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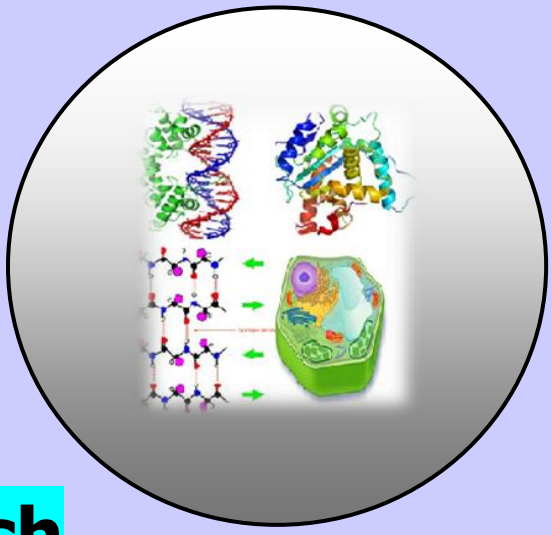

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RESEARCH PAPER

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Efficacy of bio-pesticide obtained from the blend of seeds' extract of *Azadirachta indica and* leaves' extract of C*alotropis procera* on the 2nd larval instars of *Plutella xylostella* (Lepidoptera: Plutellidae)

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ABSTRACT

Plutella xylostella (Lepidoptera: Plutellidae) is the major insect pest which causes damage to all the crucifers and especially to cauliflower every year. Brassica oleracea var. botrytis (cauliflower) is an important vegetable crop grown for its edible inflorescence (curd). 488 hectares land is under cultivation in Ajmer (acc. to 2014 records of krishi vigyan Kendra, Ajmer). And Brassica oleracea var. botrytis (cauliflower) is one of the most cultivable crops in Ajmer.

Management of Plutella is heavily reliant on the use of chemical insecticides. Farmers use chemical pesticides which are toxic to human health. According to World Health Organisation reports, the developing countries experience three million poisoning and 1800 deaths each year from the ill effects of chemical pesticides. These chemicals are also a threat for a sustainable world because the insecticides sprayed not only cause harm to the target species but also to the non-target species including the natural enemies of Plutella. Application of plant extracts as alternative to commercial insecticides provide an eco-friendly and a non-hazardous control method.

The present study deals with the combined effect of seed extracts of Azadirachta indica (Neem) with that of Calotropis procera (Akk) on the 2nd larval instar of Plutella. The larvicidal activity of the bio-pesticide mixture was tested and also compared with the individual plant extracts.

The results revealed that the bio-pesticide mixture showed significant larvicidal and antifeedant properties. The mortality rate was low after 24 hrs of exposure but showed considerable larvicidal property as compared to the control. The mortality rate increased significantly after 48 hrs when the 2nd instar larva of P. xylostella was fed with the leaf

dipped in biopesticide (seed extracts of Azadirachta indica + Calotropis procera, 7gms+7gms) /100ml distill water). It showed 51.85% mortality. Whereas Azadirachta indica seed extract (alone) 14gms/100ml showed 44.44% and Calotropis Leaf Extract (alone) 14 gms /100ml showed 25.93% mortality which is significantly low as compared to larvicidal activity of biopesticide in combination.
Keywords: Biopesticide, Leaf Extracts and Larvicidal.

INTRODUCTION

Plutella xylostella (Lepidoptera: Plutellidae) is the most destructive insect pest which poses a threat to the crucifers worldwide (Vanderberg et al., 1998). It causes around 30 % crop loss each year. In India, P. xylostella was first recorded in 1914 (Fletcher, 1914) on crucifers.

Brassica oleracea var. botrytis is one of the most cultivable crucifer in Ajmer. Pushkar, Kishanpura, Bhagwanpura, Datanda, Dodiana, Makreda, Pisangan, Daurai, Tabiji, Khanpura & Sarwar are the major areas in and around Ajmer where cauliflower is cultivated. Plutella not only damage the leaves but also the edible inflorescence and makes it unmarketable.

Insecticide applicaton is the primary method used to control *Plutella xylostella.* Farmers use chemical pesticide which adversely affect human health and cause fatal diseases like cancer, hypertension, neurological disorders, birth defects and many more. On the other hand development of high tolerance to most of the insecticides and associated environmental problems results in outbreaks of the pest by destruction of its natural enemies (Liang et al., 2003 & Xu et al., 2004). Thus introduction of economically feasible and eco-safe pesticides is definitely a welcoming strategy by farmers for pest management.

Ahmad *et al.* (1992 and 1998) showed that neem-based insecticides showed hope as effective alternative insecticide to control insects that is normally difficult to control by using conventional insecticides. Azadirachtin is considered as a promising ingredient for Integrated Pest Management (Rembold, 1989). On the other hand *Calotropis procera* of family Asclepiadaceae contains some pesticidal compounds such as calotropin and calotoxin in the milkweed plant extracts (Dubey et al., 2007). Liu et al., 1999 reported that combining of plant extract enhance the bio-efficacy of the bio-pesticides as compared to individual plant extract. Considering the fact a synergistic rationale is been approached, by combining the seed extracts of *Azadirachta indica* with the leaves extracts of *Calotropis procera* to produce a ecosafe and more effective biopesticide.

Method

Collection and Rearing of *Plutella xylostella*

Plutella xylostella larvae were collected from the major fields of cauliflower of Ajmer city. They were kept in labelled plastic containers and taken to the laboratory. The containers used were lined with tissue paper to absorb excess moisture and closed with a cap containing a fine muslin cloth to facilitate ventilation. The larvae were kept at room temperature 25 ^{0}C on dark and 30 ^{0}C on light in the laboratory. They were reared using 'Rearing Method' as adopted by Dela Mondedji et al., 2015 with some modifications. The larvae were reared on cauliflower plants (6-8 weeks old) in large tansparent buckets. Each bucket containing cauliflower plant was covered with a section of untreated net stretched with elastic. Ten male *Plutella* and ten female *Plutella* were introduced in each of these buckets. After 4-7 days the 2nd larval instar of *Plutella* were collected from the buckets.

Azadirachta indica(Neem) seed extract [NSE]	**14gms/100ml**
***Calotropis leaves extract* [CLE]**	**14gms/100ml**
Azadirachta s. extract+Calotropis l. extract	**3gms+3gms/100 ml**
Azadirachta s. extract +Calotropis l. extract	**5gms+5gms/100 ml**
Azadirachta s. extract +Calotropis l. extract	**7gms+7gms/100 ml**
Control	**Distill water**

Preparation of plant extract

Seeds of *Azadirachta indica* and leaves of *Calotropis procera* were collected, rinsed with tap water and dried in shade. The leaves were then powdered in an electric blender. The powdered leaves of *Calotropis* were then weighed to obtained 3gms, 5gms and 7gms of the same. The neem seed is pounded gently in such a way that no oil comes out. The pounded neem seed powder is also weighed to obtained 3gms, 5gms and 7gms of the same. An aqueous extracts from the same was prepared using method as adopted by Zaman et al., 2012 but with some modifications. The powdered seeds of *Azadirachta indica* and leaves of *Calotropis procera* was soaked in 100 ml of distill water. To enhance the synergistic effect the weighed leaf powder so obtained of the both the plants were soaked together in 100ml of distill water in the following concentrations 3gms, 5gms and 7gms of neem seed powder with that of 3gms, 5gms and 7gms of *Calotropis procera* leaves powder respectively. The so obtained biopesticide was filtered after 24 hrs and the filtrate was stored in clean containers in a refrigerator for further use. Last but not least 2-3 drops of liquid detergent was added to each biopesticide of different concentrations as well as to the distill water which will act as the control. This liquid detergent drops acted as a surfactant.

RESULTS

Observations after 24 hours of bioassay

Conc. of extract Used	No. of alive larvae in 1st replicate out of 5 larvae	No. of alive larvae in 2nd replicate out of 5 larvae	No. of alive larvae in 3rd replicate out of 5 larvae	No. of alive larvae in 4th replicate out of 5 larvae	No. of alive larvae in 5rd replicate out of 5 larvae	No. of alive larvae in 6th replicate out of 5 larvae	mortality % calculated using Abbot's formula
NSE+CLE 7gms+ 7gms/ 100	4	4	3	3	2	4	31.03%
NSE+CLE 5gms+5gms/100	2	4	5	3	4	3	27.58%
NSE+CLE (3gms+3gms/100	5	4	4	5	4	4	10.34%
NSE(alone) 14gms/100ml	5	5	5	5	4	4	3.44%
CLE (alone) 14gms/100ml	4	4	5	4	4	4	13.79%
Control(distill water)	5	5	4	5	5	5	0%

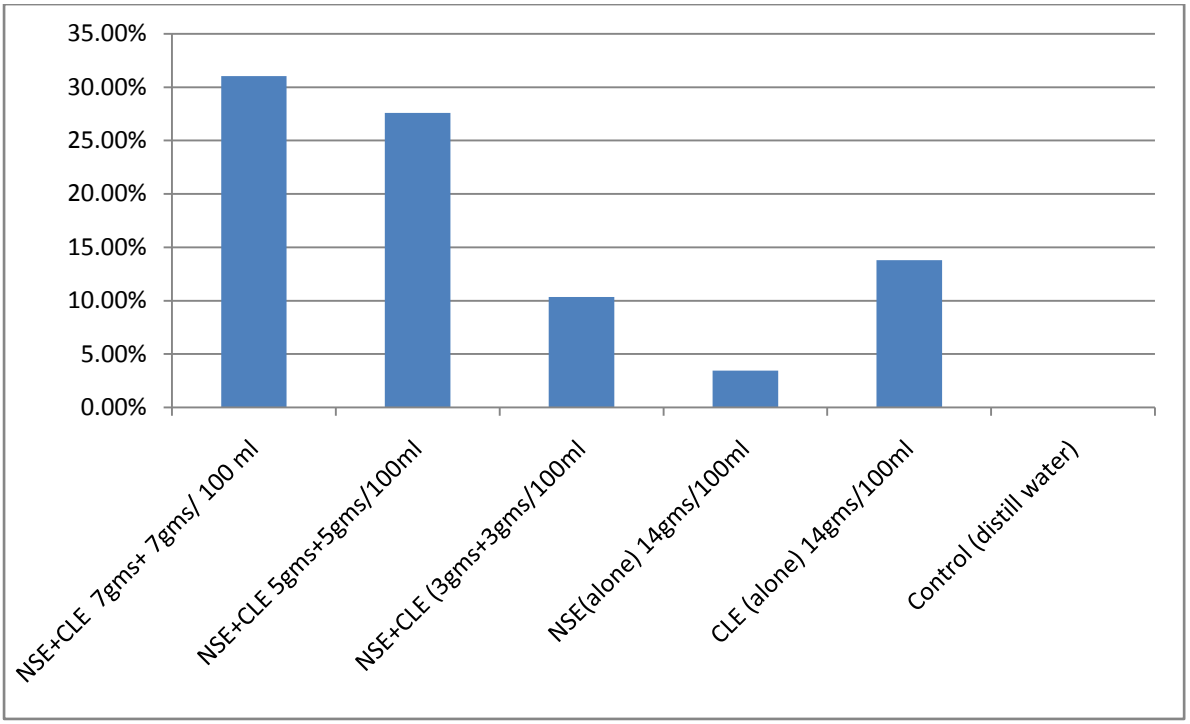

Figure 1. Showing observations after 24 hours of bioassay, NSE represents Neem Seed extracts and CLE represents *Calotropis* leaf extracts.

Observations after 48 hours, (including the no. of larva dead after 24 hrs)

Conc. of extract Used	No. of alive larvae in 1st replicate out of 5 larvae	No. of alive larvae in 2nd replicate out of 5 larvae	No. of alive larvae in 3rd replicate out of 5 larvae	No. of alive larvae in 4th replicate out of 5 larvae	No. of alive larvae in 5rd replicate out of 5 larvae	No. of alive larvae in 6th replicate out of 5 larvae	mortality % calculated using Abbot's formula
NSE+CLE (7gms+7gms/100	2	2	4	2	3	3	51.85%
NSE+CLE (5gms+5gms/100	2	2	3	2	3	2	48.15%
NSE+CLE (3gms+3gms/100	4	3	4	3	4	3	22.22%
NSE(alone) 14gms/100ml	3	2	2	3	2	3	44.44%
CLE (alone) 14 gms /100ml	3	3	4	3	3	4	25.93%
Control(distill	5	4	4	5	5	4	0%

Bioassay method

The toxicity bioassay was a leaf dip method similar to that used by Tabashnik et al., 1990.In the ingestion bioassay, cauliflower unsprayed leaves discs (8 cm diameter) were dipped in Bio-pesticides of different concentrations as mentioned in the table below:

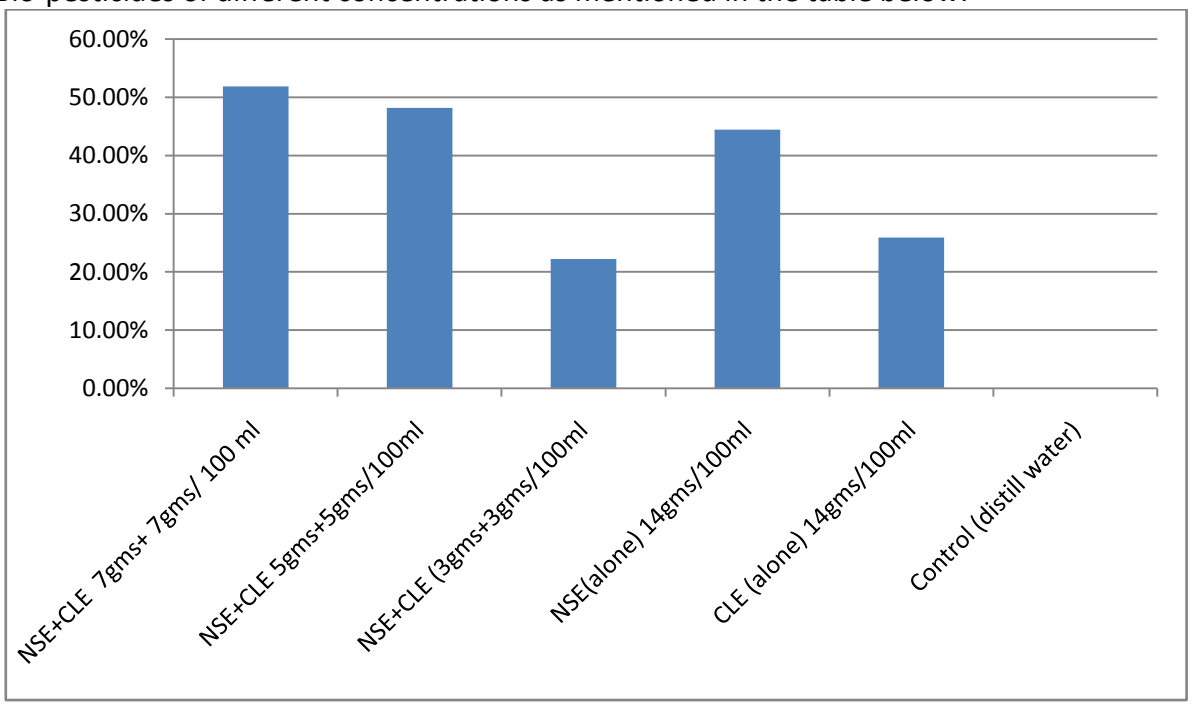

Figure 2. Showing observations after 48 hours, (including the no. of larva dead after 24 hrs), NSE represents Neem Seed extracts and CLE represents *Calotropis* leaf extracts.

Observations after 72ours, (including the no. of larva dead after 48 hrs)

Conc. Of extract Used	No. of alive larvae in 1st replicate out of 5 larvae	No. of alive larvae in 2nd replicate out of 5 larvae	No. of alive larvae in 3rd replicate out of 5 larvae	No. of alive larvae in 4th replicate out of 5 larvae	No. of alive larvae in 5rd replicate out of 5 larvae	No. of alive larvae in 6th replicate out of 5 larvae	mortality % calculated using Abbot's formula
NSE+CLE (7gms+7gms/100	1	1	2	1	1	1	73.07%
NSE+CLE (5gms+5gms/100	2	1	1	2	1	1	69.23%
NSE+CLE (3gms+3gms/100	2	2	2	1	2	1	61.53%
NSE(alone) 14gms/100ml	2	1	1	2	1	2	65.38%
CLE (alone) 14 gms/100ml	1	2	3	2	1	3	53.84%
Control(distill	4	4	4	5	5	4	0%

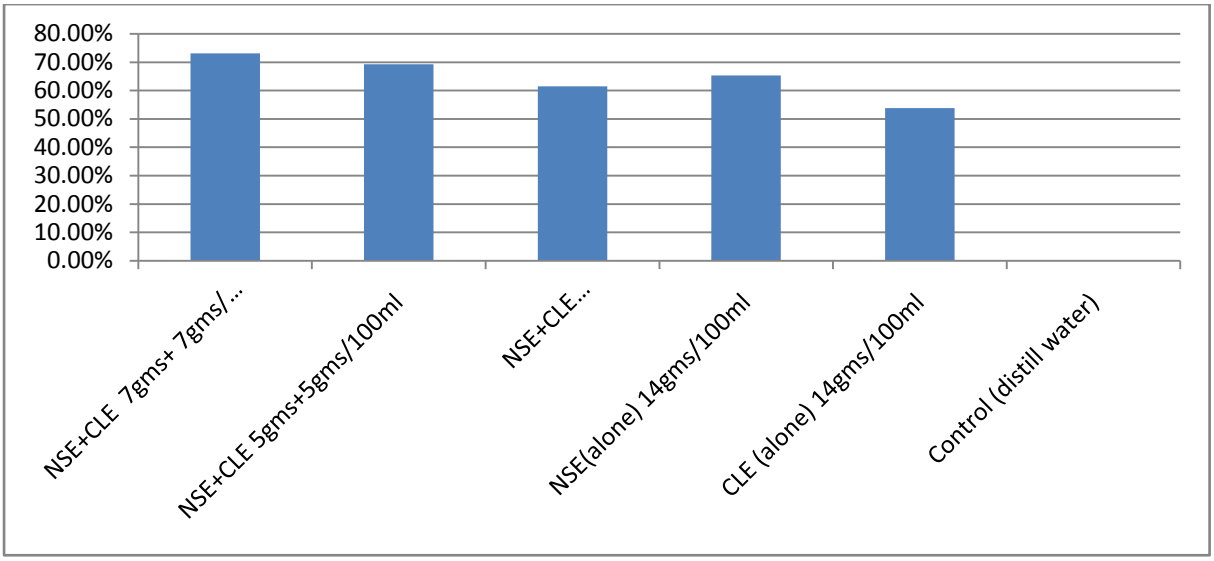

Figure 3. Showing observations after 72hours, (including the no of larva dead after 48 hrs), NSE represents Neem Seed extracts and CLE represents *Calotropis* leaf extracts.

Observations after 96 hours, (including the no. of larva dead after 72 hrs)

Conc. of extract Used	No. of alive larvae in 1st replicate out of 5 larvae	No. of alive larvae in 2nd replicate out of 5 larvae	No. of alive larvae in 3rd replicate out of 5 larvae	No. of alive larvae in 4th replicate out of 5 larvae	No. of alive larvae in 5rd replicate out of 5 larvae	No. of alive larvae in 6th replicate out of 5 larvae	mortality % calculated using Abbot's formula
NSE+CLE (7gms+7gms/100 ml)	1	1	0	1	1	0	84%
NSE+CLE (5gms+5gms/100 ml)	1	1	1	1	1	1	75.99%
NSE+CLE (3gms+3gms/100 ml)	1	1	1	2	2	1	63.99%
NSE(alone) 14gms/100ml	1	2	1	1	1	1	72.00%
CLE (alone) 14 gms/100ml	2	2	1	2	2	1	60.00%
Control (distill water)	4	4	4	5	4	4	0%

Each leaf disc was dipped in the extract for 10 seconds. After air -drying for 1 hr, leaf discs (8 cm) were placed on a moist filter paper in petri dish of 9 cm diameter. The leaf disc was placed on moist paper to avoid dessication. 5 larvae of *Plutella* were introduced in each petri disc. The petri dishes were then covered with a fine muslin cloth inorder to prevent larvae from escaping.

Each treatment had 6 replicates. The petri dishes were placed on the table randomly. Mortality was assessed at 24 hours intervals for 4 days. Mortality % was calculated using Abbot's formula. The leaf consumed was charted on graph paper. After 24 hours fresh leaf discs dipped in the extract were replaced with the older one.

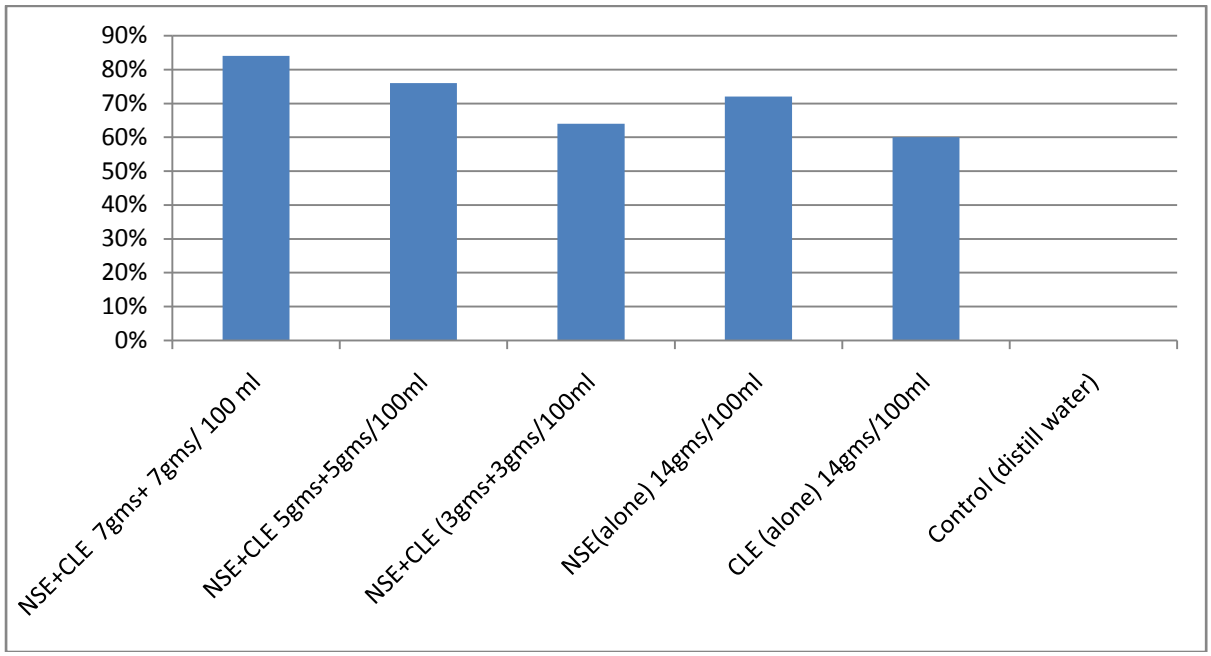

Figure 4. Observations after 96hours, (including the no. of larva dead after 72hrs), NSE represents Neem Seed extracts and CLE represents *Calotropis* leaf extracts.

DISCUSSION AND CONCLUSION

At 24 hrs of treatment the mortality percent was very low for biopesticide (3mg of *NSE*+ 3mg of CLE/100ml), it showed just 10.34% mortality of the 2 nd instar larva. Whereas it showed 3.44% mortality at 24hrs when treated with extracts of NSE (14gms/100ml), which is very low in comparison. All concentrations of the bio-pesticide in combination (*NSE + CLE)* tested showed significant antifeedant behavior and mortality as compared to individual plant extracts of NSE and *CLE* after 72 hours of exposure. The mortality of larvae of *P. xylostella* reached 84 % after 96 hours of exposure to bio-pesticide (7 mg *NSE* +7mg *CLE/100ml)*. The mortality % at 96 hrs of seeds extract of *A. indica* (alone) 14mg/100 ml was 72 %. 60 % mortality was observed when treated with *Calotropis* leaves extract (14mg/100 ml) after 72 hrs of bioassay treatment. Also, when larva treated with bio-pesticide (5mg+5mg/100ml) mortality rate of the 2nd instar significantly increased after 48hrs from 27.58% to 48.15 %. The failure to moult, blackening of the larvae and improper growth of the larvae, subsequent loss in pupal-weight and malformed larval pupal intermediaries was also observed.

The present study showed that all concentrations of the biopesticide in combination (seed extracts of *Azadirachta indica* + leaves extracts of *Calotropis procera*) found to be more effective in controlling 2nd instar larvae of *Plutella xylostella* as compared to neem and Calotropis extracts in alone. Similar result was also reported by Sinzogan and others, 2006. They found that damage to cotton by the bollworm*, Helicoverpa armigera*, can be minimized by mixtures of conventional insecticides at one half the recommended rates by combining extracts of three local plants (*Azadirachta indica, Khya senegalensis, and Hyptis suaveolens)* that provided greater efficacy than conventional products alone at their recommended rate. Yet, none of the plant extracts alone provided adequate crop protection. Such a direct demonstration of the utility and value of botanical preparations increased the farmer's confidence in indigenous technology (Isman, 2007).

The mortality of larvae of *P. xylostella* increased significantly after 96 hours of exposure to bio-pesticide (7 mg *NSE +*7mg *CLE/100ml* which also revealed the antifeedant property of pesticide along with the larvicidal property of the same, thus the biopesticide so tested is an effective growth inhibitor. Combined effect of extracts of *A. indica* with the extracts of *Calotropis procera* showed significant mortality as compared to the individual plant extracts. Thus it could be concluded that the biopesticide (NSE+CLE) has a strong potential to be incorporated in Eco-safe Pest Management strategy of *Plutella xylostella*.

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