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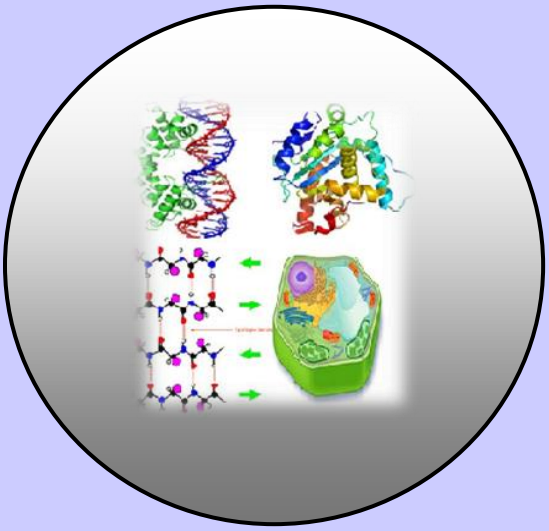

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RESEARCH PAPER

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Comparative Efficacy of *Azadirachta indica* and *Calotropis procera* Extract alone and in Combination as antifeedant against *Plutella xylostella* (L) (Lepidoptera, Plutellidae)

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ABSTRACT

In India, Plutella xylostella (L) (Lepidoptera, Plutellidae was first recorded in 1914 [5] on cruciferous vegetables and according to records, this species is distributed all over India wherever crucifers are grown. The neem tree, Azadirachta indica, is one of the most promising carries of antifeedant factors. With the aim to improve the bio-efficacy of neem based biopesticide, here in this study, the antifeedant effect of neem seeds(NSE) and Calotropis procera leaves' (CLE) extract in combination was evaluated on third instar larvae of Plutella xylostella. The toxicity bioassay was a leaf dip method and the leaves' disc of Brassica oleracea var. botrytis (cauliflower) was used for the purpose. To observe that both the plants exhibited a synergistic rationale, the antifeedant behaviour was also compaired with the results obtained from the bioassays where the third instar larva was fed with the leaves' extract of Calotropis procera and Seeds' extract of Neem in alone.This study confirms the antifeedant effect of bio-pesticide (neem seeds' extract + Calotropis procera leaves' extract). The test was conducted on labotratory reared Plutella xylostella. Observations were done after 48 hrs of bioassay. Significant feeding deterrency was exhibited at concentration (7gms NSE gms +7gms CLE/100 ml). While, the deterrent effects declined considerably with the decrease in concentration of the bio-pesticide. In case, (3gms of NSE+ 3gms of CLE/100ml), it consumed around 1.47 cm^2 leaf of cauliflower. Whereas it consumed 1.03 cm^2 area of leaf on average, when treated with extracts of NSE (14gms/100ml), which is low in comparision. Third instar larva of P. xylostella showed the least antifeedant effect in control bioassay. The Antifeedant- Index calculated for biopesticide (7gms NSE + 7gms CLE/100ml of distill water) was the highest, that is 73.94%. It was concluded that biopesticide (7gms NSE+7gms CLE/100ml of distill water) found to be effective in preventing the growth of the pest.

Keywords: Plutella xylostella (L), Biopesticide, Calotropis procera L.and Antifeedant-Index.

INTRODUCTION

The estimated area under vegetables is about 5993 thousand hectares with an annual production of 90831 thousand million tonnes **(Anonymous 2001).** Among which the winter vegetables, crucifers have their superiority over other crops and are grown throughout the country. Cauliflower, B*rassica oleracea* var. *brotrytis* Linn. is an important crop and commercially grown for its edible inflorescence. *Plutella xylostella* is one of the most notorious pest of crucifers. Studies reveal that the pest exhibits a marked preference for cauliflower and cabbage. This is probably due to the fact that both plants possess fleshy and succulent leaves compared to rest of the crucifers tested, and this probably provides olfactory and gustatory stimuli for successful host selection and development **(Dube and Chand, 1977, Singh and Singh 1993).** *Azadirachta indica,* neem has bitter compounds including nimbin, nimbinin and nimbidin as well as a complex secondary metabolite, azadirachtin, a mixture of seven isometric compounds, labelled 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).** Azadirachtin found is considered a promising ingredient for Integrated Pest Management **(Rembold, 1989).** Azadirachtin induces no accumulations in the soil, no phytotoxicity and no accumulation is seen in plants, in addition no adverse effects occur in water or groundwater [**Melhorn et al, 2011a).** Whereas' *Calotropis procera* of family Asclepiadaceae produces large quantities of latex that contains alkaloids. These alkaloids are produced by the plant as a defence strategy against organism such as virus, fungi, insects **(Larhsini et al., 1997).** *Calotropis procera* contains some pesticidal compounds such as calotropin and calotoxin in the plant extracts. Some natural additives such as garlic (*Allium sativum*) and hot pepper (*Capsicum frutescens*) exhibit synergistic effect on the neem product. Studies also reveal some relatively great effectiveness when these extracts are mixed with or applied alternatively with bio-pesticides such as *Bacillus thuringiensis* **(Adu-Acheampong 1997).** Taking in account the fact that a synergistic phenomenon among metabolites of essential oil may result in a higher bioactivity as minor constituents found in low percentages may act as synergists, enhancing the effectiveness of the major constituents through a variety of mechanisms **(Berenbaum, 1985).** consequently, reducing the dose of polluting substances and the risk of developing resistance, the antifeedant effect of neem Seeds' and *Calotropis Procera* leaves' extract in combination, at different concentrations were evaluated on third instar larva of *Plutella xylostella*.

METHODOLOGY

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 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. Ten male *Plutella* and ten female *Plutella* were introduced in each of these buckets. After 7-11 days the 3rd larval instar of *Plutella* were collected from the buckets.

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 stainless steel electric blender (Philips, India). The leaves were then weighed to obtained 3gms, 5gms and 7gms of the same. The neem seeds were pounded gently in such a way that no oil comes out. The neem seed powder is then weighed to obtained 3gms, 5gms and 7gms. An aqueous extracts from the same was prepared using method as adopted by Zaman et al., 2012 but with slight modifications. The powdered seeds of *Azadirachta indica* and leaves of *Calotropis procera* was soaked in 100 ml of distill water in 1:1 ratio. After 24 hrs, the water with soaked powdered leaves and seeds, was filtered with Whatman filter paper, the bio-pesticide so obtained was stored in clean containers in a refrigerator until further use. Last but not the least, 2-3 drops of liquid detergent was added to different concentrations of bio-pesticides so prepared. It was also added to the distill water that acted as the control. The liquid detergent drops were used as a surfactant.

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

Table 1.

***N*SE**	**(Alone**	**14gms/100ml**
CLE	**Alone)**	**14gms/100ml**
NSE+CLE	**(in combination)**	**3gms+3gms/100 ml**
NSE+CLE	**(in combination)**	**5gms+5gms/100 ml**
NSE+CLE	**(in combination)**	**7gms+7gms/100 ml**
Control		**Distill water**

(**Here NSE refers to Neem Seed Extract and CLE refers to *Calotropis leaves extract*)**

Figure 1.

Figure 2.

Antifeedant index

An antifeedant index (AI) was calculated using the formula AI = (C-T /C + T) X 100 where, C is for leaf area consumed in control and T is for leaf area consumed by the insect in treatment **(Isman er at., 1990)**.

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 filter paper to avoid dessication. 5 larvae of *Plutella* were introduced in each petri disc (Figure: 1). Petri-dishes were then covered with a fine muslin cloth in order to prevent larva from escaping. Each treatment had 6 replicates. (Figure 2 the petri dishes were placed on the table randomly. Mortality was assessed at 24 hours intervals for 4 days. After 48 hours leaf discs were collected. Each collected leaf disc was charted on a graph paper and measured **(Singh, 1982**, **Sarma and Kalita, 2001)** to calculate the leaf area consumption by the larva.

RESULTS

Antifeedant behaviour of 3rd instar larva of *Plutella xylostella of Brassica oleracea* var. *botrytis* Linn. against NSE and CLE concentrations in combination and in alone, is shown in table given below.

Table 2.

BIO-PESTICIDE used in bioassay	**Extract concentration**	**Leaf area consumption (cm2)**
***N*SE (Alone)**	**14gms/100ml**	**1.03**
CLE Alone)	**14gms/100ml**	**.59**
NSE+CLE (in combination)	**3gms+3gms/100 ml**	**1.47**
NSE+CLE (in combination)	**5gms+5gms/100 ml**	**.97**
NSE+CLE (in combination)	**7gms+7gms/100 ml**	**.31**
Control	**Distill water**	**2.07**

(Here NSE refers to Neem Seed Extract and CLE refers to *Calotropis leaves extract*)

Antifeedant index of 3rd instar larva of *Plutella xylostella against* B*rassica oleracea* var.*botrytis* Linn. When treated with NSE and CLE concentrations in combination and alone is given below

DISCUSSION AND CONCLUSIONS

Significant feeding deterrency was exhibited at 7gms NSE gms +7gms CLE/100 ml concentration. While, the deterrent effects declined considerably with decrease in concentration of biopesticide. In case, (3gms of *NSE*+ 3gms of CLE/100ml), it consumed around 1.47 cm^2 leaf of cauliflower. Whereas it consumed 1.03 cm^2 area of leaf on average, when treated with extracts of NSE (14gms/100ml), which is more in comparison, to biopesticide (7gms NSE+7gms CLE/100ml of distill water) which showed just .31cm^2 leaf consumption. In CLE (14 gms/100ml) bioassay, the larva consumed around .59 cm^2 area of leaf disc which is significant but still more as compared to the highest concentration of the biopesticide in combination. Third instar larva of *P. xylostella* showed the least antifeedant effect in control bioassay.

Table 3.

BIO-PESTICIDE used in bioassay	Extract concentration	Antifeedant index
*N*SE (Alone)	14gms/100ml	33.54
CLE Alone)	14gms/100ml	55.63
NSE+CLE (in combination)	3gms+3gms/100 ml	16.94
NSE+CLE (in combination)	5gms+5gms/100 ml	36.18
NSE+CLE (in combination)	7gms+7gms/100 ml	73.94

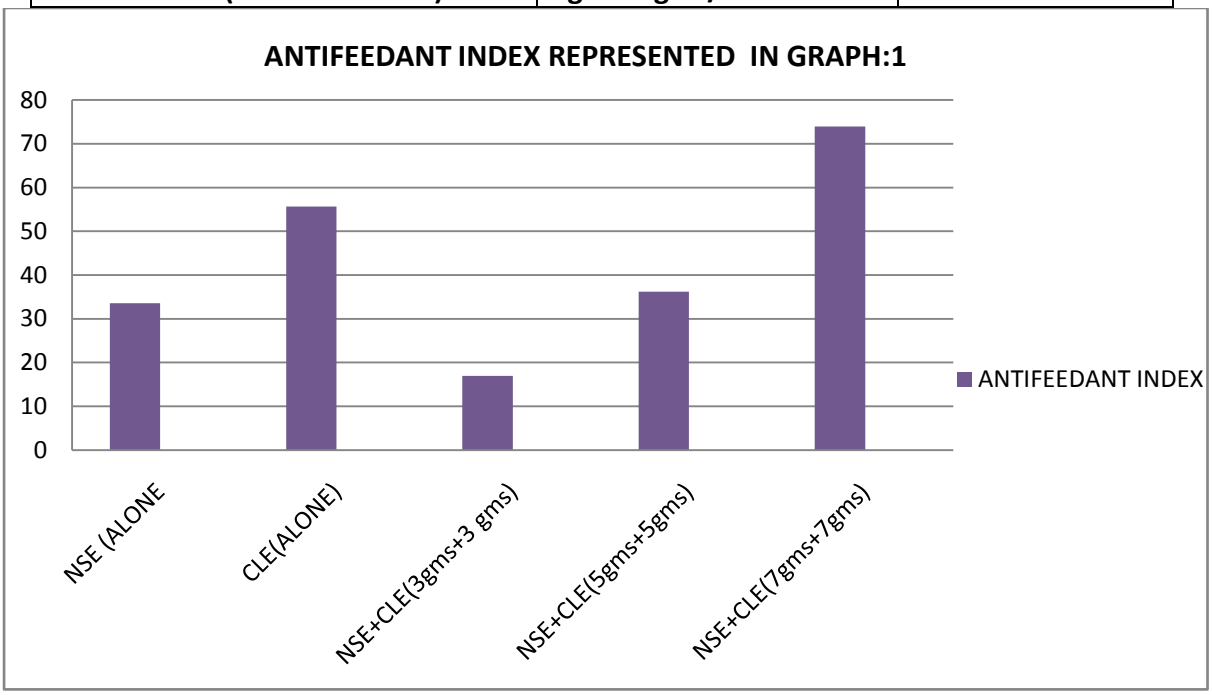

Figure 3. Showing malformed adult.

Antifeedant index (AI) of larva on the basis of leaf area consumption, increased with the increase in concentration of extract. AI index was the highest when the larva was fed with bio-pesticide (7gms NSE +7gms CLE /100).

It was 73.94 % which clearly denotes the success of using combination product against the pest. AI values for 3rd instar larvae ranged from 16.94 % to 73.94 %. Moreover, a positive correlation was recorded between the AI value and the concentrations of the NSE+CLE extract. THE AI value was low for NSE and CLE in alone as compared to the combination product.

Other than the antifeedant behavior, the prolongation in larval developmental periods, failure to moult, blackening of the larva and malformed larval, pupal, adult intermediaries was also observed.

It was concluded that biopesticide (7gms NSE+7gms CLE/100ml of distill water) found to be effective in preventing the growth of the pest and possess considerable antifeedant property against 3rd instar larva of *P. xylostella*.

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