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Residual Effect of Neem Leaf Extract on The Mortality of *Lipaphis erysimi* And Its Larvae

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***Abstract*:** *The objective was to study the mortality rate of Lipaphis erysimi and its larvae by using neem leaf extract and appropriate dose of concentrations for checking the insect population.For this cultivars were raised with three dates of sowing at an interval of 15 days each in subplots with three replications each and the clones of mustard aphids were maintained. Theeffect of neem leaf extract on different larvae stages of aphids was studied and for that neem leaf extract of different concentrations was prepared, rearing of aphid's larvae was done and then the mortality rate of the aphid larvae (i.e. 1st, 3rd and 5th instars) in presence of neem leaf extract at different time intervals with different concentrations (5%, 10%, 15%, 20%) was studied.From the study it was observed that the aphid, L. erysimi was killed with the neem leaf extract at all concentrations but there was a significant difference among different concentrations, larval stage and period of treatment. Thus the study showed that by using eco-friendly insecticide, neem leaf extract, the spread of L. erysimi can be controlled which in turn will not hamper the yield of the mustard crop.*

***Keywords**: Lipaphis erysimi, neem leaf extract, mortality, eco-friendly, insecticide*

I. Introduction

The mustard aphid, *Lipaphis erysimi*, Kaltenbach (Hemiptera: Aphididae) is undoubtedly the most destructive insect pest in India and other tropical and sub-tropical parts of the world [1]. The yield loss of mustard crop caused by*L.erysimi* is very high in India [2]. The mustard aphid has overcome the barrier of glucosinolates being involved in defense against insects by feeding on the phloem [3], [4] as well as sequestering these compounds and retaining them in the body [5]. Some are metabolized, while others are concentrated in the body for further use [6]. Aphid infestations cause decrease in yield by way of decreasing number of pods per plant, number of grains per pod and oil content of the grains.[7]Chorbandi and Bakhetia, 1987 also reported negative correlation between seed yield and aphids infestation, explaining a decrease of 22 kg. per hectare in yield with an increase of 1% infestation. They also calculated the economic injury and economic threshold levels as 55-60% and 40-45% plants infected by aphids at flowering and bud initiation stages, respectively.

Keeping this in mind a sustainable mustard aphid management program was planned using neem (*Azadirachtaindica*) belonging to the Meliaceae family, which has been identified as the most promising of all plants for its insect repellent, anti-feedent and medicinal properties by the National Research Council, Washington, USA (1992) and a safe insecticide [8]. It is of botanical origin and easily available to the farmers. The studies conducted by some researchers showed that neem based insecticides provide 95.77% of *L. erysimi* mortality as compared to endosulpan with 97.24%.The mustard aphid *L. erysimi* breed parthenogenitically and the female aphid can within 7-10 days give rise to more than 100 nymphs in the same breeding cycle. As a consequence the pest population increases within a very short time to cover the entire flowering shoot and unless there is sustained application of chemical pesticides, several times in cropping season, no crop yield of any extent materializes. Although the application of the pesticide provides temporary relief, such liberal dispersion of the chemicals is costly for the farmers and environmentally hazardous [2].Thus, use of *Azadirachtin* will be proved to be a good potential inhibitor of the growth of insect cells as compared to other phytochemical pesticides [9], [10], [11].Neem ingredients influences the hormonal system of insects and leads to feeding as well as molting inhibition, retardation of the development and fecundity reduction [12].Based on the above mentioned facts, attempt was made to use neem leaf extract which is most easily available, economically not expensive and has an impact and growth inhibitions towards aphid population.

II. Materials And Methods

2.1 Preparation of different concentrations of Neem leaf extract:

To study the effect of neem leaf extract on the mortality rate of the mustard aphid (*L. erysimi*) , different concentrations of the extract was prepared [13]. For this leaves of neem were collected from a healthy neem tree in the morning and after collection, they were washed with tap water. These leaves after partial drying were grinded in the grinder for suitable time. These grinded leaves were wrapped in muslin cloth and were

squeezed till the desirable extract was obtained. The neem leaf extract was collected in four different flasks with different concentration prepared in distilled water viz. 5%, 10%, 15% and 20% and further used to study their effect on the mortality rate of mustard aphid (*L. erysimi*) [14].

2.2 Rearing of mustard aphid's larvae:

To collect and study the different instars of the mustard aphids, they were collected from the plots and brought to the laboratory and reared in environmental chambers at a temperature of 23±1ºC.Leaves of *Brassicanapus*and *Brassica juncea* as food were provided from time to time to the insect till the 5th instar larvae changed into pupal stage. From the pupal stage after sometime emerged the adults. These adults were then released into apparatus prepared for egg laying and these eggs were collected and kept in glass jars for hatching. As the egg hatched and changed into different instars, they were used for the treatments to conduct the different experiments .The mortality percentages in different treatments were recorded in order to find out the toxic effect of the neem leaf extract. The larvae which did not form the pupae and the pupae from which adults did not emerge out to show any response to the treatment were considered as dead.

2.3 Studying the mortality rate of mustard aphid larvae :

Petri dishes of 8.5 cm in diameter were taken and blotting papers of the same size were kept in the petri dishes. Neem leaf extract of different concentrations i.e. 5%, 10%, 15%, 20% were poured in the petri dishes on blotting paper. Some partially dried food was also kept in the petri dishes to keep the larvae feeding. The petri dishes set as control only had distilled water along with some partially dried food . In every petri dish four larvae of the different instars i.e. 1st, 3rd and 5th instar were put to see the effect of different concentrations of neem leaf extract on the mortality rate of the mustard aphids for 6 hours, 12 hours and 18 hours separately. Each treatment was replicated four times.

$$\% \text{ Net mortality} = \frac{\% \text{ mortality in test} - \% \text{ mortality in normal} \times 100}{100 - \% \text{ mortality in normal}}$$

2.4 StatisticalAnalysis:

The data was statistically analyzed and interpreted usingANOVA.

III. Results And Discussion

To study the residual efficiency of neem leaf extract formulation as eco-friendly control agent against the mustard aphid(*L. erysimi),* healthy and fresh leaves were used of neem (*Azadirachtaindica*) plant for extraction. Distilled water was selected for the preparation of different concentrations of neem leaf extract and four different concentrations viz. 5%, 10%, 15% and 20% were used against the 1st, 3rd, and 5th instar larvae stages of *L. erysimi* for different time intervals viz. 6, 12 and 18 hours in the present study.As a control distilled water was used in place of harmful chemical insecticides to reduce chemical pollution and emphasis on the residual efficiency of neem leaf extract.

The effect of treatment of neem leaf extract was that it killed the larvae at all concentrations and as the concentration was increased, the mortality of mustard aphid also increased. It was reported to be highest in T_4 and T_3 and more in T_2 and T_1 as compared to control. The order of mortality recorded was-

$T_0 < T_1 < T_2 < T_3 < T_4$

Table 1: Residual effect of neem leaf extract on different instars (1st, 3rd and 5th) of *L. erysimi* when treated for 6 hours

1st Instar

Concentration	No. of larvae treated				No. of larvae dead				% mortality	% net mortality
Treatment (T)	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8		
T_1 5%	4	4	4	4	1	1	0	1	18.75	7.14
T_2 10%	4	4	4	4	1	1	1	1	25.00	14.28
T_3 15%	4	4	4	4	1	2	1	2	37.50	28.57
T_4 20%	4	4	4	4	2	2	2	2	50.00	42.85
T_0 Control	4	4	4	4	0	1	0	1	12.50	0.00
3rd Instar										
T_1 5%	4	4	4	4	1	0	0	1	12.5	6.66
T_2 10%	4	4	4	4	1	1	1	0	18.75	13.33
T_3 15%	4	4	4	4	1	1	0	1	18.75	13.33
T_4 20%	4	4	4	4	1	1	1	1	25.00	20.00
T_0 Control	4	4	4	4	0	0	1	0	6.25	0.00
5th Instar										

T_1 5%	4	4	4	4	0	0	1	1	12.50	6.66
T_2 10%	4	4	4	4	0	1	0	1	12.50	6.66
T_3 15%	4	4	4	4	1	1	0	1	18.75	13.33
T_4 20%	4	4	4	4	1	0	1	1	18.75	13.33
T_0 Control	4	4	4	4	0	0	1	0	6.25	0.00

The effect of different concentrations on the instar was that the mortality rate of 5th instar larvae was much less as compared to 3rd and 1st instar larvae. Most high effect was observed on 1st instar larvae followed by 3rd and 5th instar respectively. As the larval age was increased, the mortality rate got decreased and the order of mortality rate recorded was 1st instar > 3rd instar > 5th instar. The effect of different time interval was also recorded as highest mortality of aphids in 18 hours treatment followed by 12 hours and 6 hours respectively (6 hours < 12 hours< 18 hours).The maximum mortality was recorded with 20% leaf extract in case of 1st instar larvae when treated for 18 hours as 92.85% (Table 3) and minimum mortality 6.66% was found in case of 3rd and 5th instar larvae with 5% neem leaf extract when treated for 6 hours (Table 1).In case of control, maximum mortality 12.5% was recorded in case of 1st instar larvae of *L. erysimi* and minimum mortality was recorded as 6.25% in case of 3rd and 5th instar larvae at all-time intervals.

Table 2: Residual effect of neem leaf extract on different instars (1st, 3rd and 5th) of *L. erysimi* when treated for 12 hours

1st Instar

Concentration	No. of larvae treated				No. of larvae dead				% mortality	% net
Treatment (T)	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4		mortality
T_1 5%	4	4	4	4	2	2	2	1	43.75	35.71
T_2 10%	4	4	4	4	2	2	2	2	50.00	42.85
T_3 15%	4	4	4	4	2	3	3	2	62.50	57.14
T_4 20%	4	4	4	4	3	2	3	3	68.75	64.28
T_0 Control	4	4	4	4	0	1	1	0	12.50	0.00
3rd Instar										
T_1 5%	4	4	4	4	0	1	1	1	18.75	13.33
T_2 10%	4	4	4	4	0	1	1	1	18.75	13.33
T_3 15%	4	4	4	4	1	1	1	1	25.00	20.00
T_4 20%	4	4	4	4	1	1	2	1	31.25	26.66
T_0 Control	4	4	4	4	0	0	1	0	6.25	0.00
5th Instar										
T_1 5%	4	4	4	4	0	0	1	1	12.50	6.66
T_2 10%	4	4	4	4	0	1	1	1	18.75	13.33
T_3 15%	4	4	4	4	1	1	1	1	25.00	20.00
T_4 20%	4	4	4	4	1	1	1	2	31.25	26.66
T_0 Control	4	4	4	4	0	0	1	0	6.25	0.00

Considering the treatment of *L. erysimi* with neem leaf extract for 6 hours as mentioned in Table 1 with 1st, 3rd and 5th instar larvae, it was observed that the maximum net mortality 42.85% was recorded in case of 1st instar larvae when treated with 20% neem leaf extract and minimum net mortality 6.66% was recorded in case of 3rd and 5th instar larvae when treated with 5% of neem leaf extract. Similarly, when all the three larvae instars were treated with different concentrations of neem leaf extract for 12 hours (Table 2), the maximum net mortality was found to be 64.28% in case of 1st instar when treated with 20% extract and the minimum net mortality 6.66% was recorded with the 5th instar larvae when treated with 5% neem leaf extract [15].

Table 3: Residual effect of neem leaf extract on different instars (1st, 3rd and 5th) of *L. erysimi* when treated for 18 hours

1st Instar

Concentration	No. of larvae treated				No. of larvae dead				% mortality	% net
Treatment (T)	R_1	R_2	R_3	R_4	R_1	R_2	R_3	R_4		mortality
T_1 5%	4	4	4	4	2	2	2	2	50.00	42.85
T_2 10%	4	4	4	4	2	2	3	3	62.50	57.14
T_3 15%	4	4	4	4	3	3	3	4	81.25	78.57
T_4 20%	4	4	4	4	4	4	4	3	93.75	92.85
T_0 Control	4	4	4	4	0	1	1	0	12.50	0.00
3rd Instar										
T_1 5%	4	4	4	4	0	1	1	1	18.75	13.33
T_2 10%	4	4	4	4	1	1	1	1	25.00	20.00
T_3 15%	4	4	4	4	1	1	2	1	31.25	26.66
T_4 20%	4	4	4	4	2	2	2	2	50.00	46.66
T_0 Control	4	4	4	4	0	1	0	0	6.25	0.00
5th Instar										
T_1 5%	4	4	4	4	0	1	0	1	12.50	6.66

T_2 10%	4	4	4	4	0	1	1	1	18.75	13.33
T_3 15%	4	4	4	4	1	1	2	1	31.25	26.66
T_4 20%	4	4	4	4	1	2	2	1	37.50	33.33
T_0 Control	4	4	4	4	0	1	0	0	6.25	0.00

Table 4: Net mortality Table of *Lipaphis erysimi*

	1st Instar			3rd Instar			5th Instar			Mean
	h_1	h_2	h_3	h_1	h_2	h_3	h_1	h_2	h_3	
T_0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_1	7.14	35.71	42.85	6.66	13.33	13.33	6.66	6.66	6.66	15.44
T_2	14.28	42.85	57.14	13.33	13.33	20.00	6.66	13.33	13.33	21.58
T_3	28.57	57.14	78.57	13.33	20.00	26.66	13.33	20.00	26.66	31.58
T_4	42.85	64.28	92.85	20.00	26.66	46.66	13.33	26.66	33.33	40.73
Mean	18.56	39.99	54.28	10.66	14.66	21.33	7.99	13.33	15.99	
Mean of instars	112.83			46.65			37.31			
Mean for periods	h_1 = 37.21			h_2 = 67.98			h_3 = 91.6			

Where, h_1 = 6 hours , h_2 = 12 hours , h_3 = 18 hours
CD at 5% (Treatment) = 5.34
CD at 5% (Instars) = 4.12
CD at 5% (Periods) = 4.12

Likewise as presented in Table 3, when the three instar larvae of *L. erysimi* were treated with different concentrations of neem leaf extract for 18 hours, the maximum net mortality was found to be 92.85% in case of 1st instar larvae when treated with 20% neem leaf extract and minimum net mortality was recorded as 6.66% within 5th instar larvae, when treated with 5% neem extract solution.Considering the 1st instar larvae of *L. erysimi* when treated at different time intervals i.e. 6 hours, 12 hours and 18 hours, the maximum net mortality 92.85% was found when treated with 20% neem leaf extract for 18 hours and the minimum net mortality was noted as 7.14% when the larvae was treated with 5% solution of neem leaf extract for 6 hours [16].In the same way, if we consider the 3rd instar larvae of *L. erysimi* at different time intervals i.e. 6 hours, 12 hours and 18 hours, then the maximum net mortality 46.66% was found in case of the instar when treated with 20% solution for 18 hours and minimum net mortality 6.66% was found to be with 5% neem leaf extract when treatment was done for 6 hours .In the 5th instar larvae, when it was treated with three periods of time i.e.6 hours, 12 hours and 18 hours, maximum net mortality 33.33% was found when the larvae was treated for 18 hours with 20% neem leaf extract.The minimum net mortality was observed as 6.66% with 5% neem leaf extract when treatment was done on larvae for 6 hours .

The data in Table 4 shows that all the concentrations of neem leaf extracts viz. 5%(T_1), 10%(T_2), 15%(T_3) and 20%(T_4) gave significant mortality as compared to control (T_0) .Thehighest mortality was observed in 20%(T_4),followed by 15%, 10% and 5%. Maximum mortality was recorded in case of 1st instar larvae followed by 3rd and 5th instar larvae. 18 hours (h_3) gave significantly more mortality percentage followed by 12 hours (h_2) and 6 hours (h_1).

Table 5: ANOVA for net mortality

Source of variation	D	S.S	M.SS	F.cal.	F. tab		Result
					5%	1%	
Due to conc.	4	8729.52	2182.38	17.53	2.63	3.89	S
Due to instars	2	5651.05	2825.52	22.69	3.21	5.25	S
Due to period	2	2478.50	1939.25	15.57	3.21	5.25	S
Due to error	36	4481.45	124.48				
Total	44	21340.52					

For	SE	CD
Conc.	2.62	5.35
Instars	2.03	4.12
Period	2.03	4.12

From the above obtained results it is inferred that by increasing the concentrations of neem leaf extract and the time period, the mortality of *L. erysimi* (mustard aphid) can be increased significantly .The studies conducted by [17]showed that neem based insecticides provide 95.77% of *L. erysimi* mortality as compared toendosulfan ,an acutely neurotoxicinsecticide to both insects and mammals, including humans (97.24%). Based on the experiments conducted and the results analyzed, it can be concluded that use of neem leaf extract on the plant or on the mustard aphid will result in low percentage infestation, lowered mummy weight, low emergence rate of adult of F_1 and even of F_2[18] due to the residual effect of the neem leaf extract..

IV. Conclusion

Lipaphis erysimi (Hemiptera: Aphididae) is definitely the most destructive insect pest of *Brassica* species in India and other tropical and sub-tropical parts of the world. Aphid infestations cause decrease in yield by way of decreasing number of pods per plant, number of grains per pod and oil content of the grains.To exploit the effects of the leaves of neem tree, the leaf extract was prepared by grinding the leaves and squeezing them through a muslin cloth. Four concentrations of the neem leaf extract prepared in distilled water (5%, 10%, 15%, 20%) and a control with only distilled water were studied on three stages (1^{st}, $2^{nd}$and 5^{th}) of larvae instars of *L. erysimi*. From the study it was observed that the aphid, *L. erysimi* was killed with the neem leaf extract at all concentrations but there was a significant difference among different concentrations, larval stage and period of treatment. The highest mortality 92.85% was recorded in case of 1^{st} instar larvae at 20% concentration at 18 hours after treatment and minimum mortality 6.66% for 6 hours of the 3^{rd} and 5^{th} instar larvae.The control reflected the zero net mortality rate (Table 4) of *L. erysimi*in the absence of an insecticide meaning heavy infestation. The findings obtained from the present study suggest that by using the ecofriendly neem leaf extractof 20% concentration on the *Brassica* plantsor on the mustard aphid directly at the right time i.e. the 1^{st} larvae instar stage for 18 hours ,the infestation of*Brassica* species by*L. erysimi* can be controlled which in turn will control the damage done to the yield of the crop without being dangerous to the environment like the hazardous, neurotoxic chemical insecticides.

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