INSECTICIDAL POTENTIALS OF *DENNETTIA TRIPETALA (LINN.)* (PEPPER FRUIT-*ANNONACEAE*) APPLIED AS SINGLE AND MIXED WITH *ZINGIBER OFFICINALE (ROSC.)* (GINGER-*ZINGIBERACEAE*) CRUDE EXTRACTS AGAINST *MUSCA DOMESTICA* (HOUSEFLY) LARVAE

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ABSTRACT
Musca domestica is a common nuisance insect pest that is cosmopolitan. It also causes serious health problems to humans and livestock because it is a mechanical vector of many diseases. Crude extracts of *Dennettia tripetala* were used individually and mixed with *Zinigber officinale* in equal proportion (50:50) at various concentrations (200 to 1000ppm) to determine their toxic efficacy against newly emerged larvae of *Musca domestica*. Each concentration was replicated twice. The bioassay was done using feeding and dipping method under laboratory conditions and was monitored every 12 h for 72 h. Distilled water was used as control for the experiment. Mean mortality was recorded to obtain Median Lethal Concentration (LC_{50}) and Median Lethal Time (LT_{50}). The study also compared the efficacy of feeding or dipping the larvae in the extracts. The results showed that as concentration increased from 200 to 1000ppm, mortality was generally significant ($P<0.05$). Mortality was not significant with time ($P>0.05$), as duration of exposure increased from 12-72 h when *D. tripetala* was applied individually; but when they (*D. tipetala* and *Zingiber officinale*) were mixed in a 50:50 proportion, mortality was significant ($P<0.05$) with time, as duration of exposure increased from 12-72 h. The mixture of plant extracts (*D. tripetala* and *Z. officinale* in a 50:50 ratio) had a lower LC_{50} and LT_{50} (0.2754 mg/ml (275.4ppm) and 30 h). It was observed that dipping the insects in plant extracts caused more mortality when compared to feeding the insects with plant extracts. This result showed that plant extracts used for this study could be promising bioinsecticides especially when mixed in equal proportions and should be considered in integrated pest management (IPM) of houseflies.

Key words: *Dennettia tripetala, Zingiber officinale, Musca domestica*, LC_{50} and LT_{50}.

INTRODUCTION

Musca domestica is a common insect pest that is found everywhere. It makes up 98% of flies that are found at home and it is among the dirtiest insect pest (Stuart and Bennett, 2003; Ojianwuna *et al.* 2011). It is a nuisance species, that is, it cause annoyance to man and animals (Herve *et al*. 2008) by disturbing man during work and leisure periods; it soils human environment with its faeces. It is seen as unhygienic around man hence its negative psychological impact on man (Keiding, 1986). It also causes serious health problems to humans and livestock because it is a mechanical vector of many diseases such as cholera, dysentery, typhoid, shigellosis and salmonelllosis (Islam and Aktar, 2013); myiasis, helminthiasis (Bosly, 2013), trachoma and epidemic conjunctivitis (Keiding, 1986); as it is the mechanical carrier of various pathogens such as bacteria, protozoa and viruses (Palacios *et al.* 2009). The diseases can be found on the hairs that cover their body and legs because they crawl and feed on filthy substances. It can transmit diseases very fast within a few seconds. It transmits diseases when it feeds on human food. This is possible because when a mature adult fly feeds, it first liquefies the solid food as it has sucking mouthparts. The disease is transmitted to the food during this process and when humans or other animals feed on the contaminated food they get infected with the disease. Diseases can also be transmitted when fly makes contact with people (Keiding, 1986). The control measure commonly

used against this insect pest is the chemical control method (Herve *et al.*, 2000). The promiscuous use of chemical control method has led to pests being resistant to chemicals and the residue of this chemical affects human and the environment as they bioaccumulate (Bosly, 2013). Because of these risks, bioinsecticides especially those of plant origin are being considered as better options (Bosly, 2013). Bioinsecticides have broad range of activity and are relatively specific in their mode of action, easier to use and make, and environmentally friendly (Belmainet al., 2001; Herveet al., 2008). In recent times, lots of researches on bioinsectides against housefly and other insect pests have been documented. They include the works of Sripongpun (2008), Begum *et al.* (2010), UrzÚa et al. (2010), Sinthusiri and Soonwera (2010), Ojianwuna et al. (2011), Ahmed et al. (2013), Islam and Aktar (2013), Ghamdi et al. (2014), Pangnakorn and kanlaya (2014), Meenakshisundaram et al. (2014), Soonwera (2015), Asid et al. (2015); but information on comparism of the efficacy of some extracts applied alone and in mixed proportions with other plant extracts in equal ratio is scanty. Hence this project aims to use crude extracts, *Dennettia tripetala* applied singly and mixed with *Zingiber officinale* in equal proportion against *Musca domestica*. The efficacy of the plant extracts was also compared using feeding or dipping method.

MATERIALS AND METHODS

Insect collection and culture

Raw fish were placed in a small plastic plate and kept in a cage to attract flies into the cage. The cage was built with net and wire gauze; the floor was made with plywood. The cage was measured as 30 cm × 30 cm × 30 cm. The cage rested on raised stand of plastics which contained engine oil to prevent infes-tation from other insects and mites (Herveet al., 2008). The flies were cultured in the cage and fish samples in the cage served as food for them (flies) and for them to lay their eggs on. The eggs hatched into larvae within 24 h. The newly emerged larvae were used for the bioassay. The larvae were transferred with fine art brush into Petri dishes containing food for the larvae (the food is 2ml of powdered milk mixed with distilled water). This procedure was adopted from Bosly (2013); Herveet al. (2008), Ojianwuna *et al.* (2011), with slight modifications.

Preparation of test plant materials

Fresh *D. trip*etal*a* fruits were purchased from a local market in Abavo (Ika South Local Government Area of Delta State) while the *Z. officinale* stems were purchased from a local market in Obiaruku (Ukwuani Local Government Area of Delta state). They were identified by a botany taxonomist in Department of Botany, Delta State University, Abraka. *Dennettia tripetala* flesh was peeled off to get the seeds andrhizome of *Z. officinale* was chopped into very small pieces. They were dried at room temperature (28 ± 2^{o}c) for four weeks. The dried plant materials were ground with an electric blender (Philip) to form fine powder. The powder was sieved with a sieve of 0.1mm mesh size and kept in an airtight container to prevent the active ingredients from evaporating. This procedure was adopted from Ojianwuna and Umoru (2010) and Ojianwuna et al. (2011), with slight modifications.

Preparation of test plant extracts

Aqueous extract of the plant material was obtained by using water and ethanol as solvents. In this process, 10g of the samples (plant materials) was taken and homogenized with 100ml of the solvent (hydro – ethanol mixture (80/20, v/v)) and left for 24 h. It was then filtered with a filter paper and the filtrate was evaporated by placing it over a water bath at 40^{0}C. The crude extract was weighed and dissolved in a known volume of distilled water to obtain a final concentration. 400, 600, 800 and 1000 ppm and 0.4, 0.6, 0.8 and 1.0mg of plant extract was dissolved in a ml of distilled water (This method was adopted from Asid et al., 2015).

Larvicidal bioassay

Feeding method

This was done according to the method adopted by Asid et al. (2015). The toxicity of the plant materials was tested by adding different concentrations of crude extracts to the larvae food

in a Petri dish and its effect on them (larvae) was observed.

Dipping method

The method adopted by Asid et al. (2015) was used with some modifications. The larvae of each group was gently dipped into the extract at different concentrations for 40 s with a dip net and transferred into Petri dish with food.

For both methods, mortality rate was monitored every 6 h for 72 h and recorded. Two replicates of each group were made with each group having twenty (20) larvae. Water was used as control for both methods.

Statistical analysis

The raw data obtained from each treatment were calculated and analyzed using Analysis of Variance (ANOVA), descriptive statistics and Probit Analysis (SARS)

RESULTS

Efficiency of *D. tripetala against* freshly emerged larvae of *M. domestica*

The result presented in Table 1 shows that percentage mortality of *D. trip*etal*a* against larvae of *M. domestica* exposed to different concentrations of 0.2-1.0 mg/ml (200 – 1000 ppm) for a period of 72 h by feeding technique had a LC_{50} of 0.5888 mg/ml (Figure 1) and LT_{50} of 38 h (Figure 2). There was significant difference ($P<0.05$) as concentration increased but there was no significant difference ($P>0.05$) as the exposure period increased from 12 – 72 h. While Table 3 shows that the larvae exposed to same concentrations of *D. tripetala* and same period of exposure using dipping technique had a LC_{50} of 0.3801 mg/ml (Figure 5) and LT_{50} of 32 h (Figure 6). There was significant difference ($P<0.05$) as concentration increased but there was no significant difference ($P>0.05$) as duration of exposure increased.

Efficiency of *D. trip*etal*a mixed with Z. officinale* (50:50) against freshly emerged larvae of *M. domestica*

The result presented in Table 2 shows that percentage mortality of *D. tripetala* mixed with *Z. officinale* in a 50:50 proportion against larvae of *M. domestica* exposed to different concentrations of 0.2-1.0 mg/ml (200-1000ppm) for a period of 72 h by feeding method had a LC_{50} 0.4786 mg/ml and LT_{50} of 44 h. There was significant difference ($P<0.05$) as both concentrations and duration of exposure increased (Figures 3 and 4). While Table 4 shows that larvae exposed to same concentrations for same period of time using dipping method had an LC_{50} of 0.2754 mg/ml and LT_{50} of 30 h. There was significant difference ($P<0.05$) as both concentrations and duration of duration of exposure increased (Figures 7 and 8).

Table 1. Percent mortality and probit mortality of *M. domestica* larvae exposed to *D. tripetala* for 72 h by feeding method

Concentration (mg/ml of H_2O)	Log. conc	Period of exposure (h)						% mortality	Mean	Probit mortality
		12	24	36	48	60	72			
Control	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	1	0	1	2	2	30	1	4.48
0.4	-0.3974	0	2	0	3	2	2	45	1.15	4.87
0.6	-0.2218	1	2	2	3	0	3	55	1.833	5.13
0.8	-0.0969	2	3	5	4	2	2	90	3	6.28
1.0	0.0000	2	7	3	2	3	2	95	3.167	6.64
Mean		1	3	2	2.6	1.8	2.2			

Concentrations P = 0.02; Duration of exposure P = 0.21.

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DISCUSSION AND CONCLUSION

It was observed that that the plant crude extracts were toxic to *M. domestica* larvae as concentrations increased from 200 to 1000ppm. Toxicity of plant materials could be as a result of their constituent compound. The volatile nature of these compounds could also lead to their toxicity which causes larvae mortality and also post

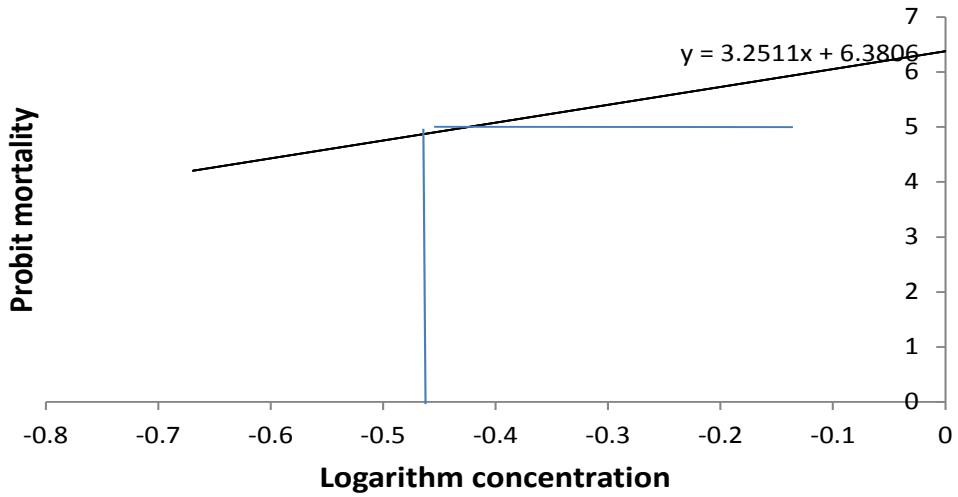

Figure 1. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were fed with *D. tripetala* .

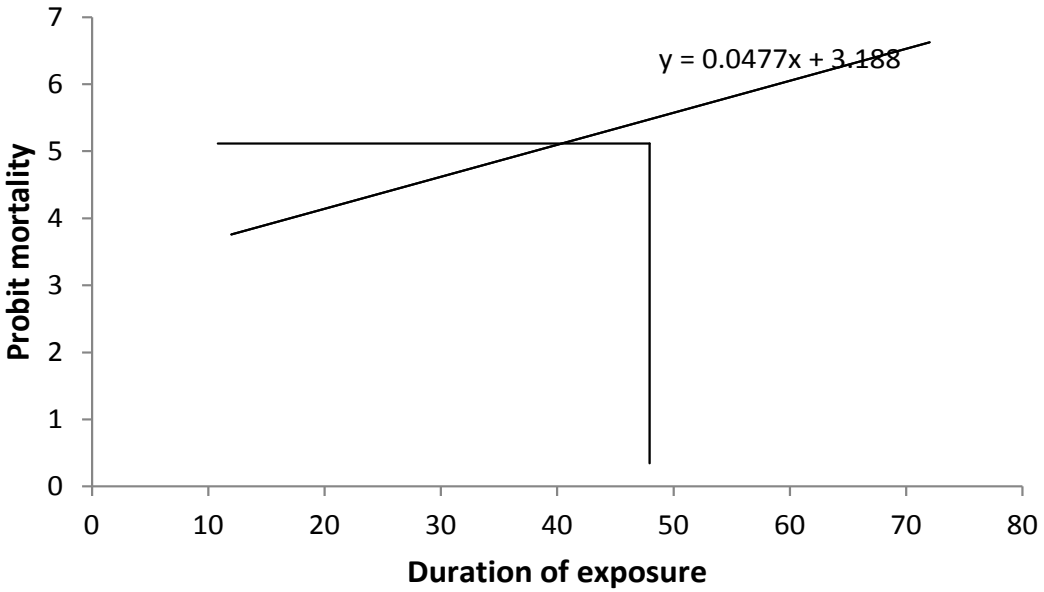

Figure 2. Relationship between probit mortality of housefly larvae and duration of exposure period to give time at LT_{50} when larvae were fed with *D tripetala.*

Table 2. Percent mortality and probit mortality of *M. domestica* larvae exposed to *D. tripetala and Z. officinale* in a 50:50 proportion for 72 h by feeding method.

Concentration (mg/ml of H_2O)	Log. Conc.	Period of exposure (h)						% mortality	Mean	Probit mortality
		12	24	36	48	60	72			
Control	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	1	1	0	0	2	20	0.667	4.16
0.4	-0.3974	0	3	0	2	0	0	25	0.833	4.33
0.6	-0.2218	2	4	0	4	2	0	60	2	5.25
0.8	-0.0969	4	5	5	2	0	0	80	2.667	5.84
1.0	0.0000	5	5	4	3	0	0	85	2.833	6.04
Mean		2.2	3.6	2	2.2	0.4	0.4			

Concentrations P = 0.05; duration of exposure P = 0.02.

Table 3. Percent mortality and probit mortality of *M. domestica* larvae exposed to *D. trip*etal*a* for 72 hours by dipping method

Concentration (Mg/ml of H_2O)	Log. Conc.	Period of exposure(h)						% mortality	Mean	Probit mortality
		12	24	36	48	60	72			
Control	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	1	2	2	2	2	45	1.5	4.87
0.4	-0.3974	2	2	4	2	1	2	65	2.167	5.39
0.6	-0.2218	2	3	2	3	2	3	75	2.5	5.67
0.8	-0.0969	2	3	2	3	3	5	90	3	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			

Concentrations P = 0.003; Duration of exposure P = 0.108.

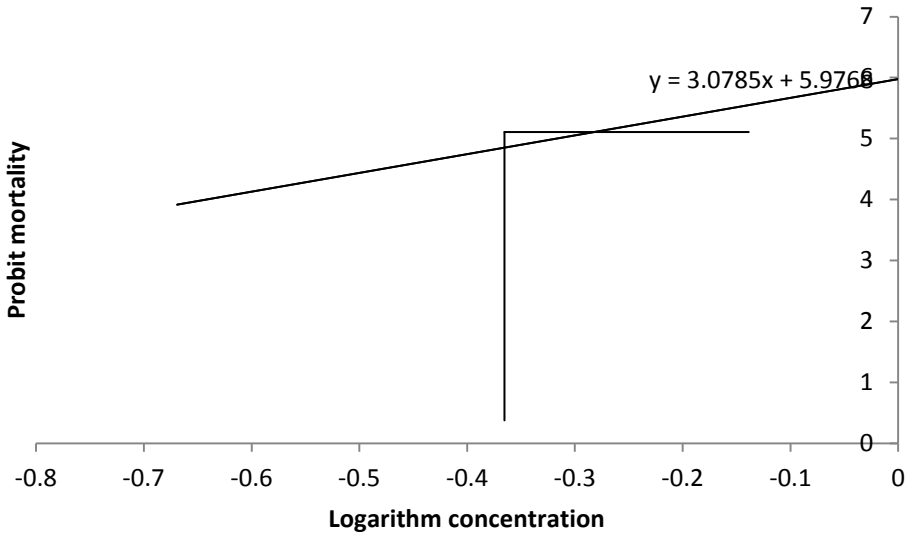

Figure 3. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were fed with *D. trip*etal*a* and *Z officinale* in equal proportions (50:50).

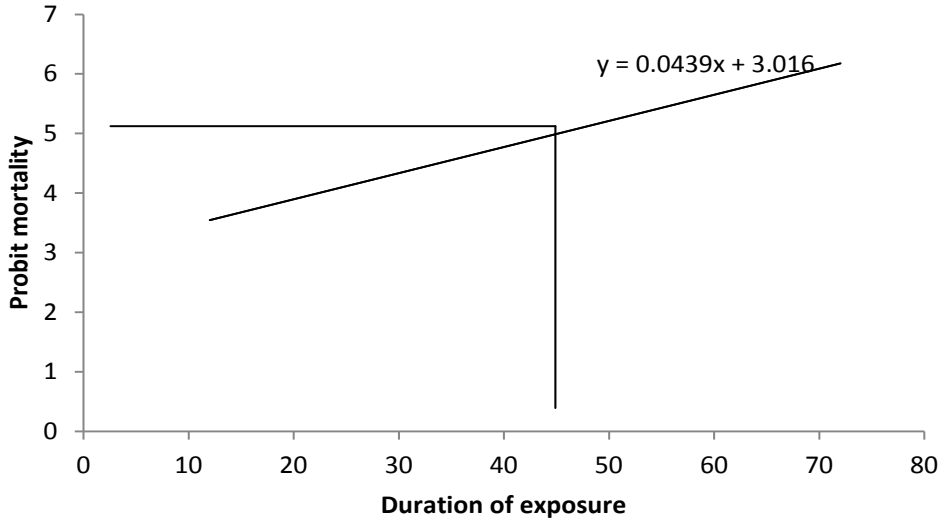

Figure 4. Relationship between probit mortality of housefly larvae and duration of exposure to give LT_{50} when larvae were fed with *D. trip*etal*a* and *Z officinale* in equal proportions (50:50).

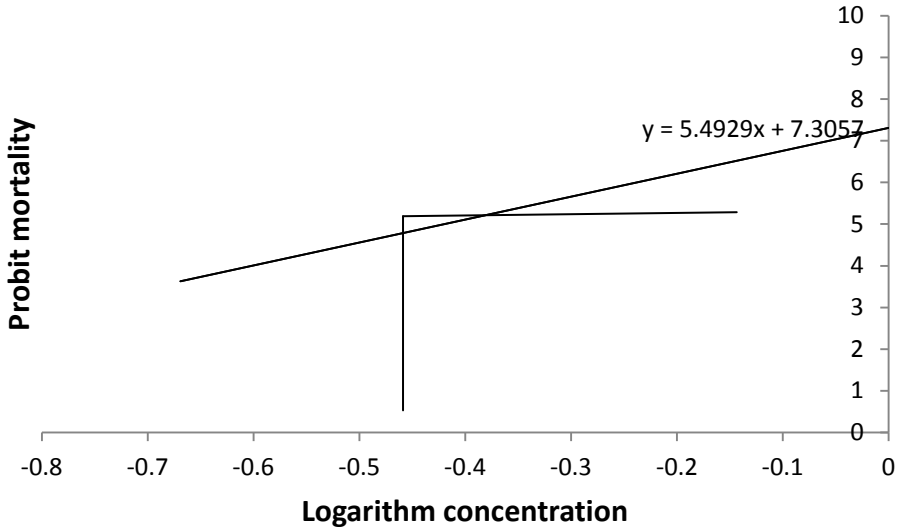

Figure 5. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were dipped in crude extracts of *D. trip*etal*a*.

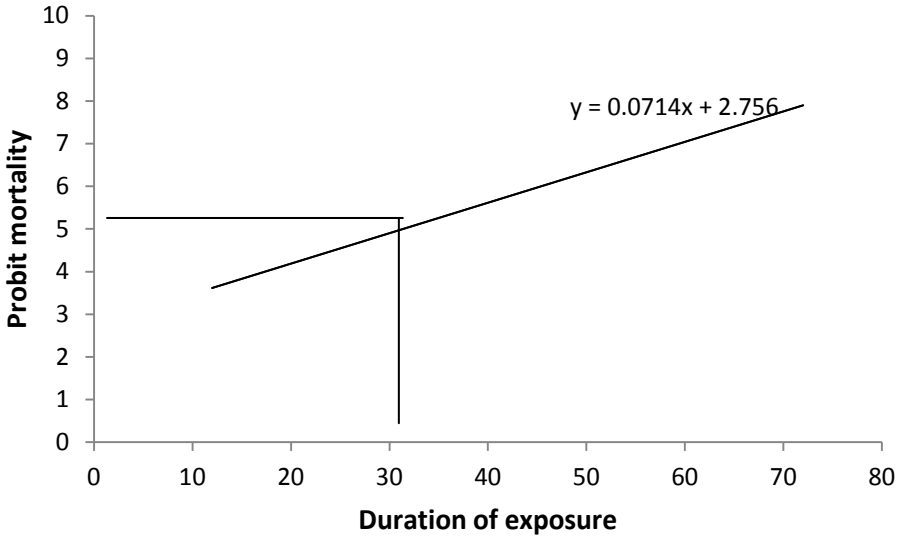

Figure 6. Relationship between probit mortality of housefly larvae and duration of exposure period to give LT_{50} when larvae were dipped in crude extracts of *D tripetala.*

Table 4. Percent mortality and probit mortality of *M. domestica* larvae exposed to *D. trip*etal*a* and *Z. officinale* in a 50:50 proportion for 72 h by dipping method.

Concentration (mg/ml of H_2O)	Log Conc.	Period of exposure(h)						% mortality	Mean	Probit mortality
		12	24	36	48	60	72			
Control	0	0	0	0	0	0	0	0	0	0
0.2	-0.6690	0	0	2	2	3	3	50	1.667	5.00
0.4	-0.3974	0	1	2	3	4	3	65	2.1667	5.39
0.6	-0.2218	1	2	2	4	4	1	75	2.5	5.67
0.8	-0.0969	2	3	3	4	3	3	95	3.1667	6.64
1.0	0.0000	12	4	4	5	3	3	100	3.333	8.71
Mean		1	2	2.8	3.6	3.4	2.6			

Concentrations P = 0.01; duration of exposure P = 0.001.

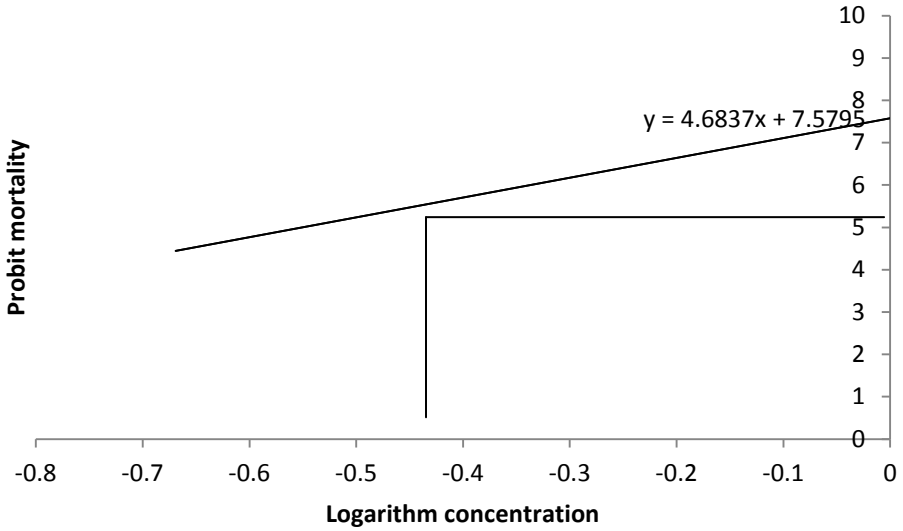

Figure 7. Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) $LC_{50}$when larvae were dipped in crude extracts of *D trip*etal*a* and *Z officinale*.

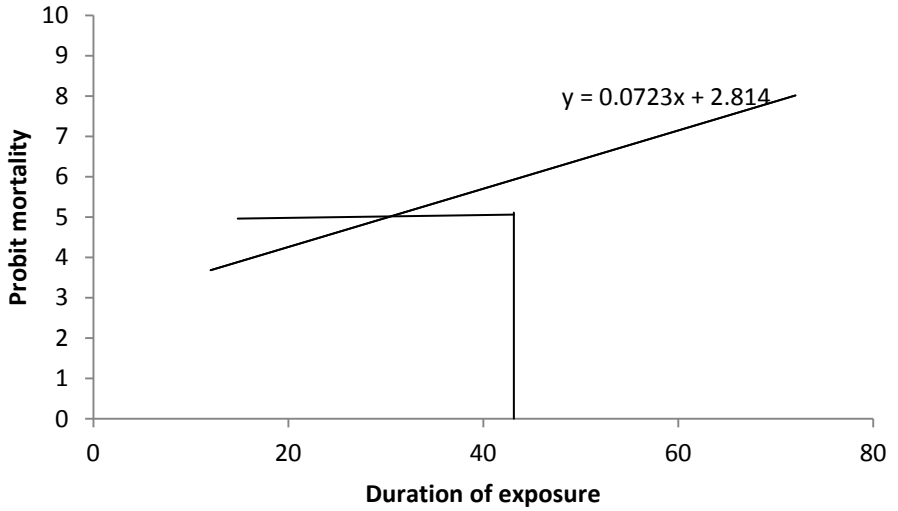

Figure 8. Relationship between probit mortality of housefly larvae and duration of exposure to give $LT_{50}$when larvae were dipped in crude extracts of *D tripetala* and *Z officinale*.

application insecticidal effects on *M. domestica* larvae.

When *D. trip*etal*a* and *Z. officinale* extract were applied individually, there was significant mortality ($P<0.05$) as concentrations increased from 200 – 1000ppm but mortality was not significant with time ($P>0.05$) as duration of exposure increased from 12 – 72 h. However, when *D. tripetala* and *Z. officinale* were mixed in a 50:50 proportion, there was significant mortality ($P<0.05$) as concentrations increased from 200-1000ppm and as duration of exposure increased from 12-72 h. There was significant difference in mortality because increase in concentration and duration of exposure led to increase in mortality. *D. tripetala* has an active compound known as β-phenylnitroethane (a nitro compound) which is responsible for its high insecticidal activity (Anyaele and Amusan, 2003). *Z. officinale* has gingerols and shogaol as its active constituents amongst other compounds (Gosh, 2011). *D. tripetala* when applied individually caused 75% mortality after 72 h and the surviving larvae pupated after 96 h but did not emerge as adult even after ten days. Meanwhile, when *D. tripetala*

and *Z. officinale* were mixed in equal proportions (50:50 ratio), it caused 86% mortality after 72 h and surviving larvae did not pupate after eight days. The failure of pupation could be because plant extracts had sublethal effect on the surviving larvae and would have disrupted the physiological process responsible for pupation. The mixture of *D. trip*etal*a* and *Z. officinale caused* more mortality because the plant extracts may have had addictive effect (synergy) when they were mixed in equal proportions. Similar findings have shown that when extracts are mixed together, there may be addictive effects. Akunne et al. (2014) reported that *Piper guinense* and *Z. officinale* had addictive effects against *C. maculatus* when mixed in a 50:50 proportion. Ojianwuna and Umoru, (2010) also reported that *C. citratus* and *O. suave* had addictive effect against *C. maculatus* in cowpea when they were mixed in a 60:40 proportion. Dawudo and Ofuya (2000) reported that the mixture of fruit powders of *Piper guineese* and *D. tripetala* in equal proportion (50:50) had addictive effects on *C. maculatus* and significantly caused their mortality, reduced their oviposition and adult emergence.

This study also showed that mortality was higher when the insects were dipped compared to when they were fed. This could be because the larvae could have inhaled the extracts which may have acted as fumigants and poison to cause mortality. The 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 may have been the reason why the dipping method caused more mortality and lower LC_{50} when compared to the feeding method. This result is in accordance with the result obtained by Asid et al. (2015) when they treated housefly larvae with *Citrullus colocynthis* (50% and 10%) using dipping and feeding method and observed that dipping method had more mortality and effects on larvicidal activity. Sripongun (2008) also observed that dipping method was very effective when he evaluated the contact toxicity of crude extract of Chinese star anise fruits to housefly larvae and their development. But it is contrary to the observation of Ghamdi et al. (2014) when they carried out potential studies of non-conventional chemicals against housefly larva; their study revealed that feeding method had more effects on larvicidal activity than dipping method. The difference in results could be because of differential mode of action of plant extracts and their effective concentration.

The mixture of *D. trip*etal*a* and *Z. officinale* (50:50 ratio) extracts had the lowest LC_{50} (0.2754 mg/ml) and LT_{50} (30 hours) using dipping method. There was no mortality in control experiment (both methods used) and their life cycle was not obstructed. This is because water is not toxic. Ojianwuna et al.(2011) reported that *Ocimum suave* (wild basil) leaf oil had a LC_{50} value of 0.09ml/50ml of water and LT_{50} value of 4.40 h 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 1-6 h. Soonwera (2015) also reported that the LT_{50} and LC_{50} values of *S. aromaticum* oil is 27.05 h and 9.83%;*C. nardus* oil has LT_{50} value of 38.99 h and LC_{50} value of 13.60%; *C. odorata* oil has LT_{50} value of 52.08 h and LC_{50} value of 29.36% respectively when he evaluated larvicidal and oviposition deterrent activities of essential oils against housefly. Asidet al. (2015) observed that *C. colocynthis* with 50% concentration against *M. domestica* larvae caused larva mortality, pupa mortality and inhibit adult emergence. Bosly (2013) observed that *Mentha piperita* and *Lavandula angustifolia* essential oils have great insecticidal effect against *M. domestica* larvae; the extracts caused mortality, prolonged larva and pupa duration and reduced adult emergence. Islam and Aktar (2013) 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* such as fecundity, percent egg hatch, larva duration, numbers of pupae and adults, female ratio and adult longevity. Meenakshisundaram et al. (2014) evaluated the effect of Sweet Flag Rhizome (*Acorus calamus*) extract for Biological activity against housefly and observed that the extract causes high mortality in housefly and offers an

alternative source for the control of houseflies.

In conclusion, results obtained showed that the plant extracts were toxic especially when mixed in equal proportions (50:50). Thus they can be considered as bioinsecticides and could be useful in integrated pest management (IPM) of housefly as the results obtained are promising. Although bioinsecticides are not as active as synthetic insecticides and do not act as fast as them (synthetic insecticides), they (bioinsecticides) are better alternatives because they are cost effective, environmental friendly and readily available in local markets.

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