPD-PCR analysis.

**Table 2. Number of bands for each isolate with different primers**

<table>
<thead>
<tr>
<th>Primers</th>
<th>Isolates</th>
<th>Total bands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>L13</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>F4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>O4</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>M20</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>C2</td>
<td>10</td>
<td>12</td>
</tr>
<tr>
<td>Z9</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>E6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>G5</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Total bands</td>
<td>53</td>
<td>64</td>
</tr>
</tbody>
</table>
DNA fragments for five selected cyanobacterial isolates that generated by the previously used primers in RAPD-PCR. Figure 2 illustrates the resulted dendrogram among five cyanobacteria isolates from RAPD-PCR analysis.

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