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Comparative Efficacy of Jatropha Curcas and Trichilia Dregeana Seed Oil on Sitophilus Zeamais in Stored Maize Grain

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ABSTRACT

Sitophilus zeamais Motschulsky continue to cause considerable damage to maize grain in most stores in Africa. In the present study, laboratory experiments was conducted to investigate the potentials of *Trichilia dregeana* and *Jatropha curcas* seed oils for the protection of stored maize against two week old adult *Sitophilus zeamais*. The results indicated that *Jatropha curcas* seed oil and combination of the two seed oils (a synergic effect) caused 100% maize weevil's mortality after one week exposure which is extremely high compared to negative control. The maize grains treated with seed oils and positive control showed a significant decrease of the number of holes compared to the negative control which indicates its protective effect against weevil from damaging the maize grains. Weevil perforation index ranged from 1.59 to 19.48 and indicate a highly positive protectant ability of the two seed oils. The computed percentage protectant ability of the two seed oils showed that 80.52-81.5% protectant ability in *Trichilia dregeana* seed oil, 96.87- 98.41% in *Jatropha curcas* seed oil, 98.41% in combination seed oils, and 98.41% in malathion powder. The results indicate that *Jatropha curcas* seed oil and combination of the two seed oils are highly effective as compared to *Trichilia dregeana* seed oil for maize seed protectant against *Sitophilus zeamais*. Thus, the order of protective ability of seed oils with various treatments on maize weevil were: *Jatropha curcas* seed oil > combination of *Trichilia dregeana* seed oil+ *Jatropha curcas* seed oil > *Trichilia dregeana* seed oil.

1. INTRODUCTION

Maize (*Zea mays* L.) is a major food security crop in Africa and it is usually stored to provide food reserves and also seed materials for planting [1]. Maize is a staple food of Ethiopia and is widely grown by smallholder farmers. Despite high maize yields, losses in storage are high due to maize weevil, *Sitophilus zeamais* (Motsch.), especially among smallholder farmers. In Ethiopia, the pest causes huge losses in storage if grain is not protected and it adversely affects food security at smallholder farmer level. As a primary pest of stored maize, *Sitophilus zeamais* is capable of penetrating and infesting intact kernels of grain, in which immature stages develop [2] leaving the maize emptied of its nutritional (lower quality and value) and seed value culminating in outright rejection of the product at the local and international markets. Declining food production, worsened by huge losses resulting from *Sitophilus zeamais* attack during maize storage expose farmers to different magnitudes of food shocks[3]. It also brings about the establishment of mould infestations including *Aspergillus* spp. which produces aflatoxins making the grain unsuitable for food and feed [4]. The maize weevil's causes weight loss of stored grain up to 18.3% [5].

Control of *Sitophilus zeamais* populations around the world is primarily dependent upon continued applications of synthetic insecticides (such as fumigation of stored products with carbon disulphide and phosphine, and dusting with pirimiphos methyl or permthrin), which are often the most effective treatments for the disinfestations of stored food, feedstuffs and other agricultural commodities from insect infestation[6]. However, the use of these chemicals is hampered by many attendant problems such as development of resistant insect strains, toxic residues in foods and humans, workers' safety and high cost of procurement[7-9]. To minimize use of synthetic pesticides and to avoid pollution of the environment, natural antifeedant, deterrent and repellent substances have been searched for pest control during recent times[10]. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Many plants that are eco-friendly and readily available in the environment can be used in the control of insects in stored products [11]. In ancient times, oils obtained from locally available plants were used for stored grain protection against insect attack. In recent years, attention has been given to use vegetable oils as stored grain protectants against insect. Oils extracted from plants have been extensively used in tropical countries for crop protection. The mode of action these oils is yet to be confirmed, but most appear to cause death of insect egg, larva or adult by suffocation [12-21].

The seed oil of *Jatropha curcas* has been shown to contain toxic substance called phorbol esters which exhibit insecticidal effects[22], efficacy of *Jatropha curcas* seed oil reported[23]. In view of encouraging results obtained by the above researchers, the present work aimed to compare protective potency (in relation to a conventional synthetic chemical, malathion dust powder) of *Jatropha curcas* and *Trichilia dregeana* seed oils against maize weevils.

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2. MATERIALS AND METHODS

2.1 Rearing of *Sitophilus zeamais*

The parent stock of *Sitophilus zeamais* used for this study was obtained from infested maize grain in the open market and reared in the laboratory at ambient temperature (27± 2^0C and 50-70% RH) in glass jars. The maize grain and all apparatus equipment were sterilized by heat to 75^0C for 1hr to protect stock culture from enemies (insects and pathogens). The sex of *Sitophilus zeamais* was identified in Addis Ababa University, Biology department. Approximately, 50 unsexed adult of *Sitophilus zeamais* adults were released in each jar containing 200g of maize seeds and the mouth was closed with a piece of nylon cloth. The *Sitophilus zeamais* were allowed for free mating and oviposition for 15days. Then the *Sitophilus zeamais* were separated from the seeds by sieving and seeds along with eggs were kept in glass container for emergence of next generation. After emergence, the newly emerged adults were collected and again allowed for further mating and oviposition with new seeds in different containers to maintain a series of stock culture of the test insect.

2.2 Extraction of seed oils

The oilseeds of *Jatropha curcas* and *Trichilia dregeana* were harvested from Tepi area, Ethiopia. They were dried in sunlight for fifteen days, shelled and the batches ground into a fine powder, three hundred grams of the powder and 2.5liters of Ethanol (95%) were used in the extraction of the oil with maceration technique. At the completion of the extraction process, the seed oils were recovered from the mixture by evaporating the residual extracting solvent.

2.3 Fatty acid methyl ester (FAME) preparation and analysis

Fatty acids are polar compounds and are not volatile. The evaluation of fatty acids in *Trichilia dregeana* and *Jatropha carcus* seed oils requires the preparation of fatty acid methyl esters (FAME) in order to improve volatility and to reduce peak tailing, and subsequent analysis by GC with good precision and reproducibility [24]. In this study, the AOCS method Ce 2-66 was used for the preparation of FAME [25]. The FAME preparations were analyzed by GC-Agilent Technologies 7820A at a temperature of 260ºC, and MS-Agilent Technologies 5977EMSD a detector temperature of 280ºC, in Addis Ababa university, department of chemistry. The mass spectra were compared with the Mass Hunter/Library/NIST11.L Mass Spectral Library.

2.4 Treatments and experimental design

There were 6 treatments arranged in completely randomized design (CRD) and the treatments were replicated three times. There were two controls in the treatments (the untreated control and the standard control- Malathion dust powder) for comparison. The treatments used were *Jatropha curcas* seed oil and *Trichilia dregeana* seed oil. Each treatment was measured and introduced (mixed with maize grain) in to 100g of clean and disinfested maize grains in each plastic bag. 0.5and 1.0ml of the test materials (seed oils) and combination of the two seed oils were applied accordingly, while that of malathion dust powder (0.5 and 1.0gm) was applied to maize grains. The seed oils were thoroughly mixed with the maize grains with the aid of a glass rod to ensure thorough admixture. Five pairs of newly emerged adult weevils were introduced into the treated and untreated maize grain in the plastic bag. Each treatment was replicated three times.

2.5 Adult mortality test

Adult mortality was assessed on 1, 2, 3, 4, 5, 10, 15, 20, 25, 30 and 35^{th} days after exposure of the *Sitophilus zeamais* to the treatments. Adults were considered dead when gently probed with sharp objects and there were no responses. Percent adult mortality was determined as per the method described by Parugrug and Roxas (2008)[26] using the following formula:

$$\text{Mortality (\%)} = \frac{\text{No. of dead insects} * 100}{\text{Total no. of insects}}$$

2.5.1 Assessment of perforated grains

Damage maize grains were assessed in each trial after 35 days exposure. Samples of 200 seeds were randomly taken from treated and untreated grains and the number of insect-damaged grains was counted. Weevil Perforation Index (WPI) was calculated using the formula below as given by Parugrug and Roxas (2008)[26]:

Weevil perforation Index (WPI):

$$\frac{\text{\% of treated grains perforated} * 100}{\text{\% control grain perforated} + \text{\% of treated grains perforated}}$$

Percent protectant Ability (PPA) = 100-WPI

2.5.2 Damage assessment

Damage was assessed by the number of exit holes. This was done using a sub-sample of 200seeds after 35days exposure and the number of damaged grains (grains with characteristic holes) and undamaged grains were counted and weighed. Seed infestation (damage) and weight loss were computed by using the following formulae:

$$\text{Seed damage (\%)} = \frac{Nb*100}{Tn} \quad \text{------------------ [27]}$$

Where, N_b = Number of bored seeds, T_n = Total number of seeds

$$\text{Weight loss (\%)} = \frac{UNd - DNu}{U(Nd + Nu)} *100 \quad \text{--------------- [28]}$$

Where, U = Weight of undamaged seeds, D = Weight of damaged seeds,

Nu = Number of undamaged seeds, Nd = Number of damaged seeds.

2.5.3 Adult mortality

The effect of *Trichilia dregeana* seed oil and *Jatropha curcas* seed oil on adult insects was evaluated by applying 0.5ml and 1.0ml of each oil and combination oils to 100g of grains in plastic bag. Eight hours after applying the plant oils, five pair adult *Sitophilus zeamais* from the stock culture were introduced into each treatment. The control experiment had no plant oil added and the experiment was replicated three times. Mortality was scored after twenty four hours and up to 35days after treatment and insects that did not move when touched was considered dead.

2.5.4 Germination Test (Seeds' Viability)

The effect of crude oils of the two seed on the germination power of the maize grains was assessed using the methodology described by Uke *et al.*, 2011[29] . Groups of 20 seeds were randomly selected from each of the concentrations and placed separately in petri dishes containing permanently moistened filter paper on the bottom. The experiment was kept at room temperature for 9 days. Subsequently, the percentage of the germination was determined in comparison with the control and the number of germinated seedlings from each petri dish was counted and recorded from 10^{th} to 14^{th} days after start. The percent germination was computed as follows:

Germination (%) = No. of maize seeds germinated *100 / Total maize seeds sampled

3. DATA ANALYSIS

The statistical tools that were used in this study are the following: the Arithmetic Mean to get the average number of dead maize weevils, one way Analysis of Variance (ANOVA) of SPSS version 20, to determine the significant difference on the mortality of weevils between the control and the experimental groups, Post-Hoc Analysis using the Tukey test to determine the degree of variability between the control and different concentrations of seed oils.

4. RESULTS

The Fatty acid composition of *Trichilia dregeana* seeds oil and *Jatropha curcas* seed oils were analyzed by GC-MS. The major long chain fatty acids present in the *Jatropha curcas* seed oil collected are palmitoleic acid (1.104%), palmitic acid (14.78%), linoleic acid (40.051%), Oleic acid (37.285%) and Methyl-18-Methylnondecanoate (6.54%). The study shows that fatty acids composition of the *Jatropha curcas* oil is rich in oleic and linoleic acids and the oil can be classified as unsaturated oil. This is in agreement with different scholar results in the literatures[31].

Trichilia dregeana seed oil contains palmitic acid (52.36%), oleic acid (36.7%) and linoleic acid (7.59%), stearic acid (1.99%) and cis-vaccenic acid (1.36%). While the fatty acid profile of *Trichilia dregeana* seed oil reported by different scholars are: plamitic acid (43-53.0%), oleic acid (51.0%), linoleic acid (16.0), linolenic acid (16.0%), stearic acid (3.0%)[32].The differences in individual contents of fatty acids when compared to the bibliographic references may be due to the cultivars used, extraction techniques, oilseeds ripening time, and to the cultivation or environmental factors [33].

4.1 Toxicity of seed oils

The results of an experiment conducted to determine the comparative effectiveness of seed oils and Malathion dust powder against *Sitophilus zeamais* with respect to percentage adult mortality, maize grain weight loss, number of perforated maize grain seeds and percentage germination are presented as follows.

4.2 Adult mortality (%)

The impact of seed oils against *Sitophilus zeamais* in terms of adult mortality in maize grains was significant (P< 0.05). After the first 48 h (two days) exposure of *Jatropha curcas* seed oil treatments showed super result in *Sitophilus zeamais* adult mortality and no mortality in *Trichilia dregeana* seed oil treatment. Standard check chemical, Malathion dust powder (1gm), *Jatropha curcas* seed oil and combination seed oils gave 100% adult mortality over time. Minimum mortality was registered from the untreated control and *Trichilia dregeana* seed oil (12.5%). The *Jatropha curcas* and combination seed oils had potent biocidal effects on *Sitophilus zeamais* with more or less the same efficacy with Malathion super dust inducing more than 100% cumulative toxicity to the test insect over 20 days exposure time. The efficacy of the treatments is in the order of Malathion dust powder, *Jatropha curcas* and combination seed oils > *Trichilia dregeana* seed oil for 35days exposure.

4.3 Maize grain damage by *Sitophilus zeamais*

Grain damage by *Sitophilus zeamais* was assessed in terms of counting perforated holes and percent weight loss caused by adult moth and larvae feeding inside the seeds on 35th days of adult moth introduction to experimental bags.

The treatments were significantly different (P<0.05) with respect to the number of perforated seeds, percent weight loss and grain viability (Table 1). Mean numbers of perforated seeds from untreated check were maximum (62 out of 200 seeds) and minimum in plastic bag received *Jatropha carcus* seed oil, combined seed oil (*Jatropha curcas* + *Trichilia dregeana* seed oils) and Malathion dust powder (1out of 200 seeds). The weevil perforation index which indicate the ability of the *Jatropha curcas* seed oil and *Trichilia dregeana* seed oil in protecting the maize seeds ranged in values from 1.59 to 18.42 compared to the control (62) values of this index above 50 indicate negative protectant ability. The effectiveness of the two oils and Malathion dust as protectant against damage by *Sitophilus zeamais* represented as percent protectant ability showed that *Trichilia dregeana* seed oil, *Jatropha curcas* seed oil and Malathion dust concentrations of 0.5ml and 1.0ml resulted in 80.52- 81.5%, 96.87-98.41% and 98.41% ability respectively. The percentages of damaged seed on *Trichilia dregeana* seed oil treated seeds was significantly lower than in the untreated control. There was no damage on *Jatropha carcus* and combination seed oils. Percentage grains weight loss was highest from untreated checks (8.6%) followed by grains treated with *Trichilia dregeana* seed oil 4.2% weight loss. No grain weight loss was recorded from the *Jatropha curcas* and combined seed oils on par with the standard check (Malathion dust powder).

Table 1. Maize grain holes, weevil perforation index and % protecting ability (after 35days exposure).

Treatments	Number of hole/200 seeds	Weevil perforation index (WPI)	Percent Protectant Ability
Control	62	-	-
Trichilia dregeana seed oil	14	19.48	80.52
Jatropha curcas seed oil	1	1.59	96.87
Jatropha + *Trichilia* seed oils	1	1.59	98.41
Malathion dust	1	1.59	98.41

Weevil Perforation Index (WPI) above 50 is an indication of negative protectant ability

Table 2. Effect of seed oils on germination of maize that were previously protected for 90 days.

Treatment	Seed germination (%)	
	0.5ml/100gm	1ml/100g
Malathion dust powder	76.65	73.33
Trichilia dregeana seed oil	80	81.65
Jatropha curcas seed oil	53.35	63.3
Combined seed oils	61.65	43.3

4.4 Viability assessment

The effect of seed oils on the viability (germination percentage) of maize seeds revealed that there was significant difference (P<0.05) among treatments. Significantly minimum germination percentage of 63% was recorded from the *Jatropha curcas* seed oil treated seeds. On the other hand, the highest germination percentage was observed from *Trichilia dregeana* seed oil 81% germination statically higher than Malathion dust powder (76%).

5. DISCUSSION & CONCLUSIONS

Results revealed that the major components of *Jatropha curcas* seed oil contain mainly palmitic acid (14.78%), linoleic acid (40.051%), Oleic acid (37.285%) and *Trichilia dregeana* seed oil contains (palmitic acid (52.36%), oleic acid (36.7%) and linoleic acid (7.59%), stearic acid (1.99%).

Oleic acid has insecticidal effective which enhances the efficacy of the microbial insecticides such as *Bacillus thuringiensis* Berliner. It also appeared that effective fatty acid that pesticide effectiveness against *Zabrotes subfasciatus* (Boheman) that stored beans[34]. Oleic acid and linoleic acid were reported with insecticide effect against fourth instar *Aedes aegyptii* larvae and exhibited potent feeding deterrent activity against larvae of *Helicoverpa zea*, *Lymantria dispar*, *Orgyia leucostigma* and *Malacosoma disstria*[35-36]. It is also appeared that effective fatty acid has insecticidal effectiveness against *Zabrotes subfasciatus* (Boheman) in contamination stored beans [34]. In recent years, interest in screening plants for novel insecticides has increased significantly .Searching for plant-derived that have potential use as crop protectants (insecticides, antifeedants, growth inhibitors) often begins with the screening of plant extracts.

The effects of two seed oils against *Sitophilus zeamais* infesting maize grains in storage were evaluated and the result reveals that there is a significant effect of the two seed oils on *Sitophilus zeamais* mortality, percentage damage and germination. The cumulative adult mortality was significantly higher over time from the *Jatropha curcas* and combined seed oils (100% mortality) on par with the standard check, Malathion dust powder application. The insecticidal effect of seed oils on *Sitophilus zeamais* in the treated maize grains might be as a result of contact toxicity. The oil extract might have blocked the spiracles of the insects, thereby leading to suffocation and death of the insect[37]. According to Copping and Menn (2000)[38] application of oils occluded seed funnels leading to the death of developing insects due to lack of oxygen. This is similar to the work of Ramzan (1994) [39] who reported that groundnut oil, soybean oil cotton seed, sunflower and mustard oils when mixed with cowpea, completely suppressed adult emergence of *C. maculatus*. Ajayi and Adedire (2003)[40] also reported that sandbox seed oil could be used as protectants on *C. maculatus*.

Shaaya *et al*, (1991)[41] reported that edible oils are potential control agents against *C. maculatus* and can play an important role in stored-grain protection. Abd El-Aziz (2001) [42] mentioned that clove and eucalyptus oil vapors impaired the fecundity of the bruchid beetles, *Callosobruchus maculatus*. Data proved promising oviposition detergence, toxicity and suppressing egg deposition and adult emergence [43-45]. Araya and Emana (2009) [46], found that more than 90% mortality of adult *Z. subfasciatus* was also observed for bean seeds treated with *Jatropha curcas*, *D. stramonium* and ***B.****dodecondra* 96 hour after treatment at the rate of 15g/ 150g of grain application. The same results were obtained by different scholars[47-48].

In conclusion, the current findings demonstrate that *Jatropha curcas* and *Trichilia dregeana* seed oils tested against *Sitophilus zeamais* had an insecticidal activity. The *Jatropha curcas* and combination seed oils are potent bio-insecticides against S*itophilus zeamaisa* similar to the standard synthetic insecticide Malathion dust powder. It was found that there was an increasing in seedling growth (length cm and root) treated with *Trichilia dregeana* seed oil over *Jatropha curcas* seed oil. This may be due to the variation of composition of oil of seed oil. Thus, the order of toxicity of seed oils with various treatments on maize weevil were: *Jatropha curcas* seed oil > combination of *Trichilia dregeana* + *Jatropha curcas* seed oils > *Trichilia dregeana* seed oil.

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