Int. J. Biol. Res., 2(1): 45-49, 2014.

COMPARATIVE TOXICOLOGICAL EFFECTS OF *AZADIRACHTA INDICA* EXTRACTS AND CYPERMETHRIN AND THEIR EFFECTS ON PUPATION AND ADULT EMERGENCE AGAINST *MUSCA DOMESTICA* L.

Habibullah Rana[1*], Muhammad Farhanullah Khan[1], M. Faheem Akbar[2], Zubair Ahmed[1], Khalid Mahmood[3], Saleem Eijaz[4] and Wali Khan[5]

[1]*Laboratory of Toxicology, Department of Zoology, University of Karachi, Karachi, Pakistan*
[2]*Assistant Director Agriculture Extension Sujawal, Pakistan*
[3]*Institute of Biochemistry, University of Balochistan, Quetta, Pakistan*
[4]*Department of Agriculture and Agribusiness Management*
[5]*Vertebrate Pest Control Institute (VPCI), Southern Zone – Agricultural Research Centre (SARC), Pakistan Agricultural Research Council (PARC), Old Block 9&10 Karachi University Campus Karachi–75270, Pakistan*
[*]*Corresponding author e-mail: habibranazoology@yahoo.com*

ABSTRACT

To investigate the effects of cypermethrin and neem extracts a series of concentration i.e., 0.0312%, 0.0625%, 0.125%, 0.25%, 0.5%, 1.0% and 0.04%, 0.08%, 0.16%, 0.32%, 0.64%, 1.285% respectively, were given to houseflies (*Musca domestica*) adults and third instar larvae. Treated larvae were keenly observed and kept separately according to their varying levels of concentrations. The treated larvae were suffering from different teratological effects respective to their increasing concentration levels. The adult female flies were suffering from the effects on reproductive potential. Inhibition in reproduction showed the inhibitory response of the neem extracts in the houseflies. Adult emergence values were changing in accordance with the increase in concentration of neem extracts. The lowest concentration posed very minute effects on adult emergence, whilst comparatively higher doses remarkably decreased the rate of adult emergence. Similarly higher doses of cypermethrin considerably decreased the rate of adult emergence in house flies. LD_{50} values of cypermethrin were 0.0249µg/insec. The LD_{50} values of the extracts of *Azadirachta indica* were found to be 8.0µg/insect. The extracts of *Azadirachta indica w*ere found to be active in terms of insecticidal potential and exhibited pronounced effects on growth, pupation and on adult emergence.

KEYWORDS: Extract concentrations, Tetatological effects, Inhibitory response, *Azadirachta indica, Musca domestica*

INTRODUCTION

House flies are very effective tool for research in laboratories, in the present studies house flies are used as test organism to determine the efficacy of neem plant extracts in comparison with cypermethrin. No doubt pesticides or insecticides are extensively used worldwide today. They show promising efficacy against the pest spp. but they are prominent agent of harming the environment in different ways and provoke resistance in pests (Azmi *et al*., 2001). Similar reports have been published by Naqvi and Tabassum, 1992 and Khan *et al*. (1996), to avoid the environmental threats biologists are working to find out alternate solutions to overcome pest problem with protecting the environment simultaneously. In this regard neem products are being used extensively. To investigate the efficacy of neem extracts many workers have reported the wide range of biological effects on variety of insects. Isman *et al*. (1990), reported neem products are much effective to restrict the rate of fecundity and fertility in insects. Neem products in different concentration are more effective to inhibit the rate of fecundity and fertility in wide range of insect species (Barnby & Klock, 1990; Schmutterer, 1990; Ali Niazee *et al.*, 1997; Ghoneim *et al.*, 2000; Vatandoost & Vaziri, 2004; Zhou *et al.*, 2005). Similar findings have also been reported by Ghoneim & Al-Dali (2002), Quiroga *et al.* (1991), Tanani (2001) and Amer *et al*. (2004). Products derived from the neem extracts have low toxicity against mammals and are short persistence in environment (Nathan *et al.*, 2005; Deota and Upadhyay, 2005).

MATERIALS AND METHODS

Rearing of *Musca domestica*: *Musca domestica L.* was reared in the laboratory of entomology, MAHQ Biological Research Center, University of Karachi. Glass manufactured cages 30 × 30 × 30 cm. were used for the rearing purpose of house flies; front and top of the cages were provided with a 14cm. hole covered with the muslin cloth to serve the insects with food and adequate aeration. Cages were placed at room temperature i.e., 27 ± 2 ºC, 60–70% relative humidity. Adult house flies were fed on soaked cotton pads with sugar and fresh milk, these milk dipped pads were also serving for oviposition sites, after 2-3 days when eggs were visible along the sides of the Petri dishes the cotton pads were shifted to the larval diet containers for the emergence of larvae. The containers of larval diet were kept in separate cages. Larval diet comprised of full cream dry milk, yeast, wheat bran and sufficient water that can give lose texture to the food. The larval medium was served in 500ml beaker. The beaker's was covered with transparent nylon mesh held with a rubber band. Larval medium were observed regularly and pupae were transferred to the empty cages for monitoring the pupae and adult emergence.

Treatment: For the investigation of neem extracts a series of concentration i.e 0.04, 0.08, 0.16, 0.32, 0.64, 1.28 % were given to house flies adults and third instar larvae and a series dilutions of cypermethrine 0.0312%, 0.0625%, 0.125%, 0.25%, 0.5%, 1.0% was given to the house flies adults and third instar larvae. For neem extracts and pesticide treatment topical method was adopted. All the treated and control specimens were reared under the same laboratory conditions. For the treatment of larvae, five replicates (10 larvae /replicate) were entertained. Similarly for the treatment of adult house flies five replicates (10 adult flies /replicate) were entertained.

RESULTS

During the present study 50 live and healthy insects were selected. Tables 1 and 2 showed that LD_{50} value of cypermethrin was 0.0249µg/insect against adult house flies after 24 hours, and it was almost same (0.0249µg/insect) against third instar larvae of house flies after 24 hours. Different concentrations were used to determine the efficacy of cypermethrin 0.0064µg/insect showed 22% mortality and 0.1996µg/insect exhibited 90% mortality against adult and third instar larvae of house flies after 24 hours. Table 5 showed 55% adults were emerged from pupae when third instar larvae of house flies were treated with cypermethrin 0.0124µg/insect. Adult emergence were varying in accordance with varying concentrations of cypermethrin, Adult emergence from pupae was the highest i.e., 81% when larvae were treated with the lowest dose i.e., 0.0064µg/insect and adult emergence was the lowest when larvae were treated with the highest dose i.e. 0.1996µg/ insect. A vast variety of deformities and morphological changes were seen in the emerged adults and larval stages. Some pupae were seen with larval morphology i.e. larval pupal intermediate stage.

Table 1. Effect of cypermethrin against adult house flies after 24 hours.

Conc.%	Dose µg/insect	Insect exposed	Insect died after 24h	Mortality after 24h %
Control	00	50	1	2
Check	100 % alcohol	50	1	2
0.0312	0.0064	50	14	20
0.0625	0.0124	50	16	32
0.125	0.0249	50	26	52
0.25	0.0499	50	28	56
0.5	0.0998	50	33	66
1.0	0.1996	50	45	90

Table 2. Effect of cypermethrin against third instar larvae of house flies after 24 hours.

Conc.%	Dose µg/insect	Insect exposed	Insect died after 24h	Mortality after 24h %
Control	00	50	1	2
Check	100 % alcohol	50	1	2
0.0312	0.0064	50	11	22
0.0625	0.0124	50	17	34
0.125	0.0249	50	26	52
0.25	0.0499	50	29	58
0.5	0.0998	50	34	68
1.0	0.1996	50	45	90

Tables 3 and 4 showed that LD_{50} value of neem extracts was 8.0µg/insect against adult and third instar larvae of house flies after 24 hours. Different concentrations were used to determine the efficacy of neem extracts. 2.0µg/insect showed 22% mortality and 64µg/insect exhibited 94% mortality against adults whilst 2.0 µg/insect showed 20% mortality and 64 µg/insect exhibited 92% mortality against third instar larvae of house flies after 24 hours. Table 6 showed that 51.28% adults were emerged from pupae when third instar larvae of house flies were treated with neem extracts 4.0µg/insect. Adult emergence were varying in accordance with varying concentrations of neem extracts, adult emergence from pupae was the highest i.e., 77% when larvae were treated with the lowest dose i.e., 2.0µg/insect and adult emergence was the lowest when larvae were treated with the highest dose i.e., 64µg/ insect. Some adults emerged from pupae were morphologically changed; some pupae found failed to emerge, some adults were found short in size.

Table 3. Effect of neem extracts against adult house flies after 24 hours.

Conc.%	Dose µg/insect	Insect exposed	Insect died after 24h	Mortality after 24h %
Control	00	50	1	2
Check	100 % alcohol	50	1	2
0.04	2	50	13	22
0.08	4	50	18	36
0.16	8	50	26	52
0.32	16	50	31	62
0.64	32	50	36	72
1.28	64	50	47	94

Table 4. Effect of neem extracts against third instar larvae of house flies after 24 hours.

Conc.%	Dose µg/insect	Insect exposed	Insect died after 24h	Mortality after 24h %
Control	00	50	1	2
Check	100 % alcohol	50	1	2
0.04	2	50	13	20
0.08	4	50	18	36
0.16	8	50	26	52
0.32	16	50	30	60
0.64	32	50	35	70
1.28	64	50	46	92

Table 5. Effect of cypermethrin on pupation and emergence of adult house flies.

Conc.%	Dose µg/insect	No. of Larvae developed in to pupa	No. of pupae emerged as adult	% of emerged adults
Control	00	50/50	48/50	96
Check	100 % alcohol	48/50	47/48	97.9
0.0312	0.0064	45/50	30/45	81
0.0625	0.0124	40/50	22/40	55
0.125	0.0249	31/50	14/31	45
0.25	0.0499	14/50	4/14	28
0.5	0.0998	7/50	1/7	14
1.0	0.1996	4/50	0/4	00

Table 6. Effect of neem extracts on pupation and emergence of adult house flies.

Conc.%	Dose µg/insect	No. of larvae developed in to pupa	No. of pupae emerged as adult	% of emerged adults
Control	00	50/50	48/50	96
Check	100 % alcohol	49/50	47/49	95.9
0.04	2	44/50	27/44	77
0.08	4	39/50	20/39	51.28
0.16	8	30/50	12/30	40
0.32	16	13/50	3/13	23
0.64	32	6/50	1/6	16.6
1.28	64	3/50	0/3	00

Rate of emergence of adults was comparatively low because larvae were treated with either with cypermethrin or neem extracts. The effects of both cypermethrin and neem extracts were exclusively dose dependent. The results suggest that neem extracts pose same prominent effects on insect's growth, pupation and adult emergence as the most powerful synthetic pesticide cypermethrin do. Considering the facts cited in table 1 to 6 it may be concluded that neem extracts are still a good alternate choice for maintaining the productivity in the fields without harming the environment.

DISCUSSION

Control of house flies is very necessary to save human population from many fatal diseases like typhoid, tuberculosis etc. (Service, 1980). To study the different aspects and varying control measures house flies has been a routine research work tool in the laboratories (Gunjima & Saito, 1992; Keiding, 1995). Pupation and adult emergence in many insects have been found altered by treating the larvae with plant extracts (Shalaby *et al*., 1997; Mohammed *et al*., 2000; El-Sheikh, 2002; Hewady *et al*., 2002). In the present study neem extracts inhibited the rate of pupation and adult emergence of when larvae were treated with different concentrations of neem extracts. Similar results were recorded by Kippal-Singh & Sharma (1987), Ayyangar and Rao (1991), Dai *et al*. (2005), Senthil *et al*. (2005) and Hassan (2002). In the present study pupation and adult emergence was decreased as the concentration of neem extract was increased with treatment of 3rd instar larvae. Similar effects have been reported by Khalaf *et al*. (2009), Ande (2001), Assar (2002), (2003). Pupal duration becomes extended after treatment (Assar, 2002, 2003). Sometimes darker puparium were observed because of the treatment of neem extracts (Khalil *et al*., 2010). In the present investigations some pupae failed to attain the adult stage, similar results were reported by Naqvi *et al.* (2007). Some adults failed to attain wings. Similar findings were reported by Aly *et al*. (2010). These results are agreement with the results of present studies. Present results suggest that the neem extracts can be used to control the house flies by treating the different developmental stages, consequently the emerging female adults will not be able to lay sufficient eggs.

REFERENCES

Ali Niazee, M.T., A. Al-Humeyri and M. Saeed. (1997). Laboratory and field evaluation of a neem insecticide against *Archips rosanus* L. (Lepi.: Tortricidae). *Canadian Entomol*., 129: 27-33.

Aly, S.A., A.S. El-Ebiarie 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.

Amer, M.S., K.S. Ghoneim, A.G. Al–Dali, A.S. Bream and K.H.S.H. Hamadah. (2004). Assessment of the bioactivity of Margosan – O and Jojoba against the house fly *Musca domestica* (Diptera: Muscidae). *Al–Azhar Bull. Sci*., 15: 9-24.

Ande, A.T. (2001). Biological activities of some plant materials against the housefly – *Musca domestica*. *Nigerian Soc. for Experimental Biol. J*., 1(4): 293-296.

Assar, A.A. (2002). Biological and histopathological effects of the water pant 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.

Ayyangar, G.S.G. and P.J. Rao. (1991). Carryover effects of azadirachtin on development and reproduction of *spodoptera litura* (Fabr). *Annu. Rev. Entomol*., 9: 55-7.

Azmi, M.A., S.N.H. Naqvi and K. Akhtar, I. Ahmed, S.A. Rizvi and S. Rais. (2001). Determination of toxicity of DDVP and cypermethrin on *Collosbruchus analis.* F and their effect on alkaline phosphatase and choinesterace activity. *Proc. Pak. Congr. Zool*., 21: 289-293.

Barnby, M.A. and J.A. Klocke. (1990). Effect of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in *Heliothis virescens* (Fabr) larvae. *J. Insect Physiol.*, 36: 125-31.

Dai, J., Z. Huag and D. Jiawei. (2005). Bioactivity of azadirachtin and its field efficacy against *Spodoptera litura*. *Ying Yong Sheng Tai Xue Bao*, 16: 1095-8.

Deota, P.T. and P.R. Upadhyay. (2005). Biological studies of azadirachtin and its derivatives against polyphagous pest, *Spodoptera litura*. *Nat. Prod. Res*., 19: 529-39.

El-Sheikh, T. (2002). Effect of application of selected insect growth regulators and plant extracts on some physiological aspects of the black cutworm, *Agrotis ipsilon* (HUF.). *Ph.D. Thesis*, Faculty of Science Ain Shams University, Cairo, Egypt.

Ghoneim, K.S., H.A. Mohamed and A.S. Bream. (2000). Efficacy of the neem seed extract, Neem Azal, on the growth and development of the Egyptian cotton leafworm, *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae). *J. Egypt Ger. Soc. Zool*., 33: 161-79.

Ghoneim, K.S. and A.G. Al-Dali. (2002). Survival and reproductive responses of *Muscina stabulans* Fallen (Muscidae: Diptera) to the neem seed extracts, Margosan-O. *Second International Conference of Plant Protection Research Inst., Cairo, Egypt*, 21-24.

Gunjima, K. and K. Saito. (1992). Oral toxicities of some pyrethroids to the house fly, *Musca domestica* L. (Diptera: Muscidae). *Appl. Entomol. Zool*., 27: 319-24.

Hassan, H.A. (2002). Biological and biochemical studies on the effects of some botanical extracts on cotton leaf worm *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae). *M. Sc. Thesis*, Faculty of Science, Ain Shams University, Cairo, Egypt.

Hewady, M.A.A., M.A. Kandell, A.A. El-Sayed and A.M. Rashad. (2002). Biological and biochemical effects of NeemAzal T/S on pink bollworm, *Pectinophora gossypiella* (Saunders) (lepidoptera: Gelechidae). *J. Egypt Acad. Soc. Environ. Develop. Entomol.*, 2: 53-65.

Isman, M.B., O. Koul, A. Lucznsk and J. Kaminski. (1990). Insecticidal and antifeedant bioactivies of neem oil and their relationship to azadirachtin. *J. Agric. Food Chem*., 38: 1406-11.

Keiding, J., (1995). Lessons provided by the house fly on evaluation of resistance (R) to insecticides. *Phytoparasitica*, 23: 97-100.

Khalaf, A.A, K.T. Hussein 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., A.A. Assar, M.M. Abo El-Mahasen 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.F., S.N.H. Naqvi, M.A. Azmi, M.I. Khan and S.S. QadriS. (1996). DDT residues on apple after spray for codling moth control. *Pak. J. Entomol. Kar*., 11: 21-22.

Kippal-Singh and P.L. Sharma. (1987). Evaluation of antifeedant and repellent qualities of various neem (*Azadirachta indica)* formulations against (*Pieris brassicae* L.) larvae on cabbage and cauliflower. *Res. Develop. Repor*., 4: 76-8.

Kocisova, A. (2001). The stability of resistance in a field house fly population, Musca domestica, over 60 generations is following the interruption of insecticides selection pressure. *Czech. J. Nim. Sci*., 46: 281-8.

Mohamed, H.A., A.S. Bream and K.S. Ghoneim. (2000). Developmental and reproductive responses of the house fly *Musca domestica* (Diptera: Muscidae) to the neem seed extract, Neem Azal. *J. Egypt Ger. Soc. Zool*., 31: 235-49.

Nathan, S.S., K. Kalaivani and K. Murugan. (2005). Effects of neem limonoids on the malaria vectors *Anopheles stephensi* Liston (Diptera: Culicidae). *Acta Trop*., 96: 47-55.

Naqvi, S.N.H., R. Tabassum, M.F. Khan, N. Yasmin, S.M. Nurulain and A.A. Burney. (2007). Toxic, residual, and teratomorphic effect of a neem extract (N-9) in comparison to Coopex 25 WP (Permethrin + Bioallethrin) against *Musca domestica* L. (Holland strain). *Turk. J. Zool*., 31:127-130.

Naqvi, S.N.H. and R. Tabassum. (1992). Probable development of resistance against neem extract (RB-A) and cyfluthrin (solfac 10% E.C.) in *Musca domestica* L. (PCSIR Strain). *Pak. J. Entomol. Kar*., 7: 9 -16.

Parkman, P. and R.L. Pienkowski. (1990). Sublethal effect On Neem seed extract on adults of *Liriomyza trifolii* (Diptera: Agromyzidae). *J. Econ. Entomol*., 83: 1246-9.

Quiroga, D., P. Arretz and J.E. Araya. (1991). Sucking insects damaging jojoba, *Simmondsia chinesis* (Link) Schneider and their natural enemies in the north central and central regions of Chile. *Crop Port.*, 10: 469-72.

Schmutterer, H. (1990). Properties and potentials of natural pesticides from the neem tree, *Azadirachta indica. Annu. Rev. Entomol*., 35: 271-97.

Senthil, N.S., K. Kalavani, K. Sehoon and K. Murugan. (2005). The toxicity and behavioural effects of neem limonoids on *Cnaphalocrocis medinalis* (Guenee), the rice leaffolder. *Chemosphere*, 62: 1381-7.

Service, M.W. (1980). *A Guide to Medical Entomology*, pp 226. MacMillan London.

Shalaby, M.A.M., A.M. Rashad, S.M. El-Gengaihi, H.M. Motawe, A.A. El-Saeady and A.I. Gadallah. (1997). Effect of *Melia azedarach* seed extract on *Pectinophora gossypiella* (Saund) and *Earias insulana* (Boisd.). *Bull. Entomol. Soc. Egypt (Econ. Ser.)*, 242: 127-42.

Tanani, M.M.A. (2001). *Study on the effects of certain IGRs and plant extracts on some physiological aspects of the red palm weevil Rhyncophorus ferrugineius (Curculionidae: Coleoptera*). *M. Sc. Thesis*, Faculty of Science, Al-Azhar University, Cairo, Egypt.

Vatandoost, H. and V.M. Vaziri. (2004). Larvicidal activity of a neem tree extract (neemarian) against mosquito larvae in the Islamic Republic of Iran. *East Mediterr. Health J.*, 10: 573-81.

(Received December 2013; Accepted March 2014)