INT. J. BIOL. BIOTECH., 13 (2): 267-271, 2016.

POTENTIAL VECTOR OF DISEASES: *MUSCA DOMESTICA* L. AND TOXIC EFFECTS OF NEEM EXTRACTS

Habiballah Rana[1,6 *], M. Farhanullah Khan[1], Hina Qasim[5], M. Faheem Akbar[2] , Jahangir Khan Achakzai[3] , M. Sohail Khan[1], S. Naveed Ahmed Hashmi[4] and Tariq Javed[1,4]

[1]*Laboratory of toxicology, Department of Zoology, University of Karachi, Karachi, Pakistan*
[2]*Department of Agriculture & Agribusiness Management, University of Karachi, Pakistan*
[3]*Institute of Biochemistry, University of Balochistan, Quetta, Pakistan.*
[4]*Department of Zoology, Government College for Men, Karachi, Pakistan.*
[5]*Department of Gynecology, Baqai Medical University Hospital, Toll plaza Karachi, Pakistan*
[6]*Department of Zoology, Government Boys Degree College ,Gulzar e Hijri, Karachi, Pakistan.*
*Corresponding author: Tel: + 92 300 7067 397, Email address: drhabibrana@gmail.com

ABSTRACT

Plants are considered an affluent reserve of phyto-chemicals for treating a wide range of diseases and for biopesticides and raw material for controlling the wide range of pests as well. Among them neem occupies a key position, it has been found suitable for carrying easily bio-degradeable and environmental friendly chemicals. Toxic effects of *Azadirachta indicia* A. Juss (leaves extract) on some biological aspects of housefly (*Musca domestica* L.) were tested. Effects of different concentrations (2 µL, 4 µL and 6 µL) on larval duration, pupation, pupal duration, percent adult emergence, longevity and fecundity were determined. Deformed larvae, pupae and adults were recorded. Extract of under test plant were found to have potential effects in controlling the cosmopolitan vector insect.

Key-words:

INTRODUCTION

Housefly *Musca domestica* L. is a common pest of home and farm environments and vector of many infectious diseases around the world (Greenberg *et al*., 1973; Iwasa *et al.*, 1999; Pangnakorn *et al*., 2012). Use of conventional pesticides is considered the short term control of this pest (Cao *et al*., 2006; Malik *et al*., 2007).

Pest control strategies include a wide range of pesticides. Pesticides are very costly and hazardous to the environment. Environmental threats of pesticides usage have encouraged the IPM workers to explore safe and cost effective botanicals (NRC, 2000). Botanicals show promising effects against a wide range of pest, safe for non targeted organisms and environment friendly with little unwanted effects (Koul *et al*., 2009). A wide variety of botanicals has been reported insecticidal action against different stages of housefly (Morsy *et al*., 2001; Sukontason, 2004; Abdelhalim and Morsy, 2006).

In the Present word plants are considered to be the key source of biologically active chemicals used as alternatives to conventional chemicals against a wide range of pest and vector insects (Hashem and Youssef, 1991; Rana *et al.*, 2015). Plant derived products are considered as comparatively non toxic to the biological systems and easily degradeable. Most of the plant derived products are reported for their particular effects to control the harmful insects (Kristensen and Jespersen, 2003). Considering such effects this study was designed to study the larvicidal effects of test plants to control the cosmopolitan vector housefly.

Azadirachta indica A. Juss. products have been extensively used to control a wide range of pests and vectors throughout the world. Even different parts of neem plant have been used in folk medicines to control a wide range of human diseases (Biswas *et al*., 2002). Different parts of the plant showed various effects as extracted in different solvent medium (Mamun-or-Rashid *et al.*, 2014). In view of the potential medicinal and toxic effects of neem, biological effects of different parts of neem were investigated on Housefly.

MATERIALS AND METHODS

Sample Collection and identification

Leaves and berries from *Azadirachta indica* A. Juss. were collected from the Premises of Campus University of Karachi, Karachi, Sindh, Pakistan. Collected samples were identified by taxonomist in the Department of Botany, University of Karachi, Karachi.

Sample preparation

The collected neem leaves and berries were properly washed and air dried at room temperature for twenty days. The dried leaves were minced and ground through a manual grinding machine. Exactly 50g of leaves and berry were dipped in 200ml of 100% alcohol separately and homogenized. After 48 hours supernatants were separated and sediments were further dipped in 150ml 100% alcohol and homogenized. After 48hours again supernatants were separated. Supernatants were filtered and evaporated in evaporating chamber, till it remained 50ml each and put in separate stoppered volumetric flasks.

Experimental Insect rearing

Housefly *Musca domestica* L. were collected from the butcher shop at Faria Chowk, Gulzar-e-Hijri, Gulshan-e-Iqbal Karachi. Collected insects were brought in to the laboratory of Phytopesticide toxicology, MAHQ, Biological Research Center (BRC), University of Karachi. Adult house flies were kept in to the glass manufactured boxes, where ideal laboratory conditioned were maintained for the further experiments.

Statistical Analysis

The obtained data were analysed using Biostat 2009, and the Statistical Package for Social Sciences (SPSS) software for windows (version 13 SPSS inc.).

RESULTS AND DISCUSSION

Data in Table 1 shows that in control group larval duration of housefly larvae was 7.42 ± 0.77 days. Remarkable prolongation was observed when 3rd instar larvae were treated with neem extract 2 µL, 4 µL, and 6 µL doses.

Table 1. Effect of tested plant material on larval duration of *M domestica* 3rd instar larvae, at 29ºC**.**

Treatment	Larval duration (days)			P-value
	Min	Max	Mean	
2µL	6	10	8.42±1.17	0.01
4µL	7	10	8.92±0.99	0.00
6µL	7	11	8.02±0.87	0.00
Control	6	9	7.42±0.77	0.01

Table 2. Effect of tested plant material on Pupation percent, pupal weight and pupal duration of *M domestica* 3rd instar larvae at 29 ºC.

Treatment	% Pupation	% inhibition in Pupation	Pupal weight (mg)	% reduction in Pupal weight	Pupal duration (days)	% change in pupal duration
2µL	87.42±0.77	10.08	16.66±0.77	2.33	6.99±0.13	56.33
4µL	69.23±1.01	29.01	11.23±1.10	7.76	7.99±0.13	66.56
6µL	59.32±0.01	38.80	08.23±0.21	10.76	9.89±0.13	75.99
Control	98.01±2.01	0.00	18.99±2.99	0.00	5.99±1.13	0.00

Data in Table 2 shows significant reduction in the pupation percent under the toxic effects of neem extracts. A high rate of reduction in pupation was observed when larvae were treated with 6µL neem extract. It shows pupation percent decreased as the dose of under test extract increased as compared with the control batches.

Table 3. Effect of tested plant material on Fecundity, % Fecundity, ODI %, Hatchability and % Sterility of *M domestica* 3rd instar larvae at 29 ºC.

Treatmrnt	Fecundity (No of eggs/female)	% Fecundity	% ODI	Fertility (% egg hatched)	% Sterility
2µL	168.22±17.22	84.84	30.00	76.1±2.35	23.9
4µL	101.21±12.11	51.01	67.00	48.3±3.65	51.7
6µL	48.64±5.11	24.24	54.00	21.1±7.72	78.9
Control	198.21±19.11	100.00	0.00	96.1±0.34	0.00

Data in table 3 indicates that when *M. domestica* 3rd instar larvae were treated with different doses of neem extract, a drastic decrease observed in deposited eggs/treated female. Higher dose exhibited huge decrease infecundity of adult females. Similarly a highly decrease in egg hatchability and ultimately percent sterility.

Application of selected doses of under test plant extract exhibited a wide range of morphological abnormalities in pupae and in emerging adults of *Musca domestica.* The main malformations were observed as, larvae failed to pupate, melanized and dark brown pupae, larval pupal intermediated, shrinked and few of them extra elongated pupae, curved pupae emerging adults were deformed failed to emerge fully from the puparium, poorly developed wings, deformed exoskeleton, deformed wings and abdomen.

Neem plant extract affect the growth of target insects in a varieties of ways, which has already been reported by (Namba *et al*., 1971; Ande, 2000; Menzoni *et al.,* 2004). Many plant products have been reported to disturb the normal growth pattern of insects (Assar, 2002 and 2003; Baker *et al.,* 2003; Shaalan *et al.,* 2005).

Pupation ratios in the treated groups were drastically decreased as the concentration of extracts were increased. Similar findings were reported by Khalaf *et al.,* (2009), they observed up to 95.57% decrease when they treated 3rd instar larvae of *Synthesiomyia nudiseta* with the *Cupressus macrocarpa* oil. Extended pupal duration was observed in all treated groups, it was because of the disturbance in normal metamorphosis peocess. Similar findings under plant materials have been reported by (Assar, 2003; Khater and Shalaby, 2008) against *Musca domestica*. Percent adult emergence was also decreased in the under test insects due to the established insect growth inhibitory (IGR) effect of the under test plant extract. These results are an agreement with the findings of Khalaf *et al.,* (2009).

Egg production in the treated groups was also decreased. Researchers have extended various explanations for the reduction of insect fecundity and fertility. Treated insects physically become ill Tripathi *et al.,* (2003), suppression of insect's mating decisive factor subsequently appear as decreased number of laid eggs by the treated insects Engelmann, (1970), Partial sterilization of males of females or the inability of sperm to be transferred in to the female genital organ during copulation Ismail, (1980), Reduction in the viable number of sperms produced by the treated male insect El- Meniawi *et al.,* (1999), Blockage in the ovarian activity or disturbed oogenesis in treated female insects Khan *et al.,* (2007), delayed or reduction in egg retention in ovaries which may be due to the compromised metabolic rate Lucantoni *et al.,*(2006). Dhar *et al.,* (1996) reported that neem extract suppressed the oviposition in mosquitoes.

Deformed adults emerged from the treated groups. Adamski *et al.,* (2005) reported that intensity if deformed adults depended upon the concentration, more deformation was observed under the effects of higher concentration doses. Khalaf *et al.,* (2009) also reported the similar results when he treated the *S. nudist* with the essential oil of *C. macrocarpa,* they observed deformed and pigmented larvae, larval pupal intermediates, C shaped Pupae, elongated and balloon shaped pupae, from most of the pupae adult failed to emerge, emerged adults were suffering in severe degree of morphological deformities and abnormalities. In the present study most of the abnormalities were noticed in the treated groups of under test insects. This indicates that extract has IGR effect. Similar findings against *M. domestica* have also been pointed out by Khalil *et al.,* (2010).

Deformation, melanization and retrogressive effects were observed in the treated groups of *M. domestica*. These effects were reported by Shoukry (1996) when he applied chamomile and jasmine oil on larvae of houseflies. The melanized patches on cuticle were the outcome of inhibition in melanin synthesis during the process of metamorphosis (Gelbic and Nemec, 2001). Malformed winged adults were also obtained. This was under the toxic effect of under test, due to the hormonal misbalance during developmental process (Aly *et al.,* 2010). Josephrajkumar *et al.,* (1999) reported that hormonal misbalance and modification of ecdysteroid titre that consequently change the activity of lysosomal enzymes which in turn cause teratomorphic abnormalities in the treated groups of insects.

Data obtained from the present study showed that application of neem extract prevented the development of houseflies at almost all developmental stages. IGR effects were also noticed in the treated groups. Results suggest

that inhibition in fecundity and fertility, inhibition in larval and pupal stages, formation of larval- pupal intermediates, and reduction in adult emergence exhibit great promise to inhibit and suppress the production of *M. domestica* that act as potential vector of human diseases.

REFERENCES

Abdel Halim, A.S. and, T.A. Morsy (2006). Efficacy of *Trigonella foenumgraecum* (fenugreek) on third stage larvae and adult fecundity of *Musca domestica. J. Egypt. Soc. Parasitol*., 36: 329-334.

Adamski, Z., Niewadzi, M and K. Ziemnicki (2005). Inheritance of chorionic malformations and insecticide resistance by Spodoptera exigua. *J. Appl. Entomol*., 129: 526-533.

Aly, S. A., El Ebiarie, A. S. and K. S. Hamadah (2010). Effects of the wild plant, *Fagonia bruguieri* on the adult performance and phase transition of schistocerca gregaria (orthoptera: acrididae). *Egypt. Acad. J. Biolog. Sci*., 3 (2): 133-147.

Assar, A. A. (2002). Biological and histopathological effects of the water plant extract of oshaar and termis on the larvae of the house fly, *Musca domestica vicina* Macq. (Diptera: Muscidae). *J. Egypt. Ger. Soc. Zool*., 38 (E): 33-51.

Assar, A. A. (2003). Biological effects of the water extract of qataf Atriplex inflate (Chenopodiaceae) on the house fly, *Musca domestica* vicina Macq. (Diptera: Muscidae). *J. Egypt. Ger. Soc. Zool*., 41 (E): 15-28.

Bakr, M. E., N. E. Nassef, A. A. Assar, M. M. El-Sobky and S. A. El Din Shams (2003). Biological and morphological effects of water extracts of some plants on the house fly, *Musca domestica vicina* Macq. (Diptera: Muscidae). *J. Egypt. Ger. Soc. Zool*., 41 (E), 29-47.

Biswas, K.I., R. Chattopadhyay, K. Banerjee and I. Bandopadhyay (2002). Biological Activity and Medicinal Properties of Neem (*Azadirachta indica*). *Current. Science*, 82(11): 1336- 1345.

Cao, M.X., Song, F.L., Zhao, T.Y., Dong, Y.D., Sun, CHX and B.L, Lu (2006). Survey of Deltamethrin resistance in houseflies (*Musca domestica*) from urban garbage dumps in Northern China. *Environ. Entomol*., 35(1): 1- 9.

Dhar, R., Dawar, H., Garg, S., Basir, S. F. and G. P. Talwar (1996). Effect of volatiles from neem and other natural products on gonotrophic cycle and oviposition of *Anopheles stephensi* and An. Culicifacies (Diptera: Culicidae). *J. Med. Entomol*., 33:195–201.

El Meniawi, F. A., Hashem, M., El Mesieri, S. M. and I. A. Rawash (1999). Physiological effects of neem seed extracts on the reproductive activities of the cotton leafworm, *Spodoptera littoralis* Boisd. *Alex. J. Agric. Res*., 44(1): 79 – 96.

Engelmann, F. (1970). *The physiology of insect reproduction*. Pergamon Press, New York.

Gelbič I and V. Němec (2001). Developmental changes caused by metyrapone and azadirachtin in *Spodoptera littorallis* (Boisd.) (Lep., Noctuidae) and *Galleria mellonella* (L.) (Lep., Pyralidae). *J. Appl. Entomol*., 125: 417-422.

Greenberg, B. (1973). *Flies and Disease. Biology and Disease Transmission.* Vol. II. New Jersey: Princeton University Press, USA.

Hashem, H. O., and N. S. Youssef (1991). Developmental changes induced by methanolic extracts of leaves and fruits of *Melia azedrach* L. on the house fly *Masca domestica* Vicina Macq. *J. Egypt. Ger. Soc. Zool.*, 3: 335-352.

Ismail, I. E. (1980). *Physiological studies on the effect of juvenile hormone analogues upon the cotton leafworm Spodoptera littoralis Boisd (Lep., Noctuidae)* . Ph.D. Thesis, Cairo, Univ. Egypt.

Iwasa, M., Maniko, S., Asakura, H., Kobori, H. and Y. Morimoto (1999). Detection of *Escherichia coli* O157:H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. *J Med Entomol*., 36: 108-112.

Josephrajkumar A, Subrahmanyam B and Srinivasan (1999). Plumbagin and azadirachtin deplete haemolymph ecdysteroid levels and alter the activity profiles of two lysosomal enzymes in the fat body of *Helicoverba armigera* (Lepidoptera: Noctuidae). *Eur. J. Entomol*., 96: 347-353.

Khalaf A A, Hussein K T and K. K. Shoukry (2009). Biocidal activity of two botanical volatile oils against the larvae of *Synthesiomyia nudiseta* (Wulp) (Diptera: Muscidae). *Egypt. Acad. J. Biolog. Sci.*, 2 (1): 89-101.

Khalil M S, Assar A A, Abo El Mahasen M M and S. H. Mahmoud (2010). Morphological effects of some insect growth regulators on *Musca domestica*, (Diptera, Muscidae). *Egypt. Acad. J. biolog. Sci*., 2 (2): 29- 36.

Khan M, Hossain M A and M. S. Islam (2007). Effects of neem leaf dust and a commercial formulation of a neem compound on the longevity, fecundity and ovarian development of the melon fly, *Bactocera cucurbitae* (Coquillett) and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Pak. J. Biol. Sci.*, 10: 3656–3661.

Khater, H. F. and A. Abdel-S. Shalaby (2008). Potential of biologically active plant oils to control mosquito larvae (*Culex pipiens*, Diptera: Culicidae) from an Egyptian locality. *Revista do Instituto de Medicina Tropical de São Paulo*, 50(2): 107-112.

Koul, O. and S. Walia (2009). Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. *Pers. Agric. Vet. Sci. Nutri. Nat. Res*. CAB Reviews No. 049.

Kristensen, M. and J. B. Jespersen (2003). Larvicide resistance in *Musca domestica* (Diptera: Muscidae) populations in Denmark and establishment of resistant laboratory strains. *J.Econ. entomol.*, 96 (4): 1300-1306.

Lucantoni, L., F. Giusti, M. Cristofaro, L. Pasqualini, F. Esposito, P. Lupetti and A. Habluetzel (2006). Effects of a neem extract on blood feeding, oviposition and oocyte ultrastructure in *Anopheles stephensi* Liston (Diptera: Culicidae). *Tissue Cell*, 38: 361–371.

Malik A, Singh N and S. Satya (2007). House fly (*Musca domestica*): A review of control strategies for a challenging pest., *J. Environ. Sci.Health*, B. 42: 453-469.

Mamun-or-Rashid, A.N.M., S. Hossain, N. Hassan, B.K. Dash, A. Sapon and M.K. Sen (2014). A review on medicinal plants with antidiabetic activity. *J. of Pharma & Phytochem*. 3(4), 149-159.

Menzoni, P., A. S. Ming, L. Andree, T. H. Zehn and F. Didier (2004). Mutations of acetylcholinesterase which confer insecticide resistance in Drosophila melanogaster population. *B. M. C. Evol. Bio*., 4: 4.

Morsy, T.A., Rahem, M.A. and K.A. Allam (2001). Control of *Musca domestica* third instar larvae by the latex of *Calotropis procera* (Family: Asclepiadaceae). *J. Egypt. Soc. Parasitol*., 31(1),107- 110.

Namba, T., C. T. Nolte, J. Jackrel and D. Grob (1971). Poisoning due to organophosphate insecticides.Acute and chronic manifestations. *Am. J. Med*., 50: 475-492.

NRC (National Research Council) (2000). *The Future Role of Pesticides in US Agriculture*. Board on Agriculture and Natural Resources and Board on Environmental Studies and Toxicology.

Pangnakorn, U., Kanlaya, S. and C. Kuntha (2012). Effect of wood vinegar for controlling on housefly (*Musca domestica* L.). *Int. J. Med. Biol. Sci*., 6: 283-286.

Rana, H., Khan, M.F., Eijaz, S., Akbar, M.F., Achakzai, J.K., Khan, M.S., Ahmed, Z and T. Javed (2015). Quantitative analysis of total protein contents in larvae and adult houseflies (*Musca domestica*) under the toxic effects of azadirachta indica extracts. *Sci.Int*. (Lahore) 27: 4287-4293.

Shaalan, E. A. S., D.V. Canyon, M. W. F. Younes, H. Abdel Wahab and A. H. Mansour (2005). Effects of sub-lethal concentrations of synthetic insecticides and Callitris glaucophylla extracts on the development of *Aedes aegypti*. *J. Vector Ecol*., 30(2): 295-298.

Shoukry, I. F. I. (1996). Morphogenic and histopathological effects induced by two natural volatile oils in the house fly, Musca domestica L. (Diptera: Muscidae). *Proc. 1st Conf. on the role of science in the development of Egypt. Soc. and Environ. Benha branch, Zag*. Univ. 21 – 23.

Sukontason, K.L., N. Boonchu, K. Sukontason and W. Choochote (2004). Effects of eucalyptol on housefly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). *Rev. Inst. Med. Trop. Sao Paulo*., 46(2): 97-101.

Tripathi, A. K., V. Prajapati, S. P. S. Khanuja and S. Kumar (2003). Effect of d-limonene on three stored-product beetles. *J. Econ. Entomol.*, 96(3): 990-995.

(*Accepted for publication March 2016*)