UNDER PEER REVIEW

Original Research Article

THE EFFICACY OF *ZINGIBER OFFICINALE* (GINGER-*ZINGIBERACEAE*) CRUDE EXTRACTS APPLIED AS INDIVIDUAL AND MIXED (WITH *DENNETTIA TRIPETALA* (PEPPERFRUIT-*ANNONACEAE*)) AGAINST *MUSCA DOMESTICA* (HOUSEFLY) LARVAE.

ABSTRACT

Musca domestica 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. Crude extracts of *Zinigber officinale* was applied alone and mixed with *Dennettia tripetala* in equal proportion at various concentrations (200ppm to 1000ppm) to determine their toxic efficacy against freshly emerged larvae of *Musca domestica*. Each concentration was replicated twice. The bioassay was done using dippinging and feeding techniques under laboratory conditions and was monitored every 12 hours for 72 hours. Distill 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 results showed that mortality was generally significant ($P<0.05$) as concentration increased from 200ppm to 1000ppm,. Mortality was not significant with time ($P>0.05$) as duration of exposure increased from 12-72 hours when *Zingiber* officinale was applied alone, but when it (*Z. officinale*) was mixed with *D. tripetala* in equal proportion mortality was significant ($P<0.05$) with time as duration of exposure increased from 12-72 hours. The mixture of plant extracts (*Z. officinale* and *D. tripetala* in equal proportion) had the lowest LC_{50} and LT_{50} (0.2754 mg/ml (275.4ppm) and 30 hours). 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).

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

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. they also transmit the eggs of parasitic worms hence houseflies are seen as carries of easily communicable disease. Diseases are found on the legs and

UNDER PEER REVIEW

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. They tend to disturb men and livestock during leisure and work periods. 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 very necessary and a step to make homes and environment hygienic. Chemical insecticides which are usually used in controlling housefly (Herve *et al.,*) 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 (Scott, *et al,* 2000; Shono and Scott 2003). 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 (El-Sherbini and Hanykamel 2014) this has led to the use of bio insecticides in controlling insect pest because they are better alternatives to chemical insecticides. *Zingeber officinale* 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 (Gosh, 2011) 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 (Anyaele and Amusan, 2003). There are various works on the use of bioinsecticides in controlling housefly such the work of Asid *et al* (2015), Soonwera (2015), Meenakshisundaram *et al.*, (2014)) 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 *Zingiber officinale* applied individually and mixed with *Dennttia tripetala* in equal proportions; their insecticidal strength will also be compared using dipping and feeding techniques.

MATERIALS AND METHODS

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 (30x30x30xm) was built with wood, nets and wire gauze with a wooden door by its side, the floor was made with plywood. Freshly emerged larvae were used for the bioassay. The cage was placed on plastic stands which contained engine oil to prevent infestation of other insects and mites (Herve' *et al.,* 2008). The larvae was transferred into petri dishes containing larvae food (2ml of powdered milk mixed with distilled water) using fine art brush. This procedure was adopted from Herve *et al.,* (2008); Bosly (2013) and Ojianwuna *et al.,* (2011) with some modifications.

UNDER PEER REVIEW

Preparation of test plant materials

The plants used for this study was purchased from a local market in Ukwuani Local Government area of Delta State and identified by a plant taxonomist in the department of botany Delta state university, Abraka. *Zingiber officcinale* rhizome was sliced into tiny pieces while *Dennettia tripetala* skin was peeled off to obtain the seeds which were used for this study. They (*Z. officinale* and *D. tripetala*) were dried at ambient temperature (28 ± 2^0c) for four weeks, afterwards they were grounded with an electric blender (Philip) 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. This procedure was adopted from Ojianwuna and Umoru 2010) and Ojianwuna *et al*., (2011) with slight modifications.

Preparation of plant crude extract

The crude extracts were obtained by homogenizing 10g of dust (plant sample) with 100ml (80/20) of solvents (v/v) (hydro-ethanol) and left for 24hours thereafter, it was filtered with a filter paper and the residue (active compound) was evaporated over a water bath at 40^0c. To obtain the final concentration, the extracts were dissolved in a known volume of distil water; to obtain 200, 400, 600, 800 and 1000ppm, 0.2, 0.4, 0.6, 0.8 and 1.0 mg of extract was dissolved in a ml of distil water. This method was adopted from Asid *et al*., (2015).

Larvicidal Bioassay

Mortality and other effect on larvae were monitored every 12 hours of 72 hours and recorded. Two replicates of each concentration were made and the mean value was taken as the final result. Each group had 20 larvae and distill water was used as control experiment. The concentrations used for study are 200ppm, 400ppm, 600ppm, 800ppm, 1000ppm.

The toxicity of crude extracts was tested by these methods:

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 40seconds, and then introduced into petri dish with food.

Feeding technique

Various concentration of the crude extract was added to larvae food in petri dish and the larvae were introduced into the petri dish. This method used was adopted from Asid *et al* (2015) with slight modification.

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 (SARS). Significance was determined at $P<0.05$.

UNDER PEER REVIEW

RESULTS

Table 1 shows the mortality observed within a period of 72 hours, percent mortality and probit mortality of *Zingiber officinale* on *Musca domestica* larvae using dipping technique. The result shows that mortality was significant ($P<0.05$) as concentrations increased from 200-1000ppm but mortality was not significant as duration of exposure increased from 12-72 hours. The LC_{50} and LT_{50} are 0.4898 mg/ml and 40 hours (Figures 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 they didn't pupate.

Tables 2 and 3 shows the results obtained when *zingiber officinale* and *Dennettia tripetala* were mixed in equal proportions using dipping and feeding techniques. Mortality was generally significant as both concentrations and duration of exposure increased when the insects were dipped in crude extract and when they were fed with crude extract. When they were dipped in crude extract, a LC_{50} and LT_{50} of 0.2754 mg/ml and 30 hours was obtained (Figures 3 and 4) while LC_{50} and LT_{50} of 0.4786 mg/ml and 44 hours was obtained when they were fed with crude extract (Figures 5 and 6).

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

Conc Mg/ml of H_2O	**Log conc**	**period of exposure(hours)**						**percent mortality**	**mean**	**probit mortality**
		12	**24**	**36**	**48**	**60**	**72**			
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			

UNDER PEER REVIEW

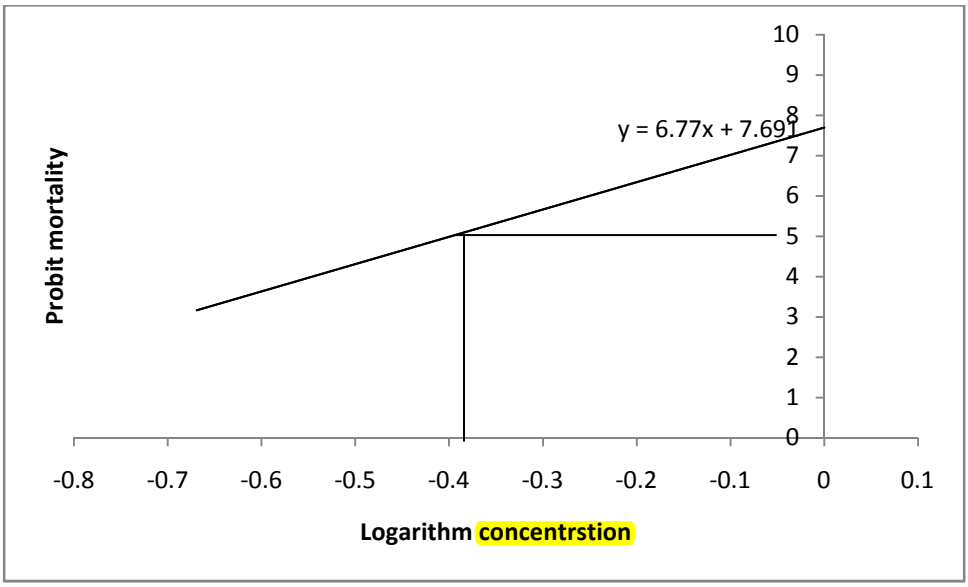

Figure 1: Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were dipped in *Z. officinale.*

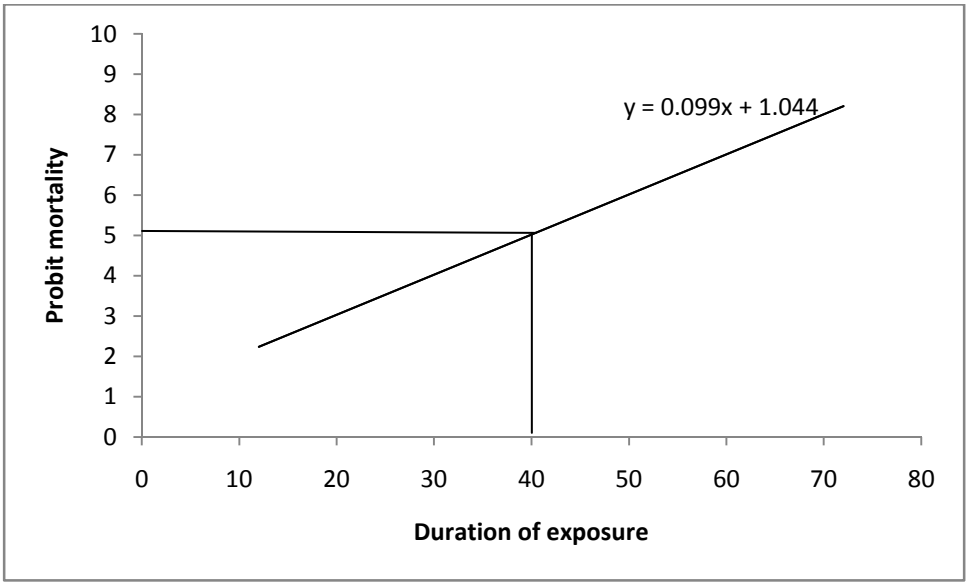

Figure 2: Relationship between probit mortality of housefly larvae and duration of exposure period to give time at LT_{50} when larvae were dipped in *Z. officinale.*

UNDER PEER REVIEW

Table 2: 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

Conc Mg/ml of H_2O	**Log conc**	**period of exposure(hours)**						**percent mortality**	**mean**	**probit mortality**
		12	**24**	**36**	**48**	**60**	**72**			
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	3	4	4	1	75	2.5	5.67
0.8	-0.0969	2	3	4	4	3	3	95	3.1667	6.64
1.0	0.0000	2	4	3	5	3	3	100	3.333	8.71
Mean		1	2	2.8	3.6	3.4	2.6			

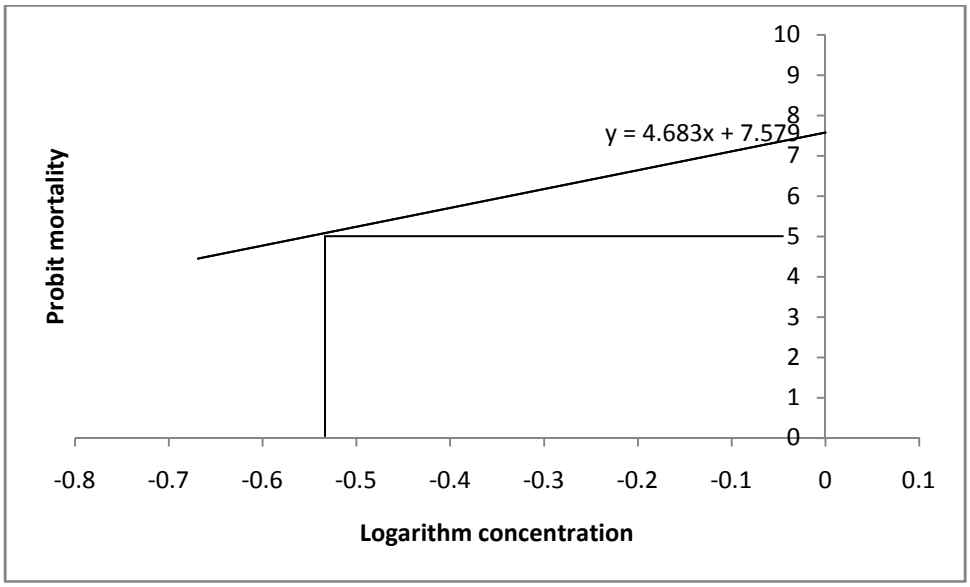

Figure 3: Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were dipped in crude extracts of *Z. officinale* and *D. tripetala*.

UNDER PEER REVIEW

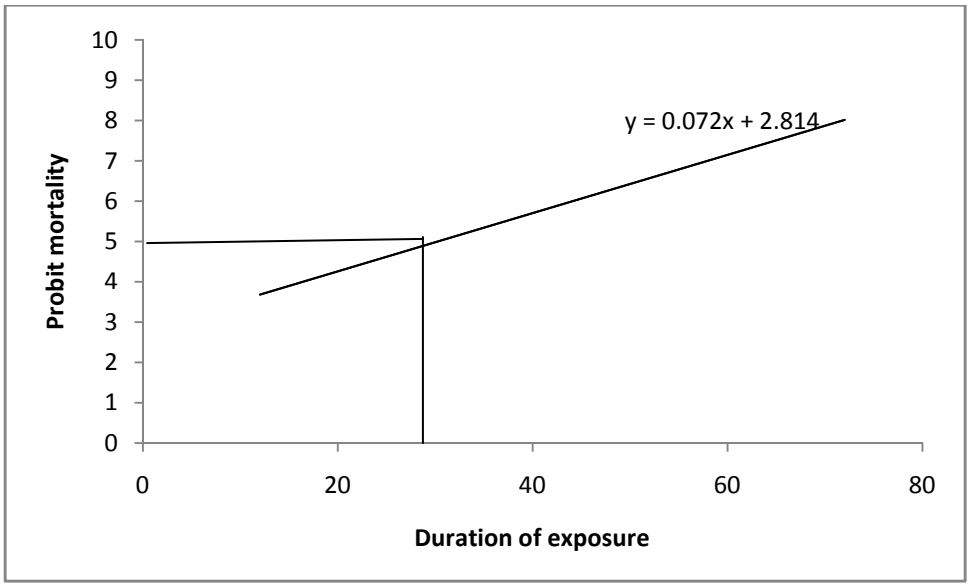

Figure 4: Relationship between probit mortality of housefly larvae and duration of exposure to give LT_{50} when larvae were dipped in crude extracts of *Z. officinale* and *D. tripetala*.

Table 3: Percent mortality and probit mortality of *M. domestica* larvae exposed to *Dennettia tripetala* and *Z. officinale* in a 50:50 proportion for 72 hours by feeding method

Conc Mg/ml of H_2O	**Log conc**	**period of exposure(hours)**						**percent mortality**	**mean**	**probit mortality**
		12	**24**	**36**	**48**	**60**	**72**			
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			

UNDER PEER REVIEW

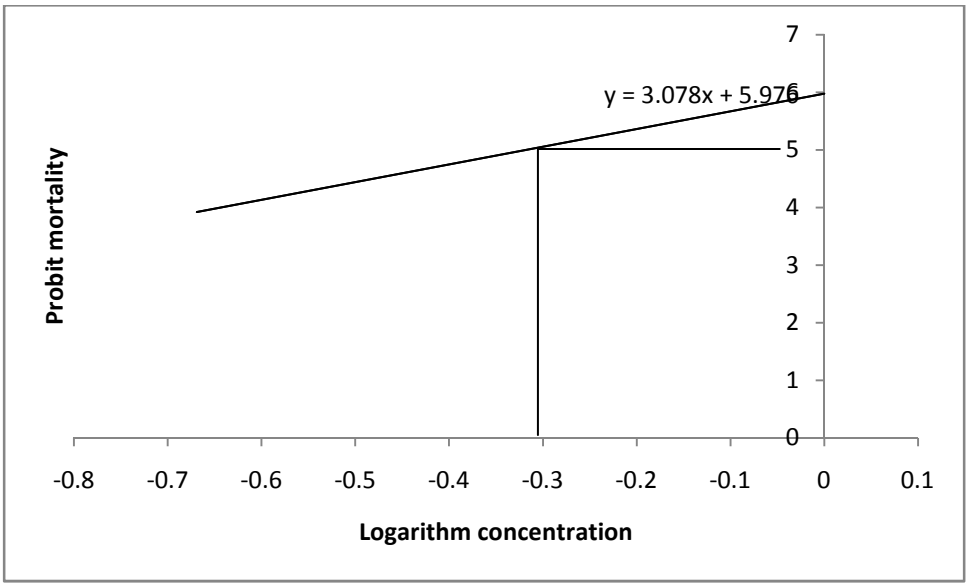

Figure 5: Relationship between probit mortality of housefly larvae and logarithm concentration to give ml concentration at (antilog) LC_{50} when larvae were fed with *Z. officinale* and *D tripetala* in equal proportions.

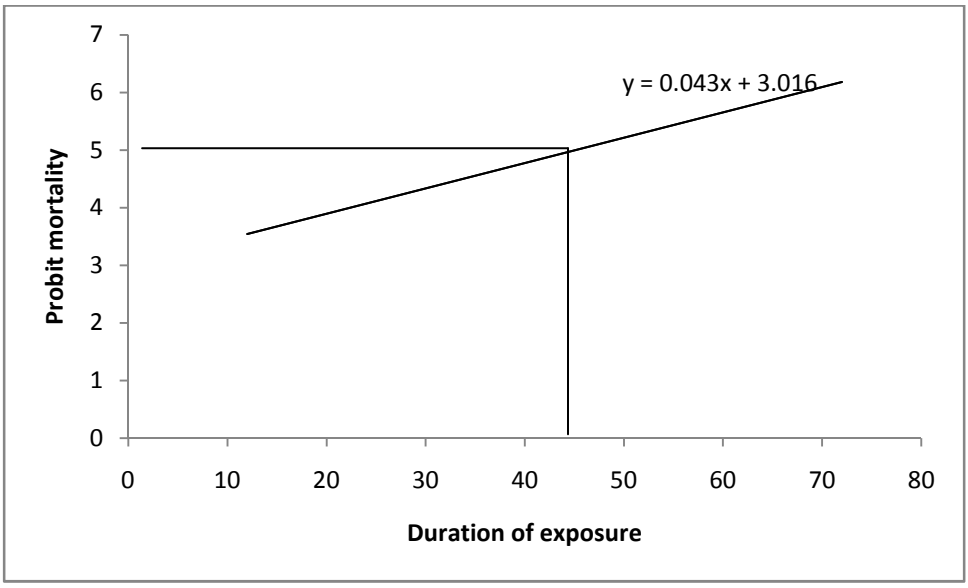

Figure 6: Relationship between probit mortality of housefly larvae and duration of exposure to give LT_{50} when larvae were fed with *Z officinale* and *D tripetala* in equal proportions

UNDER PEER REVIEW

DISCUSSION AND CONCLUSION

From the results obtained, crude extracts are toxic to freshly emerged larvae of *Musca domestica*. The high insecticidal activity and post application effect of these crude extracts is as a result of their constituent compounds; 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 synergistic effects when they were mixed in equal proportions which led to its lower LC_{50} and LT_{50}. Similar findings have shown that when extracts are mixed together, there may be synergistic effects. Akunne *et al* (2014) reported that *Piper guinense* and *Zingiber officinale* had addictive effects against *Callosobruchus 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 *Dennettia tripetala* in equal proportion (50:50) had addictive effects on *C. maculatus* and significantly caused their mortality, reduced their oviposition and adult emergence. 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 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 1-6 hours. Soonwera (2015) 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 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 Asid *et al* (2015) when he 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.

UNDER PEER REVIEW

Asid *et al.*, (2015), observed that *Citrullus 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 on *M. domestica* larvae; the extracts had 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 (*Acoruscalamus*) extract for Biological activity against housefly and observed that the extract had high mortality against housefly and offers an alternative source for the control of houseflies. From the results obtained, 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) 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. Bioinsecticides are prophylactic measure of protection against insects.

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