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# Bio-efficacy study on alcoholic extracts of fern plants against diamondback moth, *Plutella xylostella* L. in cabbage

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**Abstract**
Cabbage is an important cruciferous vegetable grown in India with an area of 4,33,870 ha. Diamond back moth is notorious pest of cabbage. Indiscriminate use of chemicals to control this pest has resulted in resurgence, resistance, replacement, impact on non-target organisms, including humans, environmental pollution. Due to this reason and the growing awareness about organic products now farmers and researchers are switching over to botanical pesticides, which overcome many problems associated with chemical insecticides especially in the vegetables. Our present study mainly focuses on efficacy of certain fern plant extracts against *P. xylostella* under laboratory condition. Three ferns (*Diplazium esculentum, Christella parasitica* and *Blechnum orientale*) extract has been tested on the larva of *P. xylostella* in the lab condition. All of these extracts showed statistically significant results over the untreated control (distil water). Increase in larval mortality with the increase in the concentrations was observed being maximum at 20% concentration. The maximum larval mortality was 36.66% after 24 hrs of treatment in case of *D. esculentum* which increased to 73.33% after 72 hrs which is well above the untreated control as 0.66% after 24 hrs and 13.33% after 72 hrs in the later case, indicating *D. esculentum* to be the most toxic to larvae of *P. xylostella*. The efficacy of *D. esculentum* altered the feeding behavior of *P. xylostella*, reduced the larval and pupal weight, prolonged the pupation period, malformed the pupa and adult were recorded under *in vitro* condition which will be briefly discussed in this paper.

**Keywords:** *Brassica oleracea*, *Plutella xylostella*, fern plant extracts

## Introduction
In India, a total of 37 insect pests have been reported to feed on cabbage (Lal, 1975) [1]. Among which diamond back moth is most notorious one. The pest was European origin but become well adopted in Indian condition causing serious threat to the cultivation of cabbage. As most of the farmers in our country mostly rely on the indiscriminate use of chemical pesticides going for at least one spray per week caused the pest to create resurgence, resistance with the cast of impact on non-target organisms, including humans and environmental pollution which raised lot of questions about the current pest management tactics and increased the awareness about the deleterious effects of insecticides. Now the use of plant products in the pest control is gaining importance. In nature more than 1800 plant species are reported to have biopesticidal properties. Plants are the richest source of bioactive phytochemicals such as alkaloids, terpenoids, poly acetylene, unsaturated isobutlyamide and retinoides etc. which may act as toxicant, repellent and behaviour modifiers (Verma and Chaurasia, 2003) [2]. Hence, the search for new and potent botanicals would be appropriate in the current agricultural scenario. Ferns are generally not eaten by herbivores insects (Soo and Fraenkel, 1964) [3]. Ferns are of great significance for application as new pesticides due to their particular status in plant taxonomy and co-evolution with insects. The condensed tannins present in ferns are one of the most important factors in plant chemical defences. They are potent antifungal, antibacterial, antiviral and powerful feeding inhibitions in ferns (Lawton, 1976; Rhoades and Cates, 1976; Cooper *et al*., 1977) [4, 5,6 ]. The ecdysone or insect moulting hormones are found in many ferns which induce metamorphosis in arthropods (Herout, 1970) [7]. The phytochemical constituents of a fern plant, *Christella parasitica* (L.) showed toxic effects as well as growth disrupting responses against *Spodoptera litura* and *Helicoverpa armigera* (Balasubramaniam *et al*., 2008) [8].

**Materials and methods**

Three species of fern namely *Diplazium esculentum* (Ratz.), *Christella parasitica*, and *Blechnum orientale* (L.) available in and around the vicinity of College of agriculture, Central Agricultural University, Imphal were collected and identifitied by the Department of Life Science, Manipur University, Imphal. The collected leaves of three plants species viz., *Diplazium esculentum* (Ratz.), *Christella parasitica*, and *Blechnum orientale* (L.) were washed in running tap water and thereafter with distilled water. The leaves were wrapped in blotting paper to absorb the excess water and shade dried at room temperature for a week. The dried leaves were grinded in a domestic grinder to fine powder and kept air tight in reagent bottles in refrigerator. From the stock, 250 gm of powder of each plant were used for the preparation of extraction. The powders were dissolved in 100ml of 80% ethanol in air tight glass container for about five days. On the fifth day the content was filtered through eight fold muslin cloth and the filtrate was kept in air tight reagent bottle in refrigerator and the final volume was measured and considered as 100% (Anuradha *et al*., 2010) [9]. From this condensed extract, different concentrations such *viz*., 1%, 5%, 10%, 15% and 20% were prepared by adding required quantity of distilled water.

The larvae and pupae of DBM were collected from the cabbage field. The larvae were reared on cabbage leaf pieces in glass petridish (21cm diameter) at room temperature till pupation. The pupae were kept over moist blotting paper in petridish for moth emergence. The emerged moths were released in insect rearing cage containing 4-5 leaf stage of cabbage plant grown in disposable plastic cups daily for oviposition. The larvae hatched were reared on pieces of cabbage leaf in petridishes. The larvae of the same age were used in the evaluation of effectiveness of extract prepared from different plant species.

Leaf disc of cabbage leaf were obtained using a 15cm long cylindrical cutter made of galvanised iron with a diameter of 3.5cm. The leaf discs obtained were dipped in the plant extracts for 10 minutes. The treated leaf discs were kept in petriplates at room temperature for 30 minutes in order to dry up the excess moisture on the leaf disc. Twenty larvae of 5 days old starved for four hrs were released on the leaf disc for feeding in the petriplates for evaluation of plant extracts. The leaf disc were renewed daily with treated leaf disc for larval feeding, consecutively for three days and thereafter untreated leaf disc were provided to the larvae till pupation. All the treatments were set in completely randomized design with four replications. Similarly, the leaf discs were dipped in distilled water and were used as a control treatment in the manner mentioned above. The effect of different concentrations of alcoholic extracts the fern plants on the larval mortality of *P. xylostella* after 24, 48 and 72 hrs was observed. Pupation success, percent adult emergence, growth index in the treatments were calculated by following the method of Srivastava (1959) [10] as follows:

$$\text{Pupation success \%} = \frac{\text{Number of pupae}}{\text{Total number of larvae}} \text{X100}$$

$$\text{The percent adult emergence} = \frac{\text{Number of adult emerged}}{\text{Total number of pupae}} \text{X100}$$

$$\text{Growth index} = \frac{\text{Number of adult emerged (n)}}{\text{Total number of pupae (p)}}$$

The percentage data of the experiment was transformed wherever necessary by using suitable transformation in order to make the analysis of variance valid and feasible. The data thus transformed was statistically analysed by Fischer's method of analysis of variance (Snedecor and Cochran, 1967) [11].

**Results**

The data presented in the table.1 indicated that larval mortality of *P. xylostella* were highly significant in case of alcohol extracts of all the fern plants viz. *D. esculentum*, *C. parasitica* and *B. orientale* under test in comparison to treatment under control. The larval mortality was low at low concentration of all the fern plants after 24 hrs of treatment but showed increase in the larval mortality with the increase in the concentration of oil and was recorded maximum at 20% concentration of all the three fern plants. In case of alcoholic extracts of *D. esculentum*, the lowest mortality was 23.33% at lowest concentration of 1% and highest mortality was 36.66% at highest concentration of 20% after 24% hrs of treatment. In case of *C. parasitica* also the lowest mortality (16.66%) was recorded at lowest concentration of 1% after 24 hrs of treatment. *B. oreintale* also gave similar result. There was 0.66% mortality in control after 24 hrs of treatment. The larval mortality increased after 48 hrs of treatment in case of all the three plant extracts. The larval mortality was 46.66%, 43.33% and 40.00% respectively for *D. esculentum*, *C. parasitica* and *B. orientale* at highest concentration of 20%. The mortality gradually showed decreasing trend with the decrease in concentration of extract and was minimum of 30.00%, 26.66% and 23.33% respectively for *D. esculentum*, *C. parasitica* and *B. orientale* at 1% concentration. Maximum larval mortality was observed after 72 hrs of treatment which was 73.33%, 63.33% and 60.00% respectively for *D. esculentum*, *C. parasitica* and *B. orientale* at 20% concentration. In comparison to this the larval mortality was only 13.33% n case of control after 72 hrs of treatment.

Significant difference in the larval and pupal size in various treatments of plant extracts in comparison to control is also observed. The larval length (5.50mm) and width (1.00mm) and pupal length (6.23mm) and width (1.23mm) was minimum at 20% of *D. esculentum.* This was followed by 20% concentration of *C. parasitica* show larval length (5.63mm) and width (1.06mm) and pupal length (6.40mm) and width (1.26mm) showing its moderate effectiveness. Similarly, *B. orientale* at 20% concentration showed larval length (5.80mm) and width (1.10mm) and pupal length (6.43mm) and width (1.30mm) which indicated least effect on the size of larva and pupa. In comparison, the larval length (6.63mm) and width (1.43mm) and pupal length (6.83mm) and width (1.53mm) was maximum in case of untreated control. The study indicated adverse effect of fern plants particularly *D. esculentum* on the size of larva and pupa of *P. xylostella.*

Based on the data presented in the table.1 it is also clear that the plant extracts have the significant effect on the pupation success, adult emergence and growth index of insects. The pupation success, adult emergence and growth index at 20% concentration of *D. esculentum* was respectively 13.33%, 33.00% and 1.68 in comparison to untreated control. The pupation success, adult emergence and growth index in the untreated control was 40.00%, 88.40% and 5.64 respectively which was considerably high.

**Table 1:** Effect of alcoholic extracts of some fern plants on the larva of *P. xylostella*

| Fern plants Conc. (%) | Larval mortality (%) | | | Larval size | | Pupal size | | Larval Duration (days) | Pupal Duration (days) | Pupation Success (%) | Adult Emergence (%) | Growth index |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | After 24 hrs | After 48 hrs | After 72 hrs | length | width | length | width | | | | | |
| *D. esculentum* @1% | 23.33(28.86) | 30.00(32.21) | 46.66(43.05) | 6.06 | 1.36 | 6.60 | 1.43 | 14.83 | 6.66 | 23.33 (28.86) | 33.33 (35.24) | 3.13 |
| *D. esculentum* @ 5% | 26.66(31.05) | 33.33(35.24) | 53.33(46.89) | 6.20 | 1.26 | 6.56 | 1.40 | 15.03 | 6.83 | 21.66 (27.69) | 33.32 (35.24) | 2.88 |
| *D. esculentum* @10% | 30.00(32.21) | 40.00(39.23) | 60.00(50.77) | 6.03 | 1.23 | 6.40 | 1.30 | 15.23 | 7.00 | 18.33 (25.33) | 33.30 (35.24) | 2.42 |
| *D. esculentum* @15% | 33.33(35.24) | 43.33(42.15) | 63.33(52.71) | 5.76 | 1.13 | 6.36 | 1.26 | 15.40 | 7.06 | 16.66 (24.04) | 33.00 (36.27) | 2.13 |
| *D. esculentum* @20% | 36.66(37.23) | 46.66(43.05) | 73.33(58.89) | 5.50 | 1.00 | 6.23 | 1.23 | 15.50 | 7.26 | 13.33 (21.39) | 33.00 (36.27) | 1.68 |
| *C.parasitica1*@1% | 16.66(24.04) | 26.66(31.05) | 40.00(39.23) | 6.43 | 1.40 | 6.66 | 1.46 | 14.83 | 6.56 | 25.00 (30.00) | 50.00 (45.00) | 3.14 |
| *C. parasitica* @5% | 20.00(26.57) | 30.00(32.21) | 46.66(43.05) | 6.23 | 1.33 | 6.60 | 1.43 | 15.00 | 6.73 | 23.33 (28.86) | 50.00 (45.00) | 3.08 |
| *C. parasitica* @10% | 26.66(31.05) | 36.66(37.23) | 50.00(45.00) | 6.13 | 1.30 | 6.50 | 1.36 | 15.10 | 6.83 | 20.00 (26.57) | 53.33 (46.89) | 2.65 |
| *C. parasitica* @15% | 30.00(32.21) | 40.00(39.23) | 56.66(48.79) | 5.96 | 1.16 | 6.46 | 1.33 | 15.16 | 7.00 | 18.33 (25.33) | 60.00 (50.77) | 2.37 |
| *C. parasitica* @20% | 33.33(35.24) | 43.33(42.15) | 63.33(52.71) | 5.63 | 1.06 | 6.40 | 1.26 | 15.33 | 7.16 | 16.66 (24.04) | 60.00 (50.77) | 2.09 |
| *B. orientale* @1% | 13.33(21.39) | 23.33(28.86) | 36.66(37.23) | 6.43 | 1.40 | 6.76 | 1.50 | 14.77 | 6.56 | 26.66 (31.05) | 60.05 (50.77) | 3.57 |
| *B. orientale* @5% | 20.00(26.57) | 30.00(32.21) | 43.33(42.15) | 6.23 | 1.36 | 6.56 | 1.46 | 14.96 | 6.66 | 25.00 (30.00) | 60.05 (50.77) | 3.31 |
| *B. orientale* @10% | 20.00(26.57) | 33.33(35.24) | 50.00(45.00) | 6.20 | 1.30 | 6.53 | 1.40 | 15.06 | 6.73 | 23.33 (28.86) | 60.03 (50.77) | 3.02 |
| *B. orientale* @15% | 26.66(31.05) | 36.66(37.23) | 56.66(48.79) | 5.96 | 1.20 | 6.36 | 1.36 | 15.16 | 6.80 | 20.00 (26.57) | 60.00 (50.77) | 2.76 |
| *B. orientale* @20% | 33.33(35.24) | 40.00(39.23) | 60.00(50.77) | 5.80 | 1.10 | 6.43 | 1.30 | 15.03 | 6.96 | 18.33 (25.33) | 60.00 (50.77) | 2.39 |
| Control --- | 0.66(4.44) | 0.66(4.44) | 13.33(21.39) | 6.63 | 1.43 | 6.83 | 1.53 | 14.76 | 6.33 | 40.00 (39.23) | 88.40 (70.09) | 5.64 |
| S.E.(d) | 0.50 | 0.60 | 0.70 | 0.35 | 0.09 | 0.11 | 0.10 | ----- | 0.26 | 3.33 | 1.20 | 0.37 |
| C.D. (p=0.05) | 1.20 | 1.27 | 1.47 | 0.73 | 0.19 | 0.23 | 0.21 | NS | 0.54 | 6.78 | 2.40 | 0.53 |

No. of larvae released are 20 larvae /replication. Data represented are based on three replications. Figures in parenthesis are in arc sine angular transformation

## Discussion

Present study is in line with Marangmei (2010) [12] who reported maximum larval mortality in case of *D. esculentum*, *C. parasitica and B. orientale* which is also similar into the result obtain in the present investigation. Various ferns contain saponins derivatives which inhibit the larval growth and development and the tannin derivatives in the fern plants combines with protein and thereby inhibits the enzyme activities and reduces the availability of protein in haemolymph (Swain, 1978) [13]. Therefore the saponins and tannins in the fern have adverse effects on the growth of larva in terms of their size as obtain in the present investigation. The role of phytoecdysone in fern is to induce metamorphosis in arthropods causing anomalous development (Herout, 1970) [7]. The parameters on pupation success, adult emergence and growth index significantly affected by the extracts of fern plants, might be due to the saponnins, tannins and other defensive compound present in the fern extract. With this study we conclude that there is a possibility of using this natural extracts from the ferns. There is also need for further studies on the compounds of the ferns and their concentration which leaded to the disruption in the biology of DBM.

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