International Journal of Advanced Research in Arts, Science, Engineering & Management (IJARASEM)

| ISSN: 2395-7852 | www.ijarasem.com | Impact Factor: 5.379 |Bimonthly, Peer Reviewed & Referred Journal|

| Volume 9, Issue 4, July 2022 |

Impact of bio-pesticides obtained from the blend of plant extracts of *Azadirachta indica* with that of the extracts of C*alotropis procera* on the ovipositioning behavior of female *Plutella xylostella* (Lepidoptera:Plutellidae).

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ABSTRACT: Biopesticides are the most promising alternative for a sustainable and a healthy environment. Looking at the hazards of use of chemical pesticides in farms, it is the need of the hour to approach for some eco-friendly management strategies. Biopesticides perfectly fills the lacunae and have a wide prospect. Here in this research paper impact of the Biopesticide on the ovipositioning behaviour of female *Plutella xylostella* (L.) is been investigated.

Plutella xylostella (L.) commonly called as diamondback moth (DBM) is the most threatening pest of cruciferous crops world-wide. It can cause 30- 90% of crop loss leaving farmers in great despair. The maximum damage caused to the crops is caused by the larvae of the moth. So, as to reduce the percent of larval infestation, one has to check the factors affecting the egg laying capacity of the moth. In pursuit of the aforesaid, and to develop a eco-safe biopesticide with enhanced efficacy against pest, the present research study was undertaken. The study deals with the impact of the Biopesticide (combination of plant extracts of *Azadirachta indica* (Neem) and *Calotropis procera* (Akk) on the ovipositioning behaviour of female *Plutella xylostella*. The plant extracts of Neem and *Calotropis procera* was combined in 1:1 ratio.

20 mated female moths were introduced into a net cage. In the net cage (16X3) treatments were analysed. After 48 hours the number of eggs oviposited on each of the treated plants was counted. The data was subjected to ANOVA using the statistical software Bio-stat pro. 5.9.8. To find out, which two treatments were significantly differed from each other, a Fishers LSD post-hoc test was also conducted.

Results revealed that the combination product (NSE 7gms + CLE 7gms/ 100ml) posses a positive syngergistic rationale and holds pesticidal property that could be developed as a potential oviposition deterrent against *Plutella xylostella*.

KEYWORDS: biopesticides, ovipositioning behavior, synergistic rationale

I.INTRODUCTION

Plutella xylostella Linnaeus (lepidoptera: plutellidae), commonly called as diamondback moth (DBM) because of the three dull whitish triangular markings visible on the hind margin of each forewings. On the male *Plutella* these diamond spots are more clearly visible rather than the female moth.

Life cycle of *Plutella xylostella*: According to Atwal (1955) female *Plutella* lay yellowish coloured eggs of pin head size either singly or in batches of 2 to 40 on the under surface of the leaves of crucifer. The larvae emerged from the eggs passes through four instars and larval period. It is the larval stage that causes the maximum damage to the crop, as it feeds on the leaves and inflorescence of the crop and renders it unmarketable.

Rembold, (1989) reported that Azadirachtin found in *Azadirachta indica* (Meliacae) is a promising ingredient to be incorporated as a tool, used under Integrated Pest Management. Azadirachtin ($C_{35}H_{44}O_{16}$) is the most complex secondary metabolite present in the plant. Azadirachtin is a mixture of seven isometric compounds, labeled as azadirachtin-A to G, with the azadirachtin-A greatest in quantity and the azadirachtin-E being the most effective insect growth regulator (Verkerk, 1993). Hanhong (2004) used extracts of leaves, seeds or other parts of *A. indica* and reported that it has a great potential as an eco-safe botanical pesticide.

Calotropis procera (Asclepiadaceae) is a xerophytic shrub. It produces large quantities of latex that contains alkaloids. Dubey et al. (2007) reported that its plant's extracts contains some pesticidal compounds such as calotropin and calotoxin. Hesse, (1950) isolated Calotropin, uscharin, calotoxin, calctin, amyrin, amyrin esters, uscharidin, calotoxin from the leaves and stalks of *C. procera* and *C. gigantae.* He also revealed that these compounds showed significant anti-fungal, anti-bacterial, nematocidal and larvicidal property.

Mohan et al. (2010) reported that though a lot of research work has been done on the coupled effects of synthetic pesticides but more work is required as far as concerned to plant-synthetic and plant-plant pesticide combinations. Liu et al., (1999) reported that plant extracts in combination show enhanced bio-efficacy as a bio-pesticide as compared to individual plant extract. Considering the above and in surge of investigating the synergistic rationale between plant extracts of *A. indica* and *C. procera*, combination product of the two plants extracts was prepared in 1:1 ratio and the pesticidal-coupled effect of the two plants was tested against the ovipositioning behavior of DBM.
The present study was carried on *Brassica oleracea var. botrytis* (cauliflower) plants. It is an important vegetable crop of the crucifer family. Cauliflower cultivators face serious threats from insect-pests to their crops and the yield loss could range about 20 to 30% (Estruch et al., 1997). Studies reveal that both cauliflower and cabbage plants possess fleshy and succulent leaves, which provides the pest an olfactory and gustatory related stimuli for successful host selection and development (Dube et al., 1977 and Singh and Singh, 1993].

II. METHOD

2.1. Collection and Rearing of *Plutella xylostella*:

P. xylostella larvae were sampled and collected from the major fields of cauliflower of Ajmer city. They were carried to laboratory in labeled plastic containers. In the laboratory, the larvae were kept at room temperature i.e. 25 ^{0}C on dark and 30 ^{0}C on light. They were reared using 'Rearing Method' as adopted by Dela Mondedji et al., 2015 with slight modifications. The larvae were reared on cauliflower plants (6-8 weeks old) in large transparent buckets. Each bucket containing cauliflower plant was covered with a section of untreated net stretched with elastic. After 22-24 days 20 mated females was collected from these rearing buckets.

2.**2. Preparation of plant extract: S**eeds, flowers and leaves of *A. indica* (Neem) and flower, shoot and leaves of *C. procera* were collected, rinsed with tap water and dried in shade. The dried leaves, flowers and shoot of both the plants were then turned to powdered form using an electric blender, while the seeds of A. indica were pounded gently in such a way that no oil comes out. These powdered plant parts were then weighed separately to obtain 3gms, 5gms, 7gms and 14gms each of both the plant parts. 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 for 24 hrs. After this, the water with soaked plant parts was filtered. Finally, 2-3 drops of liquid detergent as a surfactant was added to different concentrations of bio-pesticides prepared. It was then stored in clean and labeled glass bottles in dark and cold area for further use.

Altogether 33 combinations of *Azadirachta indica* (NSE- Neem Seeds extract, NLE – Neem leaves extract, NFE- Neem flowers extract) and *Calotropis procera* ((CSE- *Calotropis* Seeds extract, CLE –*Calotropis* leaves extract, CFE- *Calotropis* flowers extract)) was obtained.

Those 33 combinations are as follows:
(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.
Here the three doses are: 1)7gms+7gms/100ml, 2) 5gms+5gms/100ml and 3) 3gms+3gms/ 100ml.

2.3. Preparation of the control:

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

Choice of treatments to be used in net cage trials:

A pilot study was conducted on the above mentioned 33 combinations of plant extract against the ovipositioning behavior of female *Plutella*.
Among all the 33 combinations of plant extracts, only ten combinations fare well, those ten combination extracts are: the three concentrations of NSE+CLE, the high concentration (7gms+7gms)/100ml, and the medium concentration (5gms+5gms)/100ml of NSE+CSE, NLE+CLE and NLE+CSE and only one dose from the NFE+ CFE. These ten combinations were sprayed on the cauliflower plants in the net cage trials, to check the oviposition behavior of the moth in response to the treatments. For comparing the result five treatments of the plant extracts separately NSE, NLE, CLE, CSE and CFE each of 14gms/100ml concentration and a control treatment was also used in the experiment.

2.4. Study of Oviposition choice of female mated *Plutella* in Net cage trials:

A total of 48 (16x3) plants of cauliflower were placed in Randomized Block design in the net cage. The mesh size is less than 1mm. The method followed was similar to Charleston, 2004 but with some required modifications. In this

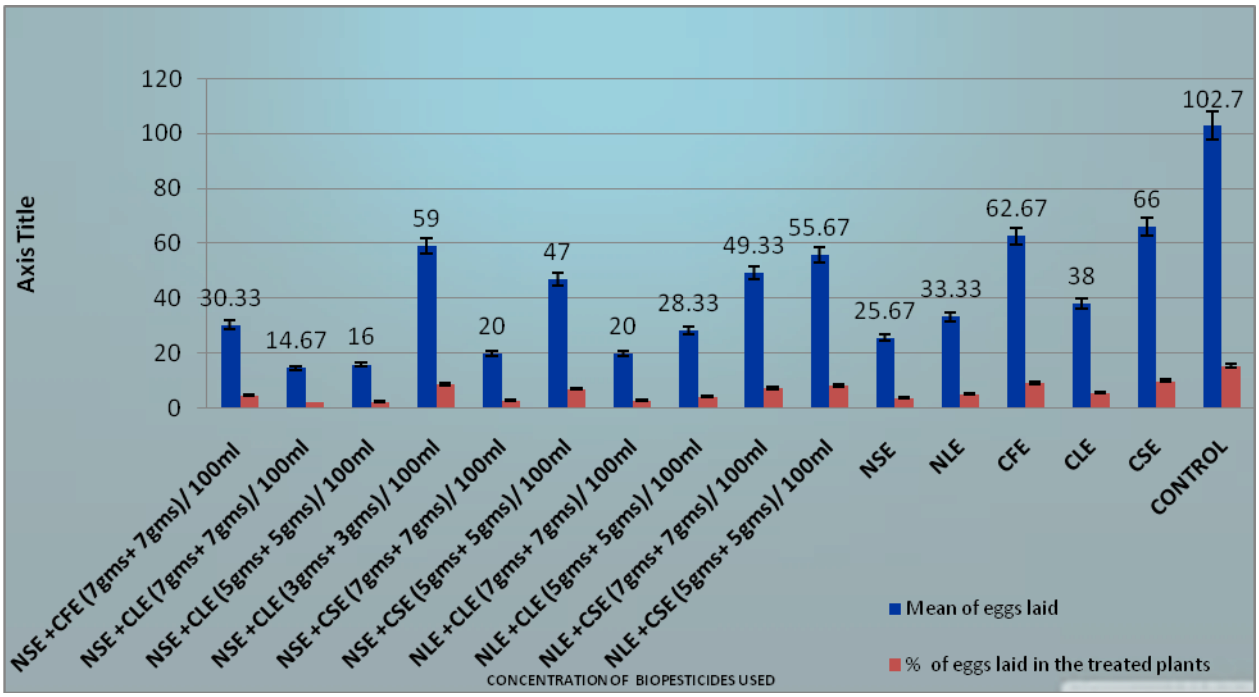
experiment female mated *Plutella's* was given freedom to choose between all the treatments and the control plants to oviposit. The data obtained was statistically analysed.

OBSERVATIONS

Evaluation of impact of the treatments on female *Plutella's* choice of oviposition in net cage trials:

Conc. of biopesticides sprayed	Mean of eggs laid	% of eggs laid in the treated plants
NSE +CFE (7gms+7gms)/100ml [T1]	30.33	4.54
NSE +CLE (7gms+7gms)/100ml [T2]	14.67	2.19
NSE +CLE (5gms+5gms)/100ml [T3]	16	2.39
NSE +CLE (3gms+3gms)/100ml [T4]	59	8.82
NSE +CSE (7gms+7gms)/100ml [T5]	20	2.99
NSE +CSE (5gms+5gms)/100ml [T6]	47	7.03
NLE +CLE (7gms+7gms)/100ml[T7]	20	2.99
NLE +CLE (5gms+5gms)/100ml [T8]	28.33	4.24
NLE +CSE (7gms+7gms)/100ml[T9]	49.33	7.38
NLE+CSE(5gms+5gms)/100ml T10]	55.67	8.33
NSE 14gms/100ml [T11]	25.67	3.84
NLE 14gms/100ml [T12]	33.33	4.99
CFE 14gms/100ml [T13]	62.67	9.37
CLE 14gms/100ml [T14]	38	5.68
CSE 14gms/100ml [T115]	66	9.87
CONTROL [T16]	102.7	15.35

Graph showing Impact of the treatments on female *Plutella's* choice of oviposition in net cage-trials:



III.RESULT AND DISCUSSION

20 mated female *Plutella* moths were introduced into the net cage. After 48 hours, the number of eggs oviposited on each plant was counted. The data was subjected to ANOVA using the statistical software Bio-stat pro. The analysis revealed that overall there was statistically significant difference between the treatments as determined by the one-way ANOVA ($F(15, 32) = 18.594$, $p = 1.12E^{-11}$).

To find out, which two treatments significantly differed from each other, a Fishers LSD post-hoc test was also conducted. The post-hoc test revealed that T1 (NSE 7gms + CFE 7gms/ 100ml) was significantly different from only T15 (CSE 14 gms) and the control (T16), whereas, the T2 (NSE 7gms + CLE 7gms/ 100ml) statistically differed from T4 (NSE 3gms+CLE 3gms/ 100ml), T6 (NSE 5gms + CSE 5gms / 100ml), T9 (NLE 7gms+CSE 7gms/ 100ml), T13 (CFE 14), T15 (CSE) and the control (T16) but was not statistically different from T11 (NSE 14 gms /100 ml). This indicates that the combination product (NSE 7gms + CLE 7gms/ 100ml) is a potential oviposition deterrent but could not statistically prove itself better than the NSE (14gms/100ml) plant extract in efficiency. However, it was better than CLE (T14) 14 gms /100ml (apart from the mixture), T4, T6, T9, T13, T15 and T16.

If we look at percentage of the eggs laid on the treated plants, it clearly shows that the lowest % of eggs was laid in Treatment 2 (NSE 7gms + CLE 7gms/ 100ml) i.e. just 2.19%. Hence the results are quite encouraging and the combination biopesticide could play a vital role in pest management.

The highest number of eggs was laid in the control (15.35%) and next to it stood the CSE (14 gms/100ml) 9.87 % followed by CFE (14 gms /100ml) 9.37%, which obviously shows that they didn't fare well as a strong oviposition deterrent.

The present research results could be corrabated with the findings of Chen et al., (1996 a), he reported that aqueous plant extracts of many plants like syringe tree showed oviposition deterrent property. Speaking of alcoholic extracts Dover, (1985) revealed that alcoholic extracts of Rosemary, sage, thyme white clove reduced egg laying capacity of *Plutella xylostella*. Similarly, here in the present research study, the NSE+CLE - binary mixtures proved to be a significant oviposition deterrent. It is possible that the spray of biopesticide (Neem +Calotropis) may have suppressed the olfactory stimulus and disrupted the pest from selecting a particular host for laying eggs. This concept holds good in the light of the findings of Reed et al. (1989), who suggested that *P. xylostella* depends on the glucosinolates as an olfactory stimulus, for host selection for oviposition.

On the other hand, results revealing the positive synergistic rationale between the plant products (NSE +CLE) can be correlated with the findings of Schoonhoven et al., (1992), that states that insect gustatory receptors response depends largely on interactions between chemicals of a mixture, he added that some chemicals apart from the mixture may not act as a stimulating factor. This holds well in the light of similar findings of Khan and Dewanda (2016) where the researchers found positive synergism in the combination product (NSE +CLE). It played a significant role as an antifeedant against *P. xylostella,* and fare better than the individual plant products as an antifeedant.

IV.CONCLUSION

Hence, it could be concluded that the synergism between plant extracts (NSE+ CLE) could play an important role in developing a potential oviposition deterrent and can be helpful in pest management, that too in eco-friendly way.

V.ACKNOWLEDGEMENT

I thank S.P.C., Govt. College, Ajmer, India, for providing facilities in the Department of Zoology, for this research work. I gratefully acknowledge Research Fellowship provided by the University Grants Commission. I am extremely thankful to Dr. Sabiha Khan, Associate Professor, Zoology, S.P.C. Govt. College, Ajmer, Rajasthan, under whose valuable guidance the research work was completed successfully.

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| ISSN: 2395-7852 | www.ijarasem.com | Impact Factor: 5.379 |Bimonthly, Peer Reviewed & Referred Journal|

| Volume 9, Issue 4, July 2022 |

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