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LABORATORY STUDIES OF LARVAL CHOICE BEHAVIOR OF PLUTELLA XYLOSTELLA'S 3RD INSTARS IN RESPONSE TO A BIOPESTICIDE DERIVED FROM THE BLEND OF CALOTROPIS PROCERA AND AZADIRHACTA INDICA EXTRACTS

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ABSTRACT

Plutella xylostella Linnaeus (Lepidoptera: plutellidae) commonly called as Diamondback Moth is one of the most notorious, cosmopolitan pest of crucifers. Studies reveal that the pest exhibits a marked preference for the Cole crops (cauliflower and cabbage) due to the presence of glucosinolates-a volatile emanant of crucifer plant. *Plutella* causes massive damage to the Cole crops, as a result farmers face huge economic loss. To overcome this situation, farmers extensively use chemical pesticides. The increasing eco- awareness about the potential adverse environmental effects as well as health hazards associated with the use of chemical pesticides and other agrochemicals has surged us to look for better eco-safe alternatives. Biopesticides developed from plant products could play a vital role in this perspective. The present research work studied the larval choice behavior of *Plutella xylostella's* 3rd instar in response to a bio pesticide that is a mixture (1:1) of *Azadirachta indica* (Meliaceae) and *Calotropis procera* (Asclepiadaceae) plant extracts. The results were also compared with the repellent behavior of *Plutella* (L3) against *A. indica* and *Calotropis* extract separately. In all 34 treatments were tested. The repellent rate was also calculated and on its basis each treatments was assigned a class (I-V). *Plutella* (L3) showed a significant repellent behaviour against all the treatments except for these treatments: NFE +CLE (3gms+3gms)/100ml, NFE +CSE (5gms+5gms)/100ml, NFE +CSE (3gms+3gms) /100ml, NLE +CFE (3gms+3gms) /100ml, CFE (14 gms/100ml) and the Control treatment. A Repellent rate was calculated and out of all the 34 treatments, NSE+CLE (7gms+7 gms/100ml) came out with flying colors as a significant repellent and larval deterrent, with a

Repellent rate 62% and classified under class IV. It should be noted that PR% for NSE (14 gms/ 100ml) and CLE (14 gms/ 100ml) separately was 52 and 46 respectively. This is quite low in comparison to the combination product and hence indicates a synergistic rationale between the two plant extracts. The calculated p-value for the combination product NSE+CLE (7gms+7gms/100ml) is less than .05, hence null hypothesis is rejected that means there is statistical significant difference between treated and the untreated counts for the treatment. Similar result was also obtained for other combination products. Thus, it can be concluded that the combination product can be used as potent larval repellent against Plutella (L3) and can play a vital role in pest management.

KEYWORDS: Biopesticide, larval choice, repellent rate.

INTRODUCTION

Plutella xylostella Linnaeus (Lepidoptera: plutellidae) commonly called as Diamondback Moth is one of the most notorious, cosmopolitan pest of crucifers. This pest is reported as the most widely distributed Lepidopteran,[7] and causes extensive damage to crucifer production in many parts of the world. The crop loss could reach up to 90%[11] leaving the farmer in despair and with huge economic loss.

Studies reveal that the pest exhibits a marked preference for the Cole crops (cauliflower and cabbage). Researchers reported that both plants possess fleshy and succulent leaves that provide gustatory and olfactory stimuli for successful host selection and development and therefore the choice is obvious.[3,4] Furthermore, it was investigated that *Plutella xylostella's* larvae are attracted by the glucosinolates-a volatile emanant of crucifer plant.[10] These glucosinolates are a class of sulphur- containing glycosides, also called as thioglycosides or mustard oil glycosides.[6] The glucosinolates are perceived by the tarsal chemo-receptors of the larva on coming in contact with crucifer crop and help the insect in finding their preferred host-plant.

Farmers mainly depend on the chemical pesticides for combating the threat from *Plutella* to their crop. Indiscriminate and extensive use of chemical pesticides has shown path to many problems like health hazards, pesticides residue retaining in the food chain, adverse environmental impacts. On the other hand, *Plutella* has evolved resistance to most of the chemical pesticides in use and is becoming difficult for the farmers to control its increasing population, especially in Southeast Asia and the Far East.[9]

The increasing eco- awareness about the potential adverse environmental effects as well as health hazards associated with the use of chemical pesticides and other agrochemicals has surged us to look for better eco-safe alternatives. Bio-pesticides developed from plant products could play a vital role in this perspective. Moving a step ahead, researchers are investigating larvicidal property of combined formulations of different essential oils and have found that the mixtures have more active substances than the individuals.

Keeping this in view, the present study deals with the deterrent property of a bio pesticide that is a mixture (1:1) of *Azadirachta indica* (Meliaceae) and *Calotropis procera* (Asclepiadaceae) plant extracts.

In all 34 treatments were used. The treatments used were.

(I) three doses from NFE+CFE, (II) three doses from NFE+CSE, (III) three doses from NFE+CLE, (IV) three doses from NSE+CFE, (V) three doses from NSE+CSE, (VI) three doses from NSE+CLE, (VII) three doses from NLE+CFE, (VIII) three doses from NLE+CSE, (IX) three doses from NLE+CLE, one dose prepared each from NFE, NSE, NLE also one dose prepared each from CFE, CSE, CLE and one control treatment. The 3rd instar larval choice behavior was assessed in perspective of these 34 treatments in comparison to the untreated.

Here NFE=Neem flower extract, NSE= Neem seed extract, NLE= Neem leaf extract, CFE= *Calotropis* flower *extract*, CSE= *Calotropis* shoot extract, CLE= *Calotropis* leaves extract.

METHOD

Rearing of Plutella xylostella (L3)

The 3rd instars of *Plutella* were reared in the laboratory in a controlled environment (25^{0}C, 65% r.h., L (16): D (8). The method followed similar to[5] but with slight modifications.

Large transparent buckets containing cauliflower plants (±6 weeks old) were used for rearing. Ten male and ten female *adult P. xylostella* were introduced in each of these buckets for mating. After 7-11 days the 3rd larval instars of *Plutella* were collected from the buckets. To avoid escape of *Plutella,* each bucket was covered with a section of untreated net stretched with elastic.

Preparation of the treatments

Flower, seeds and leaves of *Azadirachta indica* and flower, shoot and leaves of *Calotropis procera* were collected, rinsed with tap water and dried in shade. The leaves, flowers and shoot of both the plants were then powdered in an electric blender, while the seeds of A. indica were pounded gently in such a way that no oil comes out. The so obtained powdered plant parts were then weighed to obtain 3gms, 5gms, 7gms and 14 gms each of both the plant parts. An aqueous extracts from the same was prepared using method as adopted by[12] but with slight modifications. The powdered leaves, seed and flower of *Azadirachta indica* and flowers, leaves and shoot of *Calotropis procera* were soaked in 100 ml of distill water in 1:1 ratio in the combination as shown in table 3.1. After 24 hrs, the water with soaked leaves was filtered with Whatman filter paper, the bio-pesticide so obtained was stored in clean containers in a refrigerator for further use. Last but not least 2-3 drops of liquid detergent as a surfactant was added to different concentrations of bio-pesticides so prepared.

Preparation of the control

Control treatment was prepared by adding 2-3 drops of liquid detergent to100 ml of distill water.

Study of Larval choice behavior by choice method

The choice method was similar to that used by[1] but with minor modifications. Freshly excised cauliflower leaves were obtained from plants of the same age (± 6 weeks after transplant). These leaves were washed with water and then cut into leaf disc (± 8 cm diameter). Considering the main vein as a reference that divides the leaf in to two equal parts, one half of the leaf was treated with combined plant extract mixture or the control and the other half was left untreated. A 'T' was marked on the treated half with a pen. The treated half was dipped into the treatment and dried in air for around one hour.

After air-drying, the leaf was placed into a Petri dish (9 cm diameter) on a moist filter paper. One pre-starved 3rd instar larva was placed on this leaf disc in the Petri dish. To avoid escaping of the larva the Petri dishes were covered with fine muslin cloth, fasten with elastic rubber bands. The treatments were arranged randomly on the laboratory bench. The position of the larva was observed after every one hour and noted down. The observations continued for a period of 10 hours. The experiment was repeated 10 times.

The data were analyzed as paired comparisons,[8] as it deals with same individual larva both on the treated and the untreated side of the leaf disc. Most importantly, in each observation, the larva cannot be at both halves of the leaf at a single point of time, it can be either on treated half of the leaf disc, or on the untreated half.

To determine the significance of difference in the number of times the larva observed on the treated or the untreated side of the leaf disc, the data was statistically analyzed. A split plot design was adopted for the experiment, where the 32 treatments were divided between leaves, while a single leaf disc was divided into two sub-plots- the treated and the untreated respectively. As the total number of individuals was small a Wilcoxson matched pair sign test[8] at the 5% level was conducted using OriginPro 2017 software.

Calculation of Repellent rate (PR) and assigning of Class to each treatment

Repellent rate (PR) was calculated using the formula similar to.[2] The formula is as follows: PR= (NUt-NT) X 100/ (NUt+NT), here NT refers to the total no of times the larva was observed on the treated half of the leaf disc and NUt refers to the total no of times the larva was observed on the untreated half.

On the basis of the calculated PR, each of the treatments was assigned to different repellent classes ranging from 0 to V[2] Class 0 (PR<0.1%), class I (PR=0.1 to 20%), class II (PR= 20.1 to 40%), class III (PR=40.1 to 60%), class IV (PR=60.1 to 80%), class V (PR=80.1 to 100%.

RESULT AND DISCUSSION

Table 3.1:

Larval Choice Test (Pooled table)			Wilcoxson signed paired test results		
Treatments	**NT**	**Nut**	**W**	**Z**	**Exact prob>lWl**
1. NFE +CFE(7gms+7gms)/100ml	44	56	7	-1.539	0.1718 *
2. NFE +CFE(5gms+5gms)/100 ml	46	54	9	-1.3356	0.289*
3. NFE +CFE(3gms+3gms)/100ml	49	51	12	-0.2834	1*
4. NFE +CLE(7gms+7gms)/100ml	40	60	0	-2.3412	0.0156
5. NFE +CLE(5gms+5gms)/100ml	43	57	0	-2.222	0.0312
6. NFE +CLE(3gms+3gms)/100ml	46	54	3.5	-1.5163	0.2187*
7. NFE +CSE(7gms+7gms)/100ml	42	58	4.5	-2.245	0.0351
8. NFE +CSE(5gms+5gms)/100ml	45	55	0	-1.7008	0.125*
9. NFE +CSE(3gms+3gms)/100ml	48	52	7	-0.6998	0.6875*
10. NSE +CFE (7gms+7gms)/100ml	28	72	0	-2.512	0.0078
11. NSE +CFE (5gms+5gms)/100ml	30	70	0	-2.8599	0.00195
12. NSE +CFE (3gms+3gms)/100ml	37	63	0	-2.8245	0.00195
13. NSE +CLE (7gms+7gms)/100ml	20	80	0	-2.8245	0.00195

14. NSE +CLE (5gms+5gms)/100ml	24	76	0	-2.8073	0.00195
15. NSE +CLE (3gms+3gms)/100ml	33	67	0	-2.7003	0.00391
16. NSE +CSE (7gms+7gms)/100ml	22	78	0	-2.8245	0.00195
17. NSE +CSE (5gms+5gms)/100ml	27	73	0	-2.8073	0.00195
18. NSE +CSE (3gms+3gms)/100ml	35	65	0	-2.7003	0.00391
19. NLE +CFE (7gms+7gms)/100ml	31	69	0	-2.8599	0.00195
20. NLE +CFE (5gms+5gms)/100ml	33	67	0	-2.8073	0.00195
21. NLE +CFE (3gms+3gms)/100ml	44	56	3.5	-1.807	0.1093*
22. NLE +CLE (7gms+7gms)/100ml	25	75	0	-2.8264	0.00195
23. NLE +CLE (5gms+5gms)/100ml	28	72	0	-2.7904	0.00195
24. NLE +CLE (3gms+3gms)/100ml	40	60	0	-2.3412	0.01563
25. NLE +CSE (7gms+7gms)/100ml	27	73	0	-2.8073	0.00195
26. NLE +CSE (5gms+5gms)/100ml	30	70	0	-2.8207	0.00195
27. NLE +CSE (3gms+3gms)/100ml	47	53	0	-2.5663	0.00781
28. NFE (14 gms/100ml)	38	62	0	-2.5128	0.00781
29. NSE (14 gms/ 100ml)	24	76	0	-2.8382	0.00195
30. NLE (14 gms/100ml)	32	68	0	-2.8245	0.00195
31. CFE (14 gms/ 100ml)	45	55	4	-1.7953	0.125*
32. CLE (14 gms/ 100ml)	27	73	0	-2.8073	0.00195
33. CSE (14 gms/ 100 ml)	30	70	0	-2.7003	0.00391
34. Control	49	51	2	-2.8868	1*

NT refers to the total no of times the larva was observed on the treated half of the leaf disc. Nut refers to the total no of times the larva was observed on the untreated half of the leaf disc. * indicates that the calculated p-value >.05 therefore null hypothesis is accepted, this means that the no. of larvae observed on the treated is not statistically significantly different from the no of larvae observed on the untreated half of the leaf disc, The above data is also graphically represented in graph 1 depicting the difference more clearly.

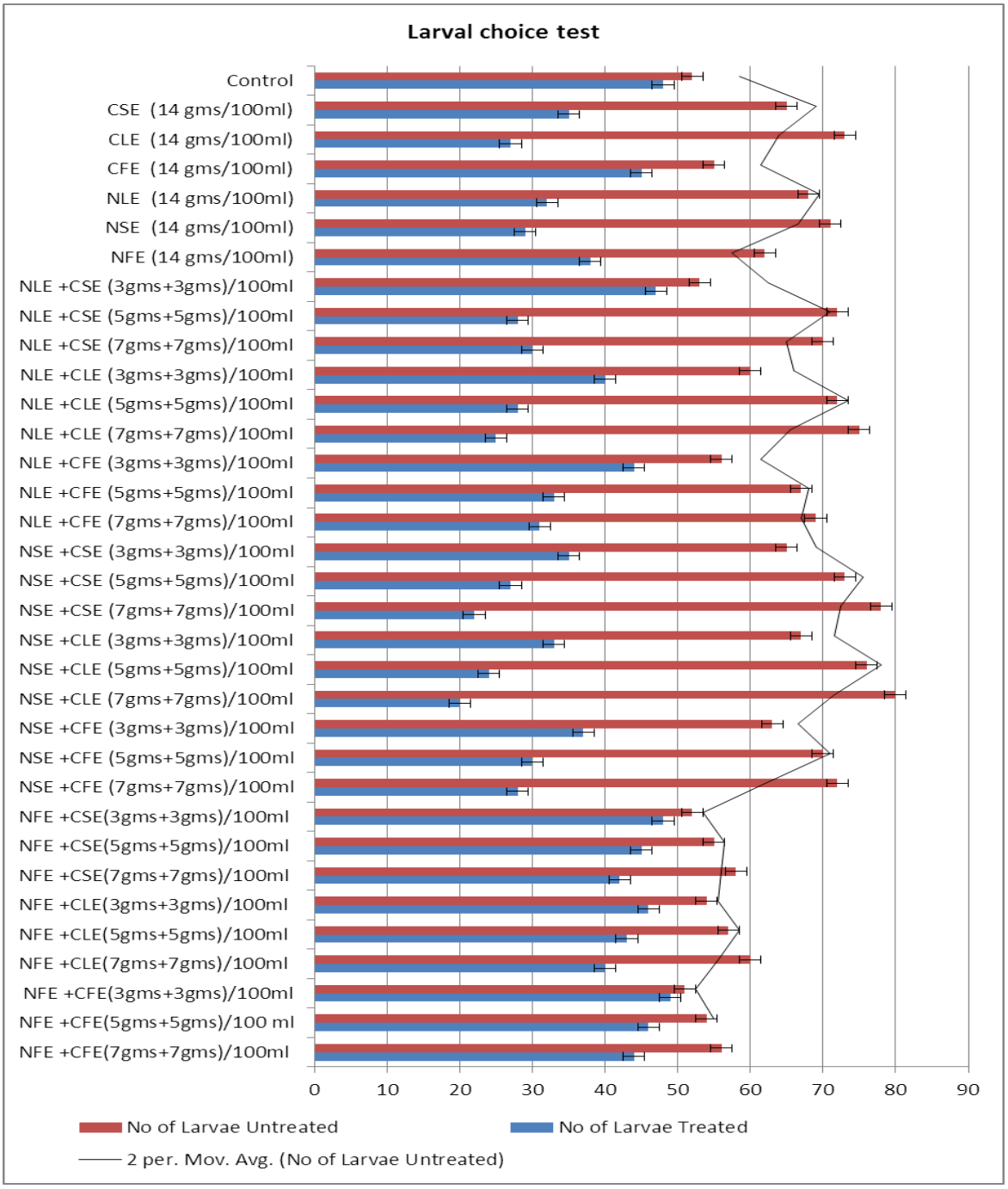
The table: 1 give us the glimpse that all three doses of NFE+CFE failed to prove itself as a good deterrent against larvae of *Plutella* (L3). The calculated P-value > α, where α is .05. Similar was the case with NFE+CLE (3gms+3gms)/100ml, NFE +CSE (5gms+5gms)/100ml, NFE +CSE (3gms+3gms) /100ml, NLE +CFE (3gms+3gms) /100ml and the Control treatment.

The CFE (14 gms/100ml) with Exact prob>lWl = .125 at 5 % level also could not prove itself as a larval repellent, but when CFE combined with Neem extracts, it showed significant difference between treated and the untreated counts indicating a synergistic rationale. Overall, in response to all the treatments (except those mentioned above), L (3) of *Plutella* showed a significant repellent behaviour.

To be more precise, a Repellent rate was calculated (Table: 2) and the treatments were assigned different class on its basis. Out of all the 34 treatments, NSE+CLE (7+7 gms/100ml) came out with flying colors as a significant repellent and a larval deterrent, with R. rate 62% and classified under class IV. It should be noted that PR% for NSE (14 gms/ 100ml) and CLE (14 gms/ 100ml) was 52 and 46 respectively. Talking about the control vs untreated, the repellent rate calculated was just 2%, the lowest of all.

Table 2:

S. no.	Treatments	PR%	Class	S. no.	Treatments	PR%	Class
1	NFE +CFE (7gms+7gms)/100ml	12	I	18	NSE +CSE (3gms+3gms)/100ml	30	II
2	NFE +CFE (5gms+5gms)/100 ml	8	I	19	NLE +CFE (7gms+7gms)/100ml	38	II
3	NFE +CFE (3gms+3gms)/100ml	2	I	20	NLE +CFE (5gms+5gms)/100ml	34	II
4	NFE +CLE (7gms+7gms)/100ml	20	I	21	NLE +CFE (3gms+3gms)/100ml	12	I
5	NFE +CLE (5gms+5gms)/100ml	14	I	22	NLE +CLE (7gms+7gms)/100ml	50	III
6	NFE +CLE (3gms+3gms)/100ml	8	I	23	NLE +CLE (5gms+5gms)/100ml	44	III
7	NFE +CSE (7gms+7gms)/100ml	16	I	24	NLE +CLE (3gms+3gms)/100ml	20	I
8	NFE +CSE (5gms+5gms)/100ml	10	I	25	NLE +CSE (7gms+7gms)/100ml	46	III
9	NFE +CSE (3gms+3gms)/100ml	4	I	26	NLE +CSE (5gms+5gms)/100ml	40	II
10	NSE +CFE (7gms+7gms)/100ml	44	III	27	NLE +CSE (3gms+3gms)/100ml	6	I
11	NSE +CFE (5gms+5gms)/100ml	40	II	28	NFE (14 gms/100ml)	24	II
12	NSE +CFE (3gms+3gms)/100ml	26	II	29	NSE (14 gms/ 100ml)	52	III
13	NSE +CLE (7gms+7gms)/100ml	62	IV	30	NLE (14 gms/100ml)	36	II
14	NSE +CLE (5gms+5gms)/100ml	52	III	31	CFE (14 gms/ 100ml)	10	I
15	NSE +CLE (3gms+3gms)/100ml	34	II	32	CLE (14 gms/ 100ml)	46	III
16	NSE +CSE (7gms+7gms)/100ml	56	III	33	CSE (14 gms/ 100 ml)	40	II
17	NSE +CSE (5gms+5gms)/100ml	46	III	34	Control	2	I



CONCLUSION

As discussed earlier, glucosinolates present in the Cole crops provide *Plutella xylostella's* larvae gustatory and olfactory stimuli for successful host selection, food preference and development[3,4] and results of the 'choice test' clearly indicates that it successfully disrupted the stimulus. The *Plutella* larvae (L3) exhibited marked preference for the untreated leaf for food as compared to the treated leaf. However, there was no significant difference between larval choice for the control or the untreated leaf. The synergistic effect of botanicals extracts of *A. indica* and *Calotropis* as a deterrent has also been the subject of present study.

It was found that the mixture NSE+CLE (7gms+7gms/100ml) gave the best results out of all the 32 treatments and classified under 'class IV' leaving far behind the plant extracts separately. It should be noted that PR% for NSE (14 gms/ 100ml) and CLE (14 gms/ 100ml) separately was 52 and 46 respectively and classified under 'class III'. This is quite low in comparison to the combination product and hence indicates a synergistic rationale between the two plant extracts.

The Exact prob>lWl value = .0051< α, where α is .05 calculated for the combination product NSE+CLE (7gms+7gms/100ml) also indicated statistical significant difference between treated and the untreated counts. Similar result was also obtained for other combination products also.

Thus, it can be concluded that the combination product can be used as potent larval repellent against *Plutella* (L 3) and can play a vital role in pest management.

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