Effect of Azadirachtin spray on total protein content of mango midge larvae.

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Abstract:

Neem was proved to be an eco-friendly & best insecticide. Neem components show multiple effects against different insects. In present work neem leaves were used for the gall treatment. Total protein content of mango midge larvae was determined in this investigation. Different concentration of neem exacts were sprayed on the midges and protein content was analyzed after 24, 48, 72 up to 15 days. In the present study significant decrease in the total protein content was recorded.

***Index Terms*: Azaditachtin, mango leaf galls, Midge fly, Protein content of larvae.**

Introduction:

Neem is one of the botanical products used as insecticide. Various neem products have been extensively researched for their phyto-chemical & exploitation in pest control programme.Azadirachtin is the major component which is the predominant insecticidal active ingredient seeds, leaves and other parts of the tree. Neem components show multiple effects against different insects. Midge fly is the causative agent of the mango leaf gall. In present work the effect of neem extract on total protein content of midge larvae was studied. This treatment was the part of the work to control the midge infestation from the mango tree.

Materials and Methods:

In present work fresh neem leaves were used for the gall treatment fresh leaves were collected and washed thoroughly with tap water. Then it was ground into a fine paste by adding little amount of water. One gram of this paste was added to the 100 ml of distilled water. Thus 1% neem extract spray was prepared and in the same manner 2%, 3%, 4%, 5% neem sprays were prepared. Likewise alcoholic neem sprays also prepared and used for the treatment. The gall infested branches were selected and treatment was given at regular interval of time I.e. 24 hrs, 48 hrs, 72 hrs, 7 days and 15 days. Then after gall was superficially cut & larvae were separated out. Almost 30 November of same instar larvae were taken and total sugar analysis was done in laboratory by using method suggested by Sadasivam & Manickkam (1997)

Results and observations:

Table: 19: Effect of 1% Neem spray on Total Protein Content of midge larvae * (The Total Protein Content expressed in terms of mg/gm wet wt. is the average of 5 samples ± S.D.)

Period	Name of sample	Total Sugar Content ± S.D.	% Change	Student "t" Test	Level of significance
24 hrs.	Control	45.3 ± 1.08			--
	Aqueous	46.18 ± 0.40	1.94	2.40	P>0.05
	Alcoholic	46.54 ± 0.80	2.73	5.32	P>0.01
48 hrs.	Control	46.22 ± 1.28			
	Aqueous	41.44 ± 0.13	10.34	9.98	P>0.001
	Alcoholic	42.34 ± 0.26	8.39	8.59	P>0.001
	Control	44.98 ± 1.13			

72 hrs.	Aqueous	41.3 ± 0.25	8.18	8.81	P>0.001
	Alcoholic	41.2 ± 0.12	8.40	8.43	P>0.001
7 days.	Control	47.28 ± 1.30			
	Aqueous	43.46 ± 0.15	8.07	7.96	P>0.001
	Alcoholic	44.3 ± 0.31	6.30	6.68	P>0.01
15 days.	Control	50.38 ± 1.198			
	Aqueous	46.3 ± 0.14	8.09	8.92	P>0.001
	Alcoholic	47.28 ± 0.20	6.15	6.99	P>0.01

Fig. 19

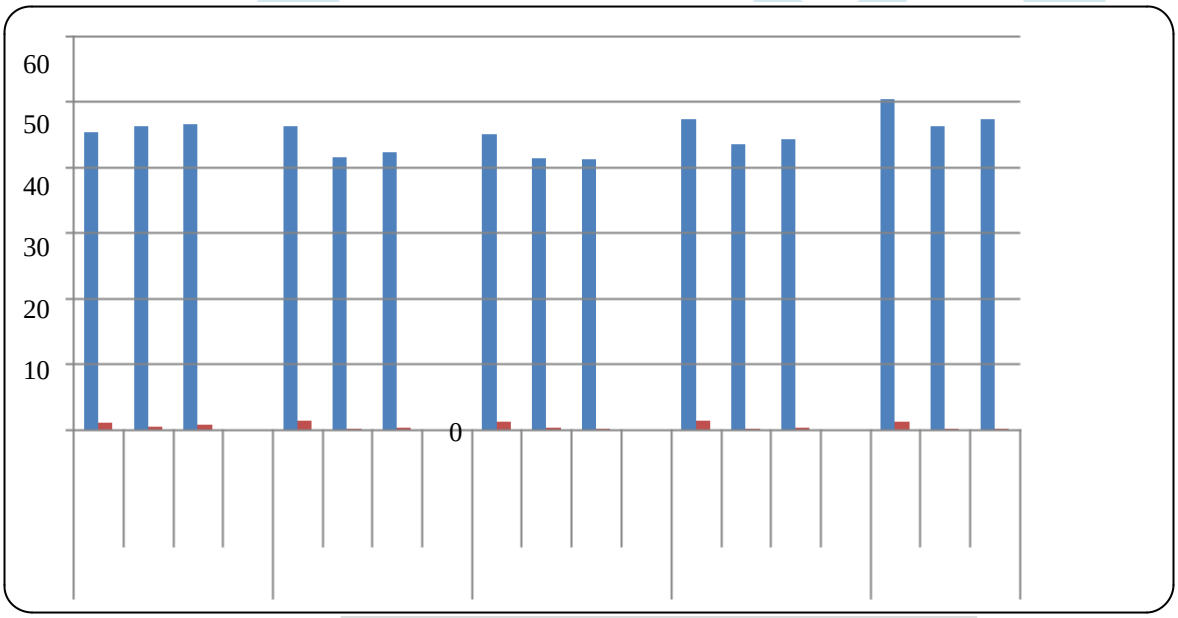

Table:20: Effect of 2% Neem spray on total Protein Content of midge larvae * (The Total Protein Content expressed in terms of mg/gm wet wt. is the average of 5 samples ± S.D.)

Period	Name of sample	Total Sugar Content ± S.D.	% Change	Student "t" Test	Level of significance
24 hrs.	Control	43.44 ± 0.85			--

	Aqueous	44.84 ± 0.96	3.22	#####	insignificant
	Alcoholic	46.16 ± 0.88	6.26	#####	insignificant
48 hrs.	Control	45.34 ± 0.71			
	Aqueous	43.88 ± 0.49	3.22	6.98	P>0.001
	Alcoholic	43.06 ± 0.42	5.02	9.57	P>0.001
72 hrs.	Control	46.04 ± 0.93			
	Aqueous	44.2 ± 0.29	3.99	5.13	P>0.01
	Alcoholic	43.68 ± 0.64	5.12	9.69	P>0.001
7 days.	Control	47.66 ± 1.59			
	Aqueous	45.1 ± 0.72	5.37	6.12	P>0.01
	Alcoholic	44.14 ± 0.62	7.38	7.98	P>0.001
15 days.	Control	48.32 ± 1.26			
	Aqueous	46.64 ± 0.57	3.47	4.53	P>0.01
	Alcoholic	44.92 ± 0.53	7.03	8.87	P>0.001

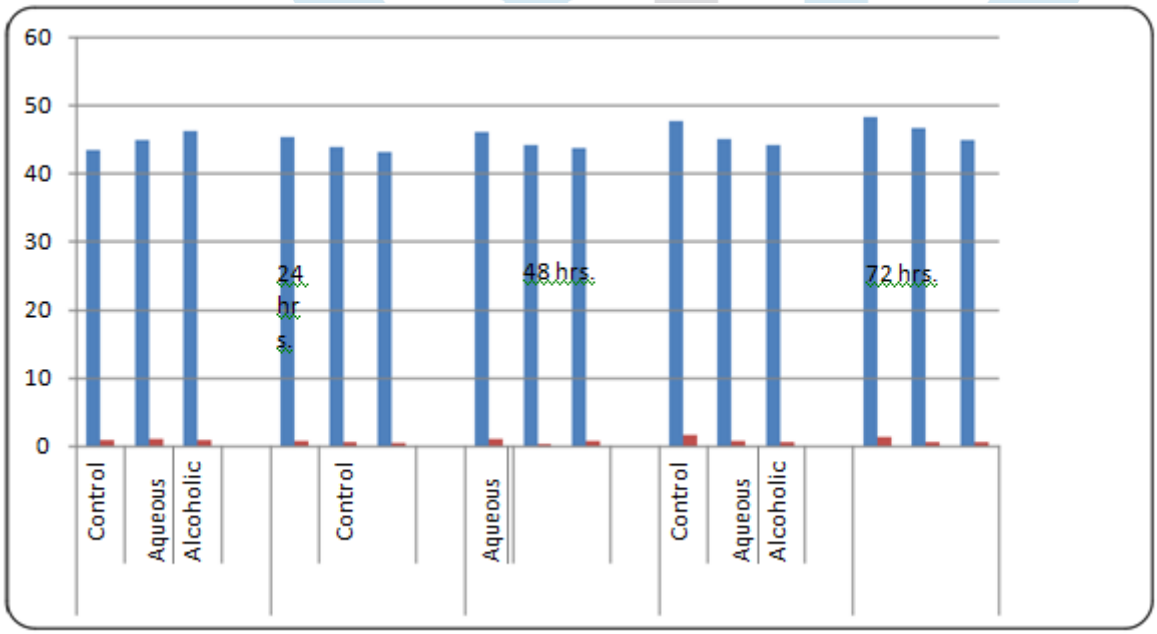

Table: 21: Effect of 3% Neem spray on total Protein Content of midge larvae* (The Total Protein Content expressed in terms of mg/gm wet wt. is the average of 5 samples ± S.D.)

Period	Name of sample	Total Sugar Content ± S.D.	% Change	Student "t" Test	Level of significance
24 hrs.	Control	45.52 ± 0.98			--
	Aqueous	44.66 ± 0.80	1.88	4.62	P>0.01
	Alcoholic	43.7 ± 0.51	3.99	5.95	P>0.001
48 hrs.	Control	47.14 ± 0.54			
	Aqueous	46.18 ± 0.48	2.03	8.43	P>0.001
	Alcoholic	44.8 ± 0.07	4.96	7.63	P>0.001
72 hrs.	Control	46.78 ± 1.35			
	Aqueous	43.12 ± 0.43	7.82	8.54	P>0.001
	Alcoholic	42.66 ± 0.15	8.80	8.41	P>0.001
7 days.	Control	48.06 ± 1.14			
	Aqueous	45.12 ± 0.43	6.11	7.76	P>0.001
	Alcoholic	44.06 ± 0.27	8.32	9.57	P>0.001
15 days.	Control	49.34 ± 1.14			
	Aqueous	48.04 ± 0.81	2.63	5.05	P>0.01
	Alcoholic	45.36 ± 0.31	8.06	9.79	P>0.001

Fig. 21

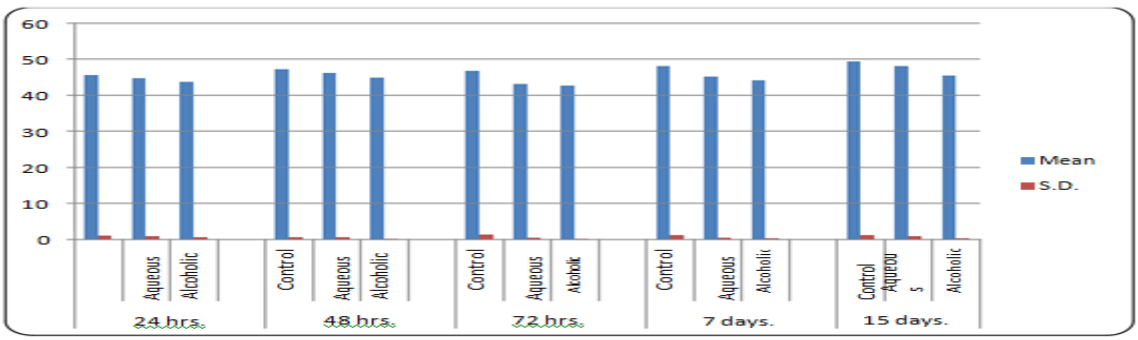

Table: 22: Effect of 4% Neem spray on total Protein Content of midge larvae * (The Total Protein Content expressed in terms of mg/gm wet wt. is the average of 5 samples ± S.D.)

Period	Name of sample	Total Sugar Content ± S.D.	% Change	Student "t" Test	Level of significance
24 hrs.	Control	44.08 ± 1.62			--
	Aqueous	42.8 ± 0.90	2.90	3.38	P>0.05
	Alcoholic	41.5 ± 1.16	5.85	8.50	P>0.001
48 hrs.	Control	46 ± 1.27			
	Aqueous	43.44 ± 0.41	5.56	6.18	P>0.001
	Alcoholic	42.54 ± 0.49	7.52	8.78	P>0.001
72 hrs.	Control	49.04 ± 1.19			
	Aqueous	45.76 ± 0.61	6.68	9.61	P>0.001
	Alcoholic	46.62 ± 0.72	4.93	7.90	P>0.001
7 days.	Control	49.38 ± 1.06			
	Aqueous	48.66 ± 0.67	1.45	2.57	P>0.05
	Alcoholic	47.72 ± 0.79	3.36	7.19	P>0.001
15 days.	Control	53.3 ± 1.23			
	Aqueous	51.12 ± 0.58	4.09	6.06	P>0.01
	Alcoholic	50.54 ± 0.38	5.17	6.68	P>0.001

Fig. 22

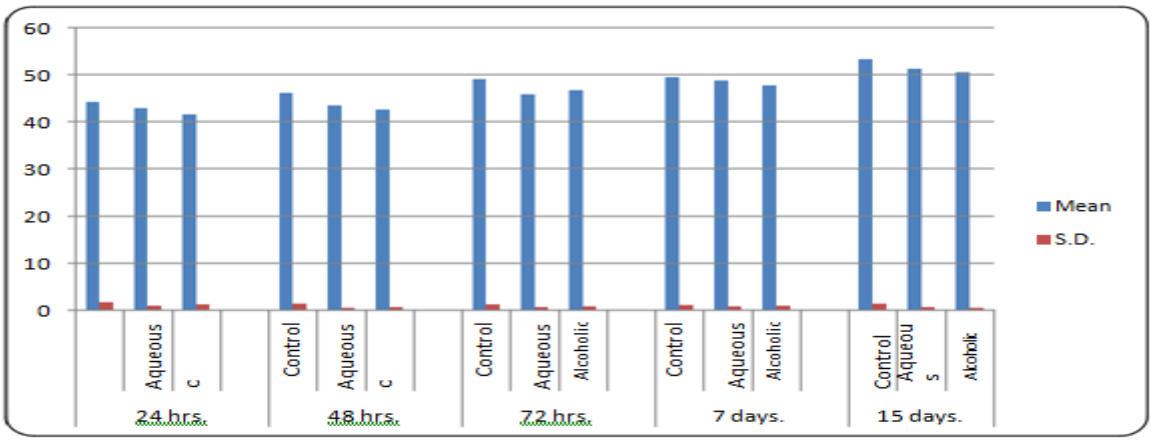

Table: 23: Effect of 5% Neem spray on total Protein Content of midge larvae * (The Total Protein Content expressed in terms of mg/gm wet wt. is the average of 5 samples ± S.D.)

Period	Name of sample	Total Sugar Content ± S.D.	% Change	Student "t" Test	Level of significance
24 hrs.	Control	44.6 ± 1.23			--
	Aqueous	43.56 ± 1.07	2.33	5.87	P>0.01
	Alcoholic	41.78 ± 0.60	6.32	7.95	P>0.001
48 hrs.	Control	47.48 ± 1.26			
	Aqueous	45.04 ± 0.81	5.13	8.15	P>0.001
	Alcoholic	43.4 ± 0.19	8.59	8.81	P>0.001
72 hrs.	Control	48.42 ± 1.90			
	Aqueous	47.02 ± 1.09	2.89	3.48	P>0.05
	Alcoholic	44.9 ± 0.60	7.26	6.92	P>0.001
7 days.	Control	49.34 ± 1.76			
	Aqueous	47.42 ± 1.03	3.89	5.03	P>0.01
	Alcoholic	46.14 ± 0.78	6.48	7.23	P>0.001
15 days.	Control	53.18 ± 1.57			
	Aqueous	51.7 ± 1.25	2.78	5.85	P>0.01
	Alcoholic	50.3 ± 0.93	5.41	8.05	P>0.001

Fig. 23

1) Effect of 1% Neem spray on total protein content of midge larvae:

Insignificant decrease in total protein content of midge larvae was observed in 1% aqueous neem spray while moderate decrease was observed in 1% alcoholic neem spray in 24 hrs.In 48 hrs and 72 hrs of both aqueous and alcoholic neem spray highly significant decrease in the total protein content was observed.After 7 days of spraying aqueous neem spray showed a highly significant decrease while moderate decrease was noted in alcoholic treatment. Such results were obtained after 15 days of spraying as well.

2) Effect of 2% Neem spray on total protein content of midge larvae:

After 24 hrs of neem extract spraying insignificant decrease was observed in both aqueous and alcoholic extracts.In 48 hrs of spraying this decrease was observed to be highly significant in both treatments ($P > 0.001$). After 72 hrs, 7 days and 15 days of aqueous spraying total protein content was found to be significantly decreased and in alcoholic spraying this decrease was observed to be highly significant.

3) Effect of 3% Neem spray on total protein content of midge larvae:

A moderate decrease was observed in 24 hrs of aqueous spraying while highly significant decrease was observed in alcoholic neem spray.But then after the decrease in the total protein was highly significant for all time durations starting from 48 hrs up to 15 days.

4) Effect of 4% Neem spray on total protein content of midge larvae: In 24 hrs of aqueous neem treatment showed a significant decrease in total protein content of midge larvae whereas alcoholic neem treatment showed highly significant decrease. After 48 hrs & 72 hrs of spraying both alcoholic and aqueous treatment showed a highly significant decrease in protein content. Significant decrease was observed after 7 days of aqueous neem spray while in alcoholic spray showed highly significant decrease. A significant decrease in total protein content was observed after 15 days of aqueous neem spray. Highly significant decrease was found in alcoholic neem treatment after 15 days.

5) Effect of 5% Neem spray on total protein content of midge larvae:

In 24 hrs of aqueous neem spraying moderate decrease in total protein of larvae was observed whereas alcoholic spray showed highly significant decrease. After 48 hrs of aqueous and alcoholic neem treatment highly significant decrease was observed. A moderate significant decrease was found in 72 hrs of aqueous spraying whereas highly significant decrease was observed in alcoholic neem treatment. In both 7 days and 15 days of neem spraying aqueous treatment showed significant decrease in total protein content and a highly significant decrease in alcoholic treatment.

Effect of neem on Protein of larvae:
In present investigation, due to the effect of neem extract total amino acid content of larvae was found to be elevated that means in less concentrations of neem i.e. 1% & 2%, but with high concentrations and increase in time duration moderate decrease and then significant decrease in the amino acid content was observed. In case of total protein content also the same trend was obtained in lower concentrations of neem extracts.
In the literature of Bita Vilizaoheu *et. al.* biochemical parameters of elm leaf beetle *Xauthogaleruca luteola* Mull were studied which shown the effect of neem-based insecticide on total amino acids and protein content of the insect larvae. In this study the amount of total protein was decreased as neem based alcoholic concentration increased. This could be due to the break-down of proteins into amino acids and their entrance to TCA cycle as keto acid. So protein depletion in tissue might play a role in compensatory mechanisms under insecticidal stress, to provide intermediate in the Krebs' cycle, by retaining free amino acid content in haemolymph (Shekari, *et. al.*, 2008 and Ziba ce, iet. al., 2008). These results are in agreement with earlier results. Khosravi *et. al.*, (2011) observed a significant decrease in the amount of total protein in larvae of *Glyphodes pyloalis* treated with *A. annua* methanolic extract.

Data obtained also showed different pattern effect on total protein content of the *Spodoptera littoralis* larvae. At the tested dose level of *A. indica*, the recorded values in pests showed marked decrease of the total protein content. Extensive work has been carried out in order to determine how various toxic agents affect protein synthesis. A diminution in the rate of ATP synthesis and inhibition of RNA synthesis also is the main cause of decreased total protein content (Dianzani, 1976; El-Beih, 1988; Nabin *et. al.*, 1989). Amer (1986), Ahmed *et. al.*, (1993) and Rawi *et. al.*, (1995) have reported that protein linkage during intoxication may arise from reduced body weight, conversion of protein to amino acids, degradation of protein to release energy or the direct effect of the tested extracts on the amino acid transport of the cell.

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