QUANTITATIVE ANALYSIS OF TOTAL PROTEIN CONTENTS IN LARVAE AND ADULT HOUSEFLIES (*MUSCA DOMESTICA*) UNDER THE TOXIC EFFECTS OF *AZADIRACHTA INDICA* EXTRACTS.

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ABSTRACT: *Crude Methanolic extracts from leaves and berry of nine plants (Azadirachta indica) were used in larvicidal biases against 3rd instar larvae and adults of housefly (Musca domestica) for 24hour exposure. Concentrations of crude extracts were 0.04%, 0.08%, 0.16%, 0.32%, 0.64% and 1.28%. LD_{50} of neem leave extract 0.7546 and 0.7404 for neem berry were recorded. Toxic effects under neem leave extract on total protein contents and mean density against 3rd instar larvae were 2.93cm and 1.70cm and 3.13cm, 2.10cm length and width wise were determined respectively. Obtained results were compared with control quantities which were promising indicators to suggest the neem plant extracts exhibit much effective insects growth regulator properties to serve as environment friendly pest insects controlling agents.*

Keywords: Larvicidal, Crude extracts, Growth regulators, Controlling agents, Environment friendly.

INTRODUCTION

Chemical pesticides are costly and much hazardous to the environment. Researchers aimed to seek economical and environmental friendly botanical extracts as pest control alternates [1]. Number of reports has been added to verify the activity of botanical products against the wide range of pests as the alternatives of synthetic chemical pesticides in the integrated pest management strategies [2]. In the literature many plants have been reported for the source of extracts and compounds for the promising insecticidal actions against a variety of pests [3-5]. In the developing countries plants have attained an excellent position in the pest management programs [6].

House fly found almost all over the world and considered as an excellent research material. It has close association with man and its environment. They also have much close association with poultry farms cattle farms, horse stables and on the heaps of garbage because they feed and reproduce on waste materials. Many reports have been published to show the toxic effects of many plant extracts against housefly [7]. Selected methanolic neem extracts against the test organism suggest that under test extracts could be used as the impending alternative to the conventional pesticides [9-10]. Crude extracts of different parts of *Calotropis procera* [8-13], *Piper* species [14-18] and *Polygonium hydropiper* [19-21] have been reported to control the dipterous flies. Among plant extracts neem extracts show promising effects to reduce feeding, reproduction and survival of pests, therefore, have been used to control the household and agricultural pests and against housefly as well.

Here we have reported the efficacy of crude extracts of different parts of neem plant that exhibited the larvicidal effects and pronounced effects on total protein contents of *Musca domestica*. These investigational evidences recommend that there is a ample scope to use plant based pesticides against different pests, because these crude extracts appear to be much potent, environment friendly, easily degradable, cost effective and much affordable tool for pest control strategies in the developing countries.

MATERIALS AND METHODS

Rearing of houseflies:

Houseflies were reared in the laboratory of entomology, MAHQ Biological Research Center, University of Karachi. For rearing insects, all the four sides mesh provided wooden cages 40 × 40 × 40 cm. were used. 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. Lab environment was maintained at 27±2º C, 60-70% relative humidity. Adult house flies were fed sugar and fresh milk while, larval diet comprised of full cream dry milk, yeast, wheat bran and sufficient water that can give lose texture to the food.

Experimental design:

Experiments were conducted on third instar larvae and adults of houseflies. Houseflies were reared in the laboratory as described in the rearing technique. For carrying out the experiments neem leave extract and neem berry extract five concentrations of each sample were used for LD_{50} determination. Contact method was applied for the treatment of larvae and adults of houseflies. A control batch (with no treatment) was kept as reference with each assay. The treated insects were left for 24 hours for the evaluation of toxic effects and for the determination of lethal dose concentration alive insects were kept for the observation and for furthering the investigations.

Statistical Analysis:

All tables contain concentration of compound, mean mortality, standard deviation, standard error and 95% confidence limit. Observed mortality was corrected through Abbot's formula. SPSS and Biostat 2009 software were used to analyze the mortality data. Solutions of selected samples were prepared in different concentrations by the help of charle's equation($C_1V_1= C_2V_2$). Density of gel was measured by software GelQuantNet.

RESULTS

Determination of toxicity:

Adults and third instar larvae of houseflies were treated for the determination of toxic effects of neem leave and neem berry extracts. Series of concentrations (0.04%, 0.08%, 0.16%, 0.32%, 0.64% and 1.28%) was prepared and applied. After 24 hours of treatment percent mortality was observed. Data values were statistically analyzed, quantitative estimation of total protein contents and electrophoretic mobility of different proteins were also analyzed.

Toxic effects of berry and neem leave extracts against houseflies:

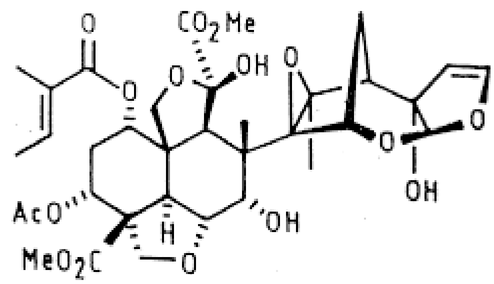
For determination of toxicity, neem leave and neem berry extracts were applied. Mean mortality after 24-hours were found to be 20.33%, 34.66%, 51.11%, 60.11%, 71.33% and 91.11% under the toxic effects of neem leave extract 0.04%, 0.08%, 0.16%, 0.32%, 0.64% and 1.28% were observed respectively (Table-1). LD_{50} and mean mortality values by probit analysis (Table- 2) were found to be 0.7546 and p value < 0.5. Concentration of neem leave extract, 0.2323% showed 22% mortality, while 0.8363% concentration caused 91% mortality. For the determination of toxicity of neem berry extract same doses were applied and mortality were observed as 19.66%, 33.33%, 50.33%, 60.33%, 71.00% and 89% respectively, table-3. Concentration of neem leave extract, 0.1881 showed 20% mortality while, 0.8730 caused 92% mortality, table-4. Probit analysis showed LD_{50} 0.7404 and p value < 0.9.

Effect of neem leave and berry extracts on total protein contents of houseflies:

Through the densitogram total protein contents in houseflies larvae under toxic effects of neem leave extract were found to be mean density lengthwise 2.93cm and widthwise 1.70cm. While, in the case of untreated/control batch total protein contents were found to be lengthwise mean density 2.50cm and widthwise 0.90cm, table-5. In adults effects of neem leave extract were found to be mean density lengthwise 3.13cm and widthwise 2.10cm. While, in the case of untreated/ control batch total protein contents were found to be lengthwise mean density 2.83cm and widthwise 1.10cm, table-6.

DISCUSSION

Toxic effects of neem leave and neem berry extracts have been studied against *Musca domestica* 3rd instar larvae and adults. Extracts had shown potential larvicidal and insecticidal effects against the under test organisms. LD_{50} (neem leave extract 0.7546 and 0.7404 for neem berry extract) of selected methanolic neem extracts against the test organism suggest that under test extracts could be used as the impending alternative to the conventional pesticides [9-10]. Larvicidal and adulticidal effects of extracts also showed interrupted growth pattern and morphogenetic aberrations in the housefly larvae [6, 22], that ensure it to be the credible option for the development bioinsecticidal control of dipterous flies and a variety of pests as well [23]. The extracts exhibited the various interrupted metabolic activities of the larvae, in the result larvae failed to feed and ultimately development was under arrest in various instar stages [24]. Azadirachtin is a well known naturally occurring insecticide [25].



Azadirachtin

In this study total protein density in treated and untreated *Musca domestica* showed that total protein contents were influenced by neem extracts and were remarkably lowered in the both NL(L) and NL(A) cases, this reduction in density was because of the effects of azadirachtin present in the extracts. This sort of lowering effect in total protein contents were reported in the desert locust by Annadurai [26]. Li, et al, (1995) [27] suggested that under the effects of azadirachtin insect modifies their capacities of protein synthesis. As a reference Paranagama, et al, (1993) [28] reported that presence of [22, 23-3 H2] dihydroazadirachtin as a trace of azadirachtin in the locust fat tissues. Another study suggested that azadirachtin reduces the protein synthesis that may appear the result of abundance of polypeptides [29]. Present study indicates that there is a potential interaction of neem extracts on the synthesis of protein pattern of insects. There are some known groups of chemical compounds are present in the extract which directly or indirectly influence the receptors of insects which ultimately cause a major damage to the growth pattern and development of insects [30-31].

CONCLUSION

On the bases of findings plant extracts may be considered as safe, environment friendly and cost effective alternative of conventional pesticides. In addition, present findings encourage the control of dipterous flies by using plant extracts as well.

Table- 1: Toxicity of neem leave extract against third instar larvae of houseflies.

Conc.% of compound	Doses µg/insect	Mean mortality	Standard deviation	Standard error	Range at 95% confidence limit	
Control	0000	2.0000	0.5400	0.1200	01.11	04.00
0.04	2	20.3333	1.52753	0.88192	15.5388	23.1279
0.08	4	34.6667	2.51661	1.45297	26.4151	38.9183
0.16	8	51.1101	1.00000	0.57735	47.5159	52.4841
0.32	16	60.1101	2.00000	1.15470	54.0317	63.9683
0.64	32	71.3333	1.52753	0.88192	56.5388	64.1279
1.28	64	91.1111	1.00000	0.57735	87.5159	91.4841

Table- 2: Probit analysis and mortality data of 3rd instar larvae of houseflies under the toxic effects of neem leave extract.

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent(%)	Insects exposed	Insects killed	E(R)	Difference	Chi-square	Probit (Y)
-1.39794	0.22	0.232363	100	22	23.23635	1.23635	0.065783	4.2280
-1.09691	0.37	0.348653	100	37	34.86525	2.13474	0.130707	4.6685
-0.79588	0.53	0.481322	100	53	48.13215	4.86784	0.492310	5.0750
-0.49485	0.59	0.616113	100	59	61.61127	2.61127	0.110673	5.2271
-0.19382	0.63	0.738072	100	63	73.80716	10.8072	1.582430	5.3314
0.10721	0.91	0.836342	100	91	83.63422	7.36577	0.648713	6.3409

Table- 3: Toxicity of neem berry extract against third instar larvae of houseflies.

Conc.% of compound	Doses µg/insect	Mean mortality	Standard deviation	Standard error	Range at 95% confidence limit	
Control	0000	1.0000	0.00000	0.00000	1.0000	1.0000
0.04	2	19.6667	1.15470	0.66667	16.7982	22.5351
0.08	4	33.3333	0.57735	0.33333	31.8991	34.7676
0.16	8	50.3333	1.15470	0.66667	47.4649	53.2018
0.32	16	60.3333	0.57735	0.33333	58.8991	61.7676
0.64	32	71.0000	1.00000	0.57735	68.5159	73.4841
1.28	64	92.0000	1.00000	0.57735	86.5159	92.4841

Table- 4: Probit analysis of mortality data of 3rd instar houseflies larvae under the toxic effects of neem berry extract.

Log10[Dose (Stimulus)]	Actual Percent (%)	Probit Percent (%)	Insects exposed	Insects killed	E(R)	Difference	Chi-square	Probit (Y)
-1.39794	0.2	0.188135	100	20	18.81354	1.186459	0.074823	4.158543
-1.09691	0.32	0.315724	100	32	31.57243	0.427568	0.00579	4.532725
-0.79588	0.48	0.470271	100	48	47.02705	0.972946	0.020129	4.94998
-0.49485	0.6	0.629492	100	60	62.94925	-2.94925	0.138176	5.252933
-0.19382	0.72	0.769016	100	72	76.90157	-4.90157	0.312417	5.582477
0.10721	0.92	0.873004	100	92	87.30043	4.699575	0.252988	6.405322

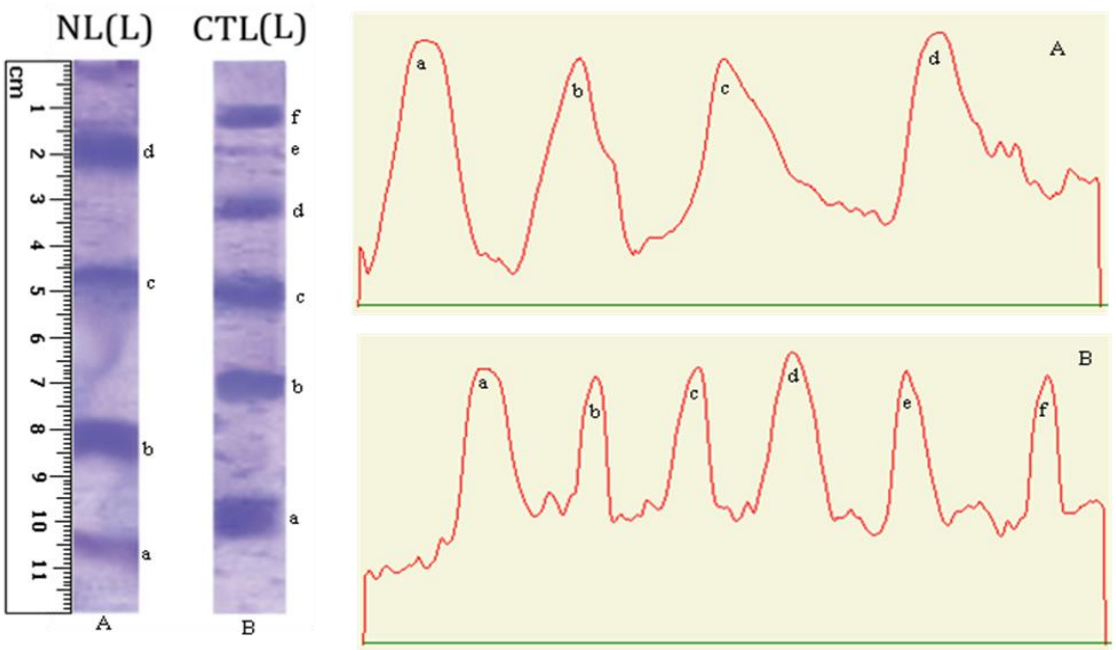
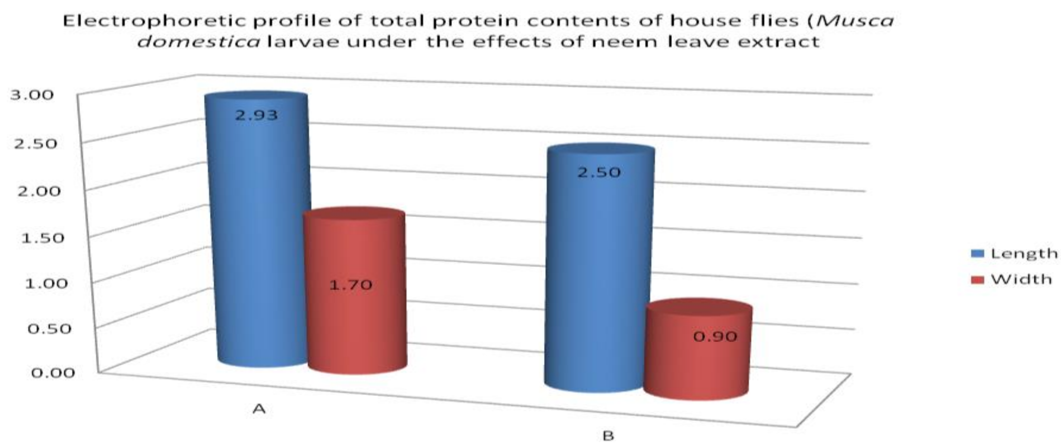


Figure-1:Densitogram uder the effects of neem leave extract on total protein pattern and electrophoretic profile of third instar houseflies (*Musca domestica*) larvae. (NL: neem leave extract, L: larvae, CTL: control)

Table-5: Effects of neem leave extract on total protein pattern and electrophoretic profile of third instar houseflies (*Musca domestica*) larvae. (NL: neem leave extract, L: larvae, CTL: control)

Gel Mark	Density	Peak a	Peak b	Peak c	Peak d	Peak e	Peak f	TOTAL density	Mean density
NL(L){A}	Length(cm)	3.8	3.3	2.5	2.1	-	-	11.70	2.93
	Width(cm)	1.7	1.7	2.1	1.3	-	-	6.80	1.70
CTL(L){B}	Length(cm)	2.5	2.4	2.4	2.7	2.6	2.4	15.00	2.50
	Width(cm)	1.1	0.8	0.8	1.1	1.0	0.6	5.40	0.90



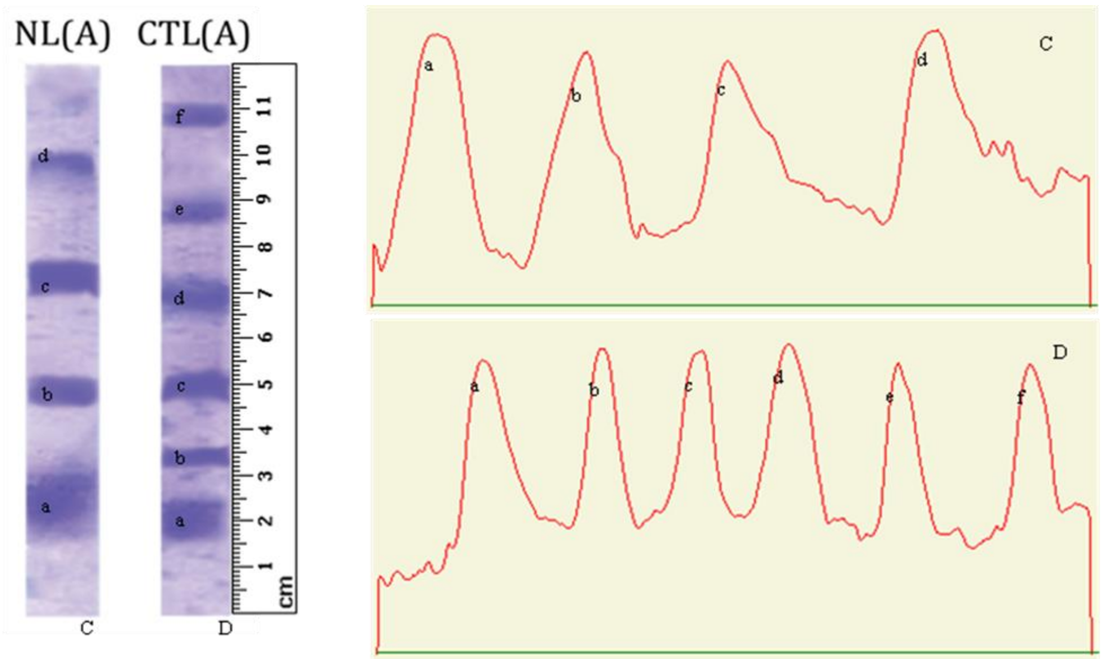
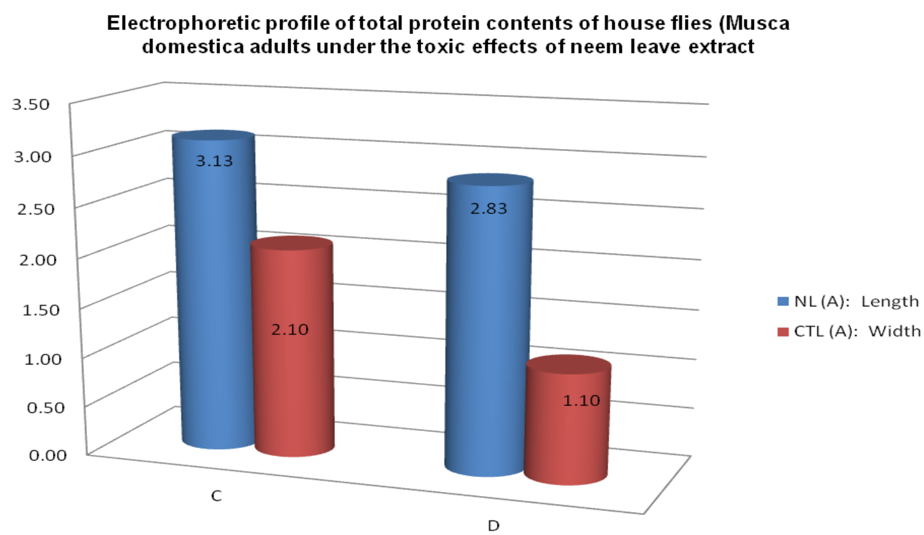


Fig-2: Densitogram under the effects of neem leave extract on total protein pattern and gel electrophoretic profile of adult houseflies (*Musca domestica*). (NL: neem leaves, A: adults, CTL: control).

Table-6: Effects of neem leave extract on total protein pattern and gel electrophoretic profile of adult houseflies (*Musca domestica*). (NL: neem leaves, A: adults, CTL: control).

Gel Mark	Density	Peak a	Peak b	Peak c	Peak d	Peak e	Peak f	TOTAL density	Mean density
NL(A){C}	Length(cm)	3.7	3.2	2.8	2.8	-	-	12.50	3.13
	Width(cm)	1.6	1.6	3	2.2	-	-	8.40	2.10
CTL(A){D}	Length(cm)	2.8	3.1	2.8	2.9	2.9	2.5	17.00	2.83
	Width(cm)	1.6	1.0	1.1	1.2	0.9	0.8	6.60	1.10



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