Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2022; 10(2): 36-42

Received: 10-01-2021
Accepted: 16-02-2021

Ernest Olaolu Dawodu
Department of Agricultural Science and Technology, Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti, Nigeria

Julius Ipadeola Olaifa
Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Timothy Abiodun Adebayo
Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Samuel Adelani Babarinde
Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Gabriel Olulakin Adesina
Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Oladele Abiodun Olaniran
Department of Crop and Environmental Protection, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Corresponding Author:
Ernest Olaolu Dawodu
Department of Agricultural Science and Technology, Bamidele Olumilua University of Education, Science and Technology, Ikere-Ekiti, Nigeria

Efficacy of *Piper guineense* (Shum and Thonn), *Dennettia tripetala* (G. Baker), and *Ocimum gratissimum* (Linnaeus) powders admixed with selected excipients for the control of *Callosobruchus maculatus* (Fabricus) infesting cowpea, *Vigna unguiculata* (L. Walp), seed

Ernest Olaolu Dawodu, Julius Ipadeola Olaifa, Timothy Abiodun Adebayo, Samuel Adelani Babarinde, Gabriel Olulakin Adesina and Oladele Abiodun Olaniran

DOI: https://doi.org/10.22271/j.ento.2022.v10.i2a.8968

Abstract
The use of synthetic insecticides to control stored product insects causes various health and Ecological challenges to man. Therefore, there is a need for alternative formulations like plant materials with the least possible side effects for the effective control of cowpea bruchid, *Callosobruchus maculatus* (Fabricius), a cosmopolitan pest of pulses. This study evaluated the efficacy of insecticidal dust formulations using three botanicals (*Piper guineense*, *Dennettia tripetala* and *Ocimum gratissimum*), three excipients (rice bran, maize husk and cowpea pod husk), admixed at ratios 70:30, 60:40, 50:50, 40:60 and 30:70 botanical to an excipient, for the control of *C. maculatus.* Four particle sizes (212, 300, 500 and 1000 *µm*) applied at dosages of 0.1- 0.5 g / 20 g cowpea seeds were assayed and untreated cowpea seeds served as controls. Ten *C. maculatus* adults (sex ratio 1:1) were used to infest the treated and untreated cowpea seeds separately in Petri dishes. Data were collected on mortality, Oviposition, F_1 progeny emergence and damaged cowpea seeds. The data were subjected to analysis of variance and means were separated with Least Significant Difference at a 5% significance level. All dosage ratios of the formulated dusts caused significantly (p< 0.05) higher mortality of bruchids, reduced the number of laid eggs, F_1 progeny emergence and damaged cowpea seeds due to *C. maculatus* infestation. Regardless of the assayed botanical, 212 *µm* particle size was outstanding and the formulation ratio of 70:30 (botanical: excipient) was significantly (p< 0.05) better than other formulation ratios. The formulation with *P. guineense* with any excipient at 0.5 g/ 20 g cowpea seeds using 212 µm was superior to other formulations as cowpea protectant against *C. maculatus*. Therefore, it is recommended as an alternative to over-dependence on synthetic insecticides.

Keywords: *Callosobruchus maculatus*, cowpea seed damage, *Dennettia tripetala,* formulations, *Ocimum gratissimum*, *Piper guineense*

Introduction
Cowpea possesses health benefits, with protein contents of about 23-28% and B-vitamins, making it a suitable component of the diet of many people in the third world countries who, in most cases, cannot afford to buy other protein sources such as meat and fish [21, 36, 16]. In the main cowpea growing areas of the tropics, processing the seeds into various edible and nutritious food forms has been the means of improving the quality of food intake of both children and adults [24]. Incidentally, despite the importance of cowpea as a staple dietary food in Africa, between 30 and 100% of total cowpea production valued at US 300 million dollars is either lost or damaged within 3-5 months of storage as a result of bruchid attack [10]. The period of food shortage can mean extreme starvation when food intake may drop below the level required for normal daily consumption. This is occasioned by the lack of proper storage facilities and methods with a high rate of depredation of available stored food commodities by insects, especially in the developing countries [10, 30 1]. Cowpea bruchid, *Callosobruchus maculatus*, (Fabricius) (Coleoptera: Chrysomelidae: Bruchinae) is among the major pests of legumes that cause high infestation both in the field and in storage [1, 4, 2, 31].

Cowpea grains stored after harvest are the favourite food of the bruchid, which causes a reduction in weight, the nutritional value of the grain and the viability of stored seeds [6]. Also, serious contamination of food commodities with frass, exuviae and dead bruchids alter the biochemical constituents of the infested cowpea seeds [37].

Although many synthetic insecticides formulated as dusts such as Pirimiphos methyl are effective protectants of stored products, they are associated with undesirable effects on the consumers, animals, non-target organisms and the environment [5, 15, 38, 12]. In some instances, these synthetic pesticides outlive their usefulness and are implicated in human poisoning, environmental contamination and pest resistance to the pesticides [5, 38, 39, 12]. Consequently, several studies [4, 14, 19, 28] have indicated the insecticidal potency of botanical pesticides, which can be produced locally with cheap materials and simple equipment without causing pollution to the environment or poison to humans and his food. For instance, the insecticidal potentials of different botanical formulations of *P. guineense*, *D. tripetala and O. gratissimum*, against stored product insects have been investigated by many workers [10, 8, 31, 25].

Powder formulations of botanicals tend to be widely accepted by resource-poor subsistence farmers since the production of botanical powders does not involve high technical knowledge. Apart from the active ingredients, it is necessary to investigate the fitness of cheaply available plant materials as excipients for the insecticidal dust formulation. Such excipients are often thrown away as agro-waste materials, which consequently constitute ecological hazards due to improper disposal methods. Therefore, this study was designed to investigate the efficacy of *Piper guineense*, *Dennettia tripetala* and *Ocimum gratissimum* admixed with selected agro-based excipients as protectants of cowpea seeds against cowpea seed bruchid, *Callosobruchus maculatus*.

Materials and Methods

Experimental site

The experiment was carried out in the laboratory of the Department of Crop and Environmental Protection, Faculty of Agricultural Sciences, Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria.

Cowpea handling and *Callosobruchus maculatus* culture

The seeds of Ife Brown, a susceptible cowpea variety, used for the experiment were collected from the Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria. The seeds were subjected to cold shock treatment, using a deeper freezer for 32 days in order to kill any current developmental stage of *C. maculatus* in the seed lot [13]. The disinfested seeds were removed from the freezer and exposed to the ambient laboratory condition for 48 hours for acclimatization before using the entomological bioassay. *Callosobruchus maculatus* used for this study was obtained from a colony originating from infested cowpea seeds obtained from Wazobia Market, Ogbomoso, Nigeria. The colony was maintained in No. 1 Kilner jars with meshed lids in the laboratory throughout the period of the study, following the standard procedure for culturing bruchid [13, 34].

Preparation of botanical insecticidal powder

Seeds of *Piper guineense* Schum and Thonn, fruits of *Dennettia tripetala* Baker and leaves of *Ocimum gratissimum* Linnaeus were used as the botanicals. They were collected from the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria. Authentication of each plant material was done at the Herbarium of the Department of Pure and Applied Biology, LAUTECH, Ogbomoso, Nigeria. The seeds of *P. guineense* were dried in an oven at 40 ºC for 24 h, while fresh fruits of *D. tripetala* were dried to a constant weight at a temperature of 40 ºC for 72 h. The leaves of *O. gratissimum* were air-dried to constant weight for seven days. Thereafter, the plant materials were separately ground with a food mill and sieved to powders of different particles sizes (212, 300, 500 and 1000 μm) using British standard sieves. The plant powder types and particle sizes were separately put in well-labeled plastic containers with tightly fitted lids and kept under ambient laboratory conditions until use.

Preparation of excipients

The excipients which acted as carriers to the botanical powders were made from dry rice (*Oryza sativa* L.) bran, dry maize (*Zea mays* L.) husk and dry cowpea (*Vigna unguiculata* L. Walp) pod husk. Each excipient material was obtained from Wazobia Market, Ogbomoso. Each excipient was dried in an oven at 40 ºC for 24 h and subsequently pulverized separately with a food mill and sieved in order to obtain powders of different particle sizes (212, 300, 500 and 1000 μm) as used for the botanicals. The milled excipients were subsequently kept in well-labelled plastic containers with tightly fitted lids in the laboratory under ambient conditions.

Formulation of botanical insecticide

The formulation was made from already prepared botanical insecticidal powders and the excipients. Formulated mixtures of ratios 30:70; 40:60; 50:50; 60:40 and 70:30 percent botanical powder to excipient were made. The particle sizes for the constituents of each formulation were the same. Each formulated botanical-excipient dust was put into a separate labelled plastic container with a tightly fitted lid and kept on the shelf under ambient laboratory conditions, until used for the entomological bioassay.

Experimental bioassay

The experiment was conducted under ambient temperature (28±2 ˚C) and relative atmospheric humidity (67±3%) in the laboratory. The formulated dusts from botanicals and excipients as stated above, were tested at the rate of 0.1, 0.2, 0.3, 0.4, and 0.5 g/20 g cowpea seeds; while the untreated seeds served as controls. Ten teneral bruchids (sex ratio 1:1) were introduced into each of the treated and untreated (control) cowpea seeds in 8.5 cm diameter Petri dishes with lids. The experiment was replicated five times. Data were collected on adult mortality at 24-72 hours after treatment (HAT), oviposition at 3 days after treatment (DAT), F_1 progeny emergence and cowpea seeds damaged by *C. maculatus* at 28 DAT.

Experimental design and statistical analysis

The experiment was arranged in a completely randomized design and the data were subjected to analysis of variance using SAS Software Package. Significant means were separated with the least significant difference at a 5% significance level.

Results

All the doses of *P. guineense* significantly ($p< 0.05$) caused higher mortality of the bruchids throughout the periods of observations and inhibited the number of eggs laid, number of F_1 progeny emergence and the number of holes bored on cowpea seeds due to *C. maculatus* when compared with the control. Application of *P. guineense* at 0.5 g/20 g seeds consistently caused the highest mean number of bruchid mortality at all observations (5.37, 6.70, 7.68, 8.41 and 8.79 at 24, 36, 48, 60 and 72 HAT, respectively) while the control steadily resulted in the lowest mean number of dead bruchids (0.17, 0.19, 0.20, 0.20 and 0.21, respectively). The control experiment recorded the highest mean number of eggs laid (107.07), emerged F_1 progenies (75.51) and a number of bored holes on cowpea seeds (81.91). At the same time, the application of *P. guineense* at 0.5 g/20 g seeds resulted in the lowest mean number of laid eggs, emerged F_1 progenies and number of bored holes on cowpea seeds (20.99, 7.61 and 12.70, respectively) (Table 1).

The excipients had a significant ($p < 0.05$) effect on the mortality of the bruchids throughout the study period. It however had no significant effect oa n number of laid eggs by the female bruchids but significantly influenced the number of emerged F_1 progenies and the number of holes bored on cowpea seeds (Table 1). The highest mortality observed in the excipients at 24, 36, 48, 60 and 72 HAT (2.98, 4.09, 4.92, 5.58 and 6.11, respectively) was observed in cowpea pod husk. All other parameters were not significantly affected by the excipients. Particle size 212 µm recorded the highest bruchid mortality in all the times of observations between 24 and 72 HAT (3.64, 5.11, 5.98, 7.11 and 7.69, respectively), while particle size 1000 µm recorded the lowest bruchid mortality (2.29, 2.99, 3.47, 3.95 and 4.36, respectively) in the same periods of observations (Table 1). Similarly, particle size 212 µm recorded the significantly lowest mean number of eggs laid, emerged F_1 progenies and number of holes bored on cowpea seeds (24.96, 14.15 and 23.66, respectively). In contrast, particle size 1000 µm resulted in the highest mean number of holes bored on cowpea seeds, emerged F_1 and number of eggs laid on cowpea seeds by female cowpea bruchids (23.66, 14.15 and 24.96, respectively).

The formulation ratio 70:30 formulation of *P. guineense* to the excipients recorded the highest mortality of bruchids at 24, 36, 48 and 60 HAT (3.86, 5.06, 5.68 and 6.16, respectively) except at 72 HATthe where raof tio 50:50 recorded the highest mortality (6.62). It was observed that formulation ratio 30:70 resulted in the significantly highest number of eggs laid (67.25) while ratio 70:30 recorded the lowest mean number (34.28). The formulation ratio 70:30 recorded the lowest mean number of emerged F_1 progenies and lowest mean number of holes bored on cowpea seeds (17.96 and 21.28, respectively) which was significantly lower than the values obtained in other ratios except for 60:40 for F_1 progeny emergence (Table 1).

For the *D. tripetala*-based formulations, its *a*pplication at 0.5 g/20 g seed consistently recorded the highest mean number of dead bruchid at 24-72 HAT (1.52, 2.18, 2.80, 3.34 and 4.19, respectively) while the control steadily resulted in the lowest bruchid mortality (0.00). The control experiment recorded the significantly highest mean number of laid eggs (116.00), emerged F_1 progenies (91.00) mean a number of holes bored on cowpea seeds (104.37). In contrast, application of *D. tripetala* at 0.5 g/20 g seeds resulted in the lowest number of laid eggs, emerged F_1 progenies and bored holes on cowpea seeds (39.21, 25.77 and 32.39, respectively) (Table 2). The excipients had no significant effect (p>0.05) on bruchid mortality, the number of eggs laid, emerged F_1 progenies and the mean number of bored holes on cowpea seeds. The particle size significantly ($p < 0.05$) caused mortality of adult bruchid, reduced the mean number of eggs laid, emerged F_1 progenies and number of bored holes on cowpea seeds. The lowest mortality at 24, 36, 48, 60 and 72 HAT (0.36, 0.51, 0.75, 1.00 and 1.42, respectively) was observed in 1000 µm while the highest mortality was observed in 212 µm at 24, 36 and 48 HAT (0.95, 1.52 and 1.96, respectively); while 500 µm particle size caused the highest mortality between 60 and 72 HAT (2.41 and 3.36, respectively). Lowest mean number of eggs laid, emerged F_1 progenies and holes bored on cowpea seeds were recorded in 212 µm particle size. The formulation ratio 70:30 of *D. tripetala*: excipient evoked highest mortality at 24, 36, 48, 60 and 72 HAT (0.92, 1.93, 2.65, 3.35 and 4.06, respectively) while 30:70 recorded the lowest mortality at the same periods of observations (0.27, 0.44, 0.80, 1.06 and 1.39, respectively). The lowest mean number of eggs laid, emerged F_1 progenies and number of holes bored on cowpea seeds (49.04, 37.07 and 42.93, respectively) were also recorded in 70:30 formulation while the highest number of eggs laid, emerged F_1 progenies and number of holes bored on cowpea seeds (74.71, 57.23 and 66.19, respectively) were recorded in 30:70 botanical to excipient (Table 2).

For the *O. gratissimum*-based formulations, application of 0.5 g/20 g at seeds consistently evoked the highest number of dead bruchid at 24, 36, 48, 60 and 72 HAT (0.29, 0.79, 1.51, 2.23 and 3.04, respectively) while the control steadily resulted in the lowest number of dead bruchids. The control experiment also recorded the highest numbers of laid eggs by female bruchids, emerged F_1 progenies and bored holes on cowpea seeds (130.05, 80.34 and 110.24, respectively) which were significantly ($p < 0.05$) higher than the values in other concentrations (Table 3). The influence of particle sizes on adult bruchids mortality, number of eggs laid, emerged F_1 progenies and number of holes bored on cowpea seeds followed similar patterns as observed in *P. guineense* and *D. tripetala*-based dusts. The formulation ratio 70:30 recorded the significantly ($p < 0.05$) highest mean mortality at 24 36, 48, 60 and 72 HAT (0.21, 0.58, 1.10, 1.67 and 2.28, respectively), the lowest number of eggs laid by female bruchids, emerged F_1 progenies and number of holes bored on the treated cowpea seeds (Table 3).

Table 1: Effects of treatment, excipient materials and the formulation ratios of *Piper guineense* on the mortality, Oviposition, F_1 emergence and hole bored on cowpea seeds by *Callosobruchus maculatus*

Treatment	Mortality at Hours After Treatment					Number of eggs laid	Emerged F_1	Number of holes
	24	36	48	60	72			
Dose (g/20g seed) 0.1	1.41	2.57	3.44	4.46	5.28	58.48	32.52	38.28
0.2	2.37	3.77	4.74	5.68	6.40	48.27	24.07	31.33
0.3	3.49	4.73	5.78	6.65	7.34	38.39	16.54	24.00
0.4	4.49	5.74	6.66	7.59	8.16	28.18	10.50	17.38
0.5	5.39	6.70	7.68	8.41	8.79	20.99	7.61	12.70
Control	0.00	0.00	0.00	0.00	0.00	107.07	75.51	81.91

LSD	0.17	0.19	0.20	0.20	0.21	2.94	1.31	1.67
Excipients								
Cowpea pod	2.98	4.09	4.92	5.58	6.11	49.52	28.15	34.51
Maize husk	2.91	3.96	4.76	5.55	6.01	50.96	27.28	33.52
Rice bran	2.67	3.71	4.49	5.27	5.88	50.21	28.09	34.76
LSD	0.12	0.14	0.14	0.14	0.15	ns	1.31	1.18
Particle size (*µm*)								
212	3.64	5.11	5.98	7.11	7.69	24.96	14.15	23.66
300	2.85	4.22	5.32	6.18	6.80	47.10	25.91	31.77
500	2.64	3.36	4.10	4.62	5.13	62.01	31.55	36.79
1000	2.29	2.99	3.47	3.95	4.36	66.85	39.85	44.83
LSD	0.14	0.16	0.16	0.16	0.18	2.40	9.16	1.36
Ratio								
30:70	2.05	2.85	3.39	4.15	4.77	67.25	28.83	47.11
40:60	2.48	3.57	4.35	4.94	5.52	52.81	30.09	38.64
50:50	2.75	3.90	4.93	5.99	6.62	53.78	27.26	31.42
60:40	3.14	4.23	5.24	6.13	6.56	43.04	25.38	32.85
70:30	3.86	5.06	5.68	6.16	6.51	34.28	17.96	21.28
LSD	0.16	0.18	0.18	0.18	0.20	2.68	9.16	1.52

ns= data not significant at 5% probability level

Table 2: Effects of treatment, excipient materials, particle sizes and the formulation ratios of *Dennettia tripetala* on mortality, Oviposition, F_1 emergence and holes bored on cowpea seeds by *Callosobruchus maculatus*

Treatment	Mortality at Hours after Treatment					Number of eggs laid	Emerged F_1	Number of holes
	24	36	48	60	72			
Dose (g/20 g seed) 0.1	0.25	0.66	1.05	1.44	1.91	65.34	53.11	59.32
0.2	0.36	0.83	1.33	1.84	2.40	60.51	47.68	54.13
0.3	0.70	1.23	1.79	2.24	2.90	53.73	40.25	46.65
0.4	1.11	1.66	2.24	2.80	3.50	46.28	32.66	39.46
0.5	1.52	2.18	2.80	3.34	4.19	39.21	25.77	32.39
Control	0.04	0.04	0.05	0.05	0.07	116.00	91.00	104.37
LSD	0.60	0.89	1.10	1.34	1.56	1.27	1.21	1.24
Excipients								
Cowpea pod	0.70	1.15	1.56	1.91	2.47	63.24	47.67	55.18
Maize husk	0.63	1.07	1.52	1.92	2.41	63.72	48.54	56.28
Rice Bran	0.66	1.08	1.55	2.01	2.61	63.72	47.86	55.79
LSD	ns	ns	Ns	ns	ns	ns	Ns	Ns
Particle size (*µm*)								
212	0.95	1.52	1.96	2.36	2.81	52.43	35.95	44.07
300	0.77	1.32	1.67	2.02	2.40	58.57	44.62	51.84
500	0.56	1.07	1.78	2.41	3.36	71.85	56.09	63.79
1000	0.36	0.51	0.75	1.00	1.42	71.38	55.43	63.30
LSD	0.10	0.14	0.18	0.21	0.25	1.42	1.36	1.39
Ratio								
30:70	0.27	0.44	0.80	1.06	1.39	74.71	57.23	66.19
40:60	0.56	0.82	1.10	1.41	1.96	71.85	54.62	63.04
50:50	0.73	1.66	1.50	1.90	2.52	65.21	48.92	57.26
60:40	0.83	1.26	1.66	2.03	2.56	56.98	42.29	49.35
70:30	0.92	1.93	2.65	3.35	4.06	49.04	37.07	42.93
LSD	0.11	0.16	0.20	0.24	0.28	1.59	1.52	1.55

ns= data not significant at 5% level of probability

Table 3: Effects of treatment, excipient materials, particle sizes and the formulation ratios of *Ocimum gratissimum* on mortality, Oviposition, F_1 emergence and holes bored on cowpea seeds by *Callosobruchus maculatus*

Treatment	Mortality at Hours after Treatment					Number of eggs laid	Emerged F_1	Number of holes
	24	36	48	60	72			
Dose (g/20 g seed) 0.1	0.00	0.06	0.30	0.72	1.37	67.99	54.11	62.14
0.2	0.00	0.14	0.60	1.37	2.06	63.25	48.14	56.91
0.3	0.05	0.30	0.88	1.60	2.35	57.12	41.14	50.16
0.4	0.13	0.54	1.25	1.93	2.61	50.22	33.97	43.11
0.5	0.29	0.79	1.51	2.23	3.04	42.80	26.52	34.76
Control	0.00	0.00	0.00	0.00	0.00	130.05	80.34	110.24
LSD	0.04	0.06	0.08	0.16	0.11	1.54	1.24	1.53
Excipients								
Cowpea pod	0.08	0.32	0.77	1.26	1.90	69.47	47.91	60.61
Maize husk	0.08	0.33	0.79	1.33	1.89	68.91	47.68	59.11
Rice Bran	0.08	0.27	0.72	1.33	1.93	67.33	46.53	58.64

LSD	Ns	0.05	0.05	ns	ns	1.09	0.87	1.08
Particle size *µm*								
212	0.11	0.42	0.97	1.53	2.12	62.38	39.48	53.10
300	0.14	0.48	0.99	1.52	2.00	67.17	44.04	58.15
500	0.03	0.16	0.54	1.08	1.75	72.37	52.98	63.48
1000	0.03	0.16	0.54	1.08	1.75	72.37	52.98	63.48
LSD	0.03	0.05	0.06	0.09	0.09	1.26	1.01	1.25
Ratio								
30:70	0.04	0.18	0.54	1.03	1.66	76.50	47.54	66.57
40:60	0.06	0.23	0.69	1.22	1.71	7.75	50.98	61.73
50:50	0.04	0.30	0.76	1.24	1.94	68.25	47.76	59.35
60:40	0.05	0.25	0.70	1.38	1.93	66.49	47.48	57.88
70:30	0.21	0.58	1.10	1.67	2.28	60.88	43.09	52.23
LSD	0.03	0.06	0.07	0.11	0.10	1.41	1.13	1.40

ns= data not significant at 5% level of probability

Discussion

Piper guineense, *D. tripetala* and *O. gratissimum* powders as insecticidal ingredients with each of the excipients (cowpea pod husk, maize husk flour and rice bran powder) protected cowpea seeds from damage by *C. maculatus.* The results agreed with earlier works [4, 31, 5, 8, 35, 17, 11] on the use of botanicals with insecticidal properties in the control of cowpea bruchid in storage. The fact that 70:30 insecticidal material: excipient evoked the highest insecticidal properties against the bruchid implies that the observed bioactivities were attributable to the bioactive compounds in the insecticidal ingredients. It was observed that dosage rate of 0.5 g/ 20 g seeds of cowpea significantly ($p< 0.05$) recorded the highest mortality at all periods of observation while the control recorded the lowest number of mortality. The dose- and exposure duration-dependent toxicity of the formulated dusts implies that the bruchids picked up sizeable amount of the insecticidal dusts in the course of their movements in the experimental units, since there were no escape routes. According to Alexander *et al.* [2] and Bayih *et al.* [9], the effectiveness of unitary botanical formulations against Mexican bean weevil, *Zabrotes subfasciatus* improved with the increase in dosage rate. Similarly, the toxicity of *P. guineense* against *Trogoderma granarium* was dose- and exposure duration-dependent [6]. The control experiment (untreated cowpea seeds), however, recorded the highest number of laid eggs by female bruchids, emerged F_1 progenies and number of holes bored on cowpea seeds, due to the conducive environment in the control experimental unit. The control (untreated) seeds had no extraneous materials which could be inimical to the physiological activities of the bruchids or induce their mortality; hence, the increased reproduction and damage done to the cowpea seeds by *C. maculatus* in the control seeds.

The particle sizes had significant effect on mortality, number of laid eggs, emerged F_1 progenies and number of holes bored on cowpea seeds, with higher mortality, lower oviposition and progeny emergence and seed damage which were observed in 212 µm than what were observed in 1000 µm. In an earlier study, the powders of *Zanthoxylum zanthoxyloides* root back and *Azadirachta indica* seed applied at particle size of 150 µm were more insecticidal than more coarse (2 mm particle size) powder [29]. Similarly, *Eugenia aromatica* and *P. guineense* powders applied at 150 µm particle size significantly inhibited oviposition by female *C. maculatus* compared to 300 µm particle size powders [18]. As postulated by previous author [18], the dispersion of botanical powder is affected by the particle size. The finer the particle, the `more uniformly distributed will the powder be on the grains; hence, the higher the tendency of the bruchids to pick up the insecticidal dusts particles. To a large extent, there were no significant difference in the efficacy of the excipient materials except in the oviposition of *C. maculatus* treated with *P. guineense*. This shows the potentials of the excipients and the superior efficacy of *P. guineense* over *D. tripetala* and *O. gratissimum*. All the excipients inhibited oviposition by female bruchids, reduced F_1 progeny emergence, caused adult mortality and reduced damage on the cowpea seeds, with no significant differences amongst the formulations. The observation was in line with earlier studies [14, 28, 17, 26, 11], on the insecticidal potentials of the plant materials admixed with leguminous seeds against *C. maculatus.*

The mechanisms of the reproduction inhibitory actions of botanical powders against *C. chinensis* include restriction of copulation, reduced oviposition by females and less fertile eggs which led to reduced progeny emergence [27]. The mechanisms for the toxicity of a botanical powder include abrasion of the insect cuticle by the powder and subsequent desiccation [1, 23]. Besides, the active compounds in each of the insecticidal plants could be responsible for the observed bioactivities. Recently, Ashamo *et al.* [3] reported the insecticidal potentials of rice husk, maize cob, groundnut and cowpea pods against *Sitotroga cerealella*, infesting paddy rice; which suggests that apart from the fact that the excipients served as the carries for the botanicals, they also served as synergists. The insecticidal ingredients and the excipients could block the spiracles and the insects died of the failure of the respiratory system. Particularly, 212 µm particle size was fine enough to block the spiracles and induce the abrasion of the cuticles when the bruchids move within the experimental unit. Piperine, octadien, glycylsarcosine, flavonoids, and polyphenols have been identified in *P. guineense*; Furanone, Pyridine and Linoleic acid in *D. tripetala* while Eugenol and thymol were identified in *O. gratissimum* [2, 20, 32, 33, 22, 11]. Reduced number of eggs laid was either due to the fact that the insects died before egg laying or that the pesticide plants prevented copulation of the insects. The resultant reduction in the number of F_1 progeny emergence implies ovicidal, larvicidal or pupicidal potentials of the botanicals.

Conclusion

The insecticidal formulation with *P. guineense* as the active ingredient with any of the excipients (maize husk flour, rice bran flour or cowpea pod husk) at particle size of 212 µm was better than other formulations for the protection of cowpea in storage. Therefore, it is recommended for practical application. The adoption of this option by the subsistence resource poor farmers would be easy since the technical knowledge (drying and pulverization of the insecticidal materials and excipients) involved is affordable for the farmers.

References

1. Adedire CO, Obembe OM, Akinkurolere RO, Oduleye SO. Response of *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae) to extracts of cashew kernels. J. Plant Dis. Protect. 2011;118(2):75-79.
2. Alexander P. Phytochemical screening and mineral composition of the leaves of *Ocimum gratissimum* (Scent leaf). Int. J. Applied Sci. Biotech. 2016;4(2):161-165.
3. Ashamo MO, Ileke KD, Onasile AI. Chemical composition and toxicity of some agro waste-derived insecticides against Angoumois grain moth, *Sitotroga ceralella* (Olivier) [Lepidoptera: Gelechiidae] infesting stored paddy grains. Bull. National Res. Centre. 2021;45(1):1-12.
4. Babarinde SA**,** Esan EO, Olatunde OZ, Ajayi DS, Olaniyi JP. Combination of *Piper guineense* essential oil with cowpea varietal resistance in control of cowpea seed beetle, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae). J Northeast Agric. Univ. 2017a;24(3):10-18.
5. Babarinde SA, Oladoye SO, Adesina GO, Adebayo MA. Chemical Composition and Bioactivity of *Lippia adoensis* Hochst ex. Walp (Verneneaceae) leaf essential oil against *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae). J Northeast Agric. Univ. 2016;23(4):8-14.
6. Babarinde SA**,** Pitan OOR, Ogunfiade AT. Bioactivity of *Piper guineense* Schum. & Thonn seed and *Moringa oleifera* Lam. leaf powder against *Trogoderma granarium* Everts (Coleoptera: Dermestidae). Arch. Phytopathol. Plant Protect. 2011;44(3):298-306.
7. Babarinde SA, Pitan OOR, Olatunde GO, Ajala MO. First report of toxicity of *Xylopia parriflora* (A. Rich) Benth (Annonaceae) root bark's essential oil against cowpea bruchid *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchidae). Nat. Prod. Res. 2015;29(4):349-352.
8. Babarinde SA, Pitan OOR, Ajala MO, Olatunde GO. Insectifugal and insecticidal potentials of two tropical botanical essential oils against cowpea seed bruchid. Environ. Sci. Pollution Res. 2017b;24(24):19785-19794.
9. Bayih T, Tamiru A, Egigu MC. Bioefficacy of unitary and binary botanical combations against Mexican bean weevil, *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae). Int. J Trop. Insect Sci. 2018;38:205-215.
10. Brisibe EA, Adugbo SE, Ekanem U, Brisibe F, Figueira GM. Controlling bruchid pests of stored cowpea seeds with dried leaves of *Artemisia annua* and two other common botanicals. Afr. J Biotechnol. 2011;10(47):9586-9592.
11. Dawodu EO, Edewor TI, Babarinde SA. Quantification of total Phenolic and Flavonoid Contents, and GC-MS Profile of Extracts of *Piper guineense*, *Dennettia tripetala* and *Ocimum gratissimum* used in the Control of Cowpea Bruchid *Callosobruchus maculatus* in Storage Grains. International Journal of Agriculture and Environmental Science. 2021;8(5):9-18. DOI: 10.14445/23942568/IJAES-V815P102
12. Dawodu EO, Olaifa JI, Babarinde SA, Adebayo TA. Traded synthetic insecticides for postharvest crop protection in Oyo State, southwestern Nigeria. LAUTECH-2021-29 Crop and Environmental Reviews (In Press).
13. Ejemen IJ. Selection for tolerance in *Callosobruchus maculatus* to sub-lethal doses of essential oils of *Eugenia aromatic* and *Piper guineense*. J Entomol. Zoo Sts. 2017;5(3):1267-1271.
14. Ekeh FN, Ovah IE, Atama CI, Njoku I, Eyo IE. Effectiveness of botanical powders against *Callosobrochus maculatus* (Coleoptera: Bruchidae) in some stored leguminous grains under laboratory conditions. Afr. J Biotech. 2013;12(12):1384-1391.
15. El-Wakeil NE. Botanical pesticides and their mode of action. Gesunde Pflanzen. 2013;(65):125-149.
16. Gondwe TM, Alamu EO, Mdziniso P, Maziya-Dixon B. Cowpea (*Vigna unguiculata* (L.) Walp) for food security: an evaluation of end-user traits of improved varieties in Swaziland. Sci. Reports. 2019;9(1):1-6.
17. Harshani HS, Karunaratne S. Chemical composition and insecticidal effect of fruit peel powders of two citrus species against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea (*Vigna unguiculata*). Intern. J Pest Manage. 2019, 1-8.
18. Idoko JE, Adesina JM. Effect of Callosobruchus maculatus (Fab.) (Coleoptera: Chysomelidae) infestation level on control using different particle sizes of *Eugenia aromatica* and *Piper guineense* powders. J Bio. Env. Sci. 2013;3(1):54-60.
19. Idoko JE, Ileke KD. Comparative evaluation of insectyicidal properties of essential oils of some selected botanicals as bio-pesticides against cowpea bruchid, Callosobruchus maculatus (Fabricius) [Coleoptera: Chrysomelidae}. Bull. Nat. Res. Cent. 2020. DOI: 10.1186/$_s$ 42269-020-00380-2.
20. Isikhuemen EM, Ogbomwan BO, Efenudu IU. Evaluation of phytochemicals and mineral constituent of *Piper guineense* Schum. & Thonn. and *Piper umbellatum* Linn: Implications for ethnomedicine. Euro. J Med. Plant 2020;31(1):84-97.
21. Jayathilake C, Visvanathan R, Deen A, Bangamuwage R, Jayawardana BC, Nammi S, Liyanage R. Cowpea: an overview on its nutritional facts and health benefits. J Sci. Food Agric. 2018;98(13):4793-806.
22. Juliani HR, Koroch AR, Giordano L, Amekuse L, Koffa S, Asante-Dartey J, *et al*. *Piper guineense* (Piperaceae): Chemistry, traditional uses, and functional properties of West African black pepper. In African natural plant products volume II: Discoveries and challenges in chemistry, health, and nutrition, American Chemical Society. 2013, 33-48.
23. Korunic Z, Fields PG. Evaluation of three new insecticide formulations based on inert dusts and botanicals against four stored-grain beetles. J Stored Prod. Res. 2020;88:101633
24. Mamiro PS, Mbwaga AM, Mamiro DP, Mwanri AW, Kinabo JL. Nutritional quality and utilization of local and improved cowpea varieties in some regions in Tanzania. Afr. J Food Agric. Nutr. Dev. 2011, 1684-5374.
25. Mkenda PA, Ndakidami PA. Pesticidal efficacy of four botanical pesticides on survival, oviposition and progeny development of bruchid, *Callosobruchus maculatus* in stored cowpea, *Vigna unguiculata*. Inter. J Plant. Soil. Sci. 2014;3(12):1504-1523.

26. Mkenda PA, Stevenson PC, Ndakidemi P, Farman DI, Belmain SR. Contact and fumigant toxicity of five pesticidal plants against *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) in stored cowpea (*Vigna unguiculata*). Int. J Trop Insect Sci. 2015;35:172-184.
27. Nizamani B, Agha MA, Ali Q, Hussain A, Manghwa H, Kamran M, *et al*. Influence of indigenous plant materials on reproductive performance of *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae) on chickpea. Int. J Trop. Insect Sci. 2000. DOI. 10.1007/s42690-020-00158-z.
28. Ofuya TI, Osadahun JM. Effect of three plant powders on behavior, mortality and reproductive fitness of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) Zoo. Res. 2005;26(6):603-608.
29. Ogunwolu EO, Idowu O. Potential of powdered *Zanthoxylum zanthoxyloides* (Rutaceae) root bark and *Azadirachta indica* (Meliaceae) seed for the control of cowpea seed bruchid, *Callosobruchus maculatus* (Bruchidae) in Nigeria. J Afr. Zool. 1994;108:521-528.
30. Olaifa JI, Adenuga AO. Neem products for protecting field cassava from grasshopper damage. Int. J Trop. Insect Sci. 1988;9(2):267-270.
31. Olaifa JI, Erhun WO. Laboratory evaluation of Piper guineense for the protection of cowpea against Callosobruchus maculatus. Int. J Trop. Insect Sci. 1988;9:55-90.
32. Omoregie EH, Augustine IE. Major compounds from the essential oil of the fruit and comparative phytochemical studies of the fruits and leaves of *Dennettia tripetala* Baker G. found in north central Nigeria. Inter. J. Pharma. Phyto. Res. 2015;7(6):1262-1266.
33. Oyetayo FL, Ogundare OL. Chemical composition of the pepper fruit (*Dennettia tripetala*) seed flour. FASEB Journal. 2018;32(1):536.13-536.13.
34. Sarwar M. Studies on resistance discrepancy in seeds of local and exotic lentil (*Lens culinari* medikus) genotype against *Callosobruchus analis* L. (*Coleoptera*: *Bruchidae*). Afr. Entomol. 2013; 21(2)273-279.
35. Saxena B, Sayyed RZ. Botanical insecticides effectively control chickpea weevil, *Callosobruchus maculatus*. Environ Sustain. 2018;1(3):295-301.
36. Tharanathan RN, Mahadevamma S. Grain legumes – a boon to human nutrition. Trends Food Sci Tech. 2003;14(12):507-518.
37. Tripathi AK. Pests of stored grains. In pests and their management. Springer. 2018, 311-359. Singapore.
38. Wilson C, Tisdell C. Why farmers continue to use pesticides despite environmental, health and sustainability costs. Working Paper on Economics, Ecology and the Environment Department of Economics University of Queensland, Australia. 2001, 1327-8231.
39. Xie H, Wang X, Chen J, Li X, Jia G, Zou Y, *et al*. Occurrence, distribution and ecological risks of antibiotics and pesticides in coastal waters around Liaodong Peninsula, China. Sci. Total Environ. 2019;656:946-951.