

Full Length Research Paper

Evaluation of botanical plants powders against *Zabrotes subfasciatus* (Boheman) (Coleoptera: Bruchidae) in stored haricot beans under laboratory condition

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Experiments were conducted to evaluate the efficacy of botanical powders of *Jatropha curcas* (L.), *Datura stramonium* (L.), *Chenopodium ambrosioides* (L.), *Phytoloca dodecondra* (L'Herit), *Azadrachta indica* (A. Juss) and *Parthenium hysterophorus* (L.) against *Zabrotes subfasciatus* (Boheman) in the laboratory under ambient condition. The test insects were reared in glass jar and tested on whole haricot bean grains. For comparison, primiphos-methyl and untreated check were used. The experiment was arranged in a completely randomized design in three replications. Hundred percent mortality of *Z. subfasciatus* was obtained with *C. ambrosioides* leaf powder at all levels of concentrations 24 hour after treatment application. More than 90% mortality of adult *Z. subfasciatus* was also observed for bean seeds treated with *J. curcas*, *D. stramonium* and *P. dodecondra* 96 hour after treatment at the rate of 15g/ 150g of grain application. Powder treatments of *C. ambrosioides* and *A. indica* at all tested rates and *D. stramonium* at higher levels (10 and 15g) gave more than 97% inhibition of F1 progeny production by *Z. subfasciatus*. Powder plant materials significantly reduced percent haricot bean grain infestation by *Z. subfasciatus*. Moreover, these plant materials had no effect on the germination capacity of haricot bean seeds. In general, the results obtained indicate that the use of these powder plant materials can be used for the control of *Z. subfasciatus*.

Key words: *Jatropha curcas*, *Datura stramonium*, *Chenopodium ambrosioides*, *Phytoloca dodecondra*, *Azadrachta indica*, *Parthenium hysterophorus*, *Zabrotes subfasciatus*.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is one of the principal food and cash crop legume grown in the tropical world (Abate and Ampofo, 1996; Songa and Rono, 1998; Schmale et al., 2002). A wide range of seed beetles attack the grain of common bean varieties (Mulungu et al., 2007). However, the predominant damaging pests of stored grain legumes mainly in the tropics are *Callosobruchus maculatus* (Fab.), *C. chinensis* (L.), *Caryedon serratus* (Oliver), *Zabrotes subfasciatus* (Boheman) and *Acanthoselids obtectus* (Say) (Nahdy and Agona, 1995; Nichimbi-Msolla and Misangu, 2002; Emana et al., 2003). In Ethiopia, *Z. subfasciatus* (Boheman) and *A. obtectus* are the major pests of stored beans

causing average grain losses of 60% within 3 - 6 months of storage period (Emana et al., 2003). To off set and/or minimize the losses, farmers use different control measures which ranges from synthetic insecticides to cultural control methods.

Synthetic insecticides may play a significant role in reducing storage losses due to insect pests (Tapondjou et al., 2002). However, their current applications for the control of storage insect pests is limited because of resistance development by the pest, consumers concern, widespread environmental hazards and increasing costs of application (Bekele et al., 1995; Bekele, 2002). For these reasons, attention was directed towards the development of alternative chemicals such as botanical pesticides (Shaaya et al., 1997). Plant products have been used for many years by the small scale farmers in parts

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Table 1. List of botanical powders tested against *Z. subfasciatus* on haricot bean seed.

Treatments	Local name	Common name	Stage of Collection	Parts used
<i>Jatropha curcas</i>	Ayderke	Physic nut	tree	seed powder
<i>Datura stramonium</i>	Atsefaris	Thorn-apple	Flowering shrub	leaf powder
<i>Phytoloca dodecondra</i>	Endod	-	Shrub	seed powder
<i>Chenopodium ambrosioides</i>	Gime	Mexican tea	inflorescences	leaf powder
<i>Azadirachta indica</i>	Kinin	Neem	tree	seed powder
<i>Parthenium hysterophorus</i>	Faramisesa	Congress weeds	inflorescences	seed powder
<i>Parthenium hysterophorus</i>	Faramisesa	Congress weeds	inflorescences	leaf powder

of Africa to protect stored products from insect infestation (Bekele et al., 1997; Bekele, 2002; Tapondjou et al., 2002). In the last two decades, many efforts have been made to screen plants with better botanical insecticides which can be used as an alternative to synthetic insecticide (Emana et al., 2003). It was reported that when mixed with stored-grains, leaf, bark, seed powder, or essential oil extracts of plants reduce oviposition rate, suppress progeny production and toxic to adults which ultimately results in low infestation and yield losses (Shaaya et al., 1997; Tunc et al., 2000; Tapondjou et al., 2002). The current study was aimed at evaluating the effectiveness of locally available botanical plants in Ethiopia in controlling *Z. subfasciatus*.

MATERIALS AND METHODS

Insect rearing

Adults of *Z. subfasciatus* were obtained from Melkasa Agricultural Research Center and reared in the Insectory of Addis Ababa University, Biology Department. The experiment was conducted at $27 \pm 3^\circ\text{C}$ and 50 - 70% RH. Haricot bean seeds, Awash-1 variety, used for the rearing were bought from local market in Melkasa and were kept in an oven at 40°C for 4 h to disinfest the seeds against internal infestation and allowed to cool for 2 h before use (Bekele, 2002). Hundred adults, comprising 50 males and 50 females, of *Z. subfasciatus* were placed in 1lt volume glass jars containing 250 g of haricot bean seeds. The parent bruchids were removed by sieving 13 days after oviposition period. Seeds were kept under laboratory condition until the emergence of F₁ progeny.

Plant material collection and extraction

Plant parts (leaf and seed) used for the study were collected from natural habitats in Addis Ababa, Wondo Genet and Melka Werer (Afar) between September and November, 2006 (Table 1). The identity of the plants used for the experiment was confirmed at the National Herbarium of the Biology Department of Addis Ababa University, where the specimens were deposited.

Dried and ground materials

Fresh plant parts (leaves and seeds) of a known weight were kept in a well-ventilated room under shade for 2 - 3 weeks depending on weather conditions. Dried plant parts were ground to fine powder using mortar and pestle. The resulting powder was passed through a 0.25 mm mesh sieve to obtain a fine dust.

Admixture toxicity assessment

Healthy disinfested haricot bean seeds (150 g) were placed in 1lt volume glass jars and treated with 5, 10 and 15 g of dried and ground leaf, and seed powder of each test plant. Primiphos methyl at the rate of 0.125/150 g grain dust was used as a standard check. Additionally, untreated grains were included as a control. After treatment application, 20, 3 - 5 days-old *Z. subfasciatus* of mixed sex were introduced to the treated and untreated seeds in the glass jar. The jars were covered with nylon mesh and held in place with rubber bands. The number of dead insects in each jar was sieved and counted after 24, 48, 72 and 96 h after treatment application and the percentage insect mortality was calculated using Abbott's formula (Abbott, 1925). The experiment was designed in a completely randomized design in three replications.

F₁ progeny assessment

The treated jars were kept for additional 10 days of oviposition time after mortality assessment. All live and dead insects were sieved and discarded after 13 days of introduction. Then the grains were kept until emergence of F₁ progeny. The number of F₁ progeny produced by the *Z. subfasciatus* was counted upon emergence until 45 days from the date of introduction to avoid overlapping generation. Percentage reduction in adult emergence or inhibition rate (% IR) was calculated using $\% \text{IR} = (C_n - T_n) \times 100 / C_n$; Where C_n is the number of newly emerged insects in the untreated (control) jar and T_n is the number of insects in the treated jar.

Damage assessment

Damage assessment was done on treated and untreated grains. To determine seed damage rate, samples of 100 grains were taken randomly from each jar of the treatment. The number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Percentage seed weight loss = $[\text{UNd DNu} / \text{U} (\text{Nd} + \text{Nu})] \times 100$ (where, U = Weight of undamaged grain, D = Weight of damaged grain, Nd = Number of damaged seeds and Nu = Number of undamaged seeds).

Germination test

For germination test, 100 seeds were taken randomly from each treatment jar. Then, 20 seeds from each treated and control group were placed separately in Petri dishes containing moistened filter paper (Whatman No. 1). Each treatment was replicated five times. Healthy untreated seeds were used as a control. The number of emerged seedlings from each Petri dish was counted and recorded after 7 days. The percent germination was computed using the following formula:

Table 2. Mean Numbers of F1 progeny produced and weight loss caused by *Z. subfasciatus* on seeds admixed with different plant materials powder at different rates.

Treatments	Dosage (g/150 grain)	Mean± SE number of F1 progeny	% mean ± SE inhibition	% mean ± SE weight loss
<i>J. curcus</i>	5	2.33±0.58a	92.56±3.83de	0.04±0.03a
	10	2.00 ±00a	93.62±6.38de	0.00±00a
	15	2.33±0.33a	92.55±1.06de	0.00±00a
<i>D. stramonium</i>	5	1.00±0.57a	96.81±1.84de	0.03±0.03a
	10	0.33±0.11a	98.94±1.06e	0.00±00a
	15	0.00±0.00a	100.00±00e	0.00±00a
<i>C. ambrosioides</i>	5	0.67±0.67a	97.87±2.12de	0.00±00a
	10	0.00±0.00a	100.00±00e	0.00±00a
	15	0.00±0.00a	100.00±00e	0.00±00a
<i>P. dodecondra</i>	5	4.33±1.67ab	86.17±5.32de	0.05±0.02a
	10	2.00±0.58a	93.62±1.84de	0.03±0.01a
	15	2.00±0.58a	93.62±1.84de	0.00±00a
<i>A. indica</i>	5	0.33±0.33a	98.94±1.06e	0.00±00a
	10	0.00±0.00a	100.00±00e	0.00±00a
	15	0.00±0.00a	100.00±00e	0.00±00a
<i>P. hysterochorus</i> (seed)	5	10.00±2.31ab	68.08±7.37cd	0.16±0.08ab
	10	9.00±5.13ab	71.28±1.61cde	0.16±0.08ab
	15	4.33±0.88ab	86.17±2.81de	0.04±0.03a
<i>P. hysterochorus</i> (leaf)	5	21.33±4.51cd	35.01±1.61b	0.13±0.1a
	10	0.67±0.67a	97.87±2.13de	0.00±00a
	15	13.33±1.45bc	54.25±4.63bc	0.10±0.05a
Primiphos- methyl	0.125	0.00±0.00a	100.00±00e	0.00±00a
Control (untreated)	0	31.33±4.81d	0.00±00a	0.35±0.07b

Means within a column followed by different letters are significantly different ($P < 0.05\%$), Tukey student test (HSD).

Viability index (%) = $(NG \times 100) / TG$

Where NG = number of seeds germinated and TG = total number of seeds tested in each Petri dish.

Data analysis

Data were analyzed using SPSS Ins., Version 13, 2004. Percentage mortality data were transformed using arcsine transformation before the analysis with one-way analysis of variance. Homogeneity of variance was determined using Levene's test. Mean values were separated using Tukey's studentized range test (HSD). Significance levels are given for $P < 0.05$.

RESULTS

Effects of botanical plants powder treatments on mortality of parent bruchids

Results of mortality of adult *Z. subfasciatus* 24 - 96 h after the application of different powders of plant materials are given in Figure 1-3. Application of botanical powders significantly ($P < 0.05$) increased adult mortality of *Z. subfasciatus* at the highest dose (15g/150g of grain) 96 h after treatment application. Treatments of *C. ambrosioides*, *J. curcas*, *D. stramonium* and *P. dodecondra* at

the rate of 15g/150g of grain were capable of inducing percent mortality ranging 90% to 100% within 96 h after treatment application. However, a similar result was recorded on seeds treated with *C. ambrosioides* and *J. curcas* at the lowest doses (5 and 10 g) 96 hr after treatment application. Significantly ($P < 0.05$), lower mortality was recorded with seeds treated with *J. curcas*, *D. stramonium*, *P. dodecondra*, *P. hysterochorus* and *A. indica* at the rates of 5, 10, and 15/150 g grain of the treatments 24 h after treatment application. *D. stramonium* (5 and 10g), *P. dodecondra* (5 g), *A. indica* (5, 10, and 15 g) and *P. hysterochorus* (5 and 10g) had no significant effect on adult mortality compared to control. Mortality effect of the powder treatments was dose and time dependent.

Effect of treatments on the F₁ progeny production, percent inhibition and percent grain weight loss

The number of F₁ progeny produced by *Z. subfasciatus* is presented in Table 2. Significantly ($p < 0.05$) lower number of F₁ progeny was produced in most of the treatments in comparison to the untreated check. Powders of *C. ambrosioides*, *A. indica*, *J. curcas* and *D. stramonium* at

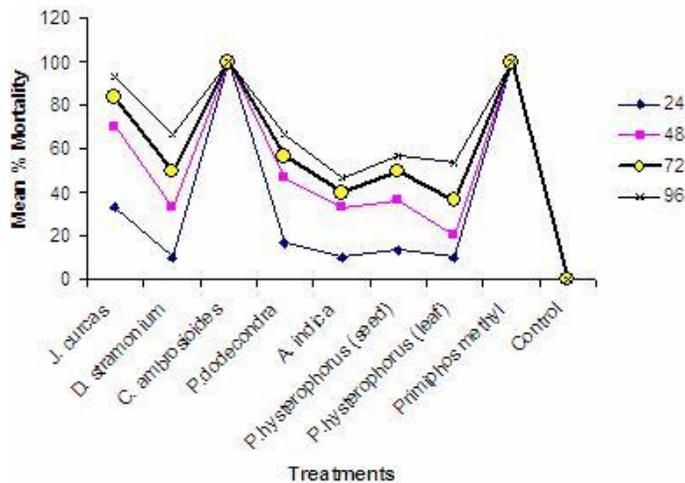


Figure 1. Effect of botanical powders of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *P. dodecondra*, *A. indica*, and *P. hysterothorus* on adult mortality of *Z. subfasciatus* applied at the dose of 5g / 150 of grain haricot bean.

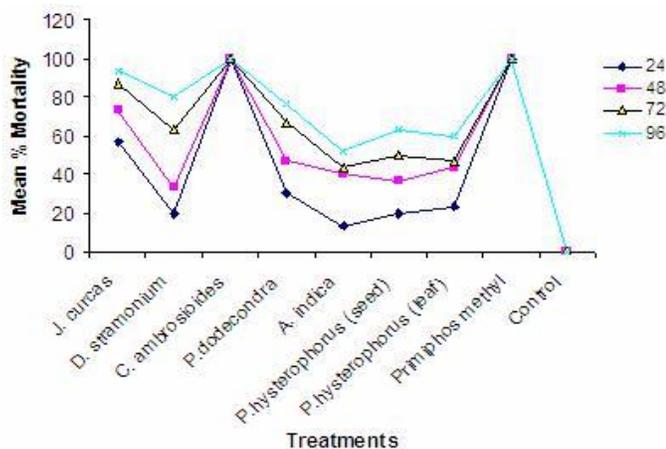


Figure 2. Effect of botanical powders of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *P. dodecondra*, *A. indica*, and *P. hysterothorus* on adult mortality of *Z. subfasciatus* applied at the dose of 10g / 150 of grain of haricot bean.

all tested rates (5, 10, and 15/150 g grain) caused more than 90% reduction in F₁ progeny production when compared to the untreated check.

Nonetheless, on seeds treated with leaf powder of *P. hysterothorus* at the rates of (5 and 15g/ 150 g grain) high number of F₁ progeny and low percent inhibition were recorded. The percent inhibition for the treatments was 35 and 54.26% respectively.

Results on the assessment of percent weight loss due to the infestation of *Z. subfasciatus* in a treated and untreated seeds are shown in Table 2. Most of the powder plant materials significantly ($P < 0.05$) reduced weight loss when compared to the untreated check 45

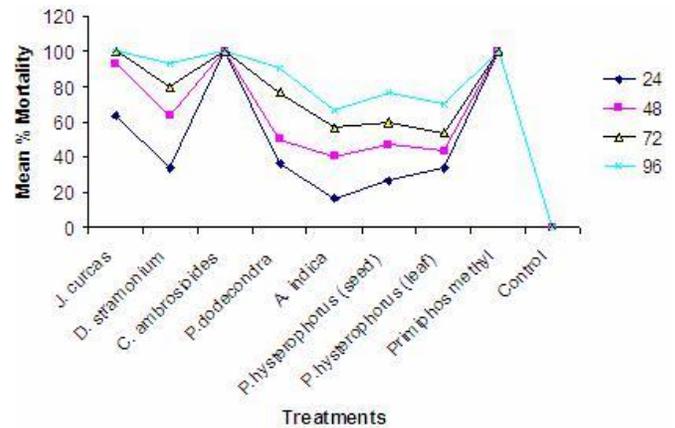


Figure 3. Effect of botanical powders of *J. curcas*, *D. stramonium*, *C. ambrosioides*, *P. dodecondra*, *A. indica*, and *P. hysterothorus* on adult mortality of *Z. subfasciatus* applied at the dose of 15g / 150 of grain of haricot bean.

days after infestation. However, *P. hysterothorus* seed powder at the lower rates (5 and 10 g) was markedly less effective in reducing the damage caused by *Z. subfasciatus*. There was no weight loss recorded on grains treated with primi-phosmethyl.

Effect of botanical plants powder treatment on the germination of haricot bean seeds

Percent germination of haricot bean seeds treated with different botanical plants powder is presented in Table 3. There was no significant ($P > 0.05$) difference in the germination capacity of haricot bean seeds treated with powder and control treatments during this investigation. Nonetheless, *P. hysterothorus* seed treatment was significantly different from control treatment. Generally, the percent germination of bean seed ranged 90 - 96% due to different botanical powder treatments.

DISCUSSION

The present study indicated that different powder plant materials screened can be used in the control of bean bruchids, especially *Z. subfasciatus* in stored beans. Among the powder plant materials tested, *C. ambrosioides* leaf powder applied at all rates (5, 10 and 15/150 g of grain) caused 100% mortality of *Z. subfasciatus* 24 h after treatment application which is more or less similar to the effects of primiphos-methyl which also induced 100% mortality of the tested insect at similar exposure time of 24 h after treatment application. Similarly, other powder plant materials admixed with haricot bean seeds at the highest doses (10 and 15 g) caused higher mortality of adult *Z. subfasciatus* 96 h after treatment application. Mortality of adult bruchids due to treatment of plant powders was directly related to the dosages used

Table 3. Effect of powder plant materials on percent germination of haricot bean seeds.

Treatments	Dosage (g / 150 grain)	% mean +SE germination of seeds
<i>J. curcas</i>	5	92.33±0.33ab
	10	91.00±0.58ab
	15	93.00±2.52ab
<i>D. stramonium</i>	5	91.67±1.45ab
	10	92.33±1.20ab
	15	95.00±0.58ab
<i>C. ambrosioides</i>	5	91.33±0.33ab
	10	91.67±0.88ab
	15	93.67±0.88ab
<i>P. dodecondra</i>	5	90.67±0.88ab
	10	92.67±0.33ab
	15	92.33±0.88ab
<i>A. indica</i>	5	90.00±1.15ab
	10	90.33±1.76ab
	15	90.67±1.45ab
<i>P. hysterothorus</i> (seed)	5	91.00±1.15ab
	10	89.00±1.20a
	15	92.00±1.00ab
<i>P. hysterothorus</i> (leaf)	5	90.00±1.15ab
	10	92.33±0.88ab
	15	91.33±0.88ab
Primiphos-methyl	0.125	90.67±1.76ab
Control (Untreated)	0	95.67±0.88b

Means within a column followed by different letters are significantly different (P 0.05%), Tukey student test (HSD).

and the exposure time of the pest to the treatment. It indicated that higher dosage and longer exposure periods are needed to achieve appreciable management of *Z. subfasciatus*.

D. stramonium, *P. dodecondra*, *A. indica* and *P. hysterothorus* grown in Ethiopia have biologically active substances which can be used in the control of *Z. subfasciatus*. The insecticidal values of some of these botanical plants were proven on different insect pests. For example, Asmare (2002) in Ethiopia reported that leaf powder of *J. curcas* applied at the rate of 4% w/w effectively controlled *S. zeamais* in sorghum. Tapondjou et al. (2002) also indicated that cowpea treated with dry ground leaf of *C. ambrosioides* at the dosage of 0.4% The present investigation demonstrated that *J. curcas*, killed 100% of *C. chinensis* and 80% of *C. maculatus* 48 h after treatment application. Shaheen (2006) also reported that dry leaf powder of *D. stramonium* applied at 3% w/w was efficient in inducing 100% mortality of *C. maculatus* 5 days after treatment application. Maize grain treated with seed powder of *P. dodecondra* at the doses of 2% and 4% w/w induced 68- 100% mortality of *S. zeamais* (Adane and Abraham, 1996; Asmare, 2002). In the pre-

sent study, there was a difference in the efficacy of Parthenium plant parts, where the seed exhibited higher toxicity. This difference probably indicates that the seed contains higher content of the active components responsible for the insecticidal properties. Hence, the current findings are in agreement with the previous works suggesting that the efficacy of botanical dust from *C. ambrosioides*, *J. curcas*, *D. stramonium*, *P. dodecondra*, *A. indica* and *P. hysterothorus* could serve as alternative bean bruchid management option.

The insecticidal activities of the botanical powders are broad and variable and dependent on different factors like the presence of bioactive chemicals which need to be identified, isolated and manufactured in the factory for pest management. The plant powders may act as fumigant, repellent, stomach poison and physical barrier (block the spiracles and impair respiration) (Law-Ogbomo and Enobakhare, 2007; Mulungu et al., 2007).

All the botanical powder treatments induced significant reduction in F1 adult emergence of *Z. subfasciatus* compared to the untreated check although the plant materials vary among themselves. Accordingly, powder treatments of *C. ambrosioides*, *A. indica*, *J. curcas*, *D. stramonium*

and *P. dodecondra* were superior in reducing the production of F1 progeny. However, *P. hysterothorus* leaf powder was less effective compared to other plant powder treatments, which is not significantly different from the control treatment. The reduction in F1 progeny emergence in the treated grains might be resulted from increased adult mortality, ovicidal and larvicidal properties of the tested leaf and seed powders. Ofuya (1990) and Tapondjou et al. (2002) have suggested that oviposition inhibition property of botanical powders on adult bruchids in terms of weakening of adults by powder treatments, which makes them, laid fewer eggs and killing the larvae hatching from eggs laid on grains.

In related studies, all these tested plant materials were shown oviposition inhibition effect on different insect pests. Tapondjou et al. (2002) found that at the dosage of 0.4 % w/w, *C. ambrosioides* dry ground leave caused 100% oviposition inhibition and subsequent progeny production by *C. chinensis*, *C. maculatus* and *A. obtectus*. El-Atta and Ahmed (2002) also reported that neem seed kernel admixed to the groundnuts at the rate of 5% gave comparable effect with BHC in reducing the adult emergence of *C. serratus*. Similar work also indicated that leaf and seed powder treatments of *D. stramonium*, *J. curcas*, *P. dodecondra* and *P. hysterothorus* were effective in reducing F1 progeny production by different storage insect pests of cereal crops (Ferdu et al., 2001; Asmare, 2002; Shaheen, 2006).

The cause for a considerable protection of haricot bean seeds against the attack by *Z. subfasciatus* by the powders of some of the botanical plants in the current investigation could be due to the presence of different chemicals which interfere with the feeding habit of the pest. Identification of the chemicals responsible should be an immediate research agenda.

The germination test demonstrated that the plant materials tested against *Z. subfasciatus* did not show any visible adverse effects on germination capacity of the seeds. Some of the treatments were infected by moulds which resulted in a reduced germination percentage ranging from 90 to 96%. Asmare (2002) showed that powders of *D. stramonium*, *J. curcas*, *P. dodecondra* and *A. indica* used in the control of *S. zeamais* did not show any significant effect on the germination capacity of sorghum.

In conclusion, the current findings demonstrated that most of the botanical plant powders tested possess insecticidal properties that can be used in the control of *Z. subfasciatus* in stored haricot bean to the same level or better than the synthetic insecticides which are expensive and environmentally less friendly. The availability of these botanical plants in the farm yard of most bean growers is another additional value for which botanical plants powders are preferred than other control methods, particularly the use of insecticides.

There are a number of botanical plants known for their insecticidal value, but there was no much progress in iso-

lating toxic substance from the plants and do some scientific work to promote them to the level of synthesizing them in the industry, so that they can be marketed to generate extra revenue to the community and country. It is widely believed that the identification of the active compounds and their modes of action against insect pest would contribute a lot to their use in stored bean protections. In such exercise (botanical plants screening) the involvement of chemists, biochemists and environmental scientists may enhance the development of products which may even play a great role in the economic development of a given country. Their transformed products may also become important supplements to imported synthetic pesticides to control stored pests. Botanical powder plants also present many farmers with large number of options for controlling insect pests that attack their products as they are cheap and based on local materials. Because, some of the botanical plants tested are considered as noxious weed by farmers and they are the target of well planned weed eradication program of a given country. For example, *P. hysterothorus* is among the top five highly targeted weed in the weed management program of the Research Institutes and Ministry of Rural Development and Agriculture of Ethiopia. Hence, in summary there is a scientific rationale for the incorporation of these botanical plants into the grain protection practice of resource-poor farmer. It is also essential that further work to isolate, improve their efficacy and reliability, and appropriate technological systems need to get priority concern.

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