Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2015; 3(5):443-451 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

An eco-friendly approach to reduce reproductive attributes in the housefly, *Musca domestica* L. using crude plant extracts

Most. Jesmin Aktar[1], M. Saiful Islam[2]*
[1]Assistant teacher, Oriental Seminary English Medium School, Dhaka, Bangladesh
[1,2]Genetics and Molecular Biology Laboratory, Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh.

***Corresponding author**
M. Saiful Islam
Email: saifulzoo.ru@gmail.com

Abstract: As an alternative to chemical insecticides, plant extracts showing insecticidal properties are being increasingly used nowadays in insect pest management programs. With a view to an eco-friendly approach, here we report some bio-active properties of whole-plant boiled extracts of three indigenous plant species *viz.*, *Calotropis procera* (Aiton), *Piper longum* L. and *Polygonum hydropiper* L., in a housefly model. Efficacies of the whole-plant aqueous extracts of three indigenous plant species *viz.*, *Calotropis procera* (Aiton), *Piper longum* L. and *Polygonum hydropiper* L., have been assayed for control of the common housefly, *Musca domestica* L. Using bioassays against the 2nd instar larvae, the estimated LC_{50} values for the three plant extracts were 557.89µL, 981.02µL and 773.27µL, respectively, suggesting *C. procera* as the most effective plant under study. Data on vital reproductive attributes of the experimental flies revealed that the egg-laying and egg-hatch ($P<0.001$), and the numbers of pupae and adults, and female ratios ($P<0.05$) were all reduced significantly by the application of the extracts in housefly culture media, whereas the apparent lengthened larval duration and reduced longevity of the adults were not statistically significant ($P>0.05$). Interestingly, *C. procera* extracts at higher concentrations yielded 21.54% deformed pupae and 11.76% deformed adults which failed to survive. Implications of these findings in terms of developing a plant-based bio-insecticide for *M. domestica* having eco-friendly, safer and economic benefits have been discussed.
Keywords: Bio-insecticides, *Calotropis procera*, *Musca domestica*, *Piper longum*, plant extracts, *Polygonum hydropiper*, reproductive attributes

INTRODUCTION

The common houseflies *Musca domestica* L. (Diptera: Muscidae) are known as one of the most serious pests at human and animal dwellings worldwide [1] which can spread a deadly pathogenic bacterium [2] as well as transmit antibiotic-resistant bacteria [3]. Adult houseflies are mechanical vectors of numerous dreadful diseases including typhoid fever, cholera, amoebic dysentery, diarrhoea, salmonellosis, anthrax and helminthic infections in man [4]. Conventional chemical and synthetic insecticides have been used extensively for many years for controlling this commensal species [5-6]. But the indiscriminate and unregulated uses of such insecticides have adverse effects like development of insect resistance and residual effects on humans, animals and the environment [7-8]. These problems, coupled with acute neuro-toxicity to man and his domesticated animals, have stimulated the search for alternative plant-derived phytochemicals, commonly known as botanicals or bio-insecticides, that have been shown to be valuable for controlling houseflies in their natural habitats [9-13]. This approach could lead to gradually decreased uses of chemical and synthetic insecticides against this important pest species.

A huge number of indigenous plants having medicinal values are available in Bangladesh [14]. Since plant-based bio-insecticides have insecticidal and/or insect repellent activities, species-specific in action, easy to manufacture and apply, and above all, they are relatively safer for animals and their environments [15-16], plant extracts have drawn considerable attention for their uses against various pest species including houseflies [17-20].

Apart from the plant species that are used in the present study, extracts from a number of other plants have been utilized against houseflies. Combined action of *Wedelia calendulacea* extracts and lambda cyhalothrin offered significant synergism, resulting in low LD_{50} value for the insecticide against *Musca domestica* [21] and topical application of eucalypol was found effective in decreasing adult emergence in this species [22]. Extracts of *Annona squamosa* exhibited strong toxicity and inhibition of acetylcholinesterase

(AchE) activity against developmental stages of the houseflies [23]. On the other hand, essential oil extracts of *Haplopappus foliosus* and *Bahia ambrosoides* were promising as a natural insecticide against *M. domestica* [10]. *Azadirachtin indica*, *Conyza aegyptiaca* and *Cichorium intybus* had larvicidal activities [24], leaf oil of *Ocimum suave* caused significant adult mortality [25] and *Artemisia nilgirica* and *Annona squamosa* had widespread activity [18] against this insect. Moreover, crude fruit extracts of *Capsicum annum* [26] and extracts of *Zingiber officinale* [27] exhibited larvicidal activities against houseflies.

A thorough investigation on the bioactivities of the crude extracts of indigenous plant species against larvae and reproductive parameters of the common housefly is lacking up till now in the country. Keeping these in mind, the present study was designed to assess the efficacies of the whole-plant boiled extracts of three plant species *viz*., *Calotropis procera* (Aiton), *Piper longum* L. and *Polygonum hydropiper* L., on larval mortality as well as on some vital reproductive attributes in *M. domestica*.

MATERIALS AND METHODS

Test insects and their mass culture

Collections of the adult houseflies were made from the kitchens of the students' residential halls of the University Campus. The collected flies were colonized in the laboratory in 50cm × 30cm × 25cm rearing cages and procedures described earlier were followed for mass rearing of the test insects [20]. In short, the culture medium was prepared by adding 6g dried full-cream milk powder to 2g yeast in the ratio of 3:1 by weight and dissolving them in 100mL boiled tap water. Cotton pads soaked in the culture medium and placed in 9-cm diameter Petri dishes were used for egg-laying by the gravid females. With the help of a fine paintbrush eggs were collected from the cotton pads and placed those onto the bottom of the 500mL beakers. The larvae were provided with the culture medium until pupation. For adult eclosion, pupae were collected in separate glass vials (*ca*. 25.80cc) to ensure virgin adults. Single-pair mating was practiced for estimating reproductive attributes narrated below.

Whole-plant boiled extracts of the test plants

Collections of *Calotropis procera* (Aiton) (Gentianales: Asclepiadacea), *Piper longum* L. (Piperales: Piperaceae) and *Polygonum hydropiper* L. (Polygonales: Polygonaceae) were made from the adjacent areas of the Rajshahi University (RU) Campus (Plate 1). For future reference, voucher specimens of the three indigenous plants were identified and preserved in the Department of Botany, RU. The whole-plant aqueous extracts were made following the procedures described elsewhere [28-29] with slight modifications. In brief, the collected whole plants were first washed in tap water and dried in the laboratory for about 21 days at 28 ± 2^{o} and 75±5% RH. Then the dried plants were chopped into tiny pieces, powdered with the help of an electric blender, sieved and kept in 250 mL flasks**.** For the preparation of extracts, 100g powder from each plant was put in separate glass container to which 1000mL distilled water was added and boiled until the final volume of each extract was reduced to *ca.* 250 mL. The extracts were then filtered into reagent bottles (Plate 2) and preserved in a refrigerator at 4°C until used. Extracts of 125 μL, 250 μL, 500 μL and 1000 μL were added separately to each 100mL fly culture media where flies were allowed to oviposit and hatch, and larvae were maintained for pupation and adult emergence. For *C. procera*, however, the highest concentration used was 750 μL. Control lines were maintained simultaneously with normal food without plant extracts. All experimental lines were replicated five times.

Larvicidal bioassays against housefly larvae

The bioassays against the 2^{nd} –instar larvae of *M. domestica* using whole-plant boiled extracts were determined in the laboratory at 28 ± 2^{o} C as per the guidelines of the World Health Organization [30]. To sum up, 25 2^{nd}-instar larvae were put into a Petri dish provided with 100mL food soaked in cotton wool. Four replicates were run simultaneously for each concentration, whereas a control group was maintained using distilled water instead of food. By counting the number of dead larvae at 24h intervals up to an exposure period of 72h, the effect of each plant extract on the experimental larvae was evaluated. The lethal effects of the extracts on the housefly larvae was calculated by using the median lethal concentration (LC_{50}) for each extract as reported previously [20-21].

Determination of reproductive attributes in post-treated *M. domestica*

Effects of the various concentrations of the whole-plant extracts on some vital reproductive attributes of *M. domestica viz*., 24h oviposition (fecundity), egg-hatch (hatchability), duration of larval period, pupae formed and adults emerged, female ratio and adult longevity were determined [31-32]. In addition, effect of the higher concentrations of *C. procera* extracts (500-750 μL/mL) on pupal and adult deformities was also monitored and recorded.

Statistical analyses

The raw data collected from each treatment group were processed for preliminary statistics such as means and standard deviations. The median lethal concentration (LC_{50}) values and the corresponding regression lines for the plant extracts were estimated by Probit analysis [33] using a *GWBASIC* software. For the reproductive attributes, ANOVA and Fisher's LSD tests [34] were performed using SPSS for Windows (version 16.0).

RESULTS AND DISCUSSION

Larvicidal bioassays against housefly larvae

Effects of the whole-plant boiled extracts on the 72h mortality in housefly larvae are shown in Table 1. Results clearly indicated that extracts of all three indigenous plants induced lethality in about 50% larvae of *M. domestica* at 557.89µL, 981.02µL and 773.27µL of *C. procera*, *P. longum* and *P. hydropiper*, respectively, suggesting *C. procera* as the most effective plant under study (Figs. 1-3).

Table 1: Estimated LC_{50} values from larvicidal bioassays using whole-plant aqueous extracts against the 2nd instar larvae of *Musca domestica* after 72h exposure

Plants	LC_{50} (µL)	95% confidence limits		Regression equations	χ^2 values (2 df)
		Lower	Upper		
C. procera	557.894	487.427	638.549	Y = -2.139306 + 2.599370 X	0.363ns
P. longum	981.024	668.020	1440.687	Y = 1.277873 + 1.244160 X	0.957ns
P. hydropiper	773.271	620.220	964.092	Y = -0.395198 + 1.867928 X	0.868ns

df= degrees of freedom; ns= not significant

Y = -2.139306 + 2.599370 X

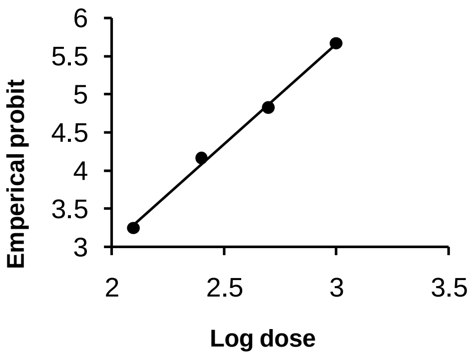

Fig. 1: Probit regression line of *C. procera* on the 2nd instar larvae of *M. domestica*

Y = 1.277873 + 1.244160 X

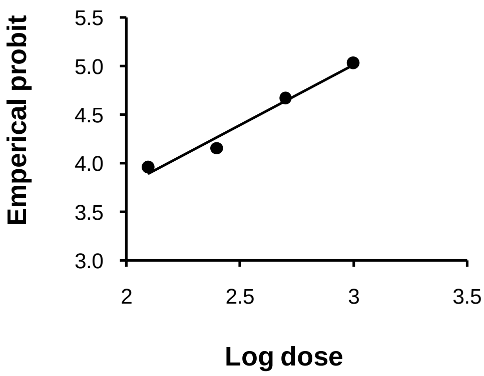

Fig. 2: Probit regression line of *P. longum* on the 2nd instar larvae of *M. domestica*

Y = -0.395198 + 1.867928 X

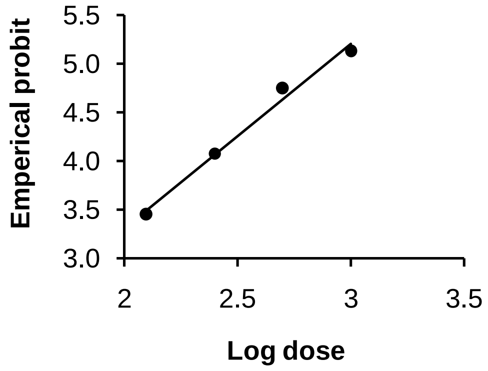

Fig. 3: Probit regression line of *P. hydropiper* on the 2nd instar larvae of *M. domestica*

Reproductive attributes

Oviposition: Twenty four-hour egg-laying by the experimental houseflies on the treated culture media resulted in a significant gradual reduction in egg numbers in a dose-dependent manner in all the three plant extracts (Table 2). Compared to 105-145 eggs in the control lines, around 47, 72 and 50 eggs were produced at the highest concentrations of *C. procera*, *P. longum* and *P. hydropiper* extracts, respectively.

Egg-hatch: Ranging from about 95% to 98% in the controls, egg-hatches in plant extract-treated lines reduced significantly to about 89% in *C. procera*, 97% in *P. longum* and 91% in *P. hydropiper* treatment groups of *M. domestica* (Table 2).

Larval duration: Unlike decreases in both oviposition and egg-hatch percentages, larval duration in the houseflies increased slightly from about 5 days in the controls to about 7-8 days in the highest treatments, indicating a reverse effect of the plant extracts, though non-significant, on this reproductive trait (Table 2)

Pupae formation: The number of pupae formed in the untreated lines ranged between 95 and 138, while those in *C. procera*, *P. longum* and *P. hydropiper* lines were reduced significantly to about 8, 21 and 9, respectively (Table 2). This clearly demonstrated a drastic effect of the plant extracts on pupae formation in *M. domestica*.

Adult emergence: The number of adults emerged in *C. procera* (about 6-41), *P. longum* (about 18-79) and *P. hydropiper* (about 7-48) was much less than those in the controls (about 91-134), which also indicated a pronounced negative impact of the extracts on adult emergence in the experimental houseflies (Table 2).

Female ratio: The female ratios in *C. procera* (0.21-0.45), *P. longum* (0.42-0.44) and *P. hydropiper* (0.37-0.45) differed significantly from those in the corresponding controls (0.47, 0.48 and 0.47, respectively), suggesting also a deleterious effect of the plant extracts on this important reproductive attribute in *M. domestica* (Table 2).

Adult longevity: Adult longevity in the houseflies was not influenced significantly by the plant extracts (Table 2), although the trait was found to fluctuate between 4 and 11 days in the treated groups compared to about 10-11 days in the controls.

Pupal and adult deformities

C. procera extracts at higher concentrations of 500 and 750 µL/mL yielded deformed pupae (n= 14, 21.54%) and adults (n=6, 11.76%) in *M. domestica* which failed to survive (Table 3; Plate 3). Such deformities were recorded neither in *P. longum* nor in *P. hydropiper* treatment lines.

It has clearly been shown by the present experimental findings that the whole-plant boiled extracts of three indigenous plant species induced a pronounced larvicidal effect on *M. domestica* under laboratory conditions. Moreover, the extracts lowered oviposition and hatchability significantly, lingered duration of the larval period, produced fewer pupae and adults, and reduced female ratios in the experimental houseflies. However, longevity of the houseflies was not affected by the treatments, but the higher concentrations of *C. procera* produced lethal deformities in over 10-20% pupae and adults.

Table 2: Effects of whole-plant aqueous extracts of *C. procera, P. longum* and *P. hydropiper* on some reproductive attributes in *M. domestica*

Plants and Doses (µL/100mL)	24h Oviposition	Percent Egg-hatch	Larval Duration (days)	No. Pupae formed	No. Adults emerged	Female Ratios[1]	Adult longevity (days)
C. procera							
Control (0)	104.60±26.81a	95.30±0.81a	4.60±0.54a	95.00±23.18a	91.20±23.72a	0.47±0.05a	10.20±0.44a
125	62.20±7.85b	94.87±2.06a	5.00±0.71a	44.20±4.21b	41.20±3.90b	0.45±0.10b	9.60±0.89a
250	54.00±9.46c	93.76±1.48a	5.80±0.84a	33.60±6.15c	31.40±5.90c	0.43±0.11b	5.80±0.84b
500	47.60±6.30c	94.04±1.87a	7.00±0.70b	14.20±2.28d	12.60±2.07d	0.37±0.10c	4.60±0.54b
750	44.60±5.77c	88.96±3.00b	7.40±0.89b	7.80±1.92e	5.80±1.64e	0.21±0.13d	4.20±0.45b
P. longum							
Control (0)	144.60±18.31a	97.74±1.00a	5.00±0.00a	137.60±17.14a	133.60±18.11a	0.48±0.03a	11.40±0.54a
125	123.20±17.71b	96.92±1.32a	5.20±0.44a	83.60±12.81b	79.20±11.94b	0.44±0.08b	11.00±0.70a
250	110.40±11.32c	97.11±1.07a	5.40±0.54a	58.20±5.80c	54.60±6.54c	0.45±0.10b	6.60±0.54b
500	94.80±15.20d	96.47±1.63a	7.20±0.44b	36.80±7.32d	33.00±6.78d	0.52±0.11c	4.80±0.83b
1000	77.20±8.40e	97.18±0.97a	7.60±0.54b	21.00±2.00e	17.80±2.58e	0.42±0.05b	4.40±0.54b
P. hydropiper							
Control (0)	105.60±18.31a	95.18±0.90a	5.00±0.00a	95.40±20.76a	91.80±19.67a	0.47±0.04a	10.40±0.45a
125	80.80±9.73b	94.44±1.26a	5.40±0.54a	91.40±6.69a	48.20±7.59b	0.45±0.07b	10.20±0.44a
250	71.80±8.16b	93.09±1.63a	6.60±0.54a	38.40±4.15b	36.00±5.09c	0.48±0.09a	6.00±0.70b
500	62.80±7.19c	90.93±3.05a	7.20±0.44b	19.80±4.26c	17.80±4.08d	0.41±0.09c	4.80±0.83b
1000	50.40±1.97c	90.99±1.01a	7.80±0.44b	8.80±2.77d	6.80±2.68e	0.37±0.17d	4.20±0.44b
F-values	24.32***	22.61***	0.90ns	4.52*	4.23*	3.20*	0.46ns

Values are mean ±SD; Dissimilar letters in the same column differ significantly by LSD tests at P<0.05; *= P<0.05; ***= P<0.001; ns= not significant; [1]Number of females ÷ total number of adults; all F-values are at 2, 72 degrees of freedom.

Table 3: Deformed pupae and adults of *Musca domestica* recovered from higher concentration lines of *C. procera*

Doses (µL/100mL)	No. Normal pupae	No. Deformed pupae	Deformed pupae (%)	No. Normal adults	No. Deformed adults	Deformed adults (%)
Control (0)	92	0	0	91	0	0
500-750	65	14	21.54	51	6	11.76

A B C

Plate 1: Indigenous plants used in the experiments; A. *C. procera* (Ait) (Milk weed), B. *P. longum* L. (Long peeper) and C. *P. hydropiper* L. (Marsh pepper).

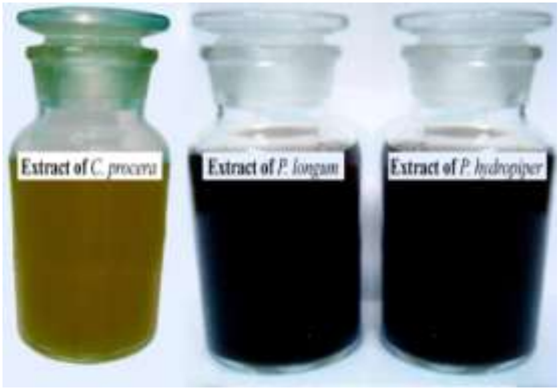

Plate 2: Whole-plant extracts of *C. procera* (left), *P. longum* (middle) and *P. hydropiper* (right) preserved in reagent bottles.

Normal pupae

Deformed and elongated pupae

Adults that failed to emerge

Deformed adult with much reduced wings

Deformed adult with curved wings and deformed legs

Plate 3: Deformed pupae and adults of *M. domestica* recovered from *C. procera* treated fly culture media.

An earlier study showed that the alkaloids, steroids and resinous substances in the latex of *C. procera* induced significant mortality in housefly larvae [35]. Subsequently, LC_{50} values of 870 mg/L for ethanolic seed extracts of *C. procera* [36] and 282.5 mg/L for crude ethanol extracts of the same plant [12] against the 3rd –instar larvae of *M. domestica* were estimated. Dose-dependent adulticidal and repellent activities of *C. procera* against *M. domestica* were also demonstrated [18]. In concert with the present results, however, insecticidal effects of the *C. procera* latex on the larval, pupal and adult stages of *M. domestica,* along with morphogenetic aberrations, were induced [19]. Whereas the methanolic leaf extracts of *C. procera* were shown to possess potentiality against *M. domestica* larvae in their natural habitats [37]. In a recent investigation, synergistic effect of the root extracts of *C. procera* and cypermethrin on the suppression of life-history traits in *M. domestica* has been reported [20]. Moreover, the effects of *Calotropis* have recently been demonstrated by a couple of studies where solvent extracts of the leaves, apical buds and flower of *C. gigantea* at 0.2-0.55 mg/mL showed toxic effects against microbial pest species [38] and *C. procera* and *C. gigantea* were found to possess enormous disease curing potential against various infectious agents such as bacteria, viruses, fungi, protozoans and worms [39]. These results corroborate nicely with the present findings in terms of the efficacy of *C. procera* against housefly reproduction, development and survival.

Reports on different species of *Piper* extracts against dipteran species are also encouraging. Thus, extracts from *Piper nigrum* exhibited synergism with pyrethroid insecticides against *M. domestica* and *Aedes aegypti* larvae [40]. On the other hand, insecticidal activity of the extracts of *P. nigrum* against four species of Coleoptera, three of Lepidoptera and one each of Hemiptera and Diptera, where both crude and semipurified extracts were more toxic than malathion to housefly adults has been demonstrated [41]. Furthermore, fruits of *Piper* species were found to contain phytochemicals that kill mosquito larvae [42]; and adult killing capabilities of three *Piper* species against Asian tiger mosquitoes [43] and adulticidal properties of *P. nigrum* against houseflies and fruit flies [44] have been reported. The insecticidal potentials of the peeper plant extracts were also noted in piperine, the active ingredient of *Piper* species [45] and *P. aduncum* oil was effective against the housefly populations [46]. Moreover, an estimated LC_{50} of 6.2 µg/fly for *P. aduncum* was very effective against adult houseflies [9], whereas *P. nigrum* (LC_{50}=50.1 ppm) worked against housefly larvae [24] and *P. nigrum* (LC_{50}=0.115 µg/insect) showed highest toxicity against *M. domestica* [47]. In recent studies, the combined effects of the root extracts of *P. longum* and cypermethrin were reported detrimental against some life-history traits in *M. domestica* [20] and water extracts of *P. nigrum* on the 2nd-instar larvae of the flesh fly *Sarcophaga haemorrhoidalis* prolonged larval duration, reduced larval weight and increased larval mortality [48]. The above findings lend support to the present data, which are indicative of the fact that plant derived bio-insecticides could be utilized against *M. domestica* in such tropical countries as Bangladesh.

A number of research on *P. hydropiper* revealed that the plant extracts have repellent actions against flies on horse wounds and sores [40], insecticidal potential against the stored grain pest of wheat *Sitophilus granaries* [49] and effective against *Exorista sorbillans*, the notorious parasitoid of the silkworm *Bombyx mori* [50]. Moreover, extracts of *P. hydropiper* were found to compact salivary gland chromosomes of *M. domestica* [51] while extracts of *P. hydropiper* and cypermethrin were effective for suppressing fecundity, hatchability, adult emergence and longevity of houseflies [20]. Recent studies showed that confertifolin from *P. hydropiper* at LC_{50} 243 ppm and 217 ppm may be considered for use in the control of human vector mosquitoes *Anopheles stephensi* and *Culex quinquefasciatus*, respectively [52], and 100% ovicidal, 98.51% oviposition deterrent and 100% adulticidal effects of *P. hydropiper* were demonstrated against the dengue vector mosquito *Aedes albopictus* using 6.25, 12.5, 25, 50 and 100 ppm doses [53]. Apart from minor fluctuations, all the aforesaid data on dipteran pest insects are in good agreement with those reported here for *M. domestica*.

CONCLUSION

Judicious uses of bio-insecticides in the integrated pest management strategies against various pest insects including houseflies may proof beneficial, because these phytochemicals appear to be pro-poor, easy to extract, less expensive, and without adverse effects on non-target organisms. These encourage the crude plant extracts to be an alternative and eco-friendly means for combating harmful insects in developing countries at affordable price. The present results clearly demonstrated the insecticidal properties of the whole-plant boiled extracts of *C. procera* (LC_{50} = 557.89 µL), *P. longum* (LC_{50} = 981.02 µL) and *P. hydropiper* (LC_{50} = 773.27 µL) against some vital reproductive attributes of houseflies. The crude extracts of some selected indigenous plant species may therefore be used for the suppression of *M. domestica* populations instead of using conventional insecticides. However, further studies on the tested plants including active ingredients, their mode of action, and synergism with commonly used insecticides under household as well as field conditions are solicited.

Acknowledgements

This research is a part of doctoral work by MJA, who is grateful to the National Science and Information & Communication Technology (NSICT), Government of the People's Republic of Bangladesh, and Dutch-Bangla Bank Limited (DBBL), for providing her with financial assistance. The authors are thankful to the Chairman, Department of Zoology, University of Rajshahi, Bangladesh, for laboratory facilities and to M. N. Haque, Laboratory Attendant, for technical assistance.

REFERENCES

1. Mündi K; Housefly, an Everyday Monster. Colorado Springs, 1994; Winstar Studios, Co, USA.
2. Sasaki T, Kobayashi M, Agui N; Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of Escherichia coli 0157:H7 to food. J. Med. Entomol. 2000; 37: 945-949.
3. Macovei L, Zurek L; Ecology of antibiotic resistance genes: Characterization of enterococci from houseflies collected in food settings. Appl. Environ. Microbiol. 2006; 72: 4028-4035.
4. Scott JG, Warren WC, Beukeboom LW, Bopp D, Clark AG, Giers SD et al.; Genome of the house fly, *Musca domestica* L., a global vector of diseases with adaptations to a septic environment. Genome Biol. 2014; 15: 466. http://genome biology.com/2014/15/10/466.
5. Cao MK, Song FL, Zhao TY, Dong YD, Sun CHX, Lu BL; Survey of deltamethrin resistance in houseflies (*Musca domestica*) from urban garbage dumps in Northern China. Environ. Entomol. 2006; 35(1): 1-9.
6. Malik A, Singh N, Satya S; House fly (*Musca domestica*): A review of control strategies for a challenging pest. J. Environ. Sci. Health B 2007; 42: 453-469.
7. Acevedo GR, Zapater M, Toloza AC; Insecticide resistance of house fly, Musca domestica (L.) from Argentina. Parasitol. Res. 2009; 105(2): 489-493.
8. Pezzi M, Lanfredi M, Chicca M, Tedeschi P, Brandolini V, Leis M; Preliminary evaluation of

insecticide resistance in a strain of *Musca domestica* (Diptera: Muscidae) from an intensive chicken farm of Northern Italy. J. Environ. Sci. Health B 2011; 46(6): 480-485.
9. Mee KC, Sulaiman S, Othman H; Efficacy of *Piper aduncum* extract against the adult housefly (*Musca domestica*). J. Trop. Med. Parasitol. 2009; 32(2): 52-57.
10. Urzua A, Santander R, Echeverria J, Cabezas N, Palacios SM, Rossi Y; Insecticidal properties of the essential oils from *Haplopappus foliosus* and *Bahia ambrosoides* against the house fly, *Musca domestica* L. J. Chil. Chem. Soc. 2010; 55(3): 392-395.
11. Khater HF; Prospects of botanical pesticides in insect pest management. J. Appl. Pharmaceut. Sci. 2012; 2(5): 244-259.
12. Begum N, Sharma B, Pandey RS; Calotropis procera and Annona squamosa: Potential alternatives to chemical pesticides. British J. Appl. Sci. Technol. 2013; 3(2): 254-267.
13. Kempraj V, Bhat SK; Acetone fraction of *Annona squamosa* seed extract inhibits mitochondrial complex II of *Musca domestica*. Nat. Prod. Chem. Res. 2014; 2(6): 1-4.
14. Ghani A; Medicinal Plants of Bangladesh: Chemical Constituents and Uses. 1998; Asiatic Society of Bangladesh, Dhaka. 238 pp.
15. Belmain SR, Neal GE, Ray D, Golob P; Insecticidal and vertebrate toxicity associated with ethnobotanicals used as post-harvest protectants in Ghana. Food Chem. Toxicol. 1981; 39: 287-291.
16. Bisseleua HBD, Gbewonyo SWK, Obebh-Ofori D; Toxicity, growth regulatory and repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidae). Afr. J. Biotechnol. 2008; 7(24): 4635-4642.
17. Issakul K, Kongtrakoon W, Dheeranupatana S, Jangsutthivorawat S, Jatisatienr A; Insecticidal effectiveness of compounds from *Mammea siamensis* Kost. against *Musca domestica* Linn. ISHS Acta Horticulturae XXVI Int. Hort. Congress: The Future food Medicinal and Aromatic Plants. 2004; 629 pp.
18. Sharma PP, Pardeshi AB, Vijigiri D; Bioactivity of some medicinal plant extracts against *Musca domestica* L. J. Ecobiotechnol. 2011; 3(9): 14-16.
19. Khatter NA; Morphological abnormalities of *Musca domestica vicina* induced by glycosidic groups from Calotropis procera plant. Life Sci. J. 2012; 9(2): 781-788.
20. Islam MS, Aktar MJ; Larvicidal efficacies of some plant extracts and their synergistic effects with cypermethrin on the life-history traits of *Musca domestica* L. Int. J. Innov. Bio-Sci. 2013; 3(3): 92-103.
21. Baki MA, Rahman MAA, Khatune NA, Zahid RA, Khalequzzaman, M, Husain MM, Sadik G; Synergistic effects of *Wedelia calendulacea* Less. plant extracts with lambda cyhalothrin on common housefly *Musca domestica* L. Online J. Biol. Sci. 2002; 2(10: 686-689.
22. Sukontason KL, Boonchu N, Sukontason K, Choochote W; Effects of eucalyptol on housefly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). Rev. Inst. Med. trop. Sao Paulo 2004; 46(2): 97-101.
23. Begum N, Sharma B, Pandey RS; Toxicity potential and anti AchE activity of some plant extracts in *Musca domestica*. J. Biofertil. Biopestici. 2010a; 2(2): 108. doi: 10.4172/2155-6202.1000108.
24. Mansour SA, Bakr RFA, Mohamed RI, Hasaneen NM; Larvicidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, *Musca domestica* L. The Open Technol. J. 2011; 4: 1-13.
25. Ojianwuna CC, Edafemakor AG, Iloh AC; Toxicity of *Ocimum suave* (wild basil) leaf oil on adult housefly (*Musca domestica*). Int. Res. J. Agric. Sci. Soil Sci. 2011; 1(10): 417-420.
26. Jesikha M; *Capsicum annum* larvicidal activity on larval stages of *Musca domestica*. Online Int. Interdisc. Res. J. 2012; 2(6): 9-11.
27. Jesikha M, Lekeshmanaswamy M, Valsalan KCV; Larvicidal effect of *Zingiber officinale* extract on *Musca domestica* L. E-J. Life Sci. 2012; 1(3): 121-125. www.e-journal.in.
28. Inam K, Zahid M, Khan GZ; Toxicity of botanic and synthetic pesticide residues to citrus psyllid *Diaphorina citri* Kuwayama and *Chrysoper lacarnea* (Stephens). Pak. J. Zool. 2012; 44(1): 197-201.
29. Ahmad F, Khan MA, Gul F, Suhail A, Ullah M, Salman M; Evaluation of different plant extracts against citrus psylla (*Diaphorina citri* Kuwayama). Pak. J. Weed Sci. Res. 2014; 20(3): 347-358.
30. WHO (World Health Organization); Guidelines for laboratory and field testing of mosquito larvicides. WHO Pesticide Evaluation Scheme. WHO/CDS/ WHOPES/GCDPP/2005.13. 2005; 39 pp.
31. Islam MS, Khan HS; Changes in reproductive attributes associated with larval rearing density in the housefly, *Musca domestica* L. Univ. j. zool. Rajshahi Univ. 2000; 19: 61-66.
32. Khan HS, Islam MS; Effect of larval rearing density on the heritability of reproductive potential in the housefly, *Musca domestica* L. Bangladesh j. genet. biotechnol. 2005; 6**:** 69-70.
33. Finney DJ; Statistical Methods in Biological Assay. 1978; Charles Griffin, London. 508 pp.
34. Steel RGD, Torrie JH; Principles and Procedures of Statistics: A Biometrical Approach. 1984; McGraw Hill, Tokyo, Japan.
35. Morsy TA, Rahem MA, Allam KA; Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). J. Egypt Soc. Parasitol. 2001; 31(1): 107-110.
36. Begum N, Sharma B, Pandey RS; Evaluation of insecticidal efficacy of *Calotropis*

procera and *Annona squamosa* ethanol extracts against *Musca domestica*. J. Biofertil. Biopestici. 2010b; 1(1)**:** 101.
37. Khatter NA, Abuldahab FF; Insecticidal activity of *Calotropis procera* extracted groups on some biochemical aspects of the house fly, *Musca domestica vicina* (Diptera: Muscidae). J. Am. Sci. 2012; 8(7): 687-693.
38. Mandepudi D, Ravuru BK, Mandepudi B; Experimental investigation for pest resistant properties of *Calotropis gigantea*. Int. J. Pharm. Appl. 2014; 3(3): 380-386.
39. Upadhyay RK; Ethnomedicinal, pharmaceutical and pesticidal uses of *Calotropis procera* (Aiton) (Family: Asclepiadaceae). Int. J. Green Pharm. 2014; 8: 135-146.
40. Jacobson M; Insecticides from Plants- A Review of the Literature, 1954-1971. Agricultural Handbook No. 461. 1975; Agricultural Research Service, USDA. 142 pp.
41. Javier PA, Marallo-Rejesus B; Insecticidal activity of black pepper (*Piper nigrum* L.) extracts. Philippine Entomol. 1986; 6(5): 517-525.
42. Lee HS; Pesticidal constituents derived from Piperaceae fruits. Agric. Chem. Biotechnol. 2005; 48(2): 65-74.
43. Choochote W, Chaithong U, Kamsuk K, Rattanachanpichai E, Jitpakdi A, Tippawangkosol P, Chaiyasit D, Champakaew D, Tuetun B, Pitasawat B; Adulticidal activity against *Stegomyia aegypti* (Diptera: Culicidae) of three *Piper* spp. Rev. Inst. Med. Trop. S. Paulo 2006; 48(1): 33-37.
44. Jensen HR, Scott IM, Sims S, Trudeau VL, Arnason JT; Gene expression profiles of Drosophila melanogaster exposed to an insecticidal extract of Piper nigrum. J. Agric. Food. Chem. 2006; 54(4): 1289-1295.
45. Barbieri Jr. E, Barreto Jr. CB, Ribeiro RC, de Oliveira VH, de Lima FME, Moya-Borja GE; Insecticide effects of natural amides from *Piper* and of the synthetic derivative tetrahydropiperine on *Lucilia cuprina* (Diptera: Calliphoridae) and *Musca domestica* (Diptera: Muscidae). Rev. Bras. Parasitol. Vet. 2007; 16(2): 87-91.
46. Leyva M, Tacoronte JE, Marquetti MC, Montada D; Insecticidal activity of three essential oils from plants on *Musca domestica* (Diptera: Muscidae). Rev. Cubana Med. Trop. 2008; 60(3): .
47. Mansour SA, Bakr RFA, Hamouda LS, Mohamed RI; Adulticidal activity of some botanical extracts, commercial insecticides and their binary mixtures against the housefly, *Musca domestica* L. Egypt. Acad. J. Biol. Sci. 2012; 5(1): 151-167.
48. Asiri BMK; The biological effects of the water extract of *Piper nigrum* (Fam: Piperaceae) seeds on the larvae of *Sarcophaga haemorrhoidalis* (Fallen) (Diptera: Sarcophagidae). Life Sci. J. 2014; 11(7): 444-454.
49. Taleb MA, Salam MA; Efficacy of plant extracts against *Sitophilus granarius* L. of wheat. J. Agric. Rural Dev. Gazipur 2005; 3(1-2): 121-126.
50. Bora D, Khanikor B, Gogoi H; Plant based pesticides: Green environment with special reference to silk worms. In: Pesticides- Advances in chemical and botanical pesticides. Chapter 8, pp.171-206. InTech 2012; http://dx.doi.org/10.5772/ 47832.
51. Prodhan ZH, Biswas M, Rahman M, Islam N, Golam F; Effects of plant extracts on salivary gland chromosomes of house fly (*Musca domestica* L.). Life Sci. J. 2012; 9(4):1930-1935.
52. Maheswaran R, Ignacimuthu S; Bioefficacy of essential oil from *Polygonum hydropiper* L. against mosquitoes *Anopheles stephensi* and *Culex quinquefasciatus*. Ecotoxicol. Environ. Safety 2013; 97(1): 26-31.
53. Maheswaran R, Ignacimuthu S; Effect of *Polygonum hydropiper* L. against dengue vector mosquito *Aedea albopictus* L. Parasitol. Res. 2014; 113(9): 3143-3150.