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Bioinsecticidal Activities of *Azadirachta indica* A. Juss (Meliaceae) Seed Oil Extracts on *Sitophilus zeamais* Motschulsky, 1855 (Coleoptera: Curculionidae) on Stored Maize

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Abstract

The preservation of stored grains against weevil infestations is critical for ensuring food security and minimizing post-harvest losses in Nigeria, where insect pests remain a major constraint to effective grain storage. This study evaluates the efficacy of seed extracts of *Azadirachta indica* in controlling *Sitophilus zeamais*. Seeds were extracted using five solvents—methanol, ethanol, acetone, petroleum ether, and n-hexane—and the resulting oils were tested for their effects on insect mortality, oviposition, adult emergence, grain damage, and long-term storage viability. Results indicate that ethanol extract was the most effective, inducing 100% mortality within 96 hours of exposure, followed by n-hexane (88.25%), petroleum ether (85.75%), methanol (82.25%), and acetone (76.15%). Extracts also significantly reduced oviposition and adult emergence, with complete suppression observed in ethanol-, petroleum ether-, and n-hexane-treated grains. These treatments also fully prevented grain damage and weight loss for three months, while methanol and acetone extracts

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allowed limited damage (9.19% and 8.18%, respectively). Germination tests showed high viability (95.25–98.25%) across all treatments, with the lowest germination recorded in petroleum ether extract (95.25%). The findings suggest that *A. indica* seed extracts are highly effective in managing *S. zeamais* infestations and represent a viable alternative to synthetic insecticides for safe and sustainable grain storage.

Keywords: *Azadirachta indica*; *Sitophilus zeamais*; Oviposition; Adult Emergence; Grain Germinability; Insect Mortality; Storage Viability

INTRODUCTION

Maize (Zea mays) is one of the major cereal grains cultivated in abundance during the raining season in West Africa most especially in Nigeria. It was first domesticated by indigenous people in southern Mexico about 10,000 years ago (Alonso and Avila, 2011).). It ranks 3rd after wheat and rice, owing to the large areas and total production output of the crop (Adiaha, 2017). The crop was introduced into Nigeria probably by the Portuguese (Olaniyan, 2015). In Nigeria, maize has many local names which include 'agbado' (Yoruba), 'ibokpot' (Efik) and 'masar' (Hausa). Annual maize production on about 5.6 million hectares out of 9 million hectares in Africa has been reported by Panchal, *et al.* (2011).

Maize is extensively cultivated in United States to the tune of about 50% production (Adiaha, 2017). It ranked fourth most edible grain after sorghum, millet and rice (FAO, 2019). The crop accounted for 19.5 % calorie being the world's highest supplier of calorie for body growth, followed by rice (16.5%) and wheat which accounted for 15.0% (FAO, 2019). Maize has become a staple food in many parts of the world and it is used for corn ethanol, animal feed and other maize products, such as corn starch and corn syrup (Listman, *et al.,* 2019). Sugar-rich varieties called sweet corn are usually grown for human consumption as kernels, while field corn varieties are used for animal feeds. Maize is a source of various corn-based human foods and it is used in producing chemical such as ethanol and other biofuels (Listman, *et al*., 2019).

The maize weevil (*Sitophilus zeamais*), is a species of beetle in the family Curculionidae found in numerous tropical areas around the world and it is a major pest of maize (Ojo and Omoloye, 2012). It is one of the most destructive stored product pests of grains, cereals and other processed and unprocessed stored products in sub-Saharan Africa

(Ojo and Omoloye, 2012). *S. zeamais* cause qualitative and quantitative damage to stored products and weight loss of between 20 to 90% to untreated stored maize, and the severity of damage depends on factors which include storage structures and physical and chemical properties of the produce (Muzemu, *et al.*, 2013). The maize weevil is a field-to-store pest of maize grains in the world (Adedire, 2001). Post-harvest losses to *S. zeamais* have been acknowledged as an increasingly important problem to food security in Africa (Tefera, *et al.*, 2011a). Generally, postharvest losses in maize grains due to maize weevil range between 20 and 30% weight losses during storage for three months on the farm in Kenya (Boxall, 2002).

Heavy infestation of adults and larvae of maize weevil which cause postharvest losses have become increasingly important constraints to storage entomology and food security in the tropics (Nta, 2019). The maize weevil commonly attacks standing crops, in particular, maize before harvest, and it is also commonly associated with rice and raw or processed cereals such as wheat, oats, barley, sorghum, rye and buckwheat (Muzemu, *et al.*, 2013).

Presently, the common management strategy used to combat weevil infestation is the use of synthetic chemical insecticides. Synthetic chemical insecticides have been used for many years to curtail the menace caused by *S. zeamais* and other stored product pests especially under large scale production (Gbaye and Holloway, 2011). However, the effectiveness of these insecticides are limited, due to the high cost of procurement of the chemicals, toxic residue effects in foods, the development of resistance to the chemical insecticides by the pest and the destruction of natural enemies and non-targeted organisms (Oni and Ileke, 2008). As alternative strategies for alleviating the problems associated with the use of synthetic chemical insecticides, the efficacies of plant materials, such as plant powders, plant extracts and plant oils to ascertain their insecticidal properties are presently under investigation (Ileke , *et al.*, 2016). This is because findings have shown that the use of botanicals have little or no effects as compared to synthetic chemical insecticides.

There exist array of plant species with insecticidal properties. About 2500 plant species from 235 families that posse pesticide properties (Ngegba *et al.*, 2022). Plants contain different chemicals with various properties, which can be used in insect pest management. Plant chemicals directly kill insect pests (Ayvaz *et al.*, 2010) or anti-feedant (Koul 2008), and fumigation effects (Trivedi *et al.*, 2017 and Mwamburi, 2022) on insects.

Essential oil derived from different parts of plant species have been utilized to tests against various arthropods, including insect pests (Lengai *et al.*, 2019, Ahmed *et al.*, 2022). Coleopterans were the most studied against essential oils of plant followed by Lepidoptera (Campolo *et al.*, 2018). These plant products have been suggested as suitable alternatives for controlling stored product pests including insecticide resistance ones worldwide because of their potential safety for the environment and human health (Regnault-Roger *et al.*, 2012). Plant essential oil also reduces effects on non-target arthropods (Tembo *et al.,* 2018) and has low mammalian toxicity (Mwamburi, 2022).

The present work investigated the efficacy of neem seeds (*Azadirachta indica*) plant for the management of maize weevils. *Azadirachta indica* A. juss, in the mahogany family Meliaceae, is a tree with attractive breed-leaves ever greens that can grow up to 30m in height and 2.5m in girth (COED, 2013). Various neem extracts are known to act on various insects in different ways including disrupting or inhibiting the development of eggs, larvae or pupae, blocking the moulting of larvae or nymphs, disrupting mating and sexual communication, sterilizing adults and deterring feeding as well as inhibiting the production of chitin and the extracts have proven to be as potent as synthetic pesticides (Srivastata et al., 2013).

MATERIALS AND METHODS

Study location and rearing *Sitophilus zeamais*

Adult *S. zeamais* used in this research work were reared in the laboratory of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti under ambient laboratory conditions (27 ± 5°C, 70 ± 5% relative humidity) and a photoperiod of 13:11 (light:darkness) hours. The parent stock of *S. zeamais* was originally obtained from naturally infested maize grains from a raw food store at Oba market, Ado-Ekiti, Nigeria. The maize grains were disinfested by storing in the refrigerator -1°C (30° F) for 48 hours.

New generations of the weevils were subsequently reared on cleaned disinfested maize grains in the laboratory.

Preparation of *Azadirachta indica* seed oil extracts

Mature *A. indica* fruits were collected from the tree in Ekiti State University, Ado Ekiti, Nigeria. The fruits were air-dried for 15 days on the laboratory tables during which

they turn brown. The dry fruits were gently rubbed against a coarse surface to remove the pulp and exposed the seeds. The seeds were rinsed in clean water to remove any remaining pulp, and allowed to dry completely. The dried seeds were stored in a cool, dry place in a cotton bag to allow air circulation. The clean dried seeds were ground into fine powder using an electric Binatone blender (Model: BLG400). The solvents used for the extraction were: Methanol, ethanol, acetone, pet-ether, n-hexane. Afterward, 50 g of ground seeds were measured separately into beakers and packed in thimbles using muslin cloth and extracted with 150 mL of solvents in soxhlet apparatus. In each case, the extraction was carried out between 40–60°C. Excess solvent was recovered using the rotary evaporator vacuum. The oil extracts were then labeled and stored as stock solution until needed.

Effects of *A. indica* seed oil extracts on mortality of adult *S. zeamais*

The experiment was conducted following the procedure of Adedire *et al.* (2011). An aliquot of 0.2 mL of ethanol oil extract of *A. indica* seeds was mixed with 20 g clean and pristine maize grains in a Petri dish (9 cm diameter) and were air-dried for 1 h. Afterwards, 20 adult *S. zeamais* (0–7 day-old) were introduced into each Petri-dish containing the treated grains. The control experiment contains untreated maize grain set up in similar manner as explained above. Similar preparation was set up for *A. indica* seeds extracted with methanol, acetone, pet-ether and n-hexane. The treatments were arranged in a completely randomized design (CRD) and were replicated four times. Weevil mortality was observed at 24 hour interval for 96 h after which the number of dead beetles were counted and recorded. The weevils were confirmed dead when there was no response to the probing of the abdomen with sharp pins.

Effects of *A. indica* seed oil extracts on oviposition by *S. zeamais*

The experiment was conducted by adopting the procedure of (Adedire *et al.*, 2011). Twenty grams (20 g) of clean and pristine maize grains were placed in each Petri dish and 0.2 mL *A. indica* seed ethanol extracts was added. The maize grains and the extracts were thoroughly mixed with a glass rod and air-dried for 1 h. Afterwards, copulating pair of newly emerged (0–24 h-old) adult *S. zeamais* were introduced into the Petri-dishes and covered with Petri plates. Similar preparation was set up for *A. indica* seeds extracted with methanol, acetone, pet-ether and n-hexane. A control experiment containing untreated grains was also set up. The treatments were arranged in a completely randomized design (CRD) and were replicated four times. The treatments were left for 7 days in the

laboratory. After seven days, the total number of eggs laid was counted and recorded. This was done by identifying the egg plugs of *S. zeamais* after staining with acid fuchsin dye solution (Frankenfeld, 1948).

Assessment of grain damage after treatment with *A. indica* oil extracts

The experiment was conducted following the procedure of Adedire *et al.* (2011). Fifty grams (50 g) of clean and wholesome maize grains were counted into transparent plastic container (10 cm diameter and 12 cm depth) and admixed with 0.5mL ethanol oil extracts. Similar preparation was set up for *A. indica* seeds extracted with methanol, acetone, pet-ether and n-hexane. A control experiment containing untreated grains was also set up. Thereafter, 10 copulating pairs of adult *S. zeamais* were introduced into each of the treatments (ethanol, methanol, acetone, pet-ether and n-hexane). The maize grains in the control set up were also infested as above. The plastic containers were covered with muslin screen held in place with rubber bands so as to prevent the exit of the insects and entrance of other insects and parasitoids. All treatments were arranged in a completely randomized design and replicated four times, and then stored in a wooden cage in the laboratory for 90 days. After 90 days, the extent of damage was observed, counted and recorded using the procedure of Fatope *et al.* (1995).

Effects of *A. indica* oil extracts on germination of maize grains

Twenty grams (20 g) of disinfested maize grains were weighed into transparent plastic containers treated with 0.2 mL of each of the different extracts and allowed to air dry. Each solvent extract were replicated four times. The control comprised four replicate samples of untreated maize. The seeds were treated with Apron plus to prevent fungal growth The treatments were covered and left in the laboratory for 90 days. After 90 days ten grains were randomly selected from each treatment and grown on a moistened filter paper in 9 cm diameter Petri-dishes. Maize grains germination was thereafter determined and expressed as percentage of total grains planted (Adedire *et al.* 2011).

Data analysis

Data collected were subjected to analysis of variance (ANOVA), and means were separated using the new Duncan's Multiple Range Test (DMRT)

RESULTS

Effects of *A. indica* seed oil extracts on mortality of *S. zeamais*

Mortality of *S. zeamais* in grains treated with different solvent extract of *A. indica* seed oil extracts was significantly ($p \geq 0.05$) higher than mortality in untreated grains (Table 1). Adult weevil mortality increased with increased in time of exposure to the extracts. The highest weevil mortality of 100% was recorded in maize grains treated with ethanol extract by 96 h post treatment, and it was significantly different from weevil mortality of 82.25 %, 76.15%, 85.75.25 %, and 88.25 % in grains treated with methanol extract, acetone extract, pet-ether and n-hexane extract of *A. indica* seed respectively. Acetone extract recorded the least mortality of 76.15 % by 96 hours of exposure.

Effects of *A. indica* seed oil extracts on oviposition and adult emergence by *S. zeamais*

The different solvent of *A. indica* seed extracts tested in this study caused reduction in the number of eggs laid by *S. zeamais* (Table 2). Oviposition by *S. zeamais* was significantly lower ($p \geq 5$ 0.05) in extract-treated grains than in untreated grains. There was no significant difference ($p \geq 0.05$) in number of eggs laid on methanol and pet-ether of *A. indica* seed extracts. There was no adult emergence in maize grains treated with ethanol and n-hexane extracts, while 51.45% adult emergence was observed in untreated maize grains. Oviposition was suppressed in maize grains treated with methanol extract (10.75 %) and acetone extract (13.25 %).

Protection of maize grains with *A. indica* seed oil extracts

A. indica seed extracts of ethanol, n-hexane pet-ether completely prevented grain damage for a period of 3 months (Table 3). There were neither weight loss nor grain damages recorded in the maize grains protected with these 3 aforementioned *A. indica* extracts. There was a high reduction in the weight of the untreated grains compared with the treated grains. The methanol and acetone extracts also significantly reduced infestation.

Effects of *A. indica* seed oil extracts on germination of maize grains

The percentage of maize grains that germinated after treatment with 0.2 mL dosage of *A. indica* seed extracts are presented in Table 5. After 7 days of germination, all the treated grains recorded high germinability of between 95.25 % to 98.25 %. The untreated (control) maize grains had the highest percentage germination of 100%. The least

percentage germination was recorded in pet-ether (95.25 %) extract. However, this value was not significantly different from the percentage germination observed in grains treated with extracts of acetone (95.50 %) and n-hexane (96.75%), respectively.

Table1. **Effect of 0.2 mL of different solvent oil extracts of *A. indica* seeds on the mortality of adult *Sitophilus zeamais.***

	% mortality at hours after Treatment			
A. indica extract	24	48	72	96
Ethanol	23.25 ± 1.61^{a}	52.25 ± 2.15^{b}	85.50 ± 3.73^{a}	100.00 ± 0.00^{a}
Methanol	18.50 ± 1.12^{c}	43.18 ± 1.23^{c}	67.25 ± 2.16^{c}	82.25 ± 3.15^{d}
Acetone	17.25 ±1.13^{c}	38.25 ± 2.18^{d}	62.75 ± 2.66^{b}	76.15 ± 2.71^{e}
Pet-ether	19.15 ± 1.17^{c}	41.25 ± 1.78^{c}	67.25 ± 1.92^{c}	85.75 ± 4.12^{c}
N-hexane	21.50 ± 1.44^{bc}	48.50 ± 2.32^{a}	73.25 ± 3.17^{b}	88.25 ± 3.51^{b}
Control	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{f}

Each value is a mean + standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different.

Table 2. **Effect of 0.2 mL of different solvent oil extracts of *A. indica* seed on the oviposition and adult emergence of *Sitophilus zeamais*.**

***A. indica* extract**	**Mean Number of eggs laid**	**Percentage adult emergence**
Ethanol	8.30 ± 0.49^{d}	0.00 ± 0.00^{d}
Methanol	11.25 ± 0.74^{cd}	10.75 ± 0.51^{c}
Acetone	15. 67 ± 1.12^{b}	13.25 ± 0.77^{b}
Pet-ether	10.25 ± 0.63^{cd}	0.00 ± 0.00^{d}
N-hexane	9.50 ± 0.67^{d}	0.00 ± 0.00^{d}
Control	34.25 ± 1.44^{a}	51.45 ± 2.13^{a}

Each value is a mean + standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different.

Table 3. **Effect of 0.2 mL of different solvent oil extracts of *A. indica* on long term storage of maize grains.**

***A. indica* extract**	**Mean number of grans**	**Mean number of damage grains**	**Mean %grains Damage**	**Mean %weight Loss**
Ethanol	181.25	0.00 ± 0.00	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Methanol	179.50	16.50 ± 1.57	9.19 ± 0.46^{b}	6.75 ± 0.21^{b}
Acetone	180.25	14.75 ± 0.78	8.18 ± 0.55^{b}	6.50 ± 0.34^{b}
Pet-ether	178.00	0.00 ±0.00	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
N-hexane	182.50	0.00 ±0.00	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Control	178.50	144.25 ± 4.37	80.81 ± 2. 33^{a}	27.75 ± 3.44^{a}

Each value is a mean + standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different.

Table 4. **Germination of maize grains after treatment with 0.2 mL *A. indica* oil extracts and preserved for 90 days.**

***A. indica* extract**	**Germination percentage**
Ethanol	98.25 ± 2.55^{b}
Methanol	97.20 ± 2. 87bc
Acetone	95.50 ± 1. 71^{c}
Pet-ether	95.25 ± 1.84^{c}
N-hexane	96.75 ± 3.17^{c}
Control	100.00 ± 0.00^{a}

Each value is a mean + standard error of four replicates. Means within the same column followed by the same letter(s) are not significantly different.

DISCUSSION

At present, food insecurity is a major challenge faced by developing countries of the world. All people at all times should have physical, social, and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for active and healthy life. Food availability has been hampered by the activities of stored product pests in the world as a whole.

In order to eradicate the use of synthetic chemical insecticides for the management of pests, as a result of their adverse effects, the use of botanicals in various forms is gradually taking over (Adesina, 2013). The bioassay investigation in the present study revealed that all the extracts used in this study showed promising pesticidal activity against *S. zeamais* infesting stored maize grains. This is in agreement with the study by Okolo and Iledun (2019), which tested the pesticidal activity of *Azadirachta indica* extracts against several cowpea pests, including Aphids.

The ethanol extract was the most effective of all the extracts used as it caused 100% mortality of *S. zeamais* when exposed to 0.2 mL of extract by 96 h post-treatment. The insecticidal effect of the extracts on *S. zeamais* in the treated maize grains might be as a result of contact toxicity whereby the extracts that were admixed with the grains might have blocked the spiracles of the insects thereby leading to suffocation and death of the insect (Akinkurolere *et al.*, 2006; Adedire *et al.*, 2011). This is because the openings or air chambers in the abdomen might have been hindered from receiving enough oxygen into the body of the insects, preventing tissue respiration and energy production which eventually lead to death as suggested by Adedire *et al.* (2011).

The *A. indica* seed extracts used in the present research were capable of preventing oviposition and adult emergence. The ability of some plant extracts to reduce or prevent oviposition by female Coleopteran pests and mortality of the developmental stages have been studied by a number of authors and well documented (Adedire *et al.*, 2011; Ojo and Ogunleye, 2011). The effects of the extracts on oviposition of insects could be due to metabolic alteration and consequently other systems of the body of the insects (Ileke, 2014 and Obembe *et al.*, 2023). The reduction in adult emergence observed in the treated grains could be due to high insect mortality rate caused by the extracts toxicity (Obembe *et al.*, 2023). Adult emergence is an important aspect to consider in evaluating the long-term efficacy of pest control treatments. This finding showed significant variations in the levels of adult emergence among the different *A. indica* seed extracts treatments. This suggests that *A. indica* extracts may interfere with the reproductive capability and developmental stages of the beetle. Adult emergence is an important aspect to be considered in evaluating the long-term efficacy of pest control treatments. Similar results have been reported by other authors (*Barik et al.*, 2021 and Tripathi *et al.*, 2022) which showed that *A. indica* extracts are effective in the control of *Sitophilus oryzae* infestations in stored wheat.

The presence of some phytochemical compounds in plants are known to play important roles in the bioactivity of these plants. The bioactivity of the plants lies in these phytochemical compounds, such as alkaloid, tannins, saponins flavonoid, resins and glycosides, which produce definite physiological action on the body (Mobolanle *et al.*, 2015). These compounds have been reported to demonstrate biochemical functions against insects and effected insecticidal, repellent, anti-moulting, nematicidal and antimicrobial activities against stored products (Udebuani *et al.*, 2015).

The treatment of maize grains with *A. indica* seed extracts significantly reduced grain damage and weight loss compared to the untreated control. This reduction may be attributed to the presence of a volatile compound that influences the feeding behaviour of the insects (Birhanu and Emana, 2018). Weight loss is a crucial indicator of the extent of damage caused by weevil infestations in stored grains. These findings align with previous studies which reported the insecticidal properties of *Azadirachta indica* extracts against various stored grain pests (Smith *et al.*, 2020; Singh *et al.*, 2021).

The germination bioassay results showed that *A. indica* seed extracts, regardless of the solvent used to treat maize infested with *S. zeamais* had little impact on seed germination. Both the treated and untreated seed germinated at rates ranging from 95.25 % to 100 %. This result corroborates the result obtained by Adedire *et al.*, 2011.

CONCLUSION

The present study demonstrates the efficacy of *A. indica* seed extracts as bio-control agents against the maize weevil infestations in various storage materials. It was confirmed that *A. indica* extracts were highly effective in controlling the population of *S. zeamais* in treated grains. This shows that the extracts could serve as alternatives to synthetic insecticides used by resource-poor farmers who store small quantities of grains for their consumption, sale and planting.

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