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Full Length Research Paper

Toxicity, growth regulatory and repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidea)

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Housefly, Musca domestica, is a major vector for many medical and veterinary pathogenic organisms. The development of naturally occurring insecticides, represent one of the most promising approaches for their ecochemical control. Petroleum-ether extracts of Griffonia simplicifolia and Zanthoxylum xanthoxyloides were assessed for their toxicity, growth regulatory and repellency to the housefly. Percent mortality and index of repellency induced by the extracts against the insects were found to be dose-dependent. Seed extracts of G. simplicifolia and root extracts of Z. xanthoxyloides were the most effective as toxicants and repellents against the fly. The LD_{50} in 24 h topical application of seed extracts of G. simplicifolia and root extracts of Z. xanthoxyloides were 0.28 and 0.35 µg, respectively. Seed extracts of G. simplicifolia evoked a very strong regulatory effect against the second larval instar of the housefly. The RD_{50} of crude extracts of G. simplicifolia and Z. xanthoxyloides against housefly ranged from 1.0 to 6.8 and 1.3 to 1.7 µg cm^{-2}, respectively. Extracts of the two plant species may be useful as insecticides for controlling the housefly and should be exploited as a component of integrated vector control strategies or could be useful in the search of new larvicidal natural compounds.

Key words: Botanical insecticides, crude extracts, integrated vector management, larval growth inhibitor.

INTRODUCTION

Housefly, *Musca domestica* L., is a major domestic, medical and veterinary pest that causes irritation, spoils food and acts as a vector for many medical and veterinary pathogenic organisms (Wallace, 1971; Milushev, 1978; Kasprzak and Majewska, 1981; Akinboade et al., 1984; Umeche and Mandah, 1989; Graczyk et al., 1999; Agui, 2001; Graczyk et al., 2001; Nayduch and Stuzenberger, 2001) or may cause annoyance to humans and agronomic livestock, resulting in considerable economic loss in livestock business (Zumpt, 1965).

Houseflies, recognized as a public health pest, occur throughout the tropics and are also found in warm temperate regions and some cooler areas. Control measure against this insect in the short-term is the use of conventional insecticides (Cao et al., 2006; Malik et al., 2007). The indiscriminate use of chemical insecticides has given rise to many well-known and serious problems, such as the risk of developing insect resistance and insecticidal residual for humans and the environment (Ahmed et al., 1981). These problems coupled with acute neuro-toxicity to man and his domesticated animals have stimulated the search for biologically based alternatives. Accordingly, botanical insecticides based on natural compounds from plants, are expected to be a possible alternative. They tend to have broad-spectrum activity, are relatively specific in their mode of action, and easy to process and use. They also tend to be safe for animals and the environment (Belmain et al., 2001). Several reports have shown the efficacy of natural compounds on

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insects (Cetin et al., 2004; Tapondjou et al., 2005; Cetin and Yanikoglu, 2006; Kestenholz et al., 2007; Rajendran and Sriranjini, 2008). Several studies have also looked at the possibility of using plant extracts in the control of eggs, larvae, pupae and adults of *M. domestica* (Issakul et al., 2004; Malik et al., 2007).

Griffonia simplicifolia Baill. (Caesalpiniaceae) and the candlewood *Zanthoxylum xanthoxyloides* (Lam.) (Rutaceae) are two medicinal and domestic shrubs widespread in Africa (Hutchinson et al., 1973). The leaves and seeds of *G. simplicifolia* contain lectins II (GS II) (Zhu-salzman et al. 1998). Lectins are protease inhibitors that exert their toxic effect by binding to carbohydrates and glycoproteins embedded within the peritrophic matrix lining the insect midgut, hence disrupting digestive processes and nutrient assimilation, thereby causing starvation (Zhu-salzman et al., 1996). However, there are few reports on the insecticidal activities of *G. simplifolia* extracts (Janzen et al., 1976; Brousseau et al., 1999). Insecticidal activities of *Z. xantholoides* on storage insect pests have been reported by Udo et al. (2004) and Owusu et al. (2007). The plant contains alkaloids, benzoic acid derivatives (Katzer, 1996) and essential oils consist mostly of terpenes.

In the present study we investigated the bioactivity of crude extracts of *G. simplicifolia* and *Z. xanthoxyloides* against the housefly, *M. domestica*.

MATERIALS AND METHODS

Culturing of insects

A stock of adult houseflies was obtained from hunting by setting metal frame cage traps baited with sliced pieces of meat. The flies were reared in 30 x 30 x 30 cm metal frame cages covered completely on all sides with muslin. A plywood floor was fixed at the base of each cage. The muslin ended in sleeves at both the rear and front sides of each cage. The front sleeve served as an entrance for the introduction or harvesting of flies and an opening for the changing of food, water and oviposition containers. The front and rear sleeves of the cages were closed with elastic bands. The cages rested on raised stands of piled Petri dishes, which contained a layer of engine oil to provide a barrier against cross infestation from other insects and mites. Adult flies were fed on sucrose provided in shallow Petri dishes. Food and water were changed daily. Each cage could accommodate up to 700 flies. Mating occurred a few hours after emergence from the pupal cases. Pieces of liver were provided in Petri dishes for eggs to be laid on. Eggs laid on the liver were transferred into the larva-rearing medium. The larvae were reared in 17 cm x 11 cm x 4 cm transparent plastic containers with lids. Each lid had a circular opening (6.5 cm diameter) covered with muslin.

A food composition of wheat bran, grass (elephant grass) meal, bakers yeast, malt extract and water was provided for the larvae. This was prepared by adding 3.5 g of dried brewers yeast and one and half (1.5) teaspoon of marmite malt to 200 ml of warm water, and stirring to dissolve. The mixture was then poured into a bowl and 400 ml of previously boiled warm water was added and stirred. To this solution were added 61 g of dry grass meal, and then 122 g of wheat bran. The mixture was stirred to mix thoroughly to give a loose texture. Each rearing container was filled with the prepared medium to about a third of its volume and seeded with 100 - 200 eggs and/or larvae. The observed average period for larval development was six days. The mature larvae migrated to the upper layer of the medium to pupate. The pupae were carefully removed from the rearing containers with a pair of fine forceps and transferred into Petri dishes. They were kept in the Petri dishes until they began to emerge, approximately four days after pupation. The Petri dishes were placed in the oviposition cages and opened for the emerging adults to fly into the cages. Three to four-day-old flies were used for the bioassays. The age of the flies used for each experiment was counted from the day of maximum adult emergence.

Plant materials

Fresh leaves (2 kg) and fresh seeds, stem and roots (5 kg each) from *G. simplicifolia* and *Z. xanthoxyloides* were collected in October 2000 in Accra. The collected plant parts were oven dried at 40°C for 5 days and milled into powder using a Fritsch (TÜV- CERT, Germany) milling machine. The powdered materials were kept at 4°C. The plant was identified by the University Herbarium, Botany Department, University of Ghana, Legon, where a voucher specimen is deposited.

A 1:10 weight of powdered plant material to volume of extracting solvent (i.e. 30 g of powder from plant material to 300 ml of petroleum ether [Chimtex, Ghana] extract) was prepared. The extraction was done at room temperature. The mixture of solvent and various powders was stirred with a magnetic stirrer for eight hours. The extracts were then filtered with a Buchner filtration system using a vacuum pump (Edwards High Vacuum Ltd., Crawley, England). The filtrates were collected in labeled flat bottom flasks and concentrated *in vacuo* and the concentration of crude extracts was determined. Fresh leaves (500 g) of the two plant species were collected and subjected to hydro-distillation using a Clevenger – type apparatus.

Contact toxicity by topical application

A hand microapplicator (Burkard Manufacturing Co, Ltd.) was used to topically apply acetone [Chimtex, Ghana] solutions of the crude extracts to adult houseflies. 1 µl of the test solution was applied on the thorax of four-day-old adult housefly of mixed sexes selected randomly. Ten insects were used for each treatment and treatments were replicated four times. The doses vary geometrically from 0.16 to 10 µg insect^{-1}. To prepare the concentrations, the crude extracts were further concentrated by measuring 200 µg of each extract into 2 ml vial, drying with N_2 gas (obtained from Air Liquide, Ghana) and redissolving in acetone. The treatment series included four groups of 10 houseflies treated with acetone alone to serve as controls. The houseflies were anaesthetized with CO_2 and treated at the rate of 10 flies per minute. Each group of houseflies was held in a petri dish (8.5 cm diameter) for 24 h after treatment and the number of dead insects was recorded.

Growth regulatory effect of crude extracts on housefly

A treated rearing medium method was used and only housefly larvae were bioassayed. Four dietary doses were tested along with a solvent treated control for each extract. Actual doses used varied geometrically and ranged from 0.03 to 1 g of extract g^{-1} of larval rearing medium. 1 ml of each concentration of the extract was added to 1 g of milled grass in 250 ml glass bottles and allowed 15 - 30 min for the solvent to evaporate. The larval rearing medium was added to make up to 25 g and the diet thoroughly stirred to mix. 10 second instars larvae were introduced into each medium and the bottle was sealed-off with muslin. Each treatment was replicated four times.

Table 1. Lethal toxicity (LD_{50}) of crude extracts of *G. simplicifolia* and *Z. xanthoxyloides* applied topically to housefly after 24 h.

Plant	Extract	LD_{50} (µg/insect)	5 % Fiducial limits		χ^2	Slope
			Upper	Lower		
G. simplicifolia						
	Seeds	0.3	0.4	0.2	199.5φ	1.7
	Leaves	0.7	0.8	0.6	-182.0	1.7
	Stem	0.7	0.7	0.6	-140.8	2.7
	Roots	0.3	0.4	0.2	17.5φ	0.5
Z. xanthoxyloides						
	Leaves	2.4	4.2	0.6	7.42	0.5
	Stem	0.5	0.6	0.2	14.1φ	0.9
	Roots	0.4	0.6	0.2	1.1	1.4

χ^2 = Chi square values; V = variance of the RD_{50}; Φ = Heterogeneous (heterogeneity factor); h = [χ^2/degree of freedom] x V.

The morphological features of larvae, pupae and adult flies emerging from treated larval media were studied with a hand lens. Individual larva, pupa and adult flies emerging were also scored on a scale of 0 - 3 as follows: (0) Normal adults; (1) abnormal adults; (2) deformities and incomplete emergence from pupa case; (3) deformities on larvae. Larval and pupal mortalities were also recorded.

The potency (P) of the extracts in imparting deformities was determined using the formula:

$$P = [\sum (n_i \times s_i) / n_t] \times 100 \quad (1)$$

n_i = the number of emerged individuals; s_i = their numerical scores and n_t = the number of test flies.

The P values were then coded and described as follows:

- 100 – 90 (+ + + +) very good activity;
- 89 – 80 (+ + +) good activity;
- 79 - 60 (+ +) moderate activity;
- 59 – 50 (+) poor activity and
- 49 – 0 (-) no activity.

An index of more than 80 indicates a significant potential of crude extract in imparting deformities to the developmental stages of houseflies (LaBreque and Wilson, 1959).

Repellency

An inverted cone trap method (Obeng-Ofori et al., 1997) was used to evaluate the repellent action of the extracts against *M. domestica*. The crude extracts were applied uniformly via Pasteur pipette to semi-circular Whatman's No. 4 filter paper (diameter, 12.5 cm; area 61.4 cm^2). 1 ml of five different doses of crude extracts was tested. Actual concentrations varied geometrically and ranged from 0. 625 to 10 µg cm^{-2}. All the treated filter papers were allowed to dry for 5 - 10 min. Each was then rolled into a cone and joined together. An aperture, large enough to allow a fly to pass through was made at the apex of each cone. The cones were inverted over 250 ml glass bottles (diameter, 5.5 cm; height, 12 cm) to form cone traps. The filter papers for control experiments were treated with only of petroleum ether (1 ml) used for extraction. 15 g of sugar previously fed on by houseflies were placed in each bottle to serve as an attractant. In each experiment, a control and treated cone traps were exposed for 30 min in a cage (18 x 25 x 25 cm) containing 100 houseflies of mixed sexes. The flies were starved overnight before use. The number of flies trapped in the treated and untreated (control) traps, was recorded. Each experiment was replicated four times. After each count the cage was turned 90º so that at the end of the fourth count 360º was covered. This was done to minimize any bias for a particular side or bottle.

The index of reaction (IR) was determined in accordance with the method of Barzeev (1962).

The percentage repellency (R%) (Campbell, 1983) for each dose was also calculated.

$$IR = [100(T - C)/(T + C)]; \; R\% = [100(C - T)/C] \quad (2)$$

Where C = the number of flies trapped in the control trap and T = the number trapped in the treated trap. The IR is a measure of the intensity of repellency of the crude extract as compared with the control. An IR of zero indicates no preference for either the treated or control. A positive value (max = + 100) indicates that the control repels more strongly than the treated and a negative value (max = -100) indicates the contrary.

Statistical analysis

Analysis of variance (ANOVA) using Genstat 5 Release 3.2 and Excel was carried out on the data collected. Data involving counts were transformed using square root ($y = \sqrt{x+0.5}$) transformation while those involving percentages were transformed using arcsine ($y = \sin^{-1}\sqrt{x}/100$) transformation before analysis. Means were separated using LSD. Probit analysis for the determination of LD_{50} and RD_{50} was based on the methods of Finney (1971) and analyzed using SPSS for windows. Correction of natural mortality in control treatment was done using Abbott's (1925) formula.

RESULTS

Contact toxicity by topical application

Crude extracts of *G. simplicifolia* and *Z. xanthoxyloides* against adult houseflies, evaluated by LD_{50} values are presented in Table 1. For all extracts, the percentage mortality increased significantly ($P < 0.001$) with increasing dose of extracts. Toxicity of seed and root extracts of *G. simplicifolia* were not significantly different

Table 2. Mortality of *M. domestica* larvae induced by crude extracts in growth medium.

Plant crude extracts				
Concentration (g extract/g of medium growth)	**Seeds**	**Leaves**	**Stem**	**Roots**
G. simplicifolia				
0.03	82.0 ± 5.2	10.0 ± 3.0	5.0 ± 2.0	33.0 ± 4.0
0.06	97.0 ± 2.0	30.2 ± 4.0	6.0 ± 4.0	53.4 ± 5.3
0.13	84.0 ± 8.2	20.0 ± 5.3	16.0 ± 5.0	84.3 ± 2.0
0.25	80.0 ± 5.0	25.4 ± 4.1	17.3 ± 4.2	90.2 ± 4.2
0.5	80.0 ± 7.2	26.2 ± 6.3	40.4 ± 5.3	98.2 ± 3.0
1	100 ± 0.0	97.0 ± 5.3	50.4 ± 15.4	100 ± 00
Z. xanthoxyloides				
0.03		00	5.2 ± 2.0	00
0.06		00	12.3 ± 4.3	00
0.13		00	12.4 ± 4.4	00
0.25		7.4 ± 4.3	16.0 ± 5.0	00
0.5		7.4 ± 4.3	20.3 ± 6.3	00
1		15.3 ± 5.3	27.5± 10.8	00

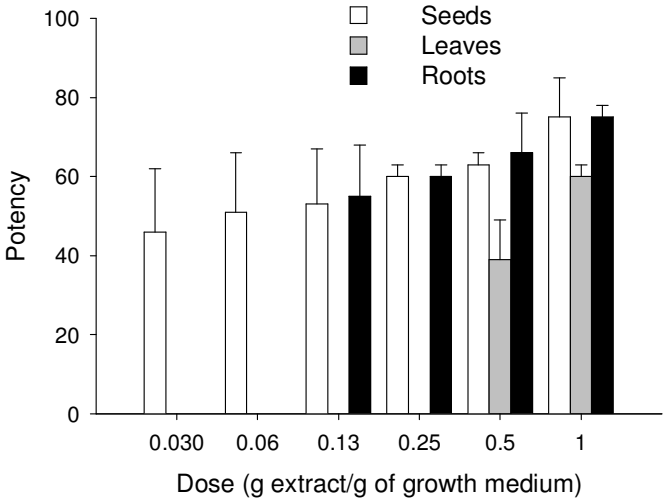

Figure 1. Potency value of *G. simplicifolia* crude extracts in imparting deformities to housefly larvae.

from that of stem and root extracts of *Z. xanthoxyloides*. Root extracts of *G. simplicifolia* and that of *Z. xanthoxyloides* have about the same potency, recording 100% *M. domestica* mortality at higher concentration (from 5 to 10 µg/µl). Leaf and stem extracts of *G. simplicifolia* gave only 50% of that potency. The leaf extracts of *Z. xanthoxyloides* had less than 12% of the activity of the root.

Growth retardation and morphogenetic abnormalities

The varying percentages of death observed at different stages of development, induced in second instar larvae of housefly following treatment with graded doses of extracts of *G. simplicifolia* and *Z. xanthoxyloides* in rearing medium are shown in Table 2. Second instar larvae of housefly were more susceptible to *G. simplicifolia* crude extracts than that of *Z. xanthoxyloides*. Deaths observed did not show any dose-dependency response with the crude extracts of the two plant species. Seed extract of *G. simplicifolia* was very potent even at the low concentration of 0.03 g causing 82% larval mortality. Larval rearing medium treated at 1 g of extract g^{-1} with seed and root extracts of *G. simplicifolia* killed all the second instar larvae exposed. Roots extracts of *Z. xanthoxyloides* was not toxic to housefly larvae. Furthermore, only 27.5% larval mortality was achieved at 1 g of extract g^{-1} of larval rearing medium treated with stem extracts of *Z. xanthoxyloides*. Most of the deaths recorded at larval stage were for larvae that did not moult to the third instar stage.

The potency (P) of crude extracts of *G. simplicifolia* in imparting deformities to the larvae is shown in Figure 1. The potency appeared to be dose-dependent. The highest potency was recorded for seed and root at a dose of 1 g of extract g^{-1} of larval rearing medium. Stem extract was not potent at doses ≤ 1 g of extract g^{-1} of larval rearing medium.

Repellency

Repellency of extracts of *G. simplicifolia* and *Z. xanthoxyloides* to *M. domestica* is shown in Figure 2. Crude extracts of *G. simplicifolia* and *Z. xanthoxyloides* significantly ($P < 0.001$) repelled the flies. In each figure, a negative index of reaction indicates that houseflies were repelled by the extracts more than the controls and a value double the standard error is considered signifi-

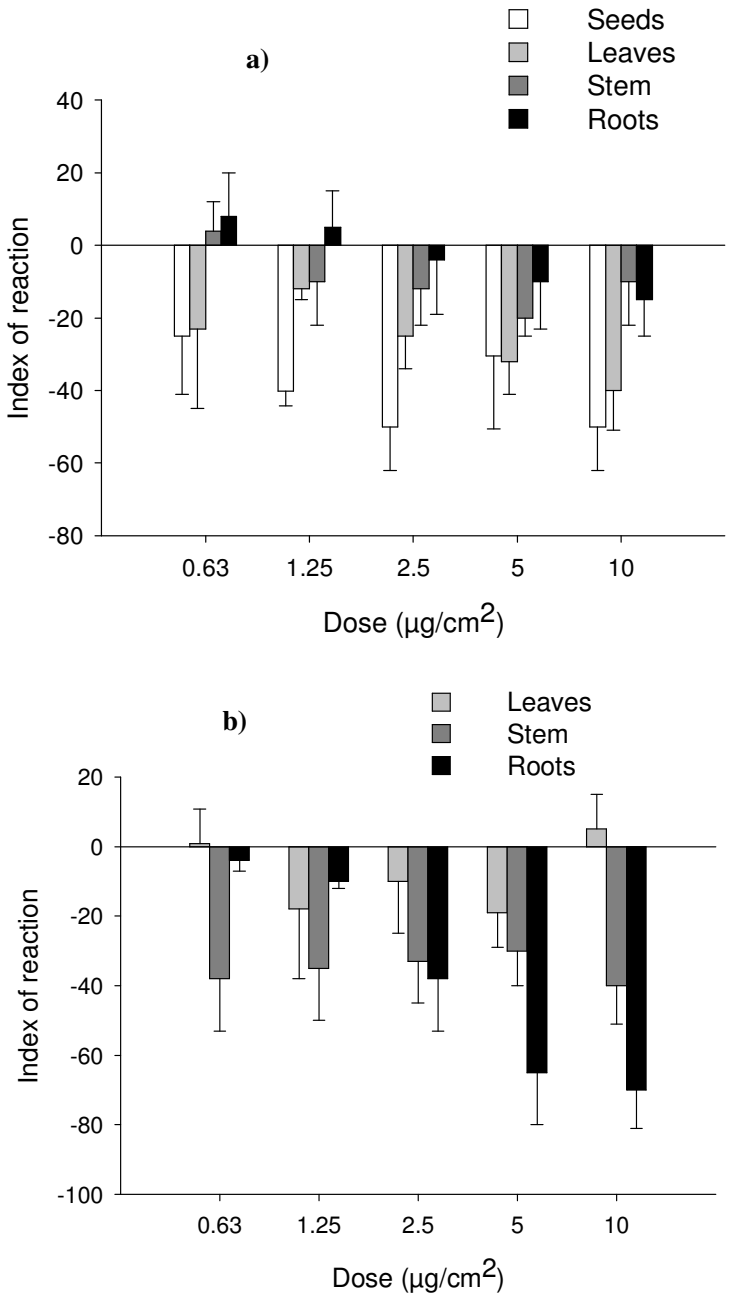

Figure 2. Repellency of graded doses of extracts of (a) *G. simplicifolia* and (b) *Z. xanthoxyloides* against *M. domestica*.

cant (Labreque and Wilson, 1959; Barzeev, 1962). Repellency of the root extracts of *Z. xanthoxyloides* was dose-dependent comparable to other extracts. Mean index of reaction values of 4.0 and 8.0 for stem and root extracts of *G. simplicifolia* and 0.8 for leaf extracts of *Z. xanthoxyloides* were obtained at 0.625 µg cm^{-2}. Also, at 1.25 µg cm^{-2} a mean index of reaction value 5.6 was obtained for root extracts of *G. simplicifolia* (Figure 2a), indicating that at these dosages they were significantly ($P < 0.001$) less repellent to the flies.

Seed extracts of *G. simplicifolia* were highly repellent to the flies at various doses recording an index of reaction value ≤ –50 at 2.5 µg cm^{-2} at higher dose. Root extracts of *Z. xanthoxyloides* were highly repellent to *M. domestica* with an index value ≤ –70 at 10 µg cm^{-2}, but its repellency decreased by more than 100 % with decreasing dose (Figure 2b).

The repellent action (RD_{50}) of crude extracts of *G. simplicifolia* and *Z. xanthoxyloides* on the housefly is shown in Table 3. The RD_{50} values indicate that seed ex-

Table 3. Repellent action (RD_{50}) of crude extracts of *G. simplicifolia* and *Z. xanthoxyloides* to the housefly.

Plant	Extract	RD_{50} (µg. cm^{-2})	95 % Fiducial limits		χ^2	Slope
			Upper	Lower		
G. simplicifolia						
	Seeds	1.0	1.4	0.7	315.7φ	2.4
	Leaves	6.0	8.6	3.4	382.1 φ	0.7
	Stem	6.8	9.5	4.1	298.9 φ	0.6
	Roots	5.2	7.7	2.7	512.3 φ	0.67
Z. xanthoxyloides						
	Stem	1.3	2.3	0.3	356.6 φ	2.3
	Roots	1.7	3.1	0.3	175.4 φ	2.3

χ^2 = Chi square values; V = variance of the RD_{50}; Φ = Heterogeneous (heterogeneity factor); h = [χ^2/degree of freedom] x V.

tracts of *G. simplicifolia* repelled the fly better than any other crude extracts with a RD_{50} value of 1.03 µg cm^{-2}. Stem and root extracts of *Z. xanthoxyloides* had similar level of repellency to *M. domestica*. Seed extracts of *G. simplicifolia* and stem extracts of *Z. xanthoxyloides* emerged as the most repellent plant parts to the flies. Leaf extract of *Z. xanthoxyloides* was the least repellent to the flies.

The RD_{50} values of all the extracts of *G. simplicifolia* and *Z. xanthoxyloides* tested were significant at 95% fiducial limits. Heterogeneity at $P < 0.001$ was recorded for all plant materials of *G. simplicifolia* and *Z. xanthoxyloides*.

DISCUSSION

Crude extracts from the various parts of *G. simplicifolia* and *Z. xanthoxyloides* applied topically to insects were highly toxic to the flies. The LD_{50} of crude extracts from various plant parts of the two plant species varied considerably. The high susceptibility of flies to the extracts may be attributed to the absence of hard and highly sclerotized thoracic cuticle which is characteristic of skeletal tissue of Coleoptera (Talukder and Howse, 1994); this may reduce the physical absorption of the active constituents of the extracts through the cuticle. The flies demonstrated hyper-excitation and paralysis prior to death. This may indicate possible contact neurotoxic action of the active constituents of the two plant species that is mainly related to the acetycholinesterase and octopaminergic levels (Isman 2000; Kostyukovsky et al., 2002) or the active constituents may transform the alcohol present into the fly body into the corresponding esters (Tsao and Coats, 1995). Many studies have drawn attention of the toxic effects of plant extracts on related Diptera (Dhar et al., 1996; Cao et al., 2004; Promsiri et al., 2006; Malik et al., 2007). Moreover, the toxic effects of crude extracts of *Z. xanthoxyloides* on storage pests have been documented by Udo et al. (2004) and Owusu et al. (2007).

Observation of the poisoning symptoms of insecticides is not only of practical importance for insect control but can also contribute to the elucidation of mode of action of insecticides. The basis for toxicity by topical application of plant extracts to houseflies has been fairly documented (Malik et al., 2007). Dongdem (1997) had reported the toxic effects of *C. anisata* and *H. spicigera* crude extracts and essential oils on *M. domestica*. Eucalyptol has been documented to be very toxic to male housefly at LD_{50} of 118 µg/fly (Sukontason et al., 2004). Issakul et al. (2004) report the insecticidal effect of *Mammea siamensis* crude extracts on the eggs of housefly.

Seed and root extracts of *G. simplicifolia* were the most active against the housefly when incorporated into the growth medium. They achieved moderate activity with regard to their potency (79 – 60 at doses ≥ 0.25 g of extracts g^{-1} of larval rearing medium). Crude extracts of *Z. xanthoxyloides* were less effective against larvae, pupae and adult housefly. This might suggest that secondary metabolites of *Z. xanthoxyloides* may be highly volatile in larval rearing medium or may act only as contact poison to the fly. Extracts from *G. simplicifolia* act more as stomach poison than contact poison to the fly, therefore affecting their growth and development. Furthermore, the potency of seed extracts of *G. simplicifolia* might also be due to its high oil content and/or to the presence of a class III chitinase – like protein that interact with carbohydrates through direct or water mediated hydrogen bonding or Van der Waals forces, therefore disrupting digestive processes and nutrient assimilation (Zhu-salzman et al., 1998). Seed extracts also induced some morphological abnormalities in larvae, pupae and adult houseflies. The abnormalities included retardation of development of larvae, failure to emerge from the pupal case, and incomplete development of wings in adults that died 12 h after emergence. This may suggest the presence of high juvenile hormone levels in the larvae (Crook et al., 2008) or else due to chemical compounds in the two plants preventing normal pupation and generating adult deformities. Zebitz (1984) reported the anti-ecdysteroid activity of neem seed kernel extract in

Aedes aegypti, resulting in growth inhibition and prolonged developmental period. The benefit of elongation is that housefly larvae numbers are reduced due to longer life cycle and would decrease the vectorial capacity of houseflies. Mohtar et al. (1999) reported an elongation of the pre-imago period of different larval instars of *Ae. Aegypti* when treated with methanol-aqueous extract of *Nerium indicum* leaf at 100 mg/L. Many studies have drawn the attention of plant extracts and essential oils on adult eclosion (Tapondjou et al., 2005; Cetin and Yanikoglu 2006; Kestenholz et al., 2007; Rajendran and Sriranjini, 2008). Crude extracts from *Z. xanthoxyloides* has been documented as suppressing the hatching and reducing the subsequent survival rate of larvae of many stored product insect pests (Udo et al., 2004; Owusu et al., 2007).

Extracts from *G. simplicifolia* and *Z. xanthoxyloides* were repellent to the fly. There was considerable variation in the repellent action of the various plant materials and this may reflect the complexity of the chemical composition of the materials. Bioassay methods used in determining the repellency of extracts against housefly was satisfactory and many flies were trapped after each test period of 30 min. This shows that granulated sugar previously fed on by flies is a good attractant to flies despite the fact that they are known to be less well equipped with organs of smell than related Diptera (Busvine, 1971). Repellents and attractants properties of natural oils and various plant extracts on *M. domestica* have been documented by Braverman and Hogsette (2001). The results of this study indicate good potential for the use of *G. simplicifolia* and *Z. xanthoxyloides* as repellents against *M. domestica*. Although it is more likely that the repellent property of *G. simplicifolia* and *Z. xanthoxyloides* is what is employed when leaves are burned in houses to repel mosquitoes and related biting flies, there is also the possibility of volatile oils playing an important role. Essential oils could not, however, be obtained in substantial quantities from the plants for bioassay.

Aqueous formulations from the two plant species may be used to control housefly eggs and larvae in cattle and poultry manure or as component of washing liquids for cleaning restaurant floors and tables to repel flies and related Diptera in tropical areas. Natural products from *G. simplicifolia* and *Z. xanthoxyloides* could be useful in the search of new larvicidal natural compounds. At present, the toxicity of *G. simplicifolia* and *Z. xanthoxyloides* to man and mammals remains unknown, although other plant – derived materials and phytochemicals have often been shown to be selective towards fish and mammals (Belmain et al., 2001; Promsiri et al., 2006; Isman, 2008). Further assays regarding the effect of these two plant extracts under field conditions should be followed.

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