ISSN: 2591-796X

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

Oladipupo SO*, Osunyemi OS, Ajayi OM, Bamgbose GG, Obimakinde ET, Lerayetan AA, Elijah OA.

Department of Biology, Federal University of Technology, Nigeria

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

Accepted on June 12, 2018

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 aboreus* has been proven to increase the quality of seeds while being effective protectants against storage insects [13,14].

Accordingly, in efforts to compliment 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 plant 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 F_1 emergence in treatments. The results were recorded and reported in percentage mean.

Data analysis

The weight loss data were computed using:

$$\%\,\text{Weight loss} = \frac{\textit{Initial weight loss} - \textit{Final weight loss}}{\textit{Initial weight loss}} \times \frac{100}{1}$$

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+pla (9-77) set up. Specifically, higher egg count was recorded in the polyethylene bag set up for both PM (766) and PM+pal (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+pal (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+pal 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 1. *Without plant material first month.*

Packaging Material	Total no of seeds	Damaged seeds	Percentage damage
Polyethylene	1053.77 ± 0.21^a	882.36 ± 0.52^b	83.28 ± 1.23^c
Jute	1055.69 ± 1.20^b	409.21 ± 0.32^a	38.74 ± 0.87^a
Hessian	1055.99 ± 1.20^b	921.14 ± 0.22^c	87.23 ± 1.43^b

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 2. *With plant material first month.*

Packaging Material	Total no of seeds	Damaged seeds	Percentage damage
Polyethylene	1046.67 ± 0.26^a	54.63 ± 3.52^c	$5.30 \pm 0.\mathbf{65}^c$
Jute	1056.00 ± 2.20^b	$6.67\ 1.32^a$	0.55 ± 0.01^a
Hessian	1056.00 ± 1.67^b	28.33 ± 1.42^b	2.75 ± 0.33^b

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.

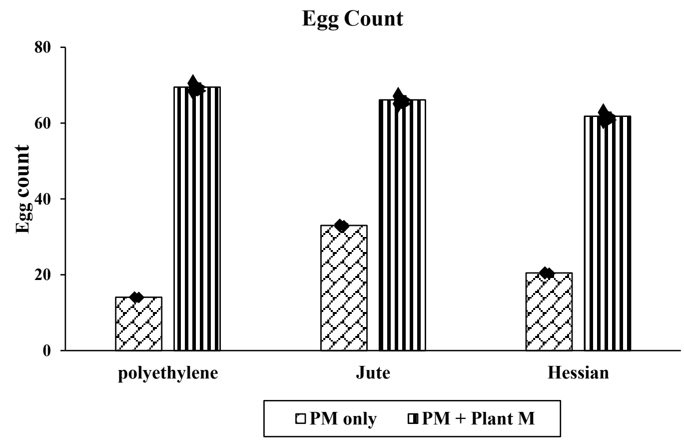

Figure 1. *Oviposition pattern in experimental set-up.*

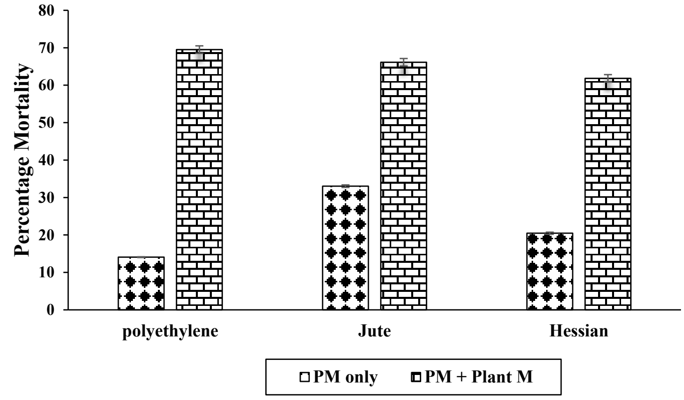

Figure 2. *Percentage mortality of Bruchids in Experimental set-up.*

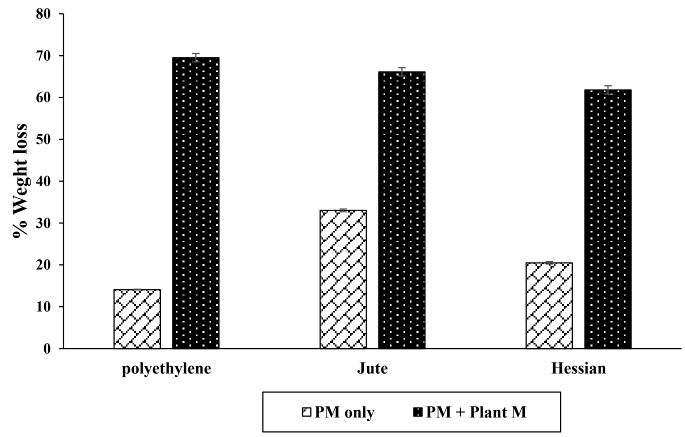

Figure 3. *Percentage weight loss of seeds in Experimental set-up.*

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)=4204, P=0.0000), percentage damage (t (4)=338.94, P=0.0000), egg count (t (4)=6219.9, P=0.0000) and mortality (t (4)=-77.81, 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* is shown on 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. arboreus (PM+Pla)*

Parameters	Pair	Mean ± S.E	SD	df	t.cal	Sig	RMKS
Damaged seeds	PM PM+Pla	88236 ± 0.20 54.63 ± 0.02	0.34 0.03	4 4	4204.	0.00	S
% damage	PM PM+Pla	83.28 ± 0.22 5.30 ± 0.06	0.39 0.10	4 4	338.94	0.000	S
Egg count	PM PM+Pla	766.47 ± 0.10 77.29 ± 0.04	0.17 0.07	4 4	6219.9	0.000	S
Morrtality	PM PM+Pla	23.21 ± 0.02 65.68 ± 0.55	0.04 0.94	4 4	-77.81	0.000	S

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

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

Parameters	Pair	Mean ± S.E	SD	df	t.cal	Sig	RMKS
Damaged seeds	PM PM+Pla	409.21 ± 0.11 6.67 ± 0.33	0.19 0.58	4 4	1150.0	0.00	S
% damage	PM PM+Pla	38.74 ± 0.01 0.55 ± 0.01	0.01 0.01	4 4	4677.3	0.000	S
Egg count	PM PM+Pla	396.21 ± 0.10 9.29 ± 0.04	0.19 0.06	4 4	3381.9	0.000	S
Mortality	PM PM+Pla	23.56 ± 0.04 48.79 ± 0.58	0.07 0.99	4 4	-43.76	0.000	S

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

Table 5. *Difference between seeds in Polyethylene packaging material (PM) and Packaging materials+M. arboreus (PM+Pla)*

Parameters	Pair	Mean ± S.E	SD	df	t.cal	Sig	RMKS
Damaged seeds	PM PM+Pla	878.29 ± 0.16 32.00 ± 0.06	0.28 0.11	4 4	4950.7	0.00	S
% damage	PM PM+Pla	83.13 ± 0.09 3.04 ± 0.09	0.15 0.15	4 4	642.18	0.000	S
Egg count	PM PM+Pla	900.26 ± 0.07 21.33 ± 0.04	0.11 0.07	4 4	10023.	0.000	S
Mortality	PM PM+Pla	19.39 ± 0.02 54.72 ± 4.06	0.04 7.04	4 4	-8.70	0.000	S

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]. Triple-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. arboreus* 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

1. Adedire CO, Lajide L. Ability of Extract of Ten Tropical Plant Species to Protect Maize Grains against Infestation by the Maize Weevil *Sitophilus zeamais* during Storage Nigerian. Journal of Experimental Biology. 2003;4:175-9.

2. Oladipupo SO Adedire CO, Gbaye OA. Fecundity and survival of *Carpohilus hemipterus* (Linnaeus 1958) (Coleoptera: Nitidulidae) on alternative hosts. Brazilian Journal of Biological Sciences. 2018;5:115-23.

3. Duke JA. Introduction to Food Legumes In: Singh SR (ed.) Insect Pests of Tropical Food Legumes Chinchester. John Wiley and Sons, 1990;5-42.

4. Adekola OF, Oluleye F. Influence of Mutation Induction on the Chemical Composition of the Cowpea *Vigna unguiculata* (L). Walp African Journal of Biotechnology. 2007;6:2143-6.

5. Kang JK, Pittendrigh BR, Onstad DW. Insect Resistance Management for Stored Product Pests: A Case Study of Cowpea Weevil (Coleoptera: Bruchidae). Journal of Economic Entomology. 2013;106:2473-90.

6. Dugje IY, Omoigui LO, Ekeleme F, et al. Farmers Guide to Cowpea Production in West Africa International Institute of Tropical Agriculture (IITA) Ibadan Nigeria. 2009;1-34.

7. Talukder FA, Howse PE. Isolation of Secondary Plant Compounds from *Aphanamixis polystachya* as Feeding Deterrents against Adults *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Stored Product Research. 2000;107:395-402.

8. Isman MB. Perspective Botanical insecticides: for richer for poorer. Pest Management Science. 2008;64:8-11.

9. Parugrug AM, Roxas AC. Insecticidal Action of Five Plants against Maize Weevil Sitophilus zeamais Motsch (Coleoptera: Curculionidae) King Mongkut's Institute of Technology Ladkrabang (KMITL). Science and Technology Journal. 2008;8:24-38.

10. Adedire CO, Obembe OO, Akinkurolere RO, et al. Response of Callosobruchus maculatus (Coleoptera: Chysomelidae: Bruchidae) to Extracts of Cashew Kernels. Journal of Plant Disease and Protection. 2011;118:75-9

11. Harrington JF, Biochemical basis of seed longevity Seed. Science and Technology. 1973;1:453-61.

12. Pusarla S, Prashant KR. Studies on effects of seed treatments polymer coating and packaging materials on seed quality parameters of stored cowpea (Vigna unguiculata) seeds. Journal of Pharmacognosy and Phytochemistry. 2017;6:677-81.

13. Asawalam EF, Emosairue SO, Hassanali A. Bioactivity of Xylopia aetiopica (Dunal) A Rich essential oil constituents on maize weevil Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) Electronic. Journal of Environmental, Agricultural and Food Chemistry. 2006;5:1195-204.

14. Akinkurolere RO, Adedire CO, Odeyemi OO, et al. Bioefficacy of Extracts of some Indigenous Nigerian Plants on the developmental stages of mosquito (Anopheles gambiae). Jordan Journal of Biological Sciences. 2011;4:237-42.

15. Oladipupo SO. Susceptibility of Anopheles gambiae in Akure metropolis to Synthetic and Botanical Insecticdes. A Masters Thesis submitted to the Federal University of Technology Akure Nigeria; 2017.

16. Jeyabalan D, Arul N, Thangamathi P. Studies on effects of Pelargonium citrosa leaf extracts on malarial vector Anopheles stephensi Liston. Bioresource Technology. 2003;89(2):185-9.

17. Tuetun B, Choochote W, Rattanachanpichai E, et al. Repellent properties of celery Apium graveolens L compared with commercial repellents against mosquitoes under laboratory and field conditions. Tropical Medicine and International Health. 2004;10:1190-8.

18. Maraddi B. Influence of growth regulators on seed yield and quality and seed treatments on storability of cowpea cv C-252. MSc (Agric) Thesis University of Agricultural Sciences Dharward; 2002.

19. Kamara EG, Massaquoi FB, James MS, et al. Effects of packaging material and seed treatment on Weevil (*Callosobruchus maculatus* (F) Coleoptera: Bruchidae) infestation and quality of cowpea seeds. African Journal of Agricultural Research. 2014;9:3313-8.

20. Sanon A, Dabire-Binso LC, Ba NM. Tripple-bagging of cowpeas within high density polyethylene bags to control cowpea beetle Callosobrunchus maculatus F (Coleoptera: Bruchidae). Journal of Stored Products Research. 2011;47:210-5.

21. Dobie P, Haines CP, Hodges RJ, et al. Insects and Arachnids of Tropical Stored Products Their Biology and Identification. Technical Report. Tropical Development and Research Institute, Berks, UK; 1991.

22. Murdock LL, Seck D, Ntoukam G, et al. Shade RE Preservation of cowpea grain in sub-Saharan Africa Bean/ Cowpea CRSP contributions. Field Crops Research. 2003;82:169-78.

***Correspondence to:**

Oladipupo SO
Department of Biology,
Federal University of Technology,
Nigeria,
Phone: +2348o67341226,
E-mail: seun_oladipupo@yahoo.com