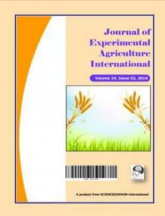

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The Efficacy of *Zingiber officinale* (Ginger-Zingiberaceae) Crude Extracts Applied as Individual and Mixed with *Dennettia tripetala* (Pepperfruit-Annonaceae) against *Musca domestica* (Housefly) Larvae

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Authors' contributions

This work was carried out in collaboration between authors CCO and FIS. Author CCO designed the study, wrote the first draft of the manuscript. Author FIS reviewed the experimental design and draft of the manuscript. Authors CCO and FIS managed the analyses of the study and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the efficacy of *Zingiber officinale* applied individually and mixed with *Dennettia tripetala* in equal proportions; their insecticidal strength was also compared using dipping and feeding techniques.
Methodology: Insects were cultured in cages (30 cmx30 cmx30 cm) built with wood, nets and gauze at the Department of Animal and Environmental Biology Laboratory. Freshly emerged larvae (1 day old) were used for the bioassay. The cages were placed on plastic stands which contained engine oil to prevent infestation of other insects and mites. 20 larvae each, were transferred into petri dishes containing larvae food (2 ml of powdered milk mixed with distilled water) using fine art brush.The plants (*Z. officinale*) rhizome *D. tripetala* fruits were purchased from

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the market. *Z officinale* rhizome was sliced into tiny pieces while *D tripetala* skin was peeled off to obtain the seeds. They were dried at ambient temperature (28±2℃) for four weeks, afterwards they were grounded with an electric blender (Philips) and sieved with 0.1mm mesh size sieve to obtain fine powder which was stored in an airtight container to prevent the active ingredients from evaporating and kept in a cupboard until the time for use. The crude extracts were obtained by homogenizing 10 g of dust (plant sample) with 100 ml (80/20) of solvents (v/v) (hydro-ethanol) and left for 24 hours thereafter, it was filtered with a filter paper and the residue (active compound) was evaporated over a water bath at 40℃. To obtain the final concentration; the extracts were dissolved in a known volume of distilled water; for example, 200, 400, 600, 800 and 1000 ppm were obtained by dissolving 0.2, 0.4, 0.6, 0.8 and 1.0 mg of extract in a ml of distilled water. Mortality and means were monitored every 12 hours for 72 hours. The experiment was replicated twice with distilled water serving as control. The toxicity was also tested using dipping and feeding techniques.

Results: Showed the mortality observed within a period of 72 hours, percent mortality and probit mortality of *Z. officinale* on *M. domestica* larvae using dipping and feeding technique. The result showed that mortality was significant ($P<0.05$) as concentrations increased from 200-1000 ppm but mortality was not significant as duration of exposure increased from 12-72 hours. The LC_{50} and LT_{50} were 0.4898 mg/ml and 40 hours. No mortality was observed when the insects were fed with crude extracts but they (insect larvae) were weakened (their movement was sluggish and they did not pupate. When *Z. officinale* and *D. tripetala* were mixed in equal proportions using dipping and feeding techniques, mortality of the larvae was generally significant ($p<0.05$) as both concentrations and duration of exposure increased. When they were dipped in the mixed crude extract, LC_{50} and LT_{50} of 0.2754 mg/ml and 30 hours were obtained while LC_{50} and LT_{50} of 0.4786 mg/ml and 44 hours were obtained when they were fed with the mixed crude extract.

Conclusion: This result showed that plant extracts used for this study could be promising bioinsecticide especially when mixed in equal proportion and may be considered in integrated pest management of housefly.

Keywords: Z. officinale; D. tripetala; M. domestica; larvae; dipping; feeding.

1. INTRODUCTION

Housefly is one of the most common household insect pests. It feeds on human food thereby transmitting disease to man and livestock since it is a vector of many diseases such as typhoid, cholera, dysentery, salmonella, etc [1,2]. It also transmits the eggs of parasitic worms hence Houseflies are seen as carriers of easily communicable disease. Diseases are found on the legs and hairs covering the body of houseflies. They come in contact with these pathogens when females lay their eggs on decaying organic matter. Houseflies transmit these pathogens to food substance or any surface they come in contact with within seconds. They are able to transmit these diseases because they liquefy solid food before feeding on them as they have sucking mouthparts and people get infested when they feed on it. Their presence in homes (around man) is irritating as they are considered as unhygienic insects [1]. They tend to disturb men and livestock during leisure and work periods [3]. Eliminating this insect pest totally maybe difficult, reducing its population below economic threshold and stopping its contact with human and livestock food and water is essential and a step to make homes and environment hygienic. Chemical insecticides which are usually used in controlling housefly [3] have great adverse effects on the ecosystem at large, as their residues left in the environment are hazardous. Chemical insecticides also make the insect pest become resistant [4,5]. Thus researchers are in continuous search of alternative methods for controlling insect pests. Plants have been used in medicine even before man understood the cause of infectious disease [6] this has led to the use of bio insecticides in controlling insect pest because they are better alternatives to chemical insecticides. Ginger, *Zingeber officinale* Roscoe is a flowering plant in the family Zingiberaceae whose rhizome or simply ginger is widely used as spice. It has gingerols and shogaol as its active constituents amongst other compounds [7] which suffocate insects. *Dennettia tripetala* (pepper fruit) is an indigenous medicinal fruit tree of the family Annonaceae known to have β-phenylnitroethane (a nitro compound) as the compound responsible for its high insecticidal activity [8]. There are many reports on the use of

bioinsecticides in controlling housefly such the work of [9-15] but less research has been done on the comparison of plant extracts applied individually and mixed in equal proportions. Thus, this work will be evaluating the efficacy of *Z. officinale* applied individually and mixed with *D. tripetala* in equal proportions; their insecticidal strength will also be compared using dipping and feeding techniques.

2. MATERIALS AND METHODS

2.1 Insect Collection and Culture

Insects were collected near a garbage site by placing fresh fish in a small plastic plate inside the rearing cages. The fish served as a culture medium for laying eggs and larvae development. The cage (30 cmx30 cmx30x cm) was built with wood, nets and wire gauze. A wooden door was fixed by its side by its side, and the floor was made with plywood. Freshly emerged larvae (1 day old) were used for the bioassay. The cage was placed on plastic stands which contained engine oil to prevent infestation of other insects and mites [3]. The larvae was transferred into petri dishes containing larvae food (2 ml of powdered milk mixed with distilled water) using fine art brush. This procedure was adopted from [3,16,17] with some modifications.

2.2 Preparation of Test Plant Materials

The plants used for this study were purchased from a local market in Ukwuani Local Government area of Delta State and identified as *Z. officinale* and *D. tripetala* by a Botanist in the Department of Botany Delta State University, Abraka. *Z. officinale* rhizome was sliced into tiny pieces while *D. tripetala* skin was peeled off to obtain the seeds which were used for this study. They were dried at ambient temperature (28±20c) for four weeks, afterwards ground with an electric blender (Philips) and sieved with 0.1mm mesh size sieve to obtain fine powder. It was stored in an airtight container to prevent the active ingredients from evaporating and kept in a cupboard until the time for use. This procedure was adopted from [17,18] with slight modifications.

2.3 Preparation of Plant Crude Extract

The crude extracts were obtained by homogenizing 10 g of dust (plant sample) with 100 ml (80/20) of solvents (v/v) (hydro-ethanol) and left for 24 hours thereafter, it was filtered with a filter paper and the residue (active compound) was evaporated over a water bath at 400C. To obtain the final concentration; the extracts were dissolved in a known volume of distilled water; for example: 200, 400, 600, 800 and 1000 ppm was obtained by dissolving 0.2, 0.4, 0.6, 0.8 and 1.0 mg of extract in a ml of distilled water. This method was adopted from [9].

2.4 Larvicidal Bioassay

Mortality of larvae were monitored and recorded every 12 hours for 72 hours. Two replicates of each concentration were made and the mean values were taken as the final result. Each group had 20 larvae and distilled water was used as control experiment. The toxicity of crude extracts was tested by these methods:

2.5 Dipping Technique

The larvae of each group were carefully dipped into various concentrations of crude extracts with a dip net and allowed to stand in it for 40 seconds, and then introduced into petri dish with food (2 ml of powdered milk mixed with distilled water).

2.6 Feeding Technique

Various concentrations of the crude extract were added to larvae food (2 ml of powdered milk mixed with distilled water) in petri dish and the larvae were introduced into the petri dish. This method used was adopted from [9] with a slight modification.

2.7 Statistical Analysis of Data

The results obtained were recorded and statistical analysis was performed using two-way ANOVA (Ms Excel), Descriptive statistics and Probit Analysis.

3. RESULTS

Table 1 shows the mortality observed within a period of 72 hours, percentage mortality and probit mortality of *Z. officinale* on M. domestica larvae using dipping technique. The result shows that mortality was significant ($P<0.05$) as concentrations increased from 200-1000 ppm but mortality was not significant as duration of exposure increased from 12-72 hours. The LC_{50} and LT_{50} were 0.489 8 mg/ml and 40 hours (Figs.

1&2). No mortality was observed when the insects were fed with crude extracts but they (insect larvae) were weakened (their movement was sluggish and did not pupate (Table 2).

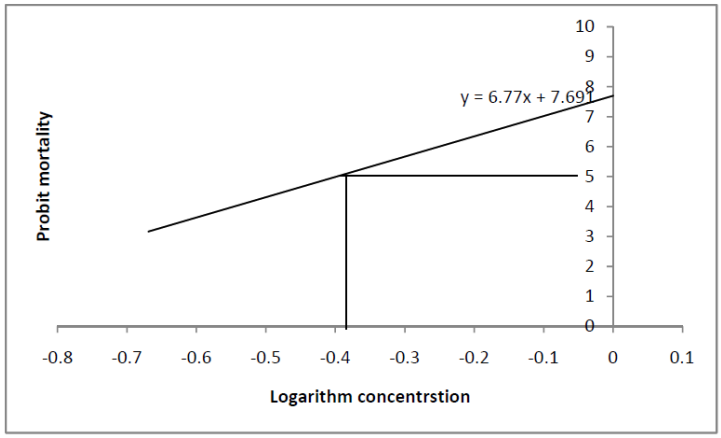

Fig. 1. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were dipped in *Z. officinale*

Table 1. Percent mortality and probit mortality of *M. domestica* larvae exposed to *Z. officinale* for 72 hours by dipping

Conc mg/ml of H_2O	Log Conc	Period of Exposure (hours)						Percent mortality after 72 hrs	Mean mortality after 72 hrs	Probit mortalty
		12	24	36	48	60	72			
0.0 (control)	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	1	2	2	2	2	45	1.50	4.87
0.4	-0.3974	2	2	4	2	1	2	65	2.17	5.39
0.6	-0.2218	2	3	2	3	2	3	75	2.50	5.67
0.8	-0.0969	2	3	2	3	3	5	90	3.00	6.28
1.0	0.0000	3	3	4	3	3	4	100	3.33	8.71
Mean		1.8	2.4	2.8	2.6	2.2	3.2			

P=0.00776(Concentration)
P=0.299727(Duration of exposure)

Table 2. Percent mortality and probit mortality of *M. domestica* larvae exposed to *Z. officinale* for 72 hours by feeding method

Conc mg/ml of H_2O	Log Conc	Period of exposure (hours)						Percent mortality after 72 hrs	Mean mortality after 72 hrs	Probit mortalty
		12	24	36	48	60	72			
0.0(control)	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	0	0	0	0	0	0	0	0
0.4	-0.3974	0	0	0	0	0	0	0	0	0
0.6	-0.2218	0	0	0	0	0	0	0	0	0
0.8	-0.0969	0	0	0	0	0	0	0	0	0
1.0	0.0000	0	0	0	0	0	0	0	0	0
Mean		0	0	0	0	0	0			

Table 3. Percent mortality and probit mortality of *M. domestica* larvae exposed to *Z. officinale* and *D. tripetala* in equal proportions for 72 hours by dipping method [19]

Conc mg/ml of H_2O	Log Conc	Period of exposure (hours)						Percent mortality after 72 hrs	Mean mortality after 72 hrs	Probit mortalty
		12	24	36	48	60	72			
0.0(control)	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	0	2	2	3	3	50	1.67	5.00
0.4	-0.3974	0	1	2	3	4	3	65	2.17	5.39
0.6	-0.2218	1	2	3	4	4	1	75	2.50	5.67
0.8	-0.0969	2	3	4	4	3	3	95	3.17	6.64
1.0	0.0000	2	4	3	5	3	3	100	3.33	8.71

P=0.002(Concentration)
P=0.001(Duration of exposure)

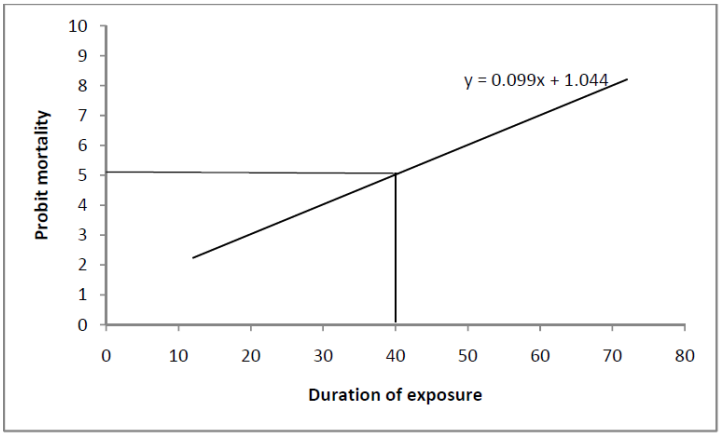

Fig. 2. Relationship between probit mortality of housefly larvae and duration of exposure period to give time at LT_{50} when larvae were dipped in *Z. officinale*

Table 4. Percent mortality and probit mortality of *M. domestica* larvae exposed to *D. tripetala* and *Z. officinale* in equal proportion for 72 hours by feeding method [19]

Conc mg/ml of H_2O	Log Conc	Period of exposure (hours)						Percent mortality after 72 hrs	Mean mortality after 72 hrs	Probit mortalty
		12	24	36	48	60	72			
0.0(control)	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	1	1	0	0	2	20	0.67	4.16
0.4	-0.3974	0	3	0	2	0	0	25	0.83	4.33
0.6	-0.2218	2	4	0	4	2	0	60	2.00	5.25
0.8	-0.0969	4	5	5	2	0	0	80	2.77	5.84
1.0	0.0000	5	5	4	3	0	0	85	2.83	6.04
Mean		2.2	3.6	2.0	2.2	0.4	0.4			

P=0.005(Concentration)
P=0.002(Duration of exposure)

Tables 3 and 4 shows the results obtained when *Z. officinale* and *D. tripetala* were mixed in equal proportions using dipping and feeding techniques. Mortality was generally significant ($p<0.05$) as concentrations increased from 200-1000 ppm. There was also significant difference ($p<0.05$) in the mortality as the duration of exposure increased from 12 to 72 hrs. These significant differences were obtained when the insects were dipped and fed in crude extract. LC_{50} and LT_{50} of 0.2754 mg/ml and 30 hours was obtained (Figs. 3 and 4) when they were dipped while LC_{50} and LT_{50} of 0.4786 mg/ml and 44 hours was obtained when they were fed with crude extract (Figs. 5 and 6).

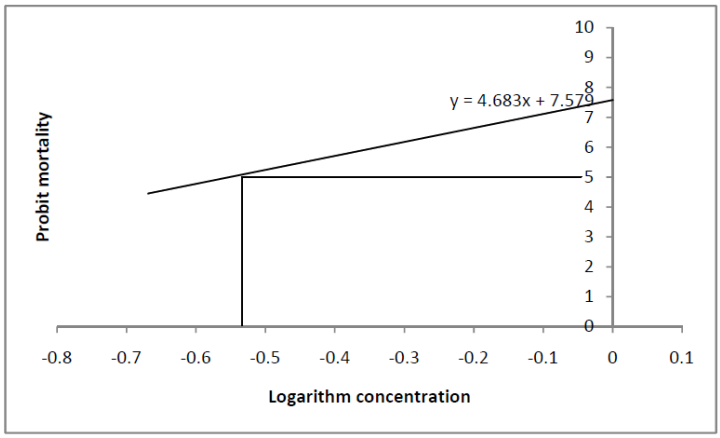

Fig. 3. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were dipped in crude extracts of *Z. officinale* and *D. tripetala*. [19]

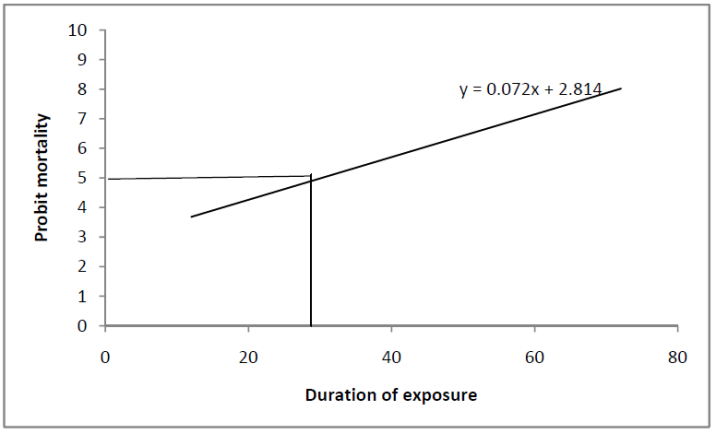

Fig. 4. Relationship between probit mortality of housefly larvae and duration of exposure to give LT_{50} when larvae were dipped in crude extracts of *Z. officinale* and *D. tripetala*. [19]

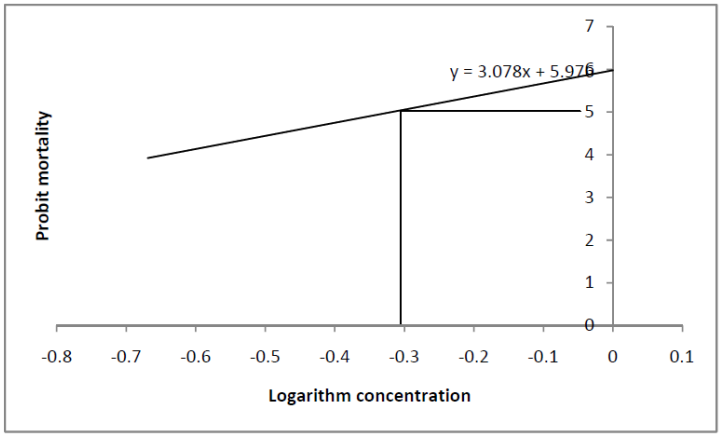

Fig. 5. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were fed with *Z. officinale* and *D tripetala* in equal proportions [19]

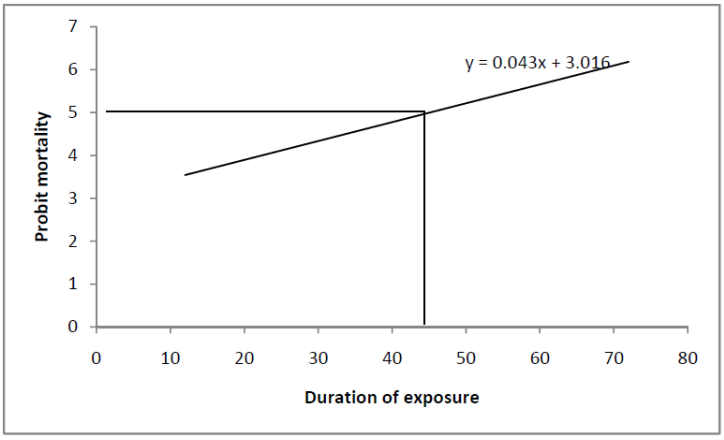

Fig. 6. Relationship between probit mortality of housefly larvae and exposure period to give LT_{50} when larvae were fed with *Z officinale* and *D tripetala* in equal proportions [19]

4. DISCUSSION AND CONCLUSION

From the results obtained, crude extracts of *Z. officinale* and *D. tripetala* are toxic to freshly emerged larvae (a day old) of *M. domestica*. The high insecticidal activity and post application effects of these crude extracts are as results of their constituent compounds gingerols, shogaol and β-phenylnitroethane; the volatile nature of the constituent compound could have also increased larvae mortality and insecticidal activities. It was also observed that test plant materials may have had synergistic effects when they were mixed in equal proportions which led to its lower LC_{50} and LT_{50}. Similar findings have also shown that when extracts are mixed together, there may be synergistic effects. [20] reported that *Piper guineense* and *Zingiber officinale* had addictive effects against *Callosobruchus maculatus* when mixed in a 50:50 proportion. [18] also reported that *C. citratus* and *O. suave* had synergistic effects against *C. maculatus* in cowpea when they were mixed in a 60:40 proportion. [21] reported that when the mixture of fruit powders of *Piper guineese* and *Dennettia tripetala* in equal proportion (50:50) were used to treat *C. maculatus*, there was addictive effects which significantly caused their mortality, reduced their oviposition and adult emerge. [17] reported that *Ocimum suave* (wild basil) leaf oil caused mortality on housefly and had a LC_{50} value of 0.09ml/50ml of water and LT_{50} value of 4.40 hours when they studied toxicity of *Ocimum suave* leaf oil on adult housefly. They also reported that there was significant difference ($P<0.05$) as both concentrations increased from 0.05-0.20ml/50ml of water and duration of exposure increased from 1 to 6 hours. [10] also reported that the LT_{50} and LC_{50} values of *S. aromaticum* oil is 27.05 hours and 9.83% while *C. nardus* oil has LT_{50} value of 38.99 hours and LC_{50} value of 13.60% and *C. odorata* oil has LT_{50} value of 52.08 hours and LC_{50} value of 29.36% respectively when he evaluated larvicidal and oviposition deterrent activities of essential oils against housefly.

Dipping technique was observed to cause higher mortality than feeding technique as shown in the results obtained, this could be because larvae inhaled the extracts which may had acted as fumigants and contact poison to cause mortality. The insecticidal potency of the extracts may have reduced when the food (mixed with plant extracts) came in contact with the digestive enzymes in the larvae. The larvae may have also been repelled and may not have fed on the food (mixed with plant extracts). This might be the reason why the dipping technique caused more mortality and had lower LC_{50} when compared to the feeding method. This result is in accordance with the result obtained by [19] when they compared the efficacy of feeding and dipping housefly larvae in crude extracts of *D. tripetala* individually and in mixed proportions with *Z. officinale*. [9] also treated housefly larvae with *Citrullus colocynthis* (50% and 10%) using dipping and feeding method and observed that dipping the insects in crude extracts caused more mortality and effects on larvicidal activity, pupa mortality and inhibited adult emergence. [22] also observed that dipping houseflies in crude extracts of Chinese star anise fruits were very effective in causing mortality and reducing development, this is contrary to the observation

of [23] when they carried out potential studies of non-conventional chemicals against housefly larva; their study showed that feeding method caused more effects on larvicidal activity than when they were dipped in chemicals. The difference in results could be because of crude extracts differential mode of action and their effective concentration. [16], observed that *Mentha piperita* and *Lavandula angustifolia* essential oils have great insecticidal effect on *M. domestica* larvae; the extracts caused mortality, prolonged larva and pupa duration and reduced adult emergence. [24] reported that *Calotropis procera, Piper longum* and *Polygonum hydropiper* had synergistic (additive) effects against *M. domestica* larvae and seven vital life-history traits of *M. domestica* which include fecundity, percent egg hatch, larva duration, numbers of pupae and adults, female ratio and adult longevity. [11] evaluated the effect of Sweet Flag Rhizome (Acoruscalamus) extract for Biological activity against housefly and observed that the extract caused high mortality against housefly and can be used as an alternative source for the control of houseflies. From the results obtained, plant extracts were toxic especially when mixed in equal proportions, thus they can be considered as bioinsecticides and could be useful in integrated pest management (IPM) as the results obtained are promising. Although, bioinsecticides may not be as active as synthetic insecticides because they do not act as fast but are better alternatives because they are cost effective, environmental friendly and readily available. Bioinsecticides are prophylactic measure of protection against insects.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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