Study of Efficacy of *Clerodendron inerme* Gaertn. Leaf extract against *Pieris brassicae* (Linnaeus)

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Abstract: The study deals with the efficacy of *Clerodendron inerme* leaf extract against *Pieris brassicae*. Larva, pupa and adult of *P.brassicae* have been reared in lab and treated with the aqueous extract of *C.inerme* leaf of different concentration. The results show that extract is quite effective against all the three stages, in general and pupa, in particular. A typical extract with 12.5% concentration shows a mortality rate of 20% for larvae which rises to 55% for pupa. The mortality rate generally increases with increase in the concentration, reaches to its maximum at 10% to 17.5% of concentration and then decrease or becomes constant for different developmental stages.

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Introduction:

India is a country where majority of population depends on agriculture produce. Farmers toil on field to get maximum output but a substantial share of their hard work is destroyed by the pests. Crop loss due to insect pests varies between 10% and 30% for major crops (Ferry *et.al*, 2004).

Use of biopesticide is an economical and ecofriendly method which has attracted the attention of scientists and the researchers. Plants are the rich source of chemicals exhibiting insecticidal properties with least side effects. Plants contain secondary metabolites that are deterrent to insects and other herbivores due to toxicity, enzyme inhibition or other ways (Wheeler and Isman, 2001). These chemicals are highly effective, safe and ecofriendly pesticides (Senthil Nathan and Kalaivani, 2005).Literature review reveals that research has been going on the toxic effect of secondary metabolites of plants on insects. (Dev and Koul, 1997; Koul and Dhaliwal, 2001; Elumalai et.al, 2008; Pugazhvendan et.al, 2009). Researchers have reported a number of plant species which possessed pesticidal properties and these species could serve as alternative to chemical pesticides (Ahmad et.al, 1984; Singh 2000; Kaushik and Kathuria 2004). Biopesticidal property of Chrystella parasitica and Ipomoea carnea on Achaea janata has been reported by Sahayaraj et.al. (2003).

Leaf extract of *Clerodendrum inerme* has many active compounds which are reported to show pesticidal properties against many yield- threatening pests. Holihosur *et.al* (2013) has reported the use of *C.inerme* plant extract against *Aedes aegypti* mosquito. The effect of crude leaf extract of *C. inerme* has shown insecticidal activity against larvae and pupae of *Culex quinquefasciatus* and the efficacy of *C. inerme* plant extract in the management of The tobacco caterpillar, *Spodoptera litura* has also been studied (Khetagoudar *et.al* 2012). Insecticidal activities of alcoholic extracts of *C.inerme* on the larvae of *Achaea janata* L. has been reported by Yankanchi *et.al* (2010).

Pieris brassicae (Linnaeus) is a polyphagus pest. In India, it is widely distributed along the entire Himalayan region. It causes severe damage to cabbage, cauliflower, radish, turnip and also mustard and rape. The young caterpillars feed gregariously on leaves. As a result of their feeding, the leaves are skeletonised; sometimes the caterpillars bore into the heads of cabbage and cauliflower (Navarajan Paul, 2007).

In the present study, efficacy of *C.inerme* plant's leaf extract on larva, pupa and adult stage of *Pieris brassicae* is investigated.

Materials and Methods:

1. Preparation of Aqueous leaf extract of C.inerme:250g of fresh leaves of C.inerme were collected from foothill plain area of Uttarakhand state in the morning hours and washed thoroughly with distilled water and dried using blotting paper. These dried leaves were cooked without water in a pressure cooker for 20 minutes. 250 ml of distilled water was added to these cooked leaves and the mixture was macerated in an electric blender to get slurry. This slurry was sieved through cheesecloth and then filtered through a Whatman filter paper No.1.The volume was made up to 500ml by using distilled water. This was considered as 50% stock solution of the extract. This solution was further diluted to make 1, 2, 5, 7.5, 10, 12.5, 15, 17.5 and 25 % of the stock solution.

2. Test organism: The leaves with egg masses of *Pieris brassicae* were collected from the fields of *Rafinus sativus* and *Brassica oleracea* in and around Nainital area, Uttarakhand state. These leaves were surface sterilised with 0.02% sodium hypochlorite solution and then dried .The dried leaves with egg masses were transferred on to the filter paper and kept in Petri dishes in laboratory under conditions $(25^{\circ}C \pm 10^{\circ}C \text{ temp.}; 70-75\% \text{ RH})$ to provide optimum conditions for the growth of larvae. Newly hatched first instar larva were transferred to plastic trays with dimensions 20cm x 20cm x 10cm on the bed of fresh cabbage leaves with a fine camel's brush. Laboratory emerged third instar larvae were used for this experiment.

3. Evaluation of Larvicidal efficacy of Bioassay:

The extract of various concentrations were prepared from the 50% stock solution. 2 ml of the extract solution were sprayed on the lab emerged third instar larvae of *Pieris brassicae* using an atomiser. At the same time untreated lab emerged third instar larvae were sprayed with 2ml of distilled water alone. The experiment with each concentration was replicated thrice. The sprayed larvae were then transferred to transparent capped plastic boxes of size 9cm x 9cm x 5cm. These boxes were covered by fine mesh net to allow proper aeration. These boxes had a bed of fresh cabbage leaves for feeding of the larvae. Twenty third instar larvae were taken per treatment .Observations were recorded at 24 hours interval.

4. Observations and Method of data analysis:

Table1. Total percent mortality of *Pieris brassicae* at larval, pupa and adult stage at various concentrations of aqueous extract of *C.inerme*

Treatment	Percent Concent- ration	Percent total mortality in larval stage	Percent total mortality in pupa stage	Percent total mortality in adult stage or deformed adults
T1	1	7	20	10
T2	2	10	28	12
T3	5	15	40	15
T4	7.5	17	50	20
T5	10	20	55	12
T6	12.5	22	62	10
Τ7	17.5	15	65	13
Т8	25.0	18	62	10
Т9	Distilled water	5	10	0
T10	Absolute control	0	0	0

Mortality in larval, pupa and adult stages was carefully observed and recorded for every 24 hours till the end of the experiment and or till the successful emergence of adults. Percentage mortality observed in the experiment was corrected by using Abbott's formula (Abbott, 1925). The experimental results obtained were analyzed and interpreted by application of Analysis of Variance (ANOVA) using a factorial Completely Randomized Design (CRD). Table 1 provides the account of the total percent mortality of Pieris brassicae at larva, pupa and adult stage at various concentrations of aqueous extract of *C. inerme.*

Graph1: Comparison of mortality data at various concentrations for larval, pupa and adult stage



Result and Discussion:

The study shows that extract is effective against different developmental stages of *Pieris brassicae*. The results reveal that the extract exhibited least effect on larval stage, as the mortality rate at this

Stage at all concentrations was lower than the mortality rates at pupa or adult stage. The mortality rate was highest for 10% and 12.5% concentration of the extract for larval stage. Even at these concentrations, the mortality percentage was 20% and 22%. It means nearly 80% of the larvae entered into pupa stage even after the use of the extract under study. In the present study, the percentage mortality in larval stage increased with the increase in the concentration of the extract, became highest at 12.5% concentration and then declined with higher concentrations of extract.

However, mortality was significantly evident in pupa stage. At 2% concentration of the extract, the percentage mortality increased from10% at larval stage to 28% in pupa and 12% in adult stage. For the 10% extract mortality was 20% for larvae and it rose to 55% for pupa. Similar trend were observed at other concentrations too. Thus the larvicidal effect of the extract was less in larval stage than the pupa stage. Similar results have been reported by Tewary *et.al*, (2005) and Holihosur *et.al*, (2013) who worked with different plants and pests. Arguably the reason may be development of biochemical strategies in larval stage against the biochemical component of the extract and also the cumulative effect in the pupa stage. Among the different concentrations of the extract used, highest mortality rate of pupa was found with 17.5% concentration. The general trend was, the percentage mortality in pupa stage increased with the increase in the concentration of the extract became maximum at 17.5% concentration and then declined with higher concentrations of extract.

The mortality rate in adult stage, though comparable to larval stage, yet substantially lower than pupa stage. It may be due to more tolerance among the emerging adults towards the leaf extract.

Although the exact mode of action of *C.inerme* extract, the compound (-)-3-Epicaryoptin isolated from the leaves of *C.inerme* was found to be responsible for growth inhibition and antifeedant activities in housefly and mosquito (Pereira and Gurudutt, 1990).Recently, new sterols have reported from *C.inerme* (Pandey *et.al*, 2003) and three new neo-clerodane diterpenoids, been found in the hexane extract of aerial parts of *C.inerme*. Arguably these may be the biochemicals responsible for the larvicidal activity at different developmental stages of *P.brassicae*.

The leaf extract of *C.inerme* is effective larvicide, economic, easy to prepare and ecofriendly. It can be used against phytophagous pests for their control.

Further research is needed to elucidate this activity against a wide range of pests and also the isolation and biomolecules characterization of the active ingredient(s) and its efficacy against the pest under field conditions and also the ecofriendliness. Exhaustive investigations can be initiated to study the integration of *C.inerme* leaf extract with other plant species' extract to check combined effect on a variety of common plant pests.

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