

Comparative efficacy of extracts from *Azadirachta indica*, *Piper guineense* and pirimiphos-methyl against *Sitophilus zeamais* Motschulsky (Coleoptera : Curculionidae) in stored maize

Mobolade Dele Akinbuluma and Francis Kolawole Ewete
University of Ibadan, Nigeria.

*E-mail of the corresponding author: delebuluma@yahoo.com

Abstract

Efficacy of ethanol seed extracts of *Azadirachta indica* A. Juss and *Piper guineense* Schum and Thonn at concentrations of 10, 20, 30 and 40 %v/v, each as protectant in the control of the maize weevil, *Sitophilus zeamais*, were compared with a conventional insecticide, pirimiphos-methyl at a rate of 0.5 ml / 50 g maize grains of Oba Super II variety. Experiment was carried out in the Entomology Research Laboratory, University of Ibadan under laboratory conditions of $27 \pm 2^\circ\text{C}$ ambient temperature and $65 \pm 5\%$ relative humidity and was arranged in a Completely Randomised Design in four replicates. Parameters assessed, including adult mortality, rate of adult emergence, grain damage and weight loss and seed viability, were subjected to analysis of variance and means separated using Duncan's Multiple Range Test. Mortality of adult *S. zeamais* increased with increasing concentration of the extracts as well as with days of exposure. All the treatments were better than the control ($P < 0.05$) in reducing the number of emerged adult *S. zeamais* and grain damage. *P. guineense* at 30 and 40 %v/v competed with pirimiphos methyl in the parameters so examined. There were no significant differences ($P > 0.05$) in the mean percentage germination of the maize seeds in all the treatments.

Keywords: *Azadirachta indica*, *Piper guineense*, pirimiphos-methyl, *Sitophilus zeamais*

Introduction

Maize, *Zea mays* (L.), is a very important cereal crop in Africa and serves as a source of dietary carbohydrate for humans (Onwueme and Sinha, 1999) and livestock. It is also utilized in starch, oil, food and feed industries. Its grains, cobs, stalks, leaves, tassels and silks all have commercial and medicinal value in most settings, though that of the grain is the greatest (Timothy *et al.*, 1988; Paliwal, 2000). Losses of food grains, such as maize, due to insect pests are a significant nutritional and economic burden (Firdisa and Abraham, 1998) as insects infest grains, resulting in qualitative and quantitative losses such as reduction of nutritive and market values, promotion of mould development and weight losses of over 30%. The maize weevil, *Sitophilus zeamais* is a serious pest of stored cereal grain, especially maize (Asawalam and Emosairue, 2006). Infestation begins in the field and is carried into the store where population builds up rapidly (Appert 1987; Adedire and Lajide, 2003). Damaged grains have reduced nutritional value, low percentage germination, reduced weight and market value. The control of *S. zeamais* is mainly centred on the use of synthetic insecticides (Menn, 1983; Redlinger *et al.*, 1988) with attendant problems such as high cost of procurement, unavailability at critical periods and may pose health hazards to man and livestock (Opareke and Dike, 2005). These problems necessitated the search for eco-friendly and cheaper insect pest control alternatives which included the use of powdered plant parts and their extracts (Ewete *et al.*, 1996; Adedire and Akinneye, 2004; Akinkurolere *et al.*, 2006). Powdered fruits of pepper and powdered neem seeds are examples of such plant products (Bekele *et al.*, 1997). They have low mammalian toxicity, cause less hazards to non-target organisms, are less expensive and are relatively specific in their mode of action and easy to process and use (Rajakakse and van Emdem, 1997). The objective of this study is to compare efficacy of extracts from the seeds of *A. indica*, *P. guineense* with Pirimiphos-methyl in the control of *Sitophilus zeamais* in stored maize.

Materials and Methods

Study Location

All experiments were conducted at the Entomology Research Laboratory, Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria under ambient temperature of $27 \pm 2^\circ\text{C}$ ambient temperature and $65 \pm 5\%$ relative humidity

Sitophilus zeamais culture

Initial culture of adult *S. zeamais* was raised from infested maize bought from Bodija market, Ibadan. Fifty pairs of adult *S. zeamais*, sexed following the reports of Odeyemi and Daramola (2000), were introduced into 1 litre Kilner jar containing 400 g Oba Super 2 maize grains. The jar was covered with wire mesh lid to allow for

aeration and replicated 4 times for ready availability of insects throughout the experiment. Jars were placed on a table whose stands were dipped in plastic bowls containing oil to prevent ants from contaminating the culture.

Source of maize grains

Maize grains (Oba Super II variety) which were bought from the Practical Year Training Programme (PYTP), University of Ibadan, Ibadan were used in this study. Prior to experiments, the grains were disinfested in a deep freezer for one week and later air-dried in the laboratory to prevent mouldiness. They were later cleaned and kept for one week under experimental conditions for acclimitization

Insecticidal Material collections and preparation

Insecticidal materials used were *Azadirachta indica* A. Juss, *Piper guineense* Schum and Thonn and a synthetic insecticide (pirimiphos-methyl). *Piper guineense* seeds were purchased from Ojoo market, Ibadan and ripe seeds of *A. indica* were picked under the neem trees along the faculty of Arts, University of Ibadan. Five hundred grammes (500 g) of each plant part was air-dried, ground and kept in separate plastic containers till the time for Soxhlet extraction. Pirimiphos - methyl was bought from Collins Group Limited, Mokola, Ibadan.

Extraction of oils from plant materials

Two hundred grammes (200 g) each of ground seed samples of *A. indica* and *P. guineense* was transferred into the soxhlet extractor using 95 % ethanol as the solvent. The oil extracts were further concentrated in a rotary evaporator under reduced pressure until a semi-liquid oil extract was obtained. Crude extracts of *A. indica* and *P. guineense* were later diluted with 95 % ethanol to obtain ten different concentrations of 5, 10, 15, 20, . . . , 50 % v/v with a control (0 %v/v) containing only ethanol.

Evaluation of extracts for contact toxicity for determination of LC₅₀

Twelve day-old *Sitophilus zeamais* were placed per petri-dish and were individually picked and treated by applying 1-2µl drops of each concentration of both extracts on their ventral sides from a micro syringe. The contents of the petri-dishes were provided with maize grains and insects were observed at 24 hours. Insects that do not respond to probing with a seeker were considered dead. The concentrations were converted into logarithmic values while mortality values were converted to probits. Probit values were plotted against logarithmic values and a regression line (of best fit) was drawn. The logarithmic dose at the median point when changed to antilogarithm was taken as the effective LC₅₀ for each extract (Finney, 1971)

Effects of plant extracts and pirimiphos-methyl on the mortality of *S. zeamais*

Fifty grammes (50 g) of maize grains was weighed into kilner jars. Using a micro-syringe, 0.5ml of the concentrations (10, 20, 30 and 40 % v/v) of each of the extracts and pirimiphos – methyl was applied to the grains and shaken to allow for coverage. Grains in the control jar were treated with ethanol only. Consequently, there were ten treatments in four replications. The grains were infested with ten (1 male: 1 female) adult *Sitophilus zeamais* per jar and jars covered with a lid of fine mesh to allow for aeration. Mortality was recorded at 24, 48, 72 and 96 hours after infestation, with insects considered dead if they did not move when probed with a camel hairbrush. Dead adults were removed at each assessment, counted and recorded. Data on percentage mortality were corrected using Abbott's (1925) formula:

$P_T = P_O - P_C / 100 - P_C$; where P_T = corrected mortality (%), P_O = observed mortality (%), P_C = control mortality (%).

Effects of plant extracts and pirimiphos-methyl on emergence of *S. zeamais*, grain damage and weight loss and seed viability

After 10 days, all adult weevils were removed and the Kilner jars left undisturbed and monitored until the emergence of F₁ progeny. Data on F₁ adult emergence were assessed from the commencement of adult emergence with emerged adults removed, counted and recorded. The grains were later sieved to remove the dust produced from adult feeding and re-weighed by using a Mettler Weighing balance and the percentage loss in weight determined as follows:

$$\text{Percentage (\% weight loss)} = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100$$

The number of grains perforated in each of the treated and control jars were counted and percentage seed damage was determined as:

$$\% \text{ seed damage} = \frac{\text{Number of perforated grains}}{\text{Total number of grains counted}} \times 100$$

In order to assess the viability of seeds, germination test was conducted using twenty (20) seeds from each jar. The seeds were placed on moist filter paper in plastic Petri dishes kept in an incubator at 25°C and the number of germinated seed was counted and recorded and percentage seed viability was calculated as:

$$\% \text{ viability} = \frac{\text{Number of germinated seed}}{\text{Number of seed sown}} \times 100$$

Statistical analysis

All percentage data were angular transformed prior to statistical analysis, in order to equalise variances. All data were analysed and significant differences were compared at 0.05 significant level using Duncan's Multiple Range Test (DMRT) (Zar, 1984).

Results

Contact Toxicity of plant extracts on *Sitophilus zeamais*

Contact effect of ethanol extracts of *Azadirachta indica* and *Piper guineense* on *Sitophilus zeamais* at various concentrations showed an increase in mortality in both extracts from 41.7% to 83.3% in *A. indica* and to 100% in *P. guineense*. The lethal concentration (LC50) of *A. indica* was 20.89 % while that of *P. guineense* was 15.49 % (Figures 1 and 2)

Effects of plant extracts and pirimiphos-methyl on the mortality of *S. zeamais*

Mortality of adult *Sitophilus zeamais* exposed to different rates of extracts of *Azadirachta indica*, *Piper guineense* and the conventional insecticide (pirimiphos - methyl) was compared in Table 1. Adult mortality increased with increase in concentration and with days of exposure to both extracts and in pirimiphos-methyl. There was no significant difference ($p < 0.05$) between *Piper guineense* at 40 %v/v and pirimiphos - methyl at 72 and 96 hours post-treatment. *Piper guineense* at 40 %v/v also caused 100 % mortality to adult *Sitophilus zeamais* even though the synthetic insecticide caused 100% mortality 24 hours earlier. All rates of application of both extracts and pirimiphos-methyl were significantly ($P < 0.05$) different from the control in all the days of the trials (Table1).

Effects of plant extracts and pirimiphos-methyl on emergence of *S. zeamais*, grain damage, weight loss and seed viability

The effect of pirimiphos-methyl and different concentrations of extracts of *A. indica* and *P. guineense* and is shown in Table 2. Mean number of emerged F₁ adults decreased with increasing concentrations of both extracts. Emergence was significantly ($P < 0.05$) reduced

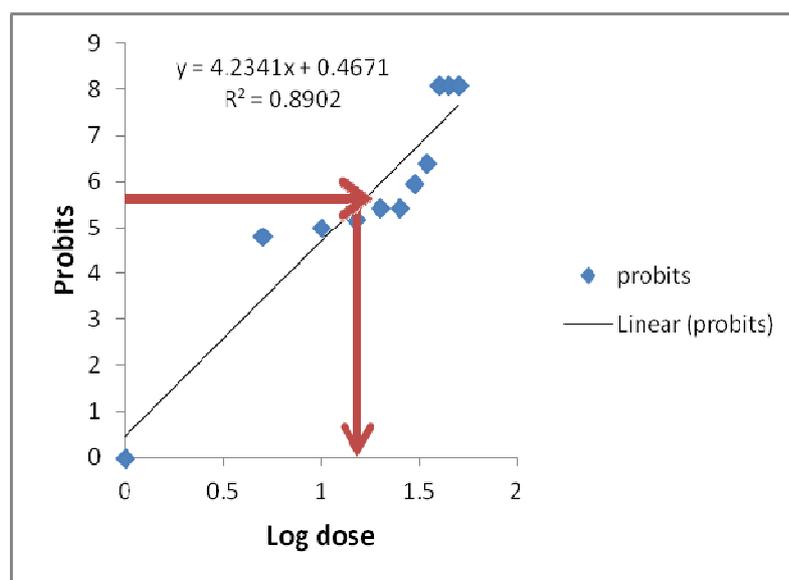
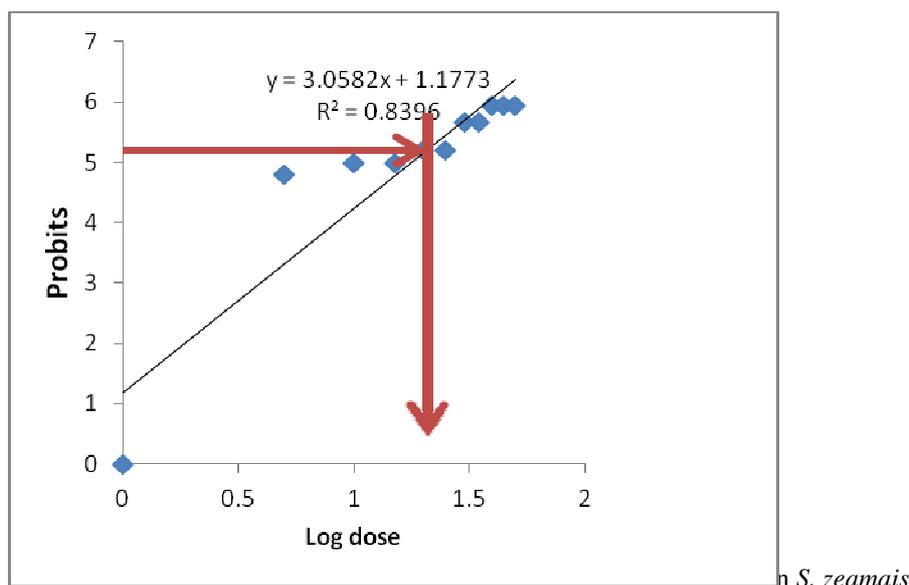


Figure 1: Determination of LC₅₀ of ethanol extract of *P. guineense* seeds on *S. zeamais*

from 52.25 in the control values to (24.25 - 2.00) in *Azadirachta indica* and (10.50 - 1.00) in *Piper guineense* with pirimiphos – methyl having the least number of emerged adults (0.75). However, treatment with pirimiphos – methyl did not significantly ($P > 0.05$) reduce emergence than those with *Piper guineense* at 30 %v/v, 40 %v/v and 40 %v/v at *Azadirachta indica*. All treatments were significantly better ($P < 0.05$) than the control in reducing adult emergence (Table 2). Damaged maize seeds in treatments of various concentrations of the extracts varied from 13.43 to 2.22% in *Azadirachta indica* and 5.97 to 0.75% in *Piper guineense* while 0.72% was recorded in treatment within pirimiphos –methyl. All treatments proved superior to control (33.33%) with pirimiphos –methyl and 40 %v/v at *Piper guineense* outstanding (Table 2).

Table 1: Effects of plant extracts and pirimiphos-methyl on mortality of *S. zeamais*

Insecticidal	Conc	Percentage mortality over 4 days post treatment (± S.E)
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Materials	% v/v	24 hr	48 hr	72 hr	96 hr
<i>Azadirachta indica</i>	10	12.5 ^g ± 2.03	17.5 ^f ± 2.05	20.0 ^{de} ± 3.02	22.5 ^d ± 3.51
	20	15.0 ^{fg} ± 2.34	22.5 ^{ef} ± 1.66	25.0 ^{cd} ± 1.92	30.0 ^d ± 2.59
	30	20.0 ^{ef} ± 0.00	32.5 ^d ± 1.51	37.5 ^c ± 2.83	47.5 ^c ± 2.89
	40	22.5 ^{ef} ± 1.66	37.5 ^d ± 1.51	42.5 ^c ± 2.82	52.5 ^c ± 2.76
<i>Piper guineense</i>	10	20.0 ^{ef} ± 3.02	30.0 ^{de} ± 3.02	40.0 ^c ± 4.14	52.0 ^c ± 4.39
	20	32.5 ^d ± 1.51	37.5 ^d ± 1.51	60.0 ^d ± 2.41	75.0 ^b ± 3.17
	30	52.5 ^c ± 1.44	57.5 ^c ± 1.44	70.0 ^b ± 2.59	87.5 ^{ab} ± 3.40
	40	70.0 ^b ± 0.00	85.0 ^b ± 5.18	95.0 ^a ± 5.32	100.0 ^a ± 0.00
Pirimiphos-methyl	0.5 ml	75.0 ^a ± 1.92	97.5 ^a ± 4.61	100.0 ^a ± 0.00	100.0 ^a ± 0.00
Control (solvent)	0.000	0.00 ^h ± 0.00	0.00 ^g ± 0.00	0.00 ^f ± 0.00	0.00 ^e ± 0.00
LSD _(0.05)		4.96	8.92	8.50	9.32

Means followed by common letters in the same column are not significantly different at 5% level

Table 2: Effects of plant extracts and pirimiphos-methyl on emergence of *S. zeamais* and grain damage

Insecticidal Materials	Concentration (% v/v)	Mean number of emerged adult (± S.E)	Percentage seed damage (± S.E)
<i>Azadirachta indica</i>	10	24.25 ± 0.15 ^b	13.43 ± 0.04 ^b
	20	20.75 ± 0.21 ^c	12.82 ± 0.01 ^c
	30	9.25 ± 0.15 ^e	5.42 ± 0.04 ^e
	40	2.00 ± 0.15 ^g	+2.22 ± 0.03 ^g
<i>Piper guineense</i>	10	10.50 ± 0.15 ^d	5.97 ± 0.02 ^d
	20	5.50 ± 0.15 ^f	3.30 ± 0.03 ^f
	30	1.75 ± 0.15 ^g	1.86 ± 0.02 ^h
	40	1.00 ± 0.15 ^h	0.73 ± 0.01 ⁱ
Pirimiphos –methyl	0.5ml	0.75 ± 0.15 ^h	0.72 ± 0.01 ⁱ
Control (solvent)	0.00	52.25 ± 0.15 ^a	33.33 ± 0.03 ^a
LSD _(0.05)		0.46	0.07

Means followed by common letters in the same column are not significantly different at 5% level

The percentage loss in weight of grains due to damage varied among treatments from 0.03% in pirimiphos -methyl to 6.68% in the control. 40 % v/v of *Piper guineense* and pirimiphos –methyl were significantly ($P < 0.05$) better than the other treatments in reducing weight loss (Table 3). Highest germination percentage (83.75) was observed in seeds treated with 10 %v/v *Azadirachta indica* and the control, followed by 82.50% in *Piper guineense* concentrations at 10, 20 and 30 %v/v. Other treatments gave the least germination of 81.25%. There were no significant differences ($p > 0.05$) in seed germination among the treatments and the control.

Table 3: Effects of plant extracts and pirimiphos-methyl on percentage weight loss and germination of maize seeds

Insecticidal Materials	Conc %v/v	Percentage weight loss (\pm S.E)	Percentage seed germination (\pm S.E)
<i>Azadirachta indica</i>	10	4.16 ^b \pm 0.08	83.75 \pm 3.75
	20	3.28 ^c \pm 0.03	81.25 \pm 1.25
	30	3.23 ^{cd} \pm 0.10	81.25 \pm 2.39
	40	1.63 ^e \pm 0.12	81.25 \pm 2.39
<i>Piper guineense</i>	10	3.35 ^c \pm 0.07	82.50 \pm 6.29
	20	3.03 ^d \pm 0.10	82.50 \pm 1.44
	30	1.44 ^e \pm 0.18	82.50 \pm 1.44
	40	0.06 ^f \pm 0.01	80.00 \pm 4.08
Pirimiphos-methyl	0.5 ml	0.03 ^g \pm 0.01	81.25 \pm 3.15
Control (solvent)	0.000	6.68 ^a \pm 0.01	81.75 \pm 4.79
LSD (0.05)		0.16	ns

Means followed by common letters in the same column are not significantly different at 5% level

Discussion and Conclusion

Oils are used in insect control because they are relatively efficacious against virtually all life stages of insects (Nezan, 1983; Adedire, 2003). Topical application of both extracts caused high mortality to *Sitophilus zeamais* suggesting that oils have contact toxicity on the insects. Pirimiphos – methyl was very effective in controlling adult *S. zeamais* which agrees with Asawalam *et al.*, (2006) who reported 100% mortality to *Sitophilus zeamais* when treated with pirimiphos –methyl in stored maize. *Piper guineense* and *Azadirachta indica* may have been potent because of the strong odours emitted, thereby disrupting normal respiratory activities of the weevils; resulting in asphyxiation and subsequent death (Adedire and Ajayi, 1996). However, their effectiveness was dependent on dosages and exposure period. Highly significant difference on the emergence of adults *S. zeamais* reared on treated and untreated maize indicates that insecticidal materials tested had significant effects on the developmental stages which in turn affected emergence. Arannilewa *et al.*, (2003) reported that the oil extract on application covered the outer layer (testa) of the seeds serving as food poison to the adult insects; while some of them penetrated the endosperm and germ layer thereby suppressing oviposition and larval development. Significantly lower number of emerged F₁ progeny relative to control suggests the presence of some active principles in the plants that had contact toxicity and fumigants action on the weevils (Adedire, 2003). Application of *A. indica* and *P. guineense* extracts with pirimiphos – methyl to grains resulted in significant reduction of percentage weight loss. The result of an earlier study by Okonkwo and Okoye, (1996) showed that percentage weight loss was related to the population of adult *S. zeamais*. Seed viability pre-treated with the extracts showed that the treatments did not negatively affect seed germination. This agrees with the report of Adedire *et al.*, (2005) which gave no significant differences in viability of seeds pre-treated with 0.5% and 2.0% of four plant extract concentrations and the control. Results obtained from this study demonstrates active potentials of these plant products as plant-derived insecticides against maize weevil and provide a scientific rationale for the use of these botanicals as alternative to synthetic insecticides in post harvest protection.

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