Synergistic effect of packaging materials and methanolic extract of *Myrianthus arboreus* on the control of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae).

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Abstract

This study was conducted to investigate the role of packaging materials in the bio efficacy of *Myrianthus arboreus* to cowpea weevils, *Callosobruchus maculatus*. 300 g of cowpea seeds was weighed into a 12 cm diameter packaging material of jute, hessian sack, and polythene bag. An aliquot of 5 ml of the plant extract were thoroughly mixed with the aid of glass rod and left for two months prior to the introduction of the cowpea bruchids. For the control, the set up was similar with the treatments except that no plant material was used. The experiment was replicated three times for both the control and the treatments. Data on seed damage, egg count, and mortality were subjected to analysis of variance (ANOVA). The relationship between packaging materials used and *M. arboreus* was investigated using independent T-test. The results demonstrated that treated seeds kept in packaging materials have the potential to protect cowpea seeds from insect damage compared to when seeds were not stored in packaging materials. Such practices should therefore be considered for inclusion in pest management strategies in grains stored on-farm areas where modern storage implements are lacking.

Keywords: *Myrianthus arboreus*, Bruchids, Packaging materials, Synergistic

Introduction

Increased understanding of the shortcomings of synthetic insecticides used in crop protection has necessitated the need for research on alternatives [1,2]. Crops such as *Vigna unguiculata* (L) Walp have been subjected to infestation from a couple of storage insects [3,4]. Infestation due to these pests have been implicated as the major constraints facing effective and efficient food storage in tropical countries [5]. According to [6], the severity of pest attacks in Nigeria, increases from the Southern Guinea Savannah towards Sahel Savannah zone of the country. Major damage results from insect infestation during storage. Such situations have further reinforced research efforts on sustainable and effective control approaches. These among other things include biological control, botanical control, environmental management and practices of integrated pest management [7,8].

In recent times, a number of studies have revealed the potential use of packaging materials in augmenting the efficacy of botanicals in the protection of cowpea against storage pests [9,10]. According to [11], packaging 0 materials are essential for prolonging the storability of food crops. Elsewhere, [12] investigated the influence of packaging materials on the protection of stored cowpea. The authors reported that the type of packaging material play a profound role in extending the longevity and maintaining the storability of cowpea seeds in storage. Among botanicals, *Myrianthus arboreus* has been proven to increase the quality of seeds while being effective protectants against storage insects [13,14].

Accordingly, in efforts to complement research on alternatives to pest control, this work was conducted to investigate the influence of three packaging; hessian sack; jute bag and polythene bag on the efficacy of *Myrianthus arboreus* in the control of cowpea bruchids. In addition, the effect of *M. arboreus* on the life stages of *C. maculatus* was explored.

Materials and Methods

**Rearing of C. maculatus culture**

Adult *C. maculatus* were collected from already infested cowpea grains, *V. unguiculata* obtained from a grocery store in Akure metropolis, Ondo state. The infested cowpea grains were kept in 1 litre kilner jar containing 500 g of clean uninfested grains. The grain was disinfected in the freezer for 96 h at -2°C. The jar was covered with muslin cloth held in place by rubber band and kept in wire mesh cage measuring (75 × 60 × 50 cm) which was maintained at a temperature of 28 ± 2°C and 75 ± 5% relative humidity. The culture of *C. maculatus* was set up and maintained by continually replacing devoured and infested cowpea grains with clean uninfested grains.

**Collection of plant material and extraction**

The stem bark of *M. arboreus* was obtained from the parent plant in a forest in Owo, Ondo State Nigeria. The plant was identified in the Herbarium unit, Department of Botany and Microbiology, University of Lagos. The plant part was washed thoroughly with tap water and air dried in the laboratory to relatively stable weights. The dried plant was pulverized using an electric grinder (Breville Model of 1500 ml capacity).

The plant extract was obtained by modifying the cold extraction method as described previously [15]. Two hundred grams of the plant powder was weighed into a conical flask and soaked
in 100 ml of absolute methanol for 72 hrs. Thereafter, it was filtered through a muslin cloth and the filtrate kept in a plastic container and used as a stock solution of the packaging extract. The solution was concentrated in a rotary evaporator (28°C at 120 rpm) under reduced pressure. The extract was stored in sterile cellophane bags in a refrigerator at 4°C until needed.

Bioassays

Effect of plant extract on egg: Three hundred grams of clean cowpea seeds of Ife brown variety was weighed into a 12 cm diameter packaging material of jute, hessian sack, and polythene bag. An aliquot of 5 ml of the plant extract obtained from serial dilution were added into each packaging material using a pipette. Concentration of 5 ml of the extract were thoroughly mixed with the aid of glass rod and left for two months prior to the introduction of the cowpea bruchids. For the control, the set up was similar except for the absence of plant material. Three replicates of each treatment and control were set up.

Ten pairs of newly emerged (0-1 day old) C. maculatus adults were introduced into the setup and sealed with a thread. The experiment was left for two months in a cage, after which the parent insects had died off. Thereafter, eggs laid were evaluated by counting the number of dead insects, eggs and F₁ emergence in treatments. The results were recorded and reported in percentage mean.

Data analysis

The weight loss data were computed using:

\[ \text{% Weight loss} = \frac{\text{Initial weight loss} - \text{Final weight loss}}{\text{Initial weight loss}} \times 100 \]

The oviposition and mortality data were subjected to analysis of variance (ANOVA) at 95% confidence interval, where significant difference occurred, the means were separated using Tukey’s test. The relationship between the packaging materials and M. arboreus was computed using independent t-test. All analyses were done with Statistical Package for Social Sciences (SPSS) version 20.

Results

Extent of seed damage in packaging materials

The total number of seeds in the uniform weight of the cowpea seeds put in each packaging materials (PM) are presented in Table 1. The highest number of damage (921.14) was observed in the hessian bag. In the same vein, seeds in Polyethylene (882) recorded more damage than jute (409). Nevertheless, significantly higher (p<0.05) level of seed damage was observed in the polyethylene bag.

Extent of seed damage in packaging materials and M. arboreus

Similarly, Table 2 shows the number of seeds in each PM imbued with aliquot concentration of M. arboreus (PM+Plant M). While the level of damage was seen to drastically reduce from 75-85% in PM (Table 1) to the range of 0-10% (Table 2). As with PM, Jute (0.55%) recorded the lowest seed damage and polyethylene (5.30), the highest.

Oviposition pattern in experimental set-up

The number of eggs laid by C. maculatus on cowpea seeds contained in each packaging material and in each packaging material containing aliquot proportion of M. arboreus is shown in Figure 1. Expectedly, higher egg count (range of 396-766) was observed in the PM as compared to the PM+plant (9-77) set up. Specifically, higher egg count was recorded in the polyethylene bag set up for both PM (766) and PM+plant (77.29). The egg count data demonstrates that jute bag reduced significantly the oviposition propensity of the bruchid as lower number of eggs were recorded in both the PM (396) and PM+plant (9.29) set up.

Mortality of Bruchids in experimental set-up

The mortality of the bruchids on the cowpea seeds in each packaging material is presented in Figure 2. Significantly higher mortality was observed in the jute bag (23.56) as compared to polyethylene (23.21) and hessian (19.39) bags. In comparison, there was an elevated increase in the mortality index in the PM+plant set up, raking above 48% bruchid mortality to the PM’s less than 24% mortality.

Weight loss in packaging materials

The percentage weight loss data showed that for the packaging materials set up only, seeds in polyethylene bag suffered the greatest loss (32.13%) in weight from the bruchids (Figure 3). Seeds contained in the Jute bag, on the other hand, recorded the lowest loss in weight (8.13). By comparison, introduction of M. arboreus into the set up significantly reduced the destructive activities of the cowpea weevil. Thus, there was a reduction in

<table>
<thead>
<tr>
<th>Packaging Material</th>
<th>Total no of seeds</th>
<th>Damaged seeds</th>
<th>Percentage damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene</td>
<td>1053.77 ± 0.21</td>
<td>882.36 ± 0.52</td>
<td>83.28 ± 1.23</td>
</tr>
<tr>
<td>Jute</td>
<td>1055.69 ± 1.20</td>
<td>409.21 ± 0.32</td>
<td>38.74 ± 0.87</td>
</tr>
<tr>
<td>Hessian</td>
<td>1055.99 ± 1.20</td>
<td>921.14 ± 0.22</td>
<td>87.23 ± 1.43</td>
</tr>
</tbody>
</table>

Values presented are means ± standard error of three replicates. Means followed by the same letters (superscript) are not significantly different (p>0.05) from one another down the column according to Tukey’s post hoc test.

<table>
<thead>
<tr>
<th>Packaging Material</th>
<th>Total no of seeds</th>
<th>Damaged seeds</th>
<th>Percentage damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyethylene</td>
<td>1046.67 ± 0.26</td>
<td>54.63 ± 3.52</td>
<td>5.30 ± 0.65</td>
</tr>
<tr>
<td>Jute</td>
<td>1056.00 ± 2.20</td>
<td>6.67 ± 1.32</td>
<td>0.55 ± 0.01</td>
</tr>
<tr>
<td>Hessian</td>
<td>1056.00 ± 1.67</td>
<td>28.33 ± 1.42</td>
<td>2.75 ± 0.33</td>
</tr>
</tbody>
</table>

Values presented are means ± standard error of three replicates. Means followed by the same letters (superscript) are not significantly different (p>0.05) from one another down the column according to Tukey’s post hoc test.
weight loss data. Seeds in polyethylene bag (PM) weight loss reduced from 32.23% to 8.13% (PM+pal).

**Differences in the Behavioural Biology of C. maculatus on Cowpea Seeds in Packaging Materials and Packaging Materials with M. arboreus**

**Polyethylene**

An independent sample T-test to check for differences in the behavioural biology of C. maculatus on cowpea seeds in packaging materials and packaging materials with M. arboreus is shown on Table 3. There was a significant difference (P<0.05) in the proportion of damaged seeds (t (4)=1150, P=0.0000), percentage damage (t (4)=4677.3, P=0.0000), egg count (t (4)=3381.9, P=0.0000) and mortality (t (4)=-43.76, P=0.0000)

**Jute**

An independent sample T-test to check for differences in the behavioural biology of C. maculatus on cowpea seeds in packaging materials and packaging materials with M. arboreus was investigated by subjecting data to independent t-test analysis (Table 4). There was a significant difference (P<0.05) in the proportion of damaged seeds (t (4)=1150, P=0.0000), percentage damage (t (4)=4677.3, P=0.0000), egg count (t (4)=3381.9, P=0.0000) and mortality (t (4)=-43.76, P=0.0000)

**Hessian**

The differences in the behavioural biology of C. maculatus on cowpea seeds in packaging materials and packaging materials with M. arboreus was investigated by subjecting data to independent t-test analysis (Table 5). There was a significant difference (P<0.05) in the proportion of damaged seeds (t (4)=4950, P=0.0000), percentage damage (t (4)=642.18, P=0.0000), egg count (t (4)=10023.0, P=0.0000) and mortality (t (4)=-8.70, P=0.0000)

**Discussion**

The recent trend in the development of tolerance to widely used insecticides, among all other things, has necessitated the need to investigate alternatives that are better adapted to pest control and at the same time sustainable. In view of this, this research work was conducted to investigate the synergistic effect of polyethylene, jute and hessian bags on the bio-efficacy of Myrianthus arboreus in controlling the bruchid weevil, Callosobruchus maculatus.

Generally, the toxicity of a phytochemical compound to insect pest depends on a number of factors [16,17]. These variations bother on the type of the plant material used, the part used, bioactive constituent, extraction solvent, insect tolerance, environmental conditions and other external factors. In our study, the toxicity of M. arboreus was heightened in the presence of an external factor – the use of packaging materials. Generally, the incorporation of the botanicals in packaging materials reduced significantly the level of seed damage as compared in every instance when plant material was just administered. Studies [18] reported that treated cowpea seeds kept in packaging materials fared better than their counter parts in control (plant material only). Although the synergistic effect of the packaging material varied from one type of material to another, it nonetheless demonstrates that packaging is essential for improving storage conditions and protection against insect attack [11,12].

In comparing the packaging materials, the result of the study clearly show that jute bag was more effective in protecting the cowpea seeds in storage. Seeds kept in Jute bag suffered the least percent damage, in comparison to polyethylene and hessian bags, by the weevil. Jute bags are more impervious, thus, providing little oxygen for the continued activity of the bruchids and proliferation of contaminants. This finding appears correlated with the works of previous study [19]. The authors highlighted that impervious packaging materials make it more unlikely for insect pest to thrive than aerated packaging materials.
Table 3. Difference between seeds in Polyethylene packaging material (PM) and Packaging materials+M. arbores (PM+Pla).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pair</th>
<th>Mean ± S.E</th>
<th>SD</th>
<th>df</th>
<th>t.cal</th>
<th>Sig</th>
<th>RMKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damaged seeds</td>
<td>PM</td>
<td>882.3 ± 0.2</td>
<td>0.34</td>
<td>4</td>
<td>4204.</td>
<td>0.00</td>
<td>S</td>
</tr>
<tr>
<td>% damage</td>
<td>PM+Pla</td>
<td>54.63 ± 0.2</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg count</td>
<td>PM</td>
<td>83.28 ± 0.22</td>
<td>0.39</td>
<td>4</td>
<td>338.44</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Mortality</td>
<td>PM+Pla</td>
<td>5.30 ± 0.06</td>
<td>0.10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Difference between seeds in Jute packaging material (PM) and packaging materials+M. arbores (PM+Pla).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pair</th>
<th>Mean ± S.E</th>
<th>SD</th>
<th>df</th>
<th>t.cal</th>
<th>Sig</th>
<th>RMKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Damaged seeds</td>
<td>PM</td>
<td>409.21 ± 0.11</td>
<td>0.19</td>
<td>4</td>
<td>1150.0</td>
<td>0.00</td>
<td>S</td>
</tr>
<tr>
<td>% damage</td>
<td>PM+Pla</td>
<td>6.67 ± 0.33</td>
<td>0.58</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg count</td>
<td>PM</td>
<td>38.74 ± 0.01</td>
<td>0.01</td>
<td>4</td>
<td>4677.3</td>
<td>0.000</td>
<td>S</td>
</tr>
<tr>
<td>Mortality</td>
<td>PM+Pla</td>
<td>9.55 ± 0.01</td>
<td>0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N: Number of Samples; RMKS: Remarks; SD: Standard deviation; df: Degree of freedom; t.cal: Calculated t value.

The experimental data recorded on various aspects of the developmental biology of the bruchids showed that, where jute bag was used, there was a significant reduction in the biological activity of the beetle. The weevil number, oviposition strength and mortality were affected. Thus, jute bags offer a better storage capability than hessian and polyethylene bags.

Previous research has shown that adding two additional layers of packaging material as an ancillary measure has been efficacious in protecting cowpea from seed damage [20]. As oxygen permeability decreases across layers, the probability that stored grains are free of insect damage is also increased [21]. Tripel-bagging was first developed in Cameroun but it was not adopted extensively due to the cost of the required packaging materials [22]. Treatment of cowpea seeds coupled with storage in triple-layered packaging could have a synergistic effect on the protection of cowpea seeds from C. maculatus attack thereby maintaining the physical integrity of the seeds.

Conclusion

As obtained from the result of this study, the combination of packaging materials with M. arbores significantly reduced the activity of the cowpea weevil. In places were high technology for preventing insect attack is unobtainable, the use of this approach could help protect cowpea seeds and maintain seed quality.

Compliance with Ethical Standards

This research does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgments

Authors are grateful to Late Dr. Rotimi Akinkurolere for the role play in making this work a success may his gentle soul rest in peace.

Conflicts of Interest

The authors declare that they have no conflict of interests behind the development and submission of this manuscript other than the desire to build our academic career as a researcher.

References


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