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EFFECT OF BOTANICALS LEAF POWDER COMBINING WITH SYNTHETIC CHEMICAL INSECTICIDE AGAINST MAIZE WEEVIL, *SITOPHILUS ZEAMAIS* MOSTCH (COLEOPTERA: CURCULIONIDAE) ON MAIZE

Adugna Gindaba[1], and Mulugeta Negeri [*1]

[1]Department of Plant Sciences, School of Agriculture, Ambo University. Guder Mamomezemir Campus, P.O.Box. 19, Ambo, Ethiopia.

E-mail: negerimulugeta@yahoo.com

ABSTRACT

This study was conducted to evaluate the efficacy of botanicals (*Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus)* leaf powder combining with 5% malathion dust against maize weevil, *Sitophilus zeamais*, under laboratory conditions. Treatments were arranged fromT1 to T7 with untreated control. All treatments were in combination or alone, showed higher maize seed protection than untreated control. T6 treatment (10% plant powder + 50% malathion) showed promising effect against maize weevil. Treatment combination that conducted using *Clausena anisata* has revealed greater effect against maize weevil than others. To conclude that in the current study reduced level of plant powder combined with 5% malathion dust showed promising effect of maize grain protection from *S. zeamais*.

Key words: Combination, Maize weevil, Botanicals, Chemicals.

1. Introduction

Maize is the main staple food crop for the majority of people in sub-Saharan Africa (SSA) including Ethiopia in which over 70% of the crop is produced by smallholder farmers and over 60 million tons of maize is produced annually (FAOSTAT, 2017). Maize is one of the major cereal crops grown for its food, feed, firewood and construction purposes in Ethiopia (Sori, 2014). Maize ranks second to tef in area coverage and first in total production of the cereal crops in Ethiopia. However, its production and yield are highly affected by an array of biotic and abiotic stresses (Tefera *et al.*, 2011). Among biotic constraints, insect pests are the main factor responsible for causing high losses (Adams and Schulter, 1978) and the most important of these pests in the field and storage are Lepidopteran stem borers and Coleopteran weevils, respectively (Emana and Tsedeke, 1999; Demissie *et al.*, 2008).

Post-harvest losses exceeding 20% have been reported in SSA mainly resulting from storage insect pest infestations (World Bank, 2011). Post-harvest losses of food grains due to insect pests are a significant nutritional and economic burden to subsistence farmers in developing countries (Firdisa and Abraham, 1998). The maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae), is one of the most serious cosmopolitan pests of stored cereal grain, especially of maize (*Zea mays* L.). To control these pests, the majority of smallholder farmers in Africa use synthetic pesticides. Although the use of pesticides is one means of protecting stored maize, the associated side effects on the environment and human health, development of genetically resistant insect strains and prohibitive costs have become major concerns and thus given impetus to the search for alternative methods of pest control (Obeng-Ofori and Reichmuth, 1999).

Farmers need to be switched to more effective and environmentally sustainable solutions of crop protection. Chemicals may be a dominant market today, but the fastest growing biopesticide inputs are the future. The use of this method provides the best alternative in maintaining the insect populations in balance without causing harm to other organisms and beneficial fauna in the ecosystem (Lv *et al.*, 2011). Among the methods, botanicals play a major role as they are easily and locally available, and having ease of manufacture, they provide employment at the rural level because botanicals had been used since the Vedic period for pest control (Koul and Walia, 2009). For instance, mixing with plant oils is an ancient Indian and African method of protecting grains against insect attack (Pereira, 1983). Therefore, the plant products could offer a solution for the problems of availability, health risks, costs and resistance in the case of synthetic pesticides, and for the lack of equipment for hermetic storage, gamma irradiation and controlled atmospheres. Due to this, botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals pose little threat to the environment or to human health. It would be easy to become familiar with locally available botanicals because medicinal plants which are normally used to cure human's and other animals' illness can also be used to protect stored food (Reddy and Chowdary, 2021).

The current methods for managing stored grain pests depend heavily on synthetic pesticides. However, their uninterrupted and indiscriminate use has not only led to the development of resistant strains (Champ and Dyte, 1976; Georghiou and Lagunes-Tejeda, 1991) but also accumulation of toxic residues on food grains used for human consumption has led to the health problems (Sharma and Meshram, 2006).

The combination of a botanicals or a plant extract with insecticidal activity is a novel approach to fight against the resistances and resurgence issues created by insect pests (Srivastava *et al*., 2011). Hence, the aim of this study was to evaluate the efficacy of combining selected botanicals powder with synthetic insecticide, Malathion against maize weevil, *Sitophilus zeamais*, under laboratory conditions.

2. Materials and Methods

2.1. Plant part collection

One kilogram of fresh leaves of *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* were collected nearby Dambi Dollo University campus in September 2022. The collected leaves were air dried in the laboratory room. Then, the drier leaves were powdered using electric grinder into fine powder and the powder packed until used for insect toxicity study.

2.2. Insect rearing

The study was conducted at Dambi Dollo University in the zoological science laboratory from October to December 2022. This study site has an altitude of 1588(m.a.s.l) above sea level, latitude 8^{0}33.011' N and longitude 34^{0}50.660' E. Test insect were collected from maize stored in various farmers' storage facilities of the study (survey) site and brought to the laboratory. Then, the test insects were cultured at 23±3°C and 64.8±9% RH laboratory conditions. Limmu variety maize seeds were bought from Dambi Dollo university research center and disinfected at -14°C for 7days from any prior infestation before using them as a substrate for insect rearing (Gemechu *et al*, 2013). The grains were used as food substrate for the test insect. Fifty unsexed test insects were placed in 16 plastic jars each of 300 ml capacity plastic jars and containing 200 g seeds. The jars were covered with nylon mesh to allow ventilation and put in place with rubber bands to prevent the escape of weevils and to protect the interference of other insects in to the jars. The set up was replicated four times. The parent maize weevils were removed by sieving after two weeks of oviposition period and transferred to another substrate. The maize grains were kept under laboratory conditions until the emergence of F1 progeny. Then, the newly emerged adults were used in toxicity experiment.

2.3. Treatment combination

Preparation of the treatment using *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* leaf powders combination with 5% malathion dust was carried out as indicated in table 1.

Table 1. Treatments prepared with different percentages of botanicals powder and 5% malathion dust for present study.

Treatment code	Treatment combination (botanical powder + Malathion 5% dust)
T1 (P100%+M0%)	Cla 30g (100%) + 0g (0%)
T2 (P50%+M10%)	Ver-15g (50%) + 0.01g (10%)
T3 (P40%+M20%)	Gra-12g (40%) + 0.02g (20%)
T4 (P30%+M30%)	Cla- 9g (30%) + 0.0.3(30%)
T5 (P20%+M40%)	Ver- 6g (20%) + 0.04g (40%)
T6 (P10%+M50%)	Gra-3g (10%) + 0.05g (50%)
T7 (P0%+M100%)	- 0g (0%) + 0.1g (100%)
Untreated control	- 0g (0%) + 0g (0%)

2.4. Effect of combined treatment on maize weevil mortality

Fifty unsexed adult maize weevils were introduced in to 200g disinfected Limmu variety maize seeds contained in 500 ml glass jars treated with each treatment and without treatment as control. The jars were covered with nylon mesh and tied with rubber band to prevent escape of test insect and entrance of new one. The experiment was laid out in a complete randomized design (CRD) and replicated three times. The number of dead insects in each jar was recorded at 2, 7, 14 and 28 days after treatment application.

2.5. F1 Progeny Assessment bioassay

The treated jars were kept for additional 28 days of oviposition time after mortality assessment.

Then assessment was started first by removing all dead and alive adult weevils from the maize by sieving. The treated and control grains were then kept until emergence of F1 progeny. Then the number of F1 progeny of the maize weevil was counted to avoid overlapping of generations. Following methods used by Araya and Emana (2009), the formula of percentage reduction in adult emergence or inhibition rate (% IR) was used to know which treatment inhibited the emergence F1 progeny.

$$(\%IR) = \frac{\text{Total F1 progeny in control} - \text{Total F1 progeny in treatment}}{\text{Total F1 progeny in control}} \times 100$$

Where, IR: Inhibition Rate

2.6. Grain Damage and Weight Loss Assessment

The percentage of weight loss of maize grains due to insect pests was calculated using a gravimetric or count and weight method (Zewde and Jembere, 2010) as follows:

$$\%\text{Weight Loss} = \frac{(Wu * Nd) - (Wd * NU)}{Wu(Nd + NU)} x100$$

Where: Wu= weight of undamaged grain
Nu= Number of undamaged grain
Wd= Weight of damaged grain
Nd= Number of damaged grain

The percentage of insect damaged seed was also calculated (Wambugu *et al*., 2009) as follows:

$$\text{Insect damaged grain } (\%) = \frac{\text{Number of insect damaged grain}}{\text{Total number of grain}} x100$$

2.7. Germination test

Twenty seeds from each treated and control group were placed separately in Petri dishes containing moistened filter paper by 10 ml distilled water. Each treatment was replicated four times (20 seeds per petri-dish) and incubated at room temperature for 4 to 7 days. The number of emerged seedlings from each Petri dish was counted and recorded. The percent germination was computed using the following Dubale *et al*. (2012) formula as follows:

$$\text{Germination } (\%) = \frac{\text{Number of germinated seed}}{\text{Number of seed planted}} x100$$

2.8. Data Analysis

The data was analyzed using IBM SPSS (version 25). Analysis of variance (ANOVA) was run to see the effect of the treatments on % mortality, number of F1 progeny reduction, % weight loss, grain damage and effect of the treatments on germination of the seed. Mean values were separated using Tukey's HSD test and significance level was set at $P < 0.05$.

3. Results

3.1. Insecticidal effect of plant powder and malathion dust

Cumulative percentage mortality by seven levels of treatment using leaf powder of *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* with 5% malathion dust and untreated control was described in table 2. Cumulative percentage maize weevil mortality by applied dosages of combined treatment was significantly different ($P<0.05$) between the three botanicals. From all, T7 (100% malathion 5% dust) caused highest maize weevil mortality. All the treatments were caused significant mortality higher than untreated control. In T1, 30g (100%)

Clausena anisata showed 86.67% mortality. T6 (3g botanicals + 0.05g malathion) caused higher mortality (88% and 72.67%) than other treatments with exception of combined treatment using *Croton macrostachyus* leaf powder (40.67%) followed by T5, T4, T2 and T3 under the mixture of *Croton macrostachyus* and malathion. Mixture of malathion and leaf powder of *Clausena anisata* showed highest percentage mortality followed by that of *Vernonia amygdalina* and *Croton macrostachyus*

Table 2. Effect of mixed leaf powder of *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* combination with 5% malathion dust on maize weevil mortality.

Treatment code	Cumulative percentage mortality after treatment application		
	Clausena anisata (Mean±SD)	*Vernonia amygdalina* (Mean±SD)	*Croton macrostachyus* (Mean±SD)
T1 (P100%+M0%)	86.67±6.11[a]	68.00±4.00[b]	48.00±2.00[c]
T2 (P50%+M10%)	74.00±2.00[a]	56.00±2.00[b]	30.00±5.29[c]
T3 (P40%+M20%)	60.00±21.17[a]	60.00±2.00[a]	26.67±2.31[b]
T4 (P30%+M30%)	78.67±3.05[a]	60.67±1.15[b]	24.67±1.15[c]
T5 (P20%+M40%)	83.33±3.05[a]	66.67±3.05[b]	30.00±2.00[c]
T6 (P10%+M50%)	88.00±2.00[a]	72.67±3.05[b]	40.67±3.05[c]
T7 (P0%+M100%)	98.67±1.15[a]	98.67±1.15[a]	99.33±1.15[a]
Untreated control	0.00±0.00[d]	0.00±0.00[d]	0.67±1.15[d]

Note: Means followed by the same letter across the row are not significantly different.

3.2. Reduction in adult emergence

The results in table 3 showed the effect of treatment combination on maize weevil F1 progeny reduction. There was no significant difference (P>0.05) among treatment combination but there was significant difference (P<0.05) between treatment combination using three botanicals. Therefore, Treatment combination using *Clausena anisata* and *Vernonia amygdalina* leaf powder was higher than that of Croton *macrostachyus* leaf powder. Inhibition rate recorded from untreated control was 0%.

Table 3. Effect of mixed leaf powder of *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* and 5% malathion dust on adult emergence inhibition rate.

Treatment code	F1 progeny emergence inhibition rate after treatment application		
	Clausena anisata (Mean±SD)	*Vernonia amygdalina* (Mean±SD)	*Croton macrostachyus* (Mean±SD)
T1 (P100%+M0%)	96.80±1.39^{a}	96.00±1.39^{a}	80.13±3.61^{b}
T2 (P50%+M10%)	97.60±0.00^{a}	96.80±1.39^{a}	88.90±2.77^{b}
T3 (P40%+M20%)	98.40±1.39^{a}	97.60±0.00^{a}	92.87±2.35^{b}
T4 (P30%+M30%)	98.40±1.39^{a}	97.60±0.00^{a}	93.67±1.33^{b}
T5 (P20%+M40%)	99.20±1.39^{a}	97.60±0.00^{a}	94.43±1.33^{b}
T6 (P10%+M50%)	99.20±1.39^{a}	97.60±0.00^{ab}	96.00±1.39^{b}
T7 (P0%+M100%)	100.00±0.00^{a}	99.20±1.39^{a}	99.20±1.39^{a}
Untreated control	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00c

Note: Means followed by the same letter across the row are not significantly different.

3.3. Weight loss and Seed damage assessment

Percentage weight loss of maize grains due to maize weevil was described in table 4. Seed weight loss in all treatments were significantly (P<0.05) lower than untreated control. The grain weight loss results showed by treatment combination using *Croton macrostachyus* leaf powder was higher than that of *Clausena anisata* and *Vernonia amygdalina*.

The results indicated in table 5 were the effects of treatment combination on maize seed damage due to test insect after treatment applications. Percentage seed damage was significantly ($P<0.05$) higher in untreated control (90.18 to 90.53%). From T1 to T6, percentage seed damage recorded from treatment combination using *Croton macrostachyus* leaf powder was significantly higher than results showed by other test plants.

Table 4. Effect of mixing test plants and 5% malathion dust on maize grain weight loss

Treatment code	Percentage seed weight loss after treatment application		
	Clausena anisata (Mean±SD)	*Vernonia amygdalina* (Mean±SD)	*Croton macrostachyus* (Mean±SD)
T1 (P100%+M0%)	0.89±0.01[c]	1.48±0.15[b]	8.31±0.79[a]
T2 (P50%+M10%)	0.91±0.01[c]	1.96±0.15[b]	9.24±0.16[a]
T3 (P40%+M20%)	0.86±0.01[b]	0.98±0.02[b]	7.25±0.15[a]
T4 (P30%+M30%)	0.84±0.01[b]	0.95±0.01[b]	6.23±0.18[a]
T5 (P20%+M40%)	0.71±0.01[c]	0.93±0.02[b]	5.25±0.14[a]
T6 (P10%+M50%)	0.64±0.03[b]	0.92±0.01[b]	5.29±1.77[a]
T7(P0%+M100%)	0.40±0.01[a]	0.40±0.03[a]	0.39±0.02[a]
Untreated control	39.69±1.46[a]	38.69±1.62[a]	40.11±0.22[a]

Note: Means followed by the same letter across the row are not significantly different.

Table 5. Effect of mixed test plants combination with 5% malathion dust on maize seed damage

Treatment code	Percentage grains damage after treatment application		
	Clausena anisata (Mean±SD)	*Vernonia amygdalina* (Mean±SD)	*Croton macrostachyus* (Mean±SD)
T1 (P100%+M0%)	10.05±0.74[b]	10.99±0.01[b]	13.85±0.52[a]
T2 (P50%+M10%)	10.78±0.19[b]	11.18±0.18[b]	15.04±0.37[a]
T3 (P40%+M20%)	8.91±0.03[c]	9.89±0.10[b]	12.79±0.17[a]
T4 (P30%+M30%)	7.93±0.06[c]	8.89±0.11[b]	11.77±0.29[a]
T5 (P20%+M40%)	6.82±0.13[c]	7.79±0.10[b]	10.94±0.05[a]
T6 (P10%+M50%)	5.68±0.29[c]	6.73±0.30[b]	9.87±0.12[a]
T7 (P0%+M100%)	1.19±0.18[a]	1.25±0.04[a]	1.14±0.19[a]
Untreated control	90.53±1.52[a]	90.18±1.53[a]	90.31±0.66[a]

Note: Means followed by the same letter across the row are not significantly different.

3.4. Seed germination Assessment

The results of seed germination after treatment applications showed in table 6. There was no significant difference (P>0.05) within and between treatment combination but there was significant difference (P<0.05) between treatments and untreated control. In T6, treatment combination using *Clausena anisata* showed percentage germination 90% which was higher than that of *Vernonia amygdalina* (81.67%) and *Croton macrostachyus* (76.67%). T7 showed significant percentage germination (91.67 to 93.33%) than other treatments using *Vernonia amygdalina* (61.67 to 81.67%) and *Croton macrostachyus* (58.33 to 76.67%).

Table 6. Effect of test plant combined with 5% malathion dust on maize seed germination.

Treatment code	Percentage seed germination after treatment application		
	Clausena anisata (Mean±SD)	*Vernonia amygdalina* (Mean±SD)	*Croton macrostachyus* (Mean±SD)
T1(P100%+M0%)	83.33±2.89[a]	80.00±0.00[a]	70.00±0.00[b]
T2(P50%+M10%)	65.00±5.00[a]	61.67±2.89[a]	63.33±2.89[a]
T3(P40%+M20%)	76.67±2.89[a]	63.33±2.89[b]	58.33±2.89[b]
T4(P30%+M30%)	68.33±7.64[a]	71.67±2.89[a]	66.67±2.89[a]
T5(P20%+M40%)	71.67±2.89[a]	76.67±2.89[a]	71.67±2.89[a]
T6(P10%+M50%)	90.00±5.00[a]	81.67±2.89[ab]	76.67±2.89[b]

T7(P0%+M100%)	93.33±2.89[a]	91.67±2.89[a]	93.33±2.89[a]
Untreated control	15.33±0.58[a]	15.33±1.53[a]	16.00±1.73[a]

Note: Means followed by the same letter across the row are not significantly different.

4. Discussion

The present study demonstrated that *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* leaf powders as mixture combination with 5% malathion dust or applied alone resulted *Sitophilus zeamais* mortality. This result is in agreement with Ahmed (2015) who reported combination treatment using malathion and neem seed powder resulted significantly higher adult weevil mortality than untreated control after 90 days infestation. The results also in line with the results reported by Yuya *et al*. (2009) that Niger seed oil combined with malathion caused maize weevil mortality. Obeng-Ofori and Amiteye (2003) also reported that vegetable oil mixed with pirimiphos-mehyl was highly toxic to adult *S. zeamais*. Leaf powder of *Clausena anisata*, *Vernonia amygdalina* and *Croton macrostachyus* as mixture combination with 5% malthion dust or alone significantly reduced F1 progeny emergence than untreated check. All treatments, in combination or alone, reduced maize seed weight loss and damage after 28 days of treatment application. Seed germination was significantly higher in all treatments than untreated control. In the current study, 10% test plants powder and 50 % malathion showed reduced seed weight loss, damage and higher seed germination than other combinations. Reduced amount of combination provided acceptable grain protection from weevils as reported by Ulrich and Mewis (2000), Yuya *et al*. (2009) and Ahmed (2015).

Conclusions

In the present study, mixed 10% plant powder combination with 50% malathion dust was effective to protect maize grains from maize weevil attack. Treatment combination or alone applied using *Clausena anisata* was effective against adult *S. zeamais* which followed by *Vernonia amygdalina and other* treatments. Therefore, reduced level of treatment combination using *Clausena anisata* has promising effect of grains protection.

Conflict of Interest

Authors declare no conflict of interest

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