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Bioefficacy of products derived from *Milletia ferruginea* (Hochst) baker against the bean bruchid, *Zabrotes subfasciatus* (bruchidae: coleoptera) in stored beans in Ethiopia

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Petroleum ether, acetone and water extracts, and fresh seed powder of *Milletia ferruginea* (Hochst) Baker (Leguminaceae) were evaluated as grain protectant against bean bruchid, *Zabrotes subfasciatus* (Boheman) in the laboratory at an ambient temperature of 28±1°C and 70% RH in a 12 h light: dark regime at the concentrations of 1, 2 and 3 ml in the case of extracts and at the rates of 5, 10 and 15 w/w in the case of seed powder. Adult mortality, F_1 progeny emergence, oviposition inhibition, insect damage and viability index of haricot bean seeds were the parameters measured. The results obtained showed that water and acetone extracts of *M. ferruginea* gave 100% mortality of the adult *Z. subfasciatus* 24 h after treatment application at the rates of 2 and 3 ml. *M. ferruginea* powder provided good protection of haricot bean seeds by reducing *Z. subfasciatus* oviposition rate, F_1 progeny emergence and seed infestation. The seed powder treatment did not show any adverse effects on the germination capacity of haricot bean seeds. This study revealed that *M. ferruginea* can be used as a botanical insecticide to protect haricot bean seeds against *Z. subfasciatus* in storage.

Key words: Botanicals, *Milletia ferruginea, Zabrotes subfasciatus*, toxicity, storage pest.

INTRODUCTION

The haricot bean, *Phaseolus vulgaris* L. (Fabaceae) is extensively grown in the lowland and medium altitude areas of Ethiopia ranging from 700 to 2000 m above sea level (Tsedeke and Ampofo, 1996). In the past, *P. vulgaris* was mainly grown by private peasant holdings under rainfed conditions. However, currently this trend has changed and big State farms and private investors are involved in the production of the crop both for domestic consumption and export market under rainfed and irrigated conditions (Shaun and Elly, 2008). *P. vulgar* is served as an important protein supplement to the cereal based Ethiopian diet. It is also a very important export commodity for the country valued at over 40 million USD annually (Shaun and Elly, 2008). Production

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varies from region to region (Ferris and Kaganzi, 2008). For example, the Oromia and the Southern Nations Nationalities region produce 70 and 60 thousand tonnes per year, respectively which is 85% of the total production.

The production of *P. vulgaris* in Ethiopia is constrained by a number of biotic and abiotic factors both under field and storage conditions (Tsedeke and Ampofo, 1996). However, pre and post harvest insect damage are the most important constraints resulting in 40 to 50% average grain losses (Tsedeke and Ampofo, 1996). *P. vulgaris* normally stored for 3-6 months in Ethiopia either to look for a better price and/or for home consumption. In the store a number of insect pests are damaging *P. vulgaris*. Among the various storage insect pests of haricot bean, *Zabrotes subfasciatus* is the most important causing over 25% losses (Tsedeke and Ampofo, 1996).

For decades, pest control strategy in developing countries has depended heavily on the use of synthetic pesticides (Tsedeke and Ampofo, 1996). Although synthetic pesticides are known to have undoubted benefits, their adoption rate and use for insect pest control in grain storage has remained remarkably low in the resource-poor farming environments of Africa including Ethiopia (Tsedeke and Ampofo, 1996). The subsistence nature of agriculture in developing countries in general and Ethiopia in particular coupled with high cost, poor information and erratic supply of synthetic pesticides accounted for farmers' reluctance to use pesticides (Tembo and Murfitt, 1995; Ogendo, 2000). Recent information about the penetration of synthetic insecticides into the stored seed and reduce the germination capacity of the seed worsen the situation (El Sheamy et al., 1988; Lalah and Wadinga, 1996). However, oils and crude powders of several plant species have been proved to have no adverse effects on the germination of maize, sorghum, pigeonpea and green gram which initiated scientists to look for botanical pesticides which are environmentally friendly (Pandey et al., 1986; Kasa and Tadese, 1995; Obeng, 1995; Saxena, 1983). *Milletia ferruginea* is one of such environmentally friendly botanical plant used for the control of stored product insect pests (Jiliani and Saxena 1988).Hence, the present study investigated the effect of seed powder and different extracts of *M. ferruginea* on the control of *Z. subfasciatus* in haricot bean seeds under storage conditions.

MATERIALS AND METHODS

Description of *M. ferruginea* and preparation of its products

M. ferruginea is a large shady tree which grows up to a length of 35 m. It is endemic to Ethiopia and widely grown at the elevation ranging from 1000 to 2500 m above sea level. There are two subspecies known to occur in Ethiopia: M.f. *ferruginea* and M. f. *darasana*. *M. f. ferruginea* is confined to the northern part of Ethiopia, while *M. f. darasana* occurs in southern region, particularly Sidamo. *M. furruginea* from central and western Ethiopia show mixture of the two species (Azene et al., 1993). *M. ferruginea* in Ethiopia is used for fish poisoning where mature pod and seed are ground to fine powder and spread over the surface of water (Siegenthaler, 1980). Furthermore, the tree is extensively used as shade for coffee in Eastern Ethiopia (Teketay and Tegineh, 1991). Seeds of *M. furruginea* were collected from matured trees in Addis Ababa and dried under shade at the room temperature of 24±1°C. Dried seeds were ground into fine powder using mortar and pestle and the powder was passed through a 25 mm-mesh sieve to obtain a fine dust.

Mass rearing of test insects

Heavily infested *P. vulgaris* seeds (over 60% infestation) were collected from the farmers' stores in the central rift valley areas of Ethiopia and stored in the laboratory for 3-6 days for the emergence of *Z. subfasciatus* adults. Two hundred unsexed adults of *Z. subfasciatus* were drawn from the stored haricot bean and reared in a 1 L jar containing 500 g of disinfested haricot bean seeds as described by Haines (1991). The top of each rearing jar was covered with nylon mesh and fastened tightly with rubber bands, and the insects were allowed to lay eggs for seven days. After seven days all adults were removed and the jars were left in the laboratory for 34 days for the emergence of F_2 generation adults which were used for the experiment. After 34 days the emerging adults of *Z. subfasciatus* were monitored and transferred to separate jars according to their age. The rearing of test insects was done in the laboratory at the ambient temperature of 28±1°C and 70% relative humidity and at 12 h light: dark condition. Seeds of susceptible haricot bean variety, "HAL-5" were obtained from Melkassa Agricultural Research Center (MARC) of Ethiopia and used for the experiment.

Disinfesting of test haricot bean seeds

Following the procedures of Lima et al. (2004) haricot bean seeds were placed in plastic bags and kept in a freezer at -5°C for one month to make them free of possible internal infestation prior to their use for various bioassays. To maintain the moisture content of the seeds to normal level, the seeds were kept in the laboratory for six days prior to the experiments.

Seed viability index

Seed powder of *M. ferruginea* at the rates of 5, 10 and 15 w/w and pirimiphos-methyl at the rates of 0.125 and 0.25 g per 250 g of haricot bean seed were used for the seed viability study which was expressed as the percent germination. Seed viability index study was conducted 90 days after treatment application by taking 25 seeds each from treated, untreated (non-infested) and infested seeds. Seeds were kept separately on a moistened filter paper (Whatman No.1) in petri dishes and arranged in a completely randomized design (CRD) in four replications. The petridishes were kept in an incubator at 25°C and at L 12: D 12 conditions. The number of germinated and un-germinated seeds in each petridish were counted after seven days.

Toxicity of *M. ferruginea* seed powder to Z. *subfasciatus*

After disinfestation, 250 g haricot bean seeds having a moisture

Table 1. Percent weight loss caused by *Z. subfasciatus* on haricot bean seeds treated with different concentrations of *M. ferruginea* seed powder at different exposure time

Treatments	Concentration (g/ 250 g bean seeds)	Exposure time (days)		
		30	60	90
Mf	5	0.11	0.17	0.33
Mf	10	0.08	0.11	0.19
Mf	15	0.0	0.0	0.0
PM	0.125	0.0	0.0	0.0
PM	0.25	0.0	0.0	0.0
C	0.0	2.17	3.80	5.40

Mf = *M. ferruginea* seed powder, PM = Pirimiphos-methyl, C = Control.

content of 10.4% were placed in 1 liter volume glass jars and treated with three rates of *M. ferruginea* (5, 10 and 15 w/w) seed powder. The treatments were thoroughly admixed with haricot bean seeds for five minutes for uniform coating. Twenty 10-day old adults of *Z. subfasciatus* (10 males and 10 females) were introduced into each jar. The glass jars were covered with nylon mesh to allow ventilation, prevent entrance and escape of insects after introduction. An untreated seeds and pirimiphos methyl treated seeds at the recommended rate of 4 ppm were used for comparison. Mortality was observed 24 and 48 h. after treatment application and the experiment was arranged in a completely randomized design in four replications.

Adult emergence, percent insect damage and oviposition rate

Following the methods of Lima et al. (2004) adults of *Z. subfasciatus* were placed in 1 L empty jars for 24 h before their release into jars containing treated and untreated haricot bean seeds. The experiment was conducted in a controlled chamber of 30°C and 40 to 50% RH. One week latter 100 treated seeds were sampled and number of eggs laid on the treated haricot bean seeds was counted. After count the adult insects were discarded. As soon as the “exit holes” were externally visible, observations were made every other day for F_2 progeny adults emergence for one month. Emerged adults were counted and removed during observation. Percent weight loss was determined by the count and weigh method as recommended by Adams and Schlten (1978).

Preparation of extracts

M. ferruginea seeds were air dried and milled into fine powder to pass through 0.5 mm mesh and extracted using water, petroleum ether and acetone in a soxhlet apparatus for 49 h or more. Before collecting the extract, excess solvents (water, petroleum ether and acetone) were evaporated and concentrated into a small volume. Then the concentrate was dissolved in 100 ml of distilled water and ready for the experiment. The extracts were kept under liquid nitrogen in a cold room until use.

Z. subfasciatus bioassay

Filtrates of *M. ferruginea* extracts at the rates of 1, 2 and 3 ml were applied to Whatman No.1 9cm diameter filter paper in a petridish. In the case of petroleum ether and acetone, the treated filter papers were exposed to open air to allow the solvent evaporate for 30 min. After evaporation 1 ml of distilled water was applied to the treated filter paper to moisten the petridish. Then 10 *Z. subfasciatus* adults were introduced into each petridish. Mortality was recorded 24, 48, 72 and 96 h after treatment application. The different solvents were used as a control and the experiment was designed in a completely randomized design (CRD) in three replications.

Data analysis

All the data collected were normalized using logarithmic and square root transformations (Gomez and Gomez, 1984) before analysis. Significant means ($p<0.05$) were separated using Tukey’s studentized range test (HSD) (Scheiner and Guvevitch, 1993; SAS Institute Inc., 1995).

RESULTS

Effect of different treatments on haricot bean seed damage due to *Z. fasciatus*

Results of percent grain loss due to *Z. subfasciatus* 90 days after storage are presented in Table 1. The results obtained showed that all the treatments significantly ($P< 0.05$) reduced weight loss due to *Z. subfasciatus* compared to the untreated check. Seeds treated with pirimiphos-methyl and *M. ferruginea* at the rate of 15 g showed no grain losses due to *Z. subfasciatus*, while the untreated grains suffered 5.4% grain losses for similar period of storage. The table further explicitly indicated that as the concentration of *M. furrignea* increase, the amount of losses due to *Z. subfasciatus* reduced by over 40%.

Effect of *M. ferruginea* seed powder on the mortality rate of *Z. subfasciatus*

The effect of *M. ferruginea* seed powder on the mortality

Table 2. Mean percent mortality of Z. *subfasciatus* exposed to different concentration of *M. ferruginea* seed powder.

Treatments	Concentration(g)	Mean % mortality ±SE at:	
		24 h	48 h
Mf	5	20.00 ± 0.58^{c}	75.00 ± 0.8^{b}
Mf	10	78.35 ± 0.88^{b}	100 ± 0.0^{a}
Mf	15	96.65 ± 0.33^{a}	100 ± 0.0^{a}
PM	0.125	100.00 ± 0.00^{a}	
PM	0.25	100.00 ± 0.00^{a}	-
C	0.0	0.00 ± 0.00^{d}	-0.00 ± 0.00^{c}

Mf = *M. ferruginea* seed powder, PM = Pirimiphos-methyl, C = Control, **-** = All *Z. subfasciatus* died, Means within a column followed by the same letter(s) are not different at 5%level (HSD).

Table 3. Mean number of eggs laid by Z. *subfasciatus* on 100 haricot bean seeds treated with different concentration of *M. ferruginea* seed powder for a week.

Treatments	Concentration (g)	Mean number of eggs ± SE
Mf	5	6.22 ± 0.98^{c}
Mf	10	2.22 ± 0.32^{b}
Mf	15	0.00 ± 0.00^{a}
PM	0.125	0.00 ± 0.00^{a}
PM	0.25	0.00 ± 0.00^{d}
C	0.0	65.6 ± 14.98^{d}

Mf = *M. ferruginea* seed powder, PM = Pirimiphos-methyl, C = Control, Means within a column followed by the same letter(s) are not different at 5% level (HSD).

of adult *Z. subfasciatus* is presented in Table 2. Results showed that mortality of Z. *subfasciatus* was significantly high ($P < 0.05$) on haricot bean seeds treated with *M. ferruginea* seed powder at the rate of 15 /250 g and pirimiphos-methyl at both concentration 24 h after treatment. High mortality rate of *Z. subfasciatus* was also recorded on haricot bean seeds treated with 10 g *M. ferruginea* 24 h after treatment.

Effect of *M. ferruginea* seed powder on oviposition of *Z. subfasciatus*

The effect of different treatments on the oviposition capacity of *Z. subfasciatus* is presented in Tables 3 and 4. There was significant ($p<0.05$) reduction in the number of eggs laid by of *Z. subfasciatus* treated with different products of *M. ferruginea*. No egg was laid by Z. *subfasciatus* in haricot bean seeds treated with 15 g of *M. ferruginea* seed powder. Table 4 shows the number of eggs laid by *Z. subfasciatus* after 30, 60, and 90 days after treatment. No eggs were also deposited on the seeds treated with pirimiphos-methyl. The number of laid eggs significantly ($p = 0.009$) increased with the increase in storage time after treatment.

Effect of *M. ferruginea* seed powder on F_1 progeny of *Z. subfasciatus*

The effect of *M. ferruginea* seed powder on F_1 progeny of *Z. subfasciatus* 30 days after treatment is presented in Table 5. All treatments markedly ($p< 0.05$) inhibited development of larvae or pupae of *Z. subfasciatus* as indicated by the low F_1 progeny emergence compared to the control. No F_1 emergence was recorded from pirimiphos-methyl treated haricot bean seeds in all the storage periods. The different concentrations of *M. ferruginea* powder except 15 g were not as effective as pirimiphos-methyl in terms of reducing the number of F_1 progeny.

Effect of *M. ferruginea* extracts on mortality of adult *Z. subfasciatus*

Results of mortality rate of *Z. subfasciatus* adults due to different treatments are presented in Table 6. Water extract of *M. ferruginea* seed showed significantly high ($p< 0.05$) mortality rate of *Z. subfasciatus* at all levels of application (1, 2 and 3 ml/filter paper) 24 h after treatment. Acetone extract of *M. ferruginea* seed induced

Table 4. Mean number of eggs laid by females of *Z. subfasciatus* on 100 seeds treated with different concentration of of *M. ferruginea* seed powder at different exposure times.

Treatments	Concentration g/250 g bean seeds	Time (day)	Mean number of eggs ± SE
	5	30	4.00 ± 0.58[b]
Mf		60	5.00 ± 1.15[b]
		90	9.67 ± 0.88[b]
	10	30	1.67 ± 0.33[b]
Mf		60	1.85 ± 0.42[b]
		90	3.33 ± 0.33[a]
	15	30	0.00 ± 0.00[a]
Mf		60	0.00 ± 0.00[a]
		90	0.00 ± 0.00[a]
	0.125	30	0.00 ± 0.00[a]
PM		60	0.00 ± 0.00[a]
		90	0.00 ± 0.00[a]
	0.25	30	0.00 ± 0.00[a]
PM		60	0.00 ± 0.00[a]
		90	0.00 ± 0.00[a]
	0.0	30	32.00 ± 2.31[c]
C		60	48.67 ± 4.13[d]
		90	93.33 ± 6.67[e]

Mf = *M. ferruginea* seed powder, PM = Pirimiphos-methyl, C = Control, Means within a column for each concentration followed by the same letter(s) are not different at 5% level (HSD).

Table 5. Mean number of *Z. subfasciatus* F_1 progeny emerged 30 days after *M. ferruginea* seed powder application.

Treatments	Concentration (g/250 g bean seeds)	Mean number of F_1 progeny ± SE
Mf	5	8.89 ± 1.16[c]
Mf	10	3.00 ± 0.41[b]
Mf	15	0.00 ± 0.[a]
PM	0.125	0.00 ± 0.00[a]
PM	0.25	0.00 ± 0.00[a]
C	0.0	62.11 ± 2.82[d]

Mf = *M. ferruginea* seed powder, PM = Pirimiphos-methyl, C = Control, Means within a column followed by the same letter(s) are not different at 5% level (HSD).

significant mortality of *Z. subfasciatus* 24 h after treatment at the rates of 2 and 3 ml. However, petroleum-ether extract of *M. ferruginea* significantly ($p < 0.05$) gave high mortality at all levels 48 h after treatment. Acetone, petroleum-ether and distilled water did not cause mortality to *Z. subfasciatus*.

Effect of different treatments on germination

The effect of *M. ferruginea* on the viability of haricot bean seeds is shown in Figure 1.There was no significant difference ($p > 0.05$) in the germination of haricot bean seeds treated with different concentrations of

Table 6. Mean percent cumulative mortality of Z*. subfasciatus* adults exposed to different extracts of *M. ferruginea* at different concentrations.

Treatments	Concentration(ml)	Hours after treatment application			
		24	48	72	96
Water extract	1	95.0±0.10^{a}	99.6± 0.1^{a}	100± 0.0 ^{a}	-
	2	100.0±0.0^{a}	-	-	-
	3	100.0± 0.0^{a}	-	-	-
Acetone extract	1	85.0± 1.0^{a}	96.6± 0.8^{a}	100±0.0^{a}	-
	2	96.6± 0.7^{a}	98.3± 0.2^{a}	100± 0.0^{a}	-
	3	100.0± 0.0^{a}	-	-	-
Petroleum ether extract	1	65.0± 5.0^{b}	75.0± 1.5^{b}	90.0± 1.7^{a}	100± 0.0^{a}
	2	73.5± 3.5^{b}	95.0± 3.4^{a}	100± 0.0^{a}	100± 0.0^{a}
	3	80.0± 1.0^{b}	86.6±0.8^{a}	100± 0.0^{a}	-
Water	1	0.0± 0.0^{c}	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}
	2	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{b}
	3	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{a}
Acetone	1	0.0± 0.0^{c}	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}
	2	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{b}
	3	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{a}
Petroleum ether	1	0.0± 0.0^{c}	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}
	2	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{b}
	3	0.0± 0.0^{c}	0.0± 0.0^{b}	0.0± 0.0^{b}	0.0± 0.0^{a}

- = 100% mortality already attained at the immediate earlier exposure time, Means within a column for similar concentration followed by the same letter(s) are not different at 5% level (HSD).

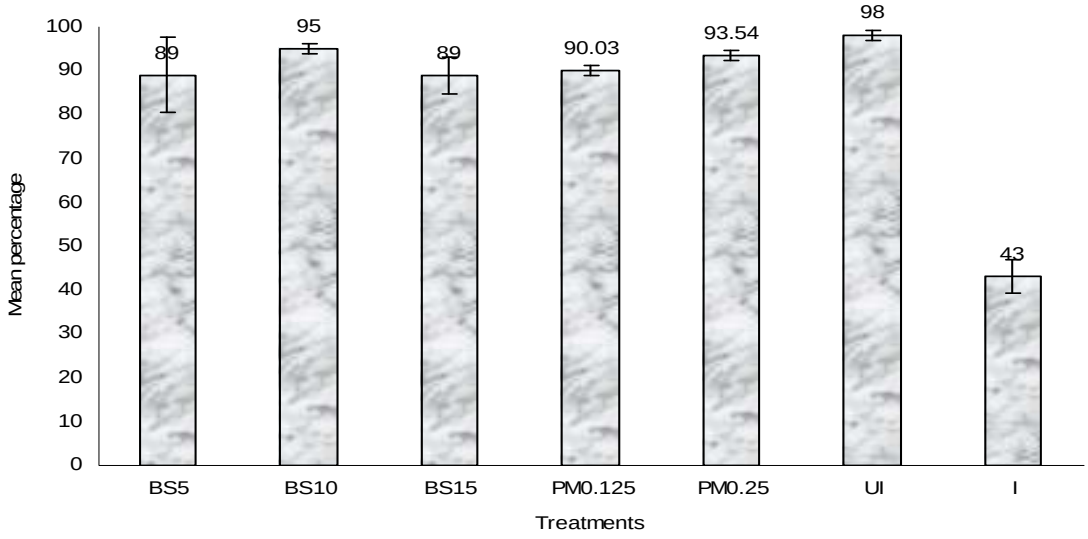

Figure 1. Effect of *M. ferruginea* seed powder on percent germination of haricot bean seeds. BS5 = Birbira seed (Mf) (5 g), PM 0.125 = Pirimiphos-methyl (0.125 g), BS10 = Birbira seed (Mf) (10 g), PM0.25 = Pirimiphos-methyl (0.25 g),BS15 = Birbira seed (Mf) (15 g),UI = Uninfested haricot bean seeds, I = Infested haricot bean seeds, Mf = *M. ferruginea.*

M. ferruginea and pirimiphos-methyl. However, the germination percentage of the treated haricot bean seeds were significantly ($p< 0.05$) higher than the germination percentage of the untreated haricot bean seeds.

DISCUSSION

Results of the present study indicated that all tested concentrations (5, 10 and 15 g) of *M. ferruginea* seed powder were comparable with primiphos-methyl in controlling *Z. subfasciatus* irrespective of exposure time. The seed powder highly reduced the number of F_1 progeny emergence, oviposition of *Z. subfasciatus* and percent weight loss. All concentrations of *M. ferruginea* seed powder extracts (acetone and water) caused very high mortality of *Z. subfasciatus* 24 h after treatment. *M. ferruginea* water extract gave more adult mortality of *Z. subfasciatus* may be because of the presence of more water soluble chemical substance in *M. ferruginea* seed powder (Bekele et al., 2005). Similar result was reported by Bekele (2002) on toxicity of *M. ferruginea* against *Sitophilus zeamais* (Motsch). Rotenone is one of the dominant chemical substance found in the seed and stem bark of *M. ferruginea* and is a well known botanical insecticide with a rat oral of LD50 = 132-1500 mg/kg through contact and stomach poisoning (Saxena, 1983; Bekele, 2002). It is also highly toxic to fish and soluble in polar solvents (Bekele, 2002). Bayeh and Tadesse (2000) reported that *M. ferruginea* and *Azadirachta indica* were able to effectively control *Callosobruchus chinensis* on faba bean by partially or completely preventing egg-laying. Tebkew and Mekasha (2002) tested *M. ferruginea* against *C. chinensis* in chickpea for six months in the laboratory. In a recent laboratory and field based study by Bekele et al. (2005), it was also investigated that all concentration levels of *M. ferruginea* seed extract filtered with cheesecloth caused very high mortality of all the termite castes comparable to Chlorpyrifos.

In general, the powder of *M. ferruginea* gave better protection at all storage periods after treatment application as compared to the check. The over-all results showed that pirimiphos-methyl can protect haricot bean seeds from *Z. subfasciatus* infestation for two to four months as less than one egg per female was laid in all storage intervals. Similarly, number of eggs laid by the female on *M. ferruginea* seed powder treated beans (that is, 10 and 15 g seed powder) was not significantly ($p>0.05$) different from pirimiphos-methyl treated seeds for all storage intervals. The reduced oviposition might be due to the reduction in egg production or inhibition of egg laying. This is in agreement with Ofuya (1990) who reported that weakening of adults by plant powder may cause insects to lay fewer eggs than normal. Bekele (2002) observed reduced F_1 progeny emergence by *S. zeamais* in maize mixed with *M. ferruginea* seed powder.

M. ferruginea seed powder used as a grain protectant for the control of *Z. subfasciatus* had no effect on the germination of haricot bean seeds. Kasa and Tadesse (1995) investigated the use of crude powders of 17 botanical plants for the control of *S. zeamais* on sorghum and indicated that the botanicals had no effect on seed germination. Similarly, Pandy et al. (1986) reported that petroleum-ether extracts of *Lantana camara* and four other plant species had no adverse effects on the germination of *Vigna radiata* (L.) Wilcz. Onu and Aliyu (1995) reported that pepper powder was effective in reducing oviposition and damage of *C. maculates* without impairing the seed quality and viability.

In conclusion, seed powder and extracts of *M. ferruginea* can be recommended for the control of *Z. subfasciatus* on stored haricot bean seed. However, some aspects such as its effect on human being, on natural enemies existing in storage ecosystem and cost-benefit analysis need to be investigated before the wide application of this research outcome.

Conflict of Interest

The authors have not declared any conflict of interest.

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