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Full length Research paper

The Role Neem Seed and Garlic Bulb Powders as Biopesticides of Adult Maize Weevil (*Sitophilus zeamais* Motsch.) in Stored Maize Grains

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Post-harvest losses are one of the major causes of food insecurity in the developing world. The present study was planned to investigate the effect of neem seed and garlic bulb powders in control of maize weevil and prolonged storage of the powders on their insecticidal activity. A 3x3 factorial experiment was laid in a Complete Randomized Design (CRD) replicated two times. The result indicated that all the treatments have shown high repellence against maize weevil at all amounts of treatments (5, 10, and 15 grams) in the whole durations. However, there was no significance difference among 5, 10, and 15 grams for all the treatments including neem seed, garlic, and combination of neem seed and garlic powders. Mean separation for maize weevil mortality rate during 48 days of treatment of maize grain with different amounts of neem seed, garlic and combination of neem seed and garlic powder was shown that there was no mortality of maize weevils recorded for control (0gram). However, significant mortality was recorded for each treatments during 48 days of storage. The highest mean mortality rate was recorded for neem seed powder than for garlic powder, and combinations of neem seed and garlic powders showing insecticidal property of neem leaves. Maize weevil progeny emergence rate during extended duration of maize grain storage as treated by neem seed, garlic, and combinations of neem- garlic leaf powders was indicated that the highest progeny emergence rate was recorded for control group in all treatments. Only after 48 days that progeny emergence was recorded for experimental groups. It can be concluded from the present study that neem and garlic bulb powders could be used as effective insect repellents. Further studies are required to evaluate the effectiveness of neem seed and garlic powders in expended storage period.

Keywords: Insecticidal activity, Mortality, Plant extracts, Repellence, Storage duration.

INTRODUCTION

Storage pests are organisms which cause economic and/or nutritional damage to stored grains by breeding in/on the grains. However, some of the insect pests do not breed on the grains but their presence in the store becomes harmful as they generate filth, noxious smell and general contamination such as segment and hair debris, excreta and other waste products. The increasing public concern over chemical pesticide safety and possible damage to the environment has resulted in increasing attention being given to natural products of mostly plant origin for the control of storage pests, globally (Radha, 2014). Apart from the negative effects of synthetic insecticides, in most remote rural areas their availability is unreliable, and are frequently adulterated (diluted to ineffective concentrations by unscrupulous traders), outdated (owing to their toxicity to people and the environment), and ineffective owing to rapid evolution of pesticide resistance. Exploring the potential to utilise the pesticidal properties of plants has become a key focus of research in pest control. Some plants are known to contain bioactive metabolites, which show antifeedant, repellent and toxic effects on a wide range of insect pests (Mondal and Khalequzzaman, 2010; Radha, 2014).

The neem tree (*Azadirachta indica* L.) is a tropical evergreen plant with wide adaptability and known resistance to insect infestation (Oparaeke *et al.,* 2006). Garlic (*Allum sativum*) is widely known for its antimicrobial

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potentials. Garlic extract (oil and powder) contains active components, such as Amino acid called alicin and an enzyme called allinase. These compounds are antibiotic, which are effective against some range of bacterial and fungal species and have been a useful plant in food storage to inhibit fungal activities. However, Garlic extract also has insecticidal properties and show considerable toxicity to a number of pest species, across all life stages (Mousa *et al.,* 2013).

The maize weevil *Sitophilus zeamais* (Motschulsky) is a major pest of stored rice and other grains (Asawalam *et al.,* 2008b). In addition, the maize weevil is difficult to control, especially because the treated grain is the marketable product and may be contaminated with undesirable pesticides (Sighamony et al., 1990). There is a need for use of botanical pesticides such as plant essential oils, which are environmental friendly (Asawalam *et al.,* 2008a). Therefore, the present study is planned to investigate the effect of ground neem seed and garilc bulb powders in control of maize weevil and prolonged storage of the powders on their insecticidal activity.

MATERIALS AND METHODS

The experiment was conducted in Biotechnology laboratory, School of Biological Sciences, Haramaya University. An open pollinated variety (OPV) was used because it has stable yields in adverse conditions such as drought due to heterogeneity and commonly grown by most smallholder farmers. 5kg maize grains was obtained from Haramaya University Rare Research Station. The grains was sieved to remove dead seed, dirty and broken particles.

Then, 2kg grains will be randomly sampled and stored in a refrigerator 2 weeks to kill any prior sources of the *S. zeamais* inoculum and eggs which might be already pre-existing in the grain as procedure followed by Parugrug and Roxas (2008). After 2 weeks in the freezer, subsamples of 200g grains was put in 375ml bottles with perforated lids to prevent weevils from escaping and for aeration.

Neem seeds were collected from neem tree found within Dire Dewa city, and garlic bulb was bought from local market at Haramaya town.

The neem seed sample was air-dried under shade at an ambient temperature to avoid photo degradation of active ingredient by ultra-violet ray in line with the recommendation of Salako (2008), and garlic bulb samples were chopped and sun dried. The dried materials was then ground into fine powder using grinding machine and sieved with a 10mm sieve. The fine powders were then kept in air-tight containers until required.

Treatment and experimental Design

A 3 (5gm, 10gm and 15gm maize grains) × 3(neem seed, garlic, neem seed +garlic powders) factorial experiment were laid in a Complete Randomized Design (CRD) in two replications.

Preparation of Insect Culture

The parent stock of *Sitophilus zeamais* were obtained from infested grains. The insects were cultured under room temperature. The food media for the insect culture was 1kg maize grains for *S. zeamais*. 500gm of maize food medium was weighed into two different glass jars. 100 adult insect pests was introduced into each culturing medium. The culturing spanned for 45 days; at the end about eighty (540) adult insect pests were randomly selected for the study.

Data collection

Test for Repellence

The method employed by Garcia (1990) with some modifications was followed. Transparent plastic tubings, 13cm long x 1.3 cm diameter as test cylinders was used in the experiment (Figure 1). Each test cylinder was plugged at one end with fine mesh tulle containing 5, 10 and 15grams of neem seed, garlic bulb, and admixture of both neem seed and garlic bulb powders, while the other end was plugged with clean cotton ball which served as control. About 30 weevils were introduced at the middle of each test cylinder through a hole at the middle portion of the cylinder. The hole was covered with nylon tulle mesh to keep the insects inside the cylinder. The cylinders was grouped accordingly to represent the treatments and replications. Each treatment consisted of three cylinders and replicated twice.

The cylinders were left undisturbed and the number of weevils that moved towards the untreated halves of the cylinders were counted and rated every hour for the first five hours and at 24, 48 and 96 hours thereafter. Repellency rating was calculated following the formula:

Repellency rating = $\frac{n(1)+n(3)+n(5)+n(7)}{N}$

Where: n **=** number of insects stayed 0, 1-2, 3-4 and 5-6 cm from the center of the cylinder towards the untreated cotton plug, respectively. 1, 3, 5 and 7 **=** rating scale on the reaction of the insects on different test materials. N**=** Total number of insects introduced per cylinder. The degree of repellency of each test material was based on the following scale (Table 1).

Test for Weevil Mortality

200g of maize grains adjusted to 10% moisture content (MC) was treated with 5, 10, & 15g of each of the test treatment in 12 cm high x 6.5 cm diameter glass jars as in figure 2. In the first treatment neem seed powder; in the second experiment garlic bulb powder, and in the third

Figure 1: Experimental set-up for repellency test

Table 1: Scale for the determination of the degree of repellency of the test materials

Rating	Distance (cm) from center of the cylinder towards the untreated plug	Description
1	0	Ineffective
3	1-2	Slightly repellant(SR)
5	3-4	Moderately repellant (MR)
7	5-6	Highly repellant(HR)

Figure 2: Experimental set-up for adult mortality test

treatment admixture of both neem seed and garlic bulb powders were used The admixtures was shaken manually for 5 minutes and then tumbled for 15 minutes in a mechanical tumbler. The treated maize grains was left undisturbed for an hour. Thereafter, mixture 30 adult maize weevils was introduced per treatment. The glass jars were covered with filter paper and sealed with molten wax to keep the insects inside. Untreated maize grains was served as control. Each treatment was replicated twice. Weevil mortality rates was measured by physically counting dead weevils at 24 and 48 days after exposure to the treatment.

The mortality counts were done during the day when the weevils were highly active due to high temperatures and

Table 2: Mean values for repellence test against with different amounts of neem seeds and garlic bulb powders

Treatment	24hrs			48hrs		
	NS	G	NS+G	NS	G	NS+G
0gm	2.15±0.21c	2.15±0.21b	2.15±0.21c	2.15±0.21b	2.15±0.21b	2.15±0.21c
5gm	3.33±0.14bc	3.03±0.38b	4.25±0.26b	4.08±1.0ab	2.82±0.59ab	3.23±0.05b
10gm	4.67±1.56ab	4.85±0.40a	3.45±0.12b	4.57±1.60ab	3.48±0.54ab	3.78±0.35ab
15gm	5.90±0.52a	4.83±0.57a	6.18±0.68a	6.03±0.14a	4.02±0.54a	4.35±0.12a
	96hrs			144hrs		
0gm	2.15±0.21b	2.15±0.21c	2.15±0.21b	2.15±0.21b	2.15±0.21c	2.15±0.21b
5gm	5.37±0.90a	3.45±0.02b	4.25±1.15a	5.82±0.21a	4.48±0.54b	2.68±0.64b
10gm	6.35±0.02a	5.85±0.40a	4.97±0.85a	6.30±0.51a	6.53±0.09a	6.53±0.19a
15gm	6.15±0.02a	5.42±0.49a	5.45±0.31a	6.67±0.19a	6.20±0.66ab	6.53±0.47a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on Tukey HSD (Honestly Significantly Different) test.

relative humidity. Percent adult mortality was determined by counting the number of dead insects divided by the total number of insects introduced multiplied by 100.

Grain loss and Germination test

Grain loss assessment was determined by using hundred Grain Method (HGM) as follow:

$$\text{HGM} = \frac{\text{initial HGM} - \text{final HGM}}{\text{initial HGM}} \text{x } 100$$

Mass of 100 grains at the beginning of the storage period was compared with mass of 100 grains after 48 days intervals during the experiment.

Progeny emergence

The treated grains and the control were kept in containers in conducive culture conditions for 48 days. At the end of the culture period, the number of emergent insect pest for each treated grain and the control was recorded.

Data Analysis

Percentages and mean mortality/migration rate of adult insect pest which occurred were calculated and the number of progeny that emerged from the treated grains after six (6) weeks were reported. Mean separation based on M Tukey HSD (Honestly Significantly Different) test and ANOVA were conducted using SAS software version 9.2.

RESULT AND DISCUSSION

Repellence test

Repellence test of maize weevil against neem seed and garlic bulb powders as indicated in Table 2. All the treatments have shown high repellence against maize weevil at all amounts of treatments (5, 10, and 15 grams) in the whole durations. However, there was no significance difference among treatments. Related study by Parugrug and Roxas, (2008) has indicated powdered leaves of neem and lantana were noted to be highly repellant. *Sitophilus zeamais* is a key pest of stored maize causing serious economic damage. The predominant control of this pest is the use of synthetic residual pesticides, which have adverse effects on consumers and environment. The use of phytochemicals for controlling storage pests constitutes an attractive alternative to synthetic products, since plant may be more biodegradable and safer.

Maize weevil mortality rate

Maize weevil mortality rate during 48 days of treatment of maize grain with different amounts of neem seed, garlic bulb and combination of neem seed and garlic bulb

Table 3: Weevil mortality rate after 24 and 48 days as maize grains treated with different amounts of neem seed and garlic powder

Treatment	24 days		
	Neem seed	**Garlic**	**Neem seed + Garlic**
0gm	0.00c	0.00c	0.00b
5gm	11.67±2.36bcA	6.67±0.00bA	5.00±2.36bA
10gm	21.67±2.36bA	13.33±0.00aB	6.67±0.00abB
15gm	53.33±4.72aA	13.33±0.00aB	16.67±4.71aB
	48 days		
Treatment	**Neem seed**	**Garlic**	**Neem seed + Garlic**
0gm	0.00d	0.00b	0.00c
5gm	11.67±2.36cA	6.07±0.85bA	13.33±4.71bA
10gm	23.33±4.71bA	13.33±4.72aA	18.33±2.36bA
15gm	31.67±2.36aA	16.67±0.01aB	28.33±2.36aA

Means followed by same letter within a column were not significantly different at 0.05 probability level based on Tukey HSD (Honestly Significantly Different) test. Small letters: significance within column; capital letters: significance within row.

powders as indicated in Table 3. It was observed that there was no mortality of maize weevils recorded for control (0gram). However, significant mortality was recorded for each treatment. The highest maize weevil mortality was recorded for 15 grams than for 10 and 5 grams in all treatments. It was also observed from Table 3 that the highest mean mortality rate was recorded for neem seed powder than for garlic bulb powder, and combinations of neem and garlic powders showing insecticidal property of neem seeds.

Population size

Maize weevil progeny emergence rate during extended duration of maize grain storage as treated by neem, garlic, and combinations of neem-garlic bulb powders as indicated in Table 4. It was observed that the highest progeny emergence rate was recorded for control group in all treatments. Only after 48 days that progeny emergence was recorded for experimental groups. The least progeny emergences were recorded for 15gm neem seed powder followed by combination of neem seed and garlic bulb powders while the highest progeny emergence was recorded for garlic bulb powders. Generally, it was observed among experimental groups that the highest progeny emergence rate was recorded for 5gram powder while the least progeny emergence was observed for 15gm powder indicating that increase in amount of treatment powders have inhibitory effect on weevil population.

Berhanu and Emana (2018) has revealed significantly higher mortality of maize weevils 4 days after treatment exposure in all treatments of integration and other tactics tested at 5 and 10% doses, the maximums that were being in integration, followed by botanicals, while relatively the minimum was in inert dusts. This finding suggests that all the tested control tactics can be used in maize management maize weevils. Similarly, Bayih (2014) also reported that the unitary and binary botanical formulations at lower and higher rates were effective against *Zabrotes subfasciatus*. Besides, it was indicated that the powders of *Plectranthus glandulosus* and *Azadirachta indica* in isolation as well as at their different proportions of their binary combinations generally caused significant mortality to adult *Callosobruchus maculatus* and *S. zeamais* compared to the controls (untreated grain) (Katamssadan, 2016). It was also reported that combinations of different rates of Malathion 5% dust and neem seed powder caused higher weevils mortality than the untreated control (Ibrahim and Sisay, 2012). In his preliminary study, Demissie (2006) also reported that the combined use of minimum rates of *Chenopodium* plant powder, botanical triplex, silicosec and filter cakes with weevil tolerant varieties has reduced grain damage at Bako of Ethiopia.

Grain Loss and Germination test

Grain loss and germination test due to damage by maize weevil (*Sitophilus zeamais*) on maize grains after 48 days of treatment with neem seed and garlic bulb powders as indicated in Table 5. It was shown that there was significance difference between control and treated groups in both grain weight loss and germination test. The highest grain loss and the least percent germination was recorded for control group (with zero treatment) indicating that neem seed and garlic bulb powders can be used to reduce grain loss during postharvest storage.

Table 4: Maize weevil progeny emergence rate during 48 days of storage period

Treatment	Neem seed	Garlic	Neem seed + Garlic
0gm	0.93±0.19a	0.93±0.19a	0.93±0.19a
5gm	-0.17±0.05b	0.30±0.05b	-0.15±0.02b
5gm	-0.30±0.05b	0.22±0.07b	-0.28±0.02b
15gm	-0.38±0.02b	0.02±0.02b	-0.32±0.02b

Means followed by same letter within a column were not significantly different at 0.05 probability level based on Tukey HSD (Honestly Significantly Different) test.

Table 5: Grain loss and germination test due to damage by maize weevil (*Sitophilus zeamais)* on maize grains after 45 days of treatment with neem seed and garlic bulb powders

Treatment	Grain weight loss(%)			Germination(%)		
	NS	G	NSG	NS	G	NSG
0gm	39.60±0.57a	39.60±0.57a	39.60±0.57a	68.50±4.95b	68.50±4.95b	68.50±4.95b
5gm	18.00±3.95b	25.23±1.44b	21.04±2.21b	90.50±2.12a	79.00±5.66ab	85.50±3.54a
10gm	8.76±1.07c	17.04±1.33c	12.71±2.07c	97.50±2.12a	82.00±2.83ab	85.00±1.41a
15gm	10.22±0.96bc	13.44±2.77c	10.97±2.53c	99.00±1.41a	87.50±2.12a	89.50±0.71a

Means followed by same letter within a column were not significantly different at 0.05 probability level based on Tukey HSD (Honestly Significantly Different) test.

Among treatments the least percentage grain loss (10.22%) and the highest percentage germination (99.00%) was recorded for neem seed powder indicating that neem seed was more effective than garlic powders, and combination of neem seed and garlic bulb powder treatments.

CONCLUSIONS

It can be concluded from the present study that neem seed and combinations of powders could be used as effective insect repellents and pesticidal activity. Based on high repellence rate, neem seed and garlic powders can be used as control for damage caused by maize weevil (*Sitophilus zeamais*). Hence, the use of neem seed and garlic powders as an alternative control option in integrated storage pest management strategies by small holder farmers. Further studies are required to check the effectiveness of repellence and pesticidal activity of neem seed and garlic powders as repellence of foliar insects. Further studies to be conducted to select the most effective botanical pesticides.

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