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Synergistic effect of *Azadirachta indica* and *Calotropis* leaves' extracts as a growth retardant for controlling *Plutella xylostella*, the most notorious pest of Crucifer

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Abstract:
Today the faunal biodiversity is at threat due to the indiscriminate use of chemical insecticides. Advanced technology in the field of agriculture has prepared a cocktail of various chemicals to fight with the problem of pest. But, for this, our ecosystem is paying the price. The non-targeted insects and other biotic fauna present in the fields are at stake. Problems like Insecticide-resistance, degraded soil quality, human food poisoning by pesticide residue and various health problems have led us to re-think and re-structure our pest management strategy.

To overcome the aforesaid, biopesticides have emerged as a better solution to all the problems resulted due to use of chemical-pesticides. The present study shows the synergistic effect of botanical bio-pesticides derived from the blend of *(Azadirachta indica)* Neem and *Calotropis* (Akk) leaves' extract as a growth retardant for controlling *Plutella xylostella* (Lepidoptera : Plutellidae, the most notorious pest of crucifers. It is also known as DBM (Diamondback Moth).

The study conducted, revealed that the biopesticides in combination (Neem Leaves' extract (NLE) + *Calotropis* leaves' extract CLE) seems to be an effective growth retardant and showed a promising path towards eco-safe management of *Plutella*. It significantly prolonged the development duration of the 3rd and 4th instars' larvae of *Plutella.* The prolonged larval stage helps in reducing infestation of *Plutella* and farmers are able to yield more healthy crops.

Keywords: *Plutella*, growth retardant, biopesticide.

1. Introduction:

The infestation of Diamondback moth, *Plutella xylostella*, is reported globally, it is widely distributed in 128 countries (Shelton, 1993). The threat from DBM was reported from India in 1914 (Fletcher, 1914). Today the faunal biodiversity is at threat due to the indiscriminate use of chemical insecticides. Advanced technology in the field of agriculture has prepared a cocktail of chemicals to fight with the problems associated with pest. Unknowingly we forgot about the non-targeted insects and other biotic fauna present in the fields. On the other hand the chemicals like chlorpyrifos, cypermethrin, Malathion, Carbaryl etc are responsible for problems like insecticide-resistance, degraded soil quality, human food poisoning by pesticide residue. In addition, farmers who are in continuous contact with the chemical insecticides are facing serious health problems, mainly diseases related to lungs, eyes and skin (Khan and Dewanda, 2020). Controlling *Plutella* has become a challenge for the farmers, as it has shown a formidable capacity to evolve resistance to pesticides (Roush, 1997). It has also developed resistance to *Bacillus thuringiensis* formulations (Tabashnik et al., 1992). The genetic adaptability of *P. xylostella* has enabled it to develop resistance against wide range of insecticides (Sakeel et al., 2017; Uesugi, 2021; Banazeer et al., 2022; Shehzad et al., 2023).

To overcome the aforesaid, biopesticides have emerged as a better solution to all the problems resulted due to use of chemical pesticides. Here, role of botanical biopesticides derived from the blend of Neem and Calotropis leaf extracts as a growth retardant for controlling *Plutella xylostella* is been studied. Synergistic effect of leaf extracts obtained from *Azadirachta indica* (NLE) blended with that of *Calotropis procera* leaves' extract (CLE) has showed a promising path towards eco-safe management of *Plutella*. Efficacy of biopesticides was studied on the life cycle of *Plutella* with special reference to growth parameters of the 3rd and 4th instars' larvae.

2. Method

2.1 Collection and Rearing of *Plutella xylostella*:

Plutella xylostella larvae were collected from the major fields of cauliflower of Ajmer city. They were taken to the laboratory in plastic containers. The plastic containers were closed with a cap containing a fine muslin cloth to facilitate ventilation. In the laboratory the larvae were reared at room temperature 25^0C on dark and 30^0C on light. They were reared using 'Rearing Method' as adopted by Dela Mondedji et al., 2015 but 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. Emerged male and female *Plutella* were introduced in glass cages. Here, the female Moths oviposited, after mating .The eggs were collected from the glass cages and reared on fresh cauliflower plants in the buckets. After 11-14 days 3rd larval instars of *Plutella* were collected from the rearing buckets. The treated 3rd instar larvae were further observed and the duration of the 4th instar larvae was also recorded till pupation occurred.

2.2. Preparation of plant extract:

Leaves of *Azadirachta indica* and *Calotropis procera* were collected, rinsed with tap water and dried in shade. The leaves were then powdered and weighed to obtained 3gms, 5gms and 7gms of leaves 'powder of each plant. An aqueous extracts from the same was prepared by soaking powdered leaves of *Azadirachta indica* and leaves of *Calotropis procera* together in 100 ml of distill water for 24 hours. To observe the synergistic effect the weighed leaf powder so obtained of the both the plants were soaked in 1:1 ratio. On the other hand 14gms /100ml of NLE and 14 gms/100 of CLE were also prepared. After 24 hrs the mixture was filtered using Whatman filter paper and the extract was stored in clean, labeled containers.

Further, as a surfactant, 2-3 drops of liquid detergent was added to the biopesticide as well as to the distill water which acted as the control.

2.3. Bioassay:

A leaf dip method (Tabashnik et al., 1990) was followed to study the efficacy of different concentrations of biopesticides on the growth of the 3rd instars larvae of *Plutella.* In the ingestion bioassay, cauliflower unsprayed leaves' discs (8 cm diameter) were dipped in Bio-pesticides (NLE+CLE) of different concentrations (3gms+3gms/100ml, 5gms+5gms/ 100ml and 7gms+7gms/ 100ml). After air-drying the treated leaf disc were placed on a moist filter paper in petri dish of 9 cm diameter. One, 3rd instars' larva of *Plutella* were introduced in each petri-dish. The petri-dishes were then covered with a fine muslin cloth to avoid escape of larva. Each treatment had 6 replicates. Growth parameters, mortality and molting behavior were assessed at 24 hours intervals for till pupation.

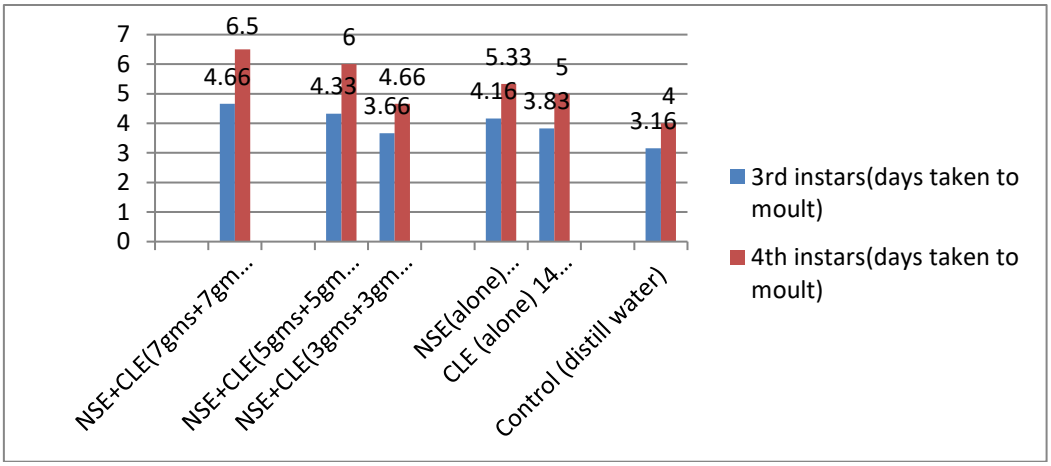
3. Result and Discussion:

Survival and development period: The development period was prolonged, when the 3rd instars' larva was fed with the biopesticides-treated leaves of cauliflower. It took significantly longer(4.66 days) to reach the fourth instars' and to the pupal stage(6.5 days), when fed with the leaves dipped in bio-pesticides (NLE

7gms+CLE 7gms/ 100ml) as compared to the control(3.6 and 4days respectively). Survival was also significantly reduced, in case of all the three concentrations of bio-pesticides as compared to the control. Only 30% larva survived up to the pupal stage, when treated with biopesticides (NLE 7gms+CLE 7gms/ 100ml.The emerged moths were malformed and were shorter in length as compared to the moths emerged from control bioassay. However when larvae fed with biopesticide (NLE 3gms +CLE 3gms/100ml) 60% could make it up to the pupal stage. The survival percent was more than 90% in case of the control bioassay. Significant prolongation in developmental period of the 3rd instars and 4th instars before pupation is clearly visible in the table and graph given below:

Table no 1: Mean larval duration (in days) of 3rd instars and 4th instars:

Conc. of extract used	3rd instars	4th instars
NSE+CLE (7gms+7gms/100ml)	4.66	6.5
NSE+CLE(5gms+5gms/100ml)	4.33	6.0
NSE+CLE(3gms+3gms/100ml)	3.66	4.66
NSE(alone) 14gms/100ml	4.16	5.33
CLE (alone) 14 gms/100ml	3.83	5.0
Control (distill water)	3.16	4.0



4. Conclusion:

Liu et al., 1999 reported that the synergistic approach of combining of plant extract enhance the bio-efficacy of the bio-pesticides as compared to individual plant extract. Considering the fact, here in this study the leaves extracts of *Azadirachta indica* is combined with the leaves extracts of *Calotropis procera* to produce a ecosafe and more effective biopesticides. The study showed that the 3rd instars' larvae moulted to 4th instars' stage after about 4.66 days, when fed with the NSE+CLE (7gms+7gms/100ml). On the other hand, when treated with NLE (alone) the development duration was 4.16 days. While in control it took only 3.16 days. It seems that the bio-pesticides in combination (NLE+CLE) are an effective growth retardant. Shorter developmental time and higher ovipositional potential of an insect on a plant are indicators of its high host suitability (Van Lenteren and Noldus, 1990). So, if application of biopesticides prolong the larval duration, it will delay pupation and slower down the infestation rate on crops due to low host suitability. Similar results were observed by Ganeshan et al., 1995. They observed prolongation in larval developmental periods and subsequent loss in pupal weight and malformed larval pupal intermediaries in *Spodoptera litura* larvae fed with crude extracts of neem + mahua + jatropha. (Sinzogan et al., 2006) also reported that the botanicals in combination act as a better bio-pesticides. It was also reported by Khan and Dewanda (2016) that different concentrations of the bio-pesticide in combination (*NSE + CLE)* tested showed

significant antifeedant behavior and mortality as compared to the individual plant extracts of NSE and *CLE* after 72 hours of exposure. On the above observations it can be concluded that incorporating synergistic rationale, biopesticide (NLE+CLE) can play an important role in the Eco-safe management tatics to combat with the threat of *Plutella.*

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