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PLANT POWDERS PROTECT STORED WHEAT FROM INFESTATION BY LESSER GRAIN BORER *Rhyzopertha dominica* (FABRICIUS) (BOSTRICHIDAE: COLEOPTERA)

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ABSTRACT
Rhyzopertha dominica (Bostrichidae: Coleoptera) is one of the most serious devastating insect pests attacking stored wheat grains in Nigeria. The most common strategy adopted in controlling this insect pest is the application of synthetic pesticides which is costly, toxic to non-target organisms and is non eco-friendly. Botanical pesticides are recently used as alternatives to synthetic pesticides due to their non-hazardous effect on the environment and non-target organisms, cost effectiveness and direct impacts on the target organisms. This study was carried out to determine the toxicity of leaf powders obtained from three species of plants: Ageratum conyzoides, Momordica charantia and Tephrosia vogelii in the control of R. dominica infestation on stored wheat grains. Five doses (0.50, 1.00, 2.00, 4.00 and 8.00 g) per 20.00 g were measured from the powdered leaves of the tested plants with ethanol treatment as the control (0.00 g 20.00 g^{-1}). The powdered doses were applied onto wheat grains before the insects were introduced. The experiment was laid down in completely randomized design (CRD) with three replications. The data obtained was analyzed using analysis of variance at 5% probability level with Least Significant Difference used to separate the means. The results showed significant ($p \leq 0.05$) differences in the magnitude of toxicity imparted by various doses of the powders on R. dominica. The treatments influence high rate of mortality, reduced number of laid eggs and emerged adults when compared with the control. The toxicity of the powders increased with increase in dosage. It was concluded that 8.00 g 20.00 g^{-1} of the powders were more toxic to R. dominica, inducing 28.00-77.30% insects' mortality thereby protecting grain damage to as low as 1.04% at 6th month of storage. T. vogelii leaf powders were recommended for use against R. dominica in stored wheat grains.

Key words: botanicals, dose, lesser grain borer, wheat

INTRODUCTION
Wheat (*Triticum aestivum*) is the world's most important cereal crop in relation to production and consumption (Ileke, 2011). It is the most important cereal crop traded in the world and the most protein us grain consumed in developing countries to combat malnutrition (Ileke, 2011). Despite the significant importance of wheat to food security in Africa in general and Nigeria in particular, its storage is threatened by several insect pests (Adedire, 2001; Ileke, 2011). These insect pests cause significant losses to stored wheat. Storage loss due to insects' infestation was estimated to be up to 70% in developing countries (Strbac, 2002; Kavita, 2004). These insect pests cause quantitative and qualitative damage to grains (Fornal *et al*., 2007). One of the most serious devastating insect pests of wheat is the lesser grain borer (*Rhyzophertha dominica*).

Rhyzopertha dominica (Bostrichidae: Coleoptera) is one of the most devastating pests of stored grains both at larval and adult stage (Raju, 1984). They impart economic losses in terms of grain mass (Subramanyam and Hagstrum, 1996) and nutrient depletion (Jood *et al*., 1996) and pose a public health risk from contamination by allergens such as uric acid (Jood and Kapoor, 1993). The most common control measure adopted in Nigeria against *R. dominica* infestation of stored wheat is the application of synthetic insecticides (Salem *et al.,* 2007). These synthetic insecticides have negative consequences on the environment and other non-target populations including man (Adedire and Lajide, 2003; Adedire *et al*., 2011; Ileke and Oni, 2011; Udo, 2011; Ileke and Bulus, 2012; Ileke and Olotuah, 2012). Alternative strategy is needed to ameliorate these problems. Botanical insecticides were recently recommended in pests management strategies as they are cheaper, ecologically friendly and specific to the target organisms (Ashamo and Odeyemi, 2001: Oni and Ileke, 2008; Akinkurolere *et al*., 2009; Ileke *et al*., 2012). *Ageratum conyzoides*, *Momordica charantia* and *Tephrosia vogelii* were selected for this study

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due to their relative abundance within the vicinity of the local communities in Nigeria and are among the plant species reported to have insecticidal properties against insect pests of stored products (Adda *et al.,* 2011; Olaniran and Adebayo, 2013; Ileke and Emmanuel, 2018; Emeasor and Ndumele, 2019). Thus, this study evaluated the insecticidal potency of three plants leaves powder against *R. dominica* infestation of stored wheat grains.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *Ageratum conyzoides* (Compositae), *Momordica charantia* (Cucurbitaceae) and *Tephrosia vogelii* (Fabaceae) were obtained from vendors at Oyingbo market in Lagos, identified and authenticated in the Herbarium of the Department of Botany, University of Lagos, Nigeria. The voucher numbers: LUH 3295, LUH 3291 and LUH 2317 were assigned to the test plants respectively.

Disinfestations of Wheat Grains

Wheat grains were obtained from Bariga market, Lagos. They were identified as *Triticum aestivum* seeds at the Nigeria Stored Products Research Institute (NSPRI) Lagos, Nigeria. All damaged seeds and debris were sorted out from the grains after which disinfestations was carried out in an oven at 50°C for six hours to kill all life stages of insects within the grains. The grains were then left for 24 hours to stabilize at ambient conditions**.**

Source of Insects

One hundred adults of *R. dominica* were obtained from an already existing culture in the Entomology Laboratory of Nigeria Stored Products Research Institute (NSPRI) Lagos. Rearing of the insects followed the method described by Dabire *et al.* (2005). The insects were introduced into 500 ml rearing bottles containing 300 g of disinfested wheat grains. The disinfested grains were then air dried in the laboratory to prevent fungal growth as described by Adedire *et al.* (2011) before introduction of insects. The bottles were covered with muslin cloth and secured with rubber bands. They were kept in an incubator for oviposition at 30°C and 70% relative humidity for 10 days after which the parents were removed. The bottles were maintained in the incubator under the same condition for adult emergence. Toxicity tests were carried out on 1st, 3rd and 5th instars larvae and adults of *R. dominica*. Wheat grains used for the experiment were also disinfested as described above before it was stored in plastic containers with tight lids.

Multiplication of Insect Culture

Rhyzopertha dominica were maintained on disinfested wheat grains. Thirty unsexed 7-14 day old adults of *R. dominica* were introduced into 20 g each of disinfested wheat grains in 1.00 L kilner jars, respectively, in three replicates in the laboratory. All adult insects were left for 10 days to allow for oviposition, after which they were removed. They were then left undisturbed until adults were observed to emerge. At each peak of emergence, the adults were removed and used to set up new cultures. Series of fresh cultures were made from these to ensure regular supply of adult insects of known ages for use in subsequent experiments.

Preparation of Plant Powders

The fresh leaves of the three test plants: *A. conyzoides, M. charantia* and *T. vogelii* were air dried at room temperature, pounded to fine powder with pestle and mortar according to the protocols described by Dabire *et al*. (2008). The powder was further sieved through 1.00 mm^2 mesh as described by Ileke and Bulus (2012). They were packed in plastic containers with tight lids and kept in the dark according to the method described by Udo (2011).

Experimental Design

The plant powders obtained from *A. conyzoides, M. charantia* and *T. vogelii* were thoroughly mixed singly with 20.00 g of disinfested wheat grains in each of the six plastic containers at 0.50, 1.00, 2.00, 4.00 and 8.00 g and manually agitated for uniform dispersal. The controls (0.00 g 20.00 g^{-1}) were treated with ethanol. Thirty newly emerged unsexed *R. dominica* were randomly picked and released into each plastic container as well as that of the control and covered. The plastic pot was covered with thick paper to provide a favorable semi-dark environment to the lesser grain borer for egg deposition. Treatments were arranged in a completely randomized design with three replications. The containers were kept in the laboratory.

After 15 days, number of eggs laid on treated seeds and control seeds were recorded and the percentage of oviposition deterrence was calculated using Singh and Jakhmola (2011) formula:

$$\% \text{ Oviposition deterrence} = \underline{Cs\text{-}Ts} \text{ x } 100 \text{ Cs}$$

where Cs is number of eggs laid on control seeds, and Ts is number of eggs laid on treated seeds. Toxicity of the powders on the insects was determined by the rate of mortality induced by the powders on the insects. Insect mortality was observed daily for 2 days. After every 24 hours, the number of dead beetles were counted and recorded. The beetles were confirmed dead when there was no response to probing with sharp pin at the abdomen as described by Adedire *et al*. (2011). At the end of day 2, all insects, both dead and alive were removed from each container. The toxicity factor (TF) is calculated using the formula:

Toxicity Factor (TF) = 48h LD_{50} value of the least toxic compound 48h LD_{50} value of the more toxic compound. The experiment was kept inside the insect cage for another 30 days. The *R. dominica* started to emerge after 30 days of oviposition. The number of adults that emerged from each container per replicate were removed, counted and recorded each day for a period of 10 days.

Insect Damage in Grains

Each wheat grains were taken out from the plastic container to determine the presence/absence of feeding hole(s) on each seed. Seeds containing hole(s) was considered as damaged seeds. The number of damaged wheat seeds was counted and recorded for each treatment per replication. Monthly insect damage in each treatment and control was determined from 20.00 g batches of grains in each plastic container as described by Odeyemi and Daramola (2000):

$$\text{Percentage weight loss} = \frac{(W_u x N_d) - (W_d x N_u)}{W_u (N_d + N_u)} \times 100$$

where W_u is weight of undamaged grains, N_u is number of undamaged grains, W_d is weight of damaged grains, and N_d is number of damaged grains.

Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA) using SPSS (11.0 versions) with Duncan's New Multiple Range Test (DNMRT) used to compare the treatment means. Lethal Dose of the powders is determined using Probit analysis.

RESULTS

The result on the relative toxicity of the three plant powders to *R. dominica* is presented in Table 1. *Tephrosia vogelii* was the most toxic and produced the best effect with LD_{50} values of 12.74 g 20.00 g^{-1} at 24 h of exposure and 9.58 g 20.00 g^{-1} at 48 h of exposure. However, the powders of *Ageratum conyzoides* had the lowest LD_{50} value 20.09 g 20.00 g^{-1} and 15.48 g 20.00 g^{-1} at 24 and 48 h of exposure respectively. The trend in the toxicity followed the following sequence *T. vogelii* > *M. charantia* > *A. conyzoides*.

The result for percentage mortality of *R. dominica* due to exposure to different doses of the powders from the three test plants is shown in Table 2. The result indicated that 8.00 g 20.00 g^{-1} of the powders obtained from *Ageratum conyzoides*, *Momordica charantia* and *Tephrosia vogelii* induced more than 50% mortality with mortality rate of 58.70%, 63.50% and 77.30% respectively. The percentage mortality increased with increase in dosage.

The result for the percentage inhibition of oviposition and progeny development induced by various doses of the plants powders is presented in Table 3. The result revealed significant differences ($p \leq 0.05$) in the mean number of eggs laid and adult emergence among the treatments. The result showed that, 28 to 65 eggs were laid in *A. conyzoides* treated grains out of which 11 to 39 adults emerged representing 38-59.75% adult emergence with decreased dosage. The number of eggs laid by the insects on grains treated with *M. charantia* was 31-67 eggs out of which 33.38-51.08% adults emerged with decreased dosage. More so, 18-53 eggs were laid on *T. vogelii* treated grains out of which 28.55-49.72% adults emerged with decrease in dosage. However, the highest percentage progeny development inhibition was found in 8.00 g 20.00 g^{-1} of *Tephrosia vogelii* powder in which only 28.55% emerged as adults while the remaining 71.45% were inhibited. Similarly, the least number of eggs laid and the percentage adult emergence were found under 8.0 g 20.00 g^{-1} of the other two test plants powders. The number of eggs laid and the percentage of adult emergence decreased with increase dosage.

The result for the average mean percentage damage induced by *R. dominica* on the treated wheat grains is shown in Table 4. Insect damage on wheat grains treated with various doses of *T. vogelii* powders for a period of six months reduced from 4.17% to as low as 0.35% with increased dosage. These values are significantly lower than that of the control (9.12%). More so, the grains treated with *M. charantia* powders reduced the infestation rate from 5.14% to as low as 0.96% with increased dosage. Similar result was found in grains treated with *A. conyzoides* powders (the infestation rate reduced from 6.14% to as low as 1.15%) with increase in dose. Complete protection of the grains for a period of three months was found in 8.00 g 20.00 g^{-1} dose obtained from *T. vogelii* while the remaining powders gave full protection for a period of two months by the higher doses.

Table 1: Relative toxicity of various plant powders on *Rhyzopertha dominica*

Plant powders	24 hr LD_{50} (g 20.00 g^{-1})	95% CL	TF	48 hr LD_{50}	95% CL	TF
Ageratum conyzoides	20.09	-	1.63	15.48	-	1.70
Momordica charantia	18.69	-	1.51	12.36	-	1.36
Tephrosia vogelii	12.74	-	1.03	9.58	-	1.05

TF - test of significance; CL - confidence limit; TF - LD_{50} values with no overlap in 95% confidence limits are significantly different

Table 2: Mortality of *Rhyzopertha dominica* due to exposure to treated grains with powders of plants

Powdered extract	0.00 g	0.50 g	1.00 g	2.00 g	4.00 g	8.00 g
Ageratum conyzoides	0.00^{a*}	28.00^{b}	38.00^{c}	49.40^{b}	52.90^{b}	58.70^{c}
Momordica charantia	0.00^{a}	35.00^{bc}	43.00^{b}	53.60^{bc}	56.20^{bc}	63.50^{b}
Tephrosia vogelii	0.00^{a}	38.00^{a}	51.50^{a}	62.30^{a}	68.90^{a}	77.30^{a}

N.B: *Mean values bearing the same letter(s) down a column are not significantly different ($p = 0.05$)

Table 3: Oviposition and progeny development of *Rhyzopertha dominica* on wheat rains

Plant powder	Dose (g 20.00 g^{-1})	Number of eggs laid	Adult emergence (%)	Percent adult emergence (%)
Ageratum conyzoides	0.00	89.76^{a*}	87.60^{a}	97.59
	1.00	65.14^{b}	38.92^{b}	59.75
	2.00	51.33^{c}	24.88^{c}	53.84
	4.00	46.21^{d}	21.32^{d}	46.17
	8.00	28.26^{e}	10.74^{e}	38.00
Momordicacharantia	0.00	90.01^{a}	86.36^{a}	95.94
	1.00	67.21^{b}	34.33^{b}	51.08
	2.00	58.11^{c}	25.79^{c}	44.38
	4.00	52.00^{d}	21.82^{d}	41.96
	8.00	30.73^{e}	10.26^{e}	33.38
Tephrosia vogelii	0.00	89.73^{a}	83.79^{a}	93.38
	1.00	52.45^{b}	26.08^{b}	49.72
	2.00	40.32^{c}	18.90^{c}	46.88
	4.00	35.11^{d}	13.05^{d}	37.17
	8.00	18.25^{e}	5.210^{e}	28.55

Key: * Means bearing the same superscripts down a column are not significantly different (p = 0.05)

Table 4: Percentage mean damage by *Rhyzopertha dominica* on treated wheat grains

Plant powder	Month	0.00 g	0.50 g	1.00 g	2.00 g	4.00 g	8.00 g
Ageratum conyzoides	1st	1.86^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
	2nd	6.52^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
	3rd	9.23^{a}	3.65^{b}	2.73^{c}	2.08^{c}	1.76^{c}	0.71^{d}
	4th	14.01^{a}	5.04^{b}	4.26^{c}	3.14^{d}	1.82^{de}	1.07^{e}
	5th	17.37^{a}	11.00^{b}	8.81^{c}	4.65^{d}	2.13^{e}	1.88^{f}
	6th	25.26^{a}	17.14^{b}	13.13^{c}	8.21^{d}	5.24^{e}	3.23^{f}
	Mean	12.38	6.14	4.82	3.01	1.83	1.15
Momordica charantia	1st	1.74^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
	2nd	5.21^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
	3rd	7.16^{a}	3.11^{b}	2.89^{bc}	2.00^{c}	1.56^{d}	0.56^{e}
	4th	12.92^{a}	4.52^{b}	3.78^{c}	2.26^{d}	1.70^{e}	0.89^{f}
	5th	15.52^{a}	9.17^{b}	5.43^{c}	3.18^{d}	2.00^{e}	1.45^{f}
	6th	22.17^{a}	14.06^{b}	11.85^{c}	6.28^{d}	4.12^{e}	2.85^{f}
	Mean	10.79	5.14	3.99	2.29	1.56	0.96
	1st	1.69^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
	2nd	4.54^{a}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}
Tephrosia vogelii	3rd	6.23^{a}	2.15^{b}	0.76^{c}	0.27^{cd}	0.04^{d}	0.00^{e}
	4th	10.16^{a}	3.68^{b}	1.44^{c}	0.98^{d}	0.26^{e}	0.08^{f}
	5th	13.25^{a}	6.92^{b}	3.91^{c}	2.83^{d}	1.89^{e}	0.95^{f}
	6th	18.84^{a}	12.27^{b}	9.85^{c}	5.94^{d}	3.64^{e}	1.04^{f}
	Mean	9.12	4.17	2.66	1.67	0.97	0.35

Key: *[1] Means bearing the same superscripts across a row are not significantly different (p = 0.05)

DISCUSSION

Botanical pesticides have proved vital in insect pests' management strategies. These botanicals serve as alternatives to commercially used synthetic insecticides and many of them have often been used against a number of species of stored product insect pests including those in the Order Coleoptera and Lepidoptera (Nathan *et al*., 2007). The three powders obtained from *Ageratum conyzoides*, *Momordica charantia* and *Tephrosia vogelii* proved to possess insecticidal potency for the protection of stored wheat grains against *R. dominica* infestation. The relative toxicity of the powders on *R. dominica* after 48 hours of exposure agrees with the findings of Ahmad *et al*. (2016) who reported similar finding with *Pipernigrum* extracts on *R. dominica* but after 120 hours of application. The present study reported highest percentage mortality induced by higher doses of the plants powders. This is in conformity with the findings of Alvi *et al*. (2018) who reported high mortality induced by the leaf and seed extracts of *Rhazya stricta* on *Rhyzopertha dominica* and *Trogoderma granarium* under laboratory conditions. The powders conferred certain degrees of toxicity to the insect by inhibiting more than 50% of the eggs laid and suppressed progeny development. This finding agrees with that of Ogendo *et al*. (2004) who reported more than 50% induced mortality by *Tephrosia vogelii* extracts on *Sitophilus zeamais*. Similar finding was also reported by Babarinde *et al.* (2001) that leaf and seeds powders of *Piper guineense* and *Moringa oleifera* were effective showed effective bioactivity against *Trogoderma granarium.* Adebayo (2003) also reported insecticidal activity of botanical insecticides in the control of insect pests of okra and cowpea. Also, dried leaves of *T. vogelii* were reported by Koona and Dorn (2005) to be effective protestants of stored legume seeds against bruchid infestation and reduced damage by *Callosobruchus maculatus. C. chinensis* and *Acanthosceli desobstectus* on the treated seeds by 7.10% compared with 99.80% recorded on grains in the control experiment. Similarly, Ileke and Bulus (2012) reported toxicity of powders and extracts of *Azadirachta indica* and *Piper guineense* seeds to *R. dominica* as reported by this study. Furthermore, botanical extracts have been reported by Amuji *et al.* (2012) to exhibit appreciable magnitude of toxicity to insects inducing mortality. Ousman *et al*. (2007) has reported that *Piper nigrum* leaf oil was toxic to stored insect pests and concluded that the extract be used as a substitute for synthetic insecticides by small scale farmers. The toxicity of the three plant extracts revealed by this study has therefore confirmed the previous findings of Oumarou *et al*. (2017) who reported the bioefficacy of plants extracts on *Anopheles gambiae*, Cheruvan and Ragesh (2018) has reported insecticidal effects of cassava leaf extracts against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst) and *Callosobruchus chinensis* (L.) under laboratory conditions whilst Ileke and Emmanuel (2018) reported high bioefficacy of *Alstonia boonei* leaf extract against the cowpea beetle (*Callosobruchus maculatus*) infestation of stored cowpea seeds. The toxicity of the plants powders can probably be attributed to the various chemical constituents present in the extracts (Mbailao *et al*., 2006).

The effect of the three powders reported by the present study in suppressing progeny development and oviposition of *R. dominica* can be attributed to the toxicity and lethality conferred on the insects thereby interfering with physiological processes of eggs development. This finding is in conformity with the report of Osawe *et al*. (2007) which opined that extracts of *Alstonia boonei* leaves adversely affected the survival and growth of *Sesamia calamistis*. Upadhyay and Jaiswal (2007) also reported that botanical insecticides significantly suppressed the progeny development of *Tribolium castaneum*. Chaubey (2011) averred that *P. nigrum* oil reduced the progeny development of *Callosobruchus chinensis*. The powders reported by this study might probably possess certain chemicals that could be responsible for the inability of the adult insects to emerge, as they are found to disrupt growth and reduce larvae survival as well as disruption of life cycle of insects. Mukanga *et al*. (2010) has earlier reported the suppression of progeny development of larger grain borer (*Prostephanus truncatus*) by *T. vogelli* extracts. The inability of these insects to emerge may be due to the death of the insect's larvae, which may occur due to inability of the larvae to fully cast off their exoskeleton, which remained linked to the posterior part of their abdomen. This agrees with the observation by Oigiangbe *et al*. (2010) who worked on insecticidal properties of an alkaloid from *Alstonia boonei*. The growth inhibition may result from toxicity or feeding deterrent properties of the plant powders used in this study. Akhtar and Isman (2004), Erturk (2006) and Suleiman *et al.* (2018) reported the insecticidal efficacy of some plant extracts in suppressing progeny development of stored insect pests.

The protection ability of the three plants powders reported by this study is in agreement with the work of Chikukura *et al*. (2011) who reported the potency of *Lippia javanica* in reducing the grain damage caused by storage pests. Duruigbo (2010) also reported that need and pepper fruit seed powders protected maize seed from damage by storage insects when stored in airtight plastic containers without affecting the seed viability. Similar finding was reported by Iliyasu and Gabriella (2015). The toxicity of the three plants powders on the test organism is an indication of the relative importance of the powders in preventing the wheat grains from damage by *R. dominica* infestation. This is in conformity with the work of Adeniyi *et al*. (2010) who reported that plant extracts obtained from *Vernonia amygdalina*, *Sida acuta*, *Osmium gratissimum* and *Telfaria occidentalis* were effective against beans weevil. This finding therefore adds to the existing data on the efficacy of plant extracts as biopesticides of stored food.

CONCLUSION AND RECOMMENDATION

Powdered extracts from the leaves of *Ageratum conyzoides, Momordica charantia* and *Tephrosia vogelii* were found to possess insecticidal potency against *Rhyzopertha dominica* by inducing 28-77.30% mortality of the insects. The effect of the powders on the mortality of *R. dominica* was dose dependent, increasing with increase in dosage rates. The powders also suppress progeny development of the insects from 97.57% to as low as 28.55%.The plants powders were also found to protect wheat grains from the insect's infestation for a period of 6 months. The sequence of insecticidal activity of the three powders is as follows: *T. vogelii* > *M. charantia* > *A. conyzoides*. Thus, 8.00 g 20.00 g^{-1} of the *T. vogelii* leaf powder is highly recommended for biological control of *R. dominica* in stored wheat grains.

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