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Insecticidal Toxicity of Goat Weed, *Ageratum conyzoides*, Linn. (Asteraceae) against Weevil, *Dermestes maculatus*, Degeer (Coleoptera: Dermestidae) Infesting Smoked Fish

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Abstract

In Nigeria and most West African countries, the most common pest of animal products which also infest smoked-dry fish is *Dermestes maculatus* (fish weevil). The bio-insecticidal potentials of *Ageratum conyzoides* (goat weed) leaf powder and extract on fish weevils was evaluated with different doses of the plant materials (0.5 – 3.0 g and 50.0-100 mg^{-ml}). Twenty unsexed adult weevils were exposed to these plant treatments which were all replicated thrice. The mortality rate was observed daily for 120 hours and 72 hours (h) with the use of the powder and extract respectively. The results of this study showed that mortality increased with the increase in gram (g) and the extract (mg^{-ml}) concentration of *A. conyzoides*. Statistically, this indicates that the application of the *A. conyzoides* powder of different concentrations had a significant effect ($P < 0.05$; F = 13.69) on the mean percentage mortality of *D. maculatus* over a 120-hour period of exposure with a Median Lethal Concentration (LC_{50}) of 0.59 g, and Median Lethal Time (LT_{50}) of 22.80 h at 3.0 g. Comparatively, the extract application had no significant effect ($P > 0.05$) on the mean percentage mortality of *D. maculatus* over a seventy-two-hour exposure ($P = 0.2573$; F = 1.7422). The minimum LC_{50} of the extract required to kill 50% of *D. maculatus* was determined as 36.86 mg^{-ml}. The overall results showed that the extract was more toxic than the powder. The effectiveness of the phytochemical components of *A. conyzoides* against *D. maculatus*, as well as the local availability of the plant make it an attractive choice in pest-management practices. Therefore, dry fish traders are advised to use *A. conyzoides* for the protection and storage of smoked-dry fish against weevil infestations.

Keywords: Insecticidal, Toxicity, Mortality, Weevil, *Dermestes maculatus, Ageratum conyzoides*

1. Introduction

Smoked fish is one of the most widely distributed and cheapest animal protein product available in Nigeria. It is also an important source of food and income for many people, especially in the Southern part of Nigeria, where over 25% of the population depend on it as a rich source of protein, essential amino acids, vitamins and minerals (Azam *et al.*, 2004; Aderolu and Akpabio, 2009 and Ito, 2017). In West Africa, particularly Nigeria; the total annual consumption of fish is 1.2 million tons (FAO, 2004 and FDF, 2005) of which 45% of the total fish catch are utilized as smoked fish (FAO, 2002).

In Nigeria and most West African countries, the most common pest of animal products, which also infest smoked fish, is the hide weevil *Dermestes maculatus* (Degeer). A large-scale deterioration in quality, and 50% losses in quantity of dried fish, due to dermestid infestation, have been reported (Egwunyenga *et al.*, 1998 and Odeyemi *et al.*, 2000). Ito and Ighere (2017a) stated that during storage, transportation and marketing, smoked fish is readily attacked by several species of insects, notably *Dermestes maculatus, D. frischii, D. ater* and *Necrobia rufipes*. These weevils form aggregations of 1 – 13 weevils around food sources where individuals feed and mate (McNamara *et al.*, 2008).

Problems of pest resistance/resurgence, residual/ vertebrate toxicity, widespread environmental hazards, and the increasing costs of the application of synthetic insecticides have created a need for the utilization of effective, ecofriendly and biodegradable botanicals such as *Ageratum conyzoides* (goat weed), a promising botanical insecticide. The use of plant products in the control of insect pests is influenced by its availability, safety and effectiveness. *A. conyzoides* is a common weed found in several countries in tropical and sub-tropical regions, including Nigeria where its control is often difficult. The aqueous extracts of the whole plant have been used by Shabana *et al.*, (1990) to cause a significant reduction of larvae emergence of root knot nematode, *Meloidogyne incognita*. Gbolade *et al.*, (1999) also confirmed the

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insecticidal properties of the volatile oil of *A. conyzoides* against *Callosobruchus maculatus* and *Tribolium cataneum* (Singh *et al.,* 2014). The aqueous extracts of the leaves or whole plants have been used to treat colic, colds and fevers, diarrhea, rheumatism, spasms; they have been used also as a tonic (Negrelle *et al.,* 1988; Oliveira *et al.,* 1993). *A. conyzoides* contains monoterpenoids, diterpenoids, sesquiterpenoids and other compounds, including alkaloids, cumarins, flavonoids, chromenes (conyzorigum), benzofurans, sterols and terpenoids, which exhibit ovicidal, larvicidal, repellent, deterrent, antifeedant, and toxic effects in a wide range of insects. This study is aimed at evaluating the bioinsecticidal potentials of the leaf powder and extracts of goat weed, (*A. conyzoides*)**,** on fish weevils (*Dermestes maculates).*

2. Materials and Methods

2.1. Preparation of Plant Powders

The leaves of *A. conyzoides* were dried under shade for several days to prevent breakdown and loss of phytochemical components. The dry leaves were ground by an electric blender and were sieved to obtain the powder which was stored in an air tight container.

2.2. Preparation of Crude Extracts

The extract of the *A. conyzoides* plant material was prepared by dissolving 50 g of the powder in 1000 mL of 95% ethanol, giving a concentration of 0.05 g/mL each. The extraction was done using the Soxhlete extractor. The process was repeated several times, and the supernatant of the plant extracts were decanted. A crude extract was obtained after the complete removal of the solvents with vacuum evaporation at temperature <40ºC to produce a thick liquid and syrupy material. From the main extract, 1.5 g, 2.25 g and 3 g were taken and dissolved in 30 mL of the solvent in a separate jar to produce a concentration of 50 mg^{-ml} 75 mg^{-ml} and 100 mg^{-ml} extract concentration which was used for the test.

2.3. Insect Culture and Maintenance

The *Dermestes maculatus* weevils used in this study were obtained from infested catfish (*Clarias gariepinus*) bought locally from Abraka market in Delta State, Nigeria. The pest was cultured in the Department of Animal and Environment Biology Laboratory, Delta State University, Abraka. Heavily infested catfish were put in different plastic containers covered with muslin cloth, and held tightly in a place to prevent the entry and exit of the weevils. A new generation of *D. maculatus* was obtained from the stock cultured by infesting clean uninfected catfish with adult *D. maculatus*. The newly emerged insects were then collected and used for the study (Egwuyenga *et al.,* 1998).

2.4. Toxicity Test of A. conyzoides Powders

The current study was carried out using four different doses (0.5, 1, 1.5, 2, 2.5 and 3 g) of *A. conyzoides* powders. Each dose was placed in a clean Petri dish and replicated three times. Ten grams of uninfested dry catfish (*C. gariepinus*) were put into the different Petri dishes containing the plant powder. This was done in the replicate of the different doses. Each Petri dish was shaken mechanically to ensure that the powder and dry catfish were thoroughly mixed. Twenty unsexed adult weevils were collected from the culture, and added to each treated catfish in the Petri dishes. The dishes were then covered to prevent the weevils from escaping. A control experiment consisting of ten grams of catfish and twenty weevils was also setup and replicated. The experimental set-up was observed for pest mortality over a period of 120 hours (5 days).

2.5. Toxicity Test of Crude Plant Extracts of A. conyzoides

This study was carried out using three different concentrations (50, 75, and 100 mg^{-ml}) of the *A. conyzoides* leaf extract. Each concentration (50, 75 and 100 mg^{-ml}) was used to treat the filter paper placed on a clean Petri dish, and was left for twenty-four hours to dry. These were replicated three times for each concentration. Ten grams of uninfected dry catfish (*C. gariepinus*) were put into the different Petri dishes containing the plant extract. This was done in the replicate of the different concentrations. Twenty unsexed adult weevils (*D. maculatus*) were collected from the culture and added to each treated Petri dish. The dishes were then covered to prevent the escaping of the weevils. A control experiment consisting of ten grams of catfish and twenty weevils was also setup and replicated. The experimental set-up was observed for a period of seventy-two hours for pest mortality.

2.6. Statistical Analysis

The data collected were analyzed by finding the average number of dead *D. maculates* during the 120-hour exposure to the different concentrations, and then converting it to mortality percentage. The mean percentage mortality obtained was then subjected to a two-way ANOVA to determine the significant difference using SPSS 17, and the results were interpreted accordingly.

3. Results

The results of the assessment of the biopesticidal efficacy of *A. conyzoides* (goat weed) at different concentrations of powders and extracts against *D. maculatus* (fish weevil) over a 120-hour period of exposure are presented in Tables 1 – 4.

3.1. Toxicity of A. conyzoides Powder on D. maculatus

Table 1 shows that the daily (24 h interval) mean mortality of the unsexed catfish weevils under the effect of the *A. conyzoides* powder over a 24 – 120-hour exposure period gave a cumulative mean percentage mortality of 43.33, 76.67, 88.33, 96.67, 98.33 and 100% at 0.5, 1, 1.5, 2, 2.5, and 3 g concentrations respectively. This study also showed that mortality increased as the gram (g) concentration of the powder of *A. conyzoides* increased from 0.5 – 3.0 g. Statistically, this indicated that the application of the *A. conyzoides* powder with different concentrations had a significant ($P < 0.05$; $F = 13.69$) effect on the mean percentage mortality of *D. maculatus* over a 120-hour period of exposure. The data presented in Table 1 revealed also that the *A. conyzoides* powder at its peak concentration (3.0 g) gave the highest mortality (100 %) of fish weevils. The first twenty-four hours of application of the plant powder recorded the highest weevil mortality with all the concentration of *A.*

conyzoides (Table 1). However, no mortality was observed in the negative control (triplicates without plant treatment).

The results of the probit analysis percentage for the median lethal concentration (LC_{50}) on the mortality of *D. maculatus* is presented in Figure 1. The minimum concentration required to kill 50% of the fish weevils was determined to be a concentration of 0.59 g (Table 1).

3.1.1. Median Lethal Time LT_{50} of A. conyzoides Powder

The respective regression equation, R^2 and LT_{50} values caused by the plant powders at different concentrations are presented in Table 2. The minimum time required to kill 50% of *D. maculatus* at 0.5 – 3.0 g of *A. conyzoides* was determined (Figures 2 – 7). The LT_{50} value for gram concentration after the treatment indicated that 3.0 g was the most toxic at the minimum time giving an LT_{50} of 22.80 h (Table 2). 0.5 g was the least effective dose causing a mortality rate of 50% of *D. maculatus* over an exposure time of 115.2 hours.

Table 1. Mean percentage mortality (Mean ± S.E) of *D. maculatus* exposed to *A. conyzoides* leaf dust in 120 hours.

Plant powder Doses (g)	Mean Mortality (%)					No. of Dead weevil	Cumulative Mean % Mortality at 120h	LC_{50} (g)
	Exposure Hours (h)							
	24h	48h	72h	96h	120h			
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	0.59
0.5	11.67 ± 1.67	11.67 ± 1.67	10.00 ± 0.00	6.67 ± 1.67	3.33 ± 1.67	26.00	43.33	
1.0	25.00 ± 2.89	18.33 ± 1.67	15.00 ± 2.89	10.00 ± 2.89	8.33 ± 3.33	46.00	76.67	
1.5	30.00 ± 2.89	25.00 ± 5.00	18.33 ± 4.41	8.33 ± 4.41	6.67 ± 1/67	53.00	88.33	
2.0	28.33± 6.01	25.00 ± 5.77	25.00 ± 2.89	13.33 ± 4.41	5.00 ± 2.89	58.00	96.67	
2.5	31.67 ± 1.67	28.33 ± 3.33	30.00 ± 5.77	5.00 ± 5.00	3.33 ± 3.33	59.00	98.33	
3.0	41.67 ± 4.41	26.67 ± 4.41	25.00 ± 2.89	6.67 ± 6.67	0.00 ± 0.00	60.00	100.00	

Percentage values are mean of triplicates observations with 20 weevils per replicate; F = 13.69; P<0.05

Table 2. Cumulative mean mortality (%) of *D. maculatus*, regression equation and median lethal time (LT_{50}) caused by *A. conyzoides* leaf dust.

Plant powder Doses (g)	Cumulative Mean Mortality (%)					Regression equation	R^2 value	Correlation (%)	LT_{50} (h)
	Exposure Hours (h)								
	24h	48h	72h	96h	120h				
0.5	11.67	23.34	33.34	40.01	43.33	y = 0.4266x + 1.12	0.9264	92.64	115.2
1.0	25.00	43.33	58.33	68.33	76.67	y = 0.5347x + 15.83	0.9732	97.32	64.20
1.5	30.00	55.00	73.33	78.33	88.33	y = 0.5972x + 22.67	0.9277	92.77	45.60
2.0	28.33	53.33	78.33	91.66	96.67	y = 0.7291x + 17.17	0.9384	93.84	45.60
2.5	31.67	60.00	90.00	95.00	98.33	y = 0.7013x + 24.50	0.8659	86.59	36.00
3.0	41.67	68.34	93.34	100.00	100.00	y = 0.618x 36.17	0.8524	85.24	22.80

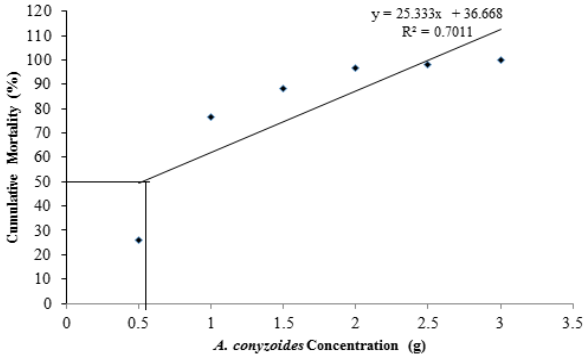

Figure 1. Percentage probit kill of *D. maculatus* exposed to *A. conyzoides* gram concentration at

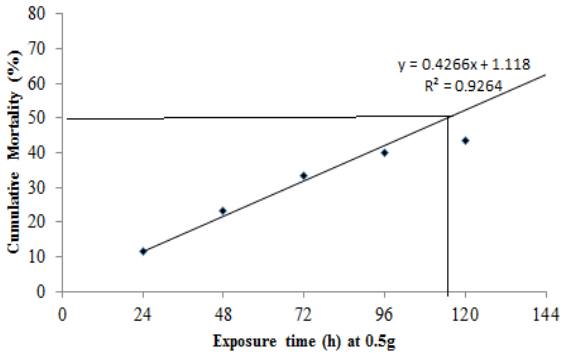

Figure 2. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 0.5 g of *A. conyzoides* dust.

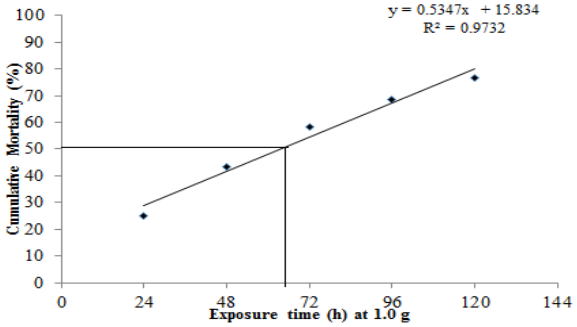

Figure 3. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 1.0 g of *A. conyzoides* dust.

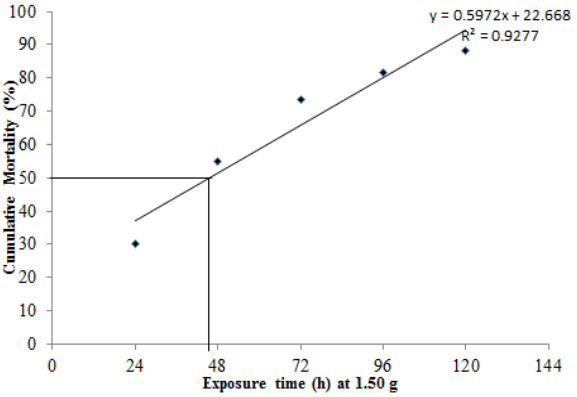

Figure 4. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 1.5 g of *A. conyzoides* dust.

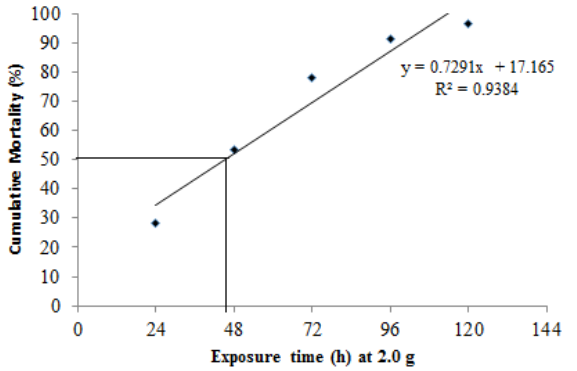

Figure 5. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 2.0 g of *A. conyzoides* dust.

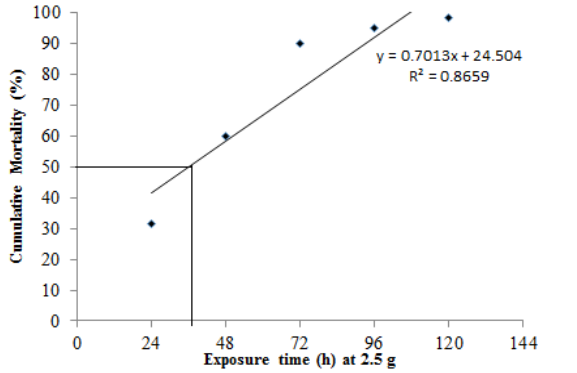

Figure 6. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 2.5 g of *A. conyzoides* dust.

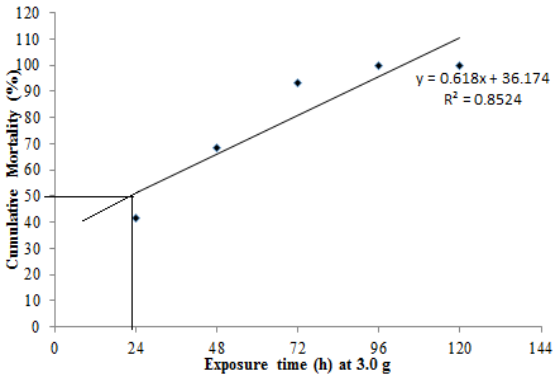

Figure 7. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 3.0 g of *A. conyzoides* dust.

3.2. Toxicity of A. conyzoides Extract on D. maculatus

The mortality caused by *A. conyzoides* also showed a similar mortality trend similar to the powder. Here, mortality of *D. maculatus* increased with increasing the concentration (mg-mL) of the extract used in the test; hence the mortality was concentration-dependent. The extract of *A. conyzoides* at 75.0 and 100.0 mg-mL per ten grams of smoked catfish exhibited the highest cumulative mean mortality of 93.34 % and 100.0% respectively (Table 3). ANOVA showed that the application of *A. conyzoides* extract at different concentrations had no significant effect ($P > 0.05$) on the mean percentage mortality of *D. maculatus* over a seventy-two-hour period of exposure ($P = 0.2573$; $F = 1.7422$). The results for the probit analysis percentage for median lethal concentration (LC_{50}) on the mortality of *D. maculatus* is presented in Figure 8. The minimum concentration required to kill 50% of the fish weevils was determined to be an extract concentration of 36.86 mg-ml (Table 3).

3.2.1. Median Lethal Time LT_{50} of the A. conyzoides Extract

The regression equation, (R^2 and LT_{50}) values caused by the leaf extract of the plant at different concentrations are presented in Table 4. The minimum time required to kill 50% of *D. maculatus* at 50.0 – 100.0 mg-mL of *A. conyzoides* was also determined and presented in Figures 9 – 11). The LT_{50} value for all the extract concentrations after the treatment indicated that 100.0 mg-mL was the most toxic with the minimum time giving an LT_{50} of 15.40 h (Table 4). The least effective dose was 50.0 mg-mL, causing 50% mortality of *D. maculatus* after 26.48 hours of exposure.

Table 3. Mean percentage mortality (Mean ± S.E) of *D. maculatus* exposed to *A. conyzoides* leaf extract in 72 hours.

Plants' Extract Conc. (mg/mL)	Mean Mortality (%)			No. of Dead weevil	Cumulative Mean % Mortality at 120h	LC_{50} (mg^{-ml})
	Exposure Hours (h)					
	24h	48h	72h			
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00	0.00	36.86
50.0	43.33 ± 0.33	33.33 ± 0.33	13.33 ± 0.33	54.00	89.99	
75.0	45.00 ± 0.58	31.67 ± 0.33	16.67 ± 0.33	56.00	93.34	
100.0	53.33 ± 0.33	36.67 ± 0.88	10.00 ± 0.58	60.00	100.00	

Percentage values are mean of triplicates observations with 20 weevils per replicate; F = 1.7422; $P > 0.05$

Table 4. Cumulative mean percentage mortality of *D. maculatus*, regression equation and median lethal time (LT_{50}) caused by *A. conyzoides* leaf extract**.**

Plants' Extract Conc. (mg/mL)	Cumulative Mean Mortality (%)			Regression equation	R^2 value	Correlation (%)	LT_{50} (h)
	Exposure Hours (h)						
	24h	48h	72h				
50.0	43.33	76.66	89.99	y = 0.9721x + 23.33	0.9423	94.23	26.48
75.0	45.00	76.67	93.34	y = 1.0071x + 23.33	0.9689	96.89	26.00
100.0	53.33	90.00	100.00	y = 0.9723x + 34.44	0.9018	90.18	15.40

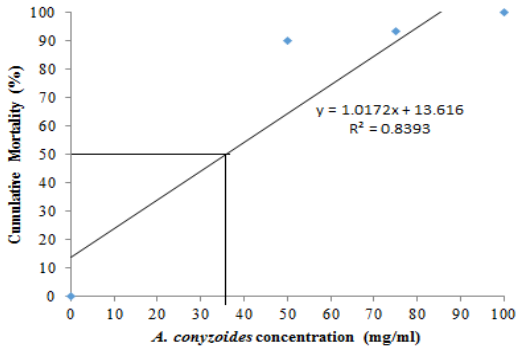

Figure 8. Percentage probit kill of *D. maculatus* exposed to *A. conyzoides* extract concentration at LC_{50}; Regression equation inclusive.

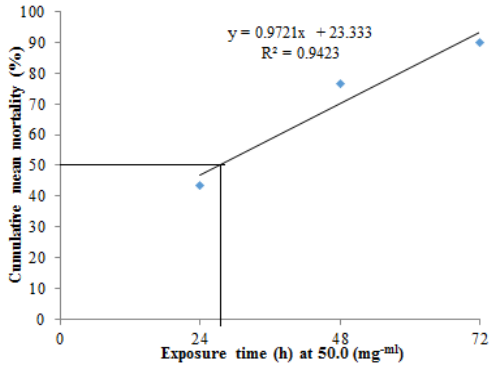

Figure 9. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 50.0 mg^{-ml} of *A. conyzoides* extract.

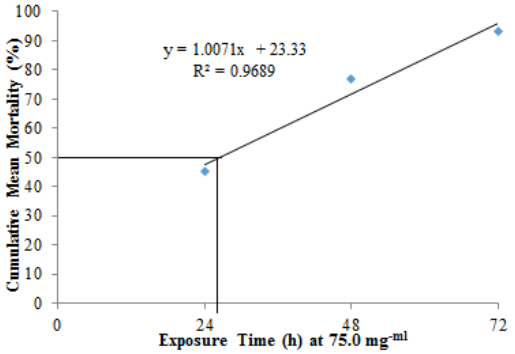

Figure 10. Time-mortality (LT_{50}) response of *D. maculatus* exposed to 75.0 mg^{-ml} of *A. conyzoides* extract.

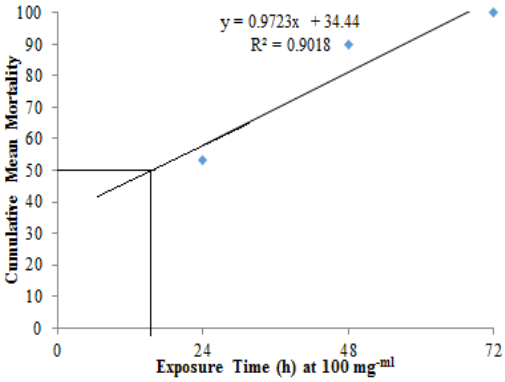

Figure 11. Time-mortality (LT_{50}) response of *D. maculatus* (fish weevil) exposed to 100.0 mg^{-ml} of *A. conyzoides* extract.

4. Discussion

The current study showed that the *A. conyzoides* powder treatment was toxic to *D. maculates*, and the application of the powder at different concentrations had a significant effect ($P < 0.05$) on the mean percentage mortality of the weevils over a 120-hour period of exposure. A high mortality rate of 100.0% of the weevils was recorded at 3.0 g of the *A. conyzoides* powder per ten grams of the substrate (dry smoked catfish). This finding is in agreement with Singh *et al*., (2014) who reported a 100.0% mortality rate of *T. castaneum* using *A. conyzoides.* Similarly, Akinwumi (2011) also documented a 100.0% rate killing *D. maculatus* with ten grams of powder per 100.0 g of fish using *Dennettia tripetala*, *Eugenia aromatic, Piper guineense*, and *Monodora myristica*. The current study also revealed that the minimum concentration required to kill 50% of the fish weevils was 0.59 g of the plant/10.0 g of smoked-dried fish (Table 1).

In the present study, there was no significant difference (P>0.05) in the percentage of mortality of *D. maculatus* using *A. conyzoides* extracts as shown by the statistical analysis. In all treatments, mortality was relatively more at higher doses (2.5 g and 3.0 g), and more with the extract concentration being (75 and 100 mg^{-ml}) than lower extract concentration (50 mg^{-ml}). This shows that *A. conyzoides* has promising bioactive properties for dry-smoked fish traders in the tropics. The crude plant extracts of the plant have also showed insecticidal and pesticidal activities against various types of insects and pests (Kamboj and Saluja 2008). The application of the ethanol extract and powder on *D. maculatus* exhibited high insecticidal activity against the weevils (Table 3). The findings of the current study are in accordance with those of other researchers, who had reported earlier that the plant powders and extracts, including those of *A. conyzoides* are toxic to insects (Singh *et al.,* 2014; Ito *et al.,* 2015; Uwamose *et al.,* 2017 and Ito and Ighere 2017b). The mortality percentage was significantly ($P < 0.05$) higher with the use of the powder of *A. conyzoides*, but not the ethanolic extract ($P > 0.05$). The extracts caused more than 80% mortality rate at all extract concentrations. Although no mortality was observed in the control set-up. The results obtained in this study are in accordance with Ito and Ighere (2017b) who reported that ethanolic extracts of the plant usually contain more active insecticidal ingredients than the powder.

The extract of *A. conyzoides* gave better mortality effects than the leaf powder (Table 1 and 3). The different effects may can be attributed to the presence of higher proportions of active chemical components in the extract than in the powder. A wide range of chemical compounds, including alkaloids, cumarins, flavonoids, chromenes, benzofurans, sterols, and terpenoids have been isolated from *A. conyzoides*. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities (Vyas and Mulchandani, 1980). *A. conyzoides* contain bioactive compounds, such as terpenics, mainly precocenes, with antijuvenile hormonal activity that may affect the growth and development of the insect, rather than being the direct cause to insect mortality. Vyas and Mulchandani (1980) reported the action of chromenes (precocenes I and II) isolated from *Ageratum* plants that accelerate larval metamorphosis resulting in maintaining the juvenile forms or producing weak and small adults. They also stated that conyzorigum is the active ingredient of chromene in *A. conyzoides* which could be the reason for the high mortality observed in this present study. The two chromenes have been reported to act synergistically, and they survived metabolism for at least twelve days (Fagoonee and Umrit, 1981).

Comparatively, the mortality of weevils fed on fish substrate, and treated with the extract of the *A. conyzoides* leaf at both concentrations was very much higher than those fed on substrate and treated with the powder. This indicates that the leaf powder of *A. conyzoides* has less bioactive substances than the extract. The cause of the high mortality of *D. maculatus* adults within three to five DAT could be due to conyzorigum substances in *A. conyzoides* which act as an antifeedant and a stomach poison. There is a possibility that the active component, conyzorigum, whose synergetic effects of precocenes I and II was at the highest concentration in the extract resulting in rapid mortality of adult *D. maculatus.*

5. Conclusion

The use of indigenous plant-based products by individuals and communities can provide prophylactic measures for the protection against various insect pest infestations. Therefore, smoked-fish traders are advised to use the *A. conyzoides* plant, commonly called "shell leaf, in Nigeria, for the better protection and storage of their products, because of its effectiveness, less hazards, availability and easy accessibility, and also for its medicinal functions. The ethanolic extract of *A. conyzoides* is a better botanical insecticide; however, further studies need to be conducted to ascertain which phytochemical is the active ingredient responsible for the *D. maculatus* mortality.

Acknowledgment

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Conflicts of interest: Nil

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