Insecticidal potential of traditionally important plant, *Zanthoxylum armatum* DC (Rutaceae) against cabbage butterfly, *Pieris brassicae* (Linnaeus)

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Cabbage butterfly, *Pieris brassicae* (Linnaeus) is one of the most destructive pests of brassicaceous crops. *Zanthoxylum armatum* DC (Rutaceae) is versatile, traditionally important and edible medicinal plant, known to have insect repellent and larvicidal properties against many pests. Thus, the insecticidal potential of *Z. armatum* was evaluated against *P. brassicae*. N-hexane fraction of different plant parts of *Z. armatum* was extracted by using standard Soxhlet extraction method. Median lethal concentrations and time (LC₅₀ and LT₅₀) were determined by contact and oral toxicity tests by using Probit analysis. Pericarp and leaf extracts were found toxic to the caterpillars by contact. LC₅₀ of pericarp extract (by contact) was found to be 0.15% and 0.22%, respectively at 72 h. The relative toxicity of pericarp extract was recorded to be 1.50, when Azadirachtin 0.15 EC was considered as a unit. With sub-lethal concentrations, the developmental time was delayed; while percentage of pupation and adult emergence was significantly affected in treated caterpillars. In semi-field study, egg-laying of the female butterfly was significantly reduced and larval mortality was considerably higher (up to 53.33%) within 72 h after treatment in plants treated with *Z. armatum* extracts. In field trial, significant reduction (67.92%) in larval population was observed within 5 days of spraying of *Z. armatum* extracts.

**Keywords**: Contact and oral toxicity, Rutaceae, Time-mortality response, *Zanthoxylum armatum*

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The large white cabbage butterfly, *Pieris brassicae* (Linnaeus) (Lepidoptera: Pieridae) is one of the most destructive and widely distributed pest of cruciferous crops across the world including hilly region of India¹–³. The gregarious caterpillars of *P. brassicae* feed voraciously on the leaves; defoliate the plants and reduce the market value of the resultant vegetables⁴. Broods of *P. brassicae* frequently defoliate host plants and compel the farmers to spray pesticides including highly hazardous class 1b organo-phosphates⁵–⁶. Furthermore, *P. brassicae* larvae are highly mobile and move from an exhausted food source to the proximal plants in the field⁷. Considering the profuse fecundity and gregariousness, the protective measures by employing chemical pesticides are usually recommended to reduce their damage. Besides cost, it can also lead to undesirable side effects to human health; since cruciferous crops are usually eaten as raw or in semi-cooked conditions. Besides a huge number of pollinators, the cruciferous ecosystem is known to harbour several important bio-control agents of crop pests⁸. Therefore, depending on synthetic chemicals can cause severe damage to on-farm biodiversity⁹. Consequently, there is an urgent need to find out some natural plant products, those can be excellent alternatives to the chemical pesticides and can effectively manage the emerging pest problems without adversely affecting the ecosystem. Recently, many plant extracts have shown their potential either sole or in combination with chemical pesticides to reduce the damage of destructive pests¹⁰–¹¹.

*Zanthoxylum armatum* DC (Rutaceae) is a versatile, edible and widely distributed plant species, commonly known as Bamboo-Leaf Prickly Ash or Nepal Pepper or Toothache tree in northeast India. Besides high medicinal values¹²–¹³, the *Zanthoxylum* species are known to have insecticidal properties against several insect pests including *Plutella xylostella* Linnaeus¹⁴–¹⁵ and *Spodoptera litura* (Fabricius)¹⁶. Chemical characterization of the plant extract is the most crucial step of making it popular in larger areas. Chemical composition of different

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fractions of plant parts of *Z. armatum* has been studied in detail\(^{14,15,17,18}\). However, the insecticidal potential of *Z. armatum* has yet not been evaluated against several agricultural pests including *P. brassicae*. Due to congenial climatic conditions, the cabbage butterflies are becoming menace in many new agricultural habitats. Considering the pesticide residue problems in brassica vegetables, the alternate management sources are urgently needed for the management of this emerging agricultural pest.

Insect herbivores usually reduce the toxicity of pesticidal compounds and their products by excretion, detoxification, or behavioral adaptations\(^ {19}\). Cabbage butterfly, *P. brassicae* is one of the best examples of Brassica specialist herbivore, since they can detoxify/tolerate higher level of certain chemical compounds, which are lethal to the other herbivores. Paul and Sohkhlet\(^ {20}\) found antifeedent effect with extended pupal period and certain level of larval mortality of *P. brassicae* fed with crude extract of *Z. armatum*. Nevertheless, detailed investigation would give more insights on interactions between this model herbivore and *Z. armatum* extract. Therefore, we studied the insecticidal potential of *Z. armatum* against *P. brassicae*. This information would have immense value in developing effective bio-pesticide based on this plant species.

**Materials and methods**

The experiments were carried out in the Biological control laboratory and entomology farm at the Division of Crop Protection, Indian Council of Agricultural Research (ICAR)- Complex for NEH Region, Umiam, Meghalaya, during the year 2016-2018.

**Mass rearing of test insects**

Caterpillars of *P. brassicae* were collected from the cabbage field (25°41’01.91” North latitude and 91°54’46.24” East longitude) of the Division of Crop Protection, ICAR Research Complex for NEH Region, Umiam, Meghalaya (India), during the year 2016 and further reared in the laboratory on cabbage and knol-khol leaves at ambient conditions (19±2°C temperature, 70±5% relative humidity and 14:10, light: dark period). *P. brassicae* were reared in the laboratory by the standard method described in Firake et al.\(^ {9}\). *P. brassicae* completes the larval growth with five instars and takes 35-38 days to complete the development period\(^ {2}\). The caterpillars from the third and subsequent generations were used for the experiments.

**Collection of plant parts of *Z. armatum* and preparation of extracts**

The fruits and leaves of *Z. armatum* were shade dried and different parts (viz., pericarp, seeds and leaves) were grind separately in a blender. The plant extract was prepared by the standard protocol and the detail procedure is available in Kaleeswaran et al.\(^ {16}\). Different fractions of plant parts of *Z. armatum* were extracted using four extraction solvents viz., hexane, ethyl acetate (non-polar), methanol, water (polar). The extraction involved the use of Soxhlet extraction method\(^ {21}\). In our previous studies, n-hexane fraction of different plant parts of *Z. armatum* was found to have maximum insecticidal potential against *S. litura*\(^ {16}\). Therefore, only n-hexane fractions have been used in this study to evaluate their insecticidal potential against *P. brassicae*.

**Toxicity of *Z. armatum* extracts against *P. brassicae* by topical application technique**

Plant extracts were evaluated against second instar larvae of *P. brassicae* by topical application technique\(^ {22}\). Based on preliminary experiments, seven different concentrations of each extract and commercial biopesticide were prepared with 5% DMSO. Bioassay of plant extracts was conducted against *P. brassicae* caterpillars by the similar procedure as described in Kaleeswaran et al.\(^ {16}\) for leaf worm, *S. litura*. Each treatment was replicated for six times and each replicate contained 10 insects. The experiment was repeated for three times. The observations on larval mortality were recorded up to 72 h at 12 h interval. Whenever needed, the mortality was corrected using Abbot’s formula\(^ {23}\) and corrected mortality was used for data analysis.

**Time-mortality response (LT\(_{50}\)) of *Z. armatum* extracts (at LC\(_{50}\)) against *P. brassicae***

Time-mortality response was determined for pericarp and leaf extract against second instar *P. brassicae* caterpillars. Similar bioassay procedure was followed as mentioned in the above sub-section for contact toxicity test; but here the test insects were treated only at ‘LC\(_{50}\)’ of respective extract (based on results of the contact toxicity test). Each treatment contained 10 insects, replicated for six times and each experiment was repeated for three times. Observations on larval mortality were recorded at every 6 h time interval up to 96 h after treatment.
Determination of oral toxicity of botanical extracts against *P. brassicae*

The oral toxicity of plant extracts was determined by leaf dip method\(^\text{24}\). Procedure for oral toxicity test was similar as described in Kaleeswaran et al.\(^\text{16}\) for *S. litura*. Except here, the test insects were reared on cabbage plants throughout the experiment. Each treatment was replicated thrice and each replicate consisted of 10 insects. The mortality obtained at 72 h was treated as final mortality. The corrected mortality data was used for the analysis.

Effects of sub-lethal concentration of *Z. armatum* extract against *P. brassicae*

To understand the effects of sub lethal concentrations of *Z. armatum* extract, the second instar larvae of *P. brassicae* were treated with 0.046% of pericarp extract (i.e., at LC\(_{10}\) recorded in contact toxicity tests) and were allowed to feed on fresh leaves of the cabbage throughout the experiment. In control, the larvae were treated only with the solvent. The experiment was carried out under controlled conditions (25±1°C temperature, 75-80% relative humidity and 14:10 light: dark period). The treatments were replicated thrice and each replicate consisted of 10 insects. The experiment was repeated for four times. Observations were recorded on developmental period, % pupation, % adult emergence and longevity of surviving adults etc.

Antifeedent effect of *Z. armatum* extract against *P. brassicae*

Since the pericarp extract (n-hexane) of *Z. armatum* has been reported to have antifeedant activity against many insects\(^\text{16}\); it was also evaluated against *P. brassicae* larvae as per the method described in Kaleeswaran et al.\(^\text{16}\) for *S. litura*. Second instar caterpillars of *P. brassicae* were treated (topical application method as mentioned in contact toxicity test) with seven different concentrations of pericarp extract (viz., 0.6%, 0.3%, 0.15%, 0.075%, 0.0375%, 0.0187% and 0.0%) and allowed to grow on cabbage leaf discs (4.5 cm diameter). Each treatment was replicated for nine times and each replicate consisted of 10 insects. Observations on leaf area consumption (%) were recorded at 24 h after the treatment.

Bio-efficacy of *Z. armatum* extracts against *P. brassicae* under caged conditions (semi-field conditions)

N-hexane fractions of pericarp and leaf of *Z. armatum* were evaluated against *P. brassicae* under cages at their LC\(_{50}\)’s (determined in lab experiments) and compared with commercially available formulation ‘Azadirachtin 0.15% EC’. Initially, 2 months old potted cabbage plants (5 no’s) were kept inside the specially developed cages (45 x 45 x 45 cm) and allowed to establish for 2 days under the cage environment. The cages were arranged inside the room in such a way that, enough sunlight and air made available to each plant inside the cages. The potted plants were sprayed with respective concentrations of extracts and Azadirachtin with the help of hand atomizer. After 30 min of spraying, 5 pairs of *P. brassicae* adults (3 days old) were released inside the cages and allowed to lay eggs. Adults were fed with water and honey *ad libitum*. After 5 days of release, observations were recorded on number of eggs laid per plant (if any). In another experiment, 30 numbers of second instar larvae were released on potted plants inside the cages and allowed to settle for 6 h. The plants (along with insects) were sprayed with respective concentrations of extracts and Azadirachtin with the help of hand atomizer. Each treatment was replicated thrice. Observations on mortality were recorded at 48 h, 72 h and 96 h after treatments.

Bio-efficacy of *Z. armatum* extracts against *P. brassicae* under field conditions

The experiment was conducted in randomized block design (RBD) at entomology farm of the institute during 2017-2018. One month old seedlings of cabbage variety ‘Wonder ball’ were transplanted in the field and recommended management practices were followed for raising healthy crop. After 45 days of transplanting, n-hexane fractions of pericarp and leaf of *Z. armatum* were sprayed at LC\(_{50}\) and LC\(_{100}\) with the help of hand sprayer. Azadirachtin 0.15% EC (Nimbecidine Plus®) was included as one of the treatments for comparison. Sticker cum surfactant was added in spray solution to improve the efficacy of the extracts. In control, only water with surfactant was sprayed on the crop. Each treatment was replicated thrice. In treatment plot (size: 3 m x 3 m), the plants were spaced at 50 cm (row to row) and 40cm (plant to plant) apart. Observations were recorded on number of larvae per plant just before spraying, 1 DAS (days after spraying), 3 DAS and 5 DAS. The experiment was repeated twice in two different fields during 2017-2018.

Statistical Analysis

Corrected mortality data were subjected to Probit analysis\(^\text{25}\) to determine the lethal concentrations and
median lethal time by using Polo Plus: Probit and Logit analysis. Data on sub-lethal effects of *Z. armatum* were subjected to independent sample ‘T’ test to determine the significant differences in two treatments. Data on leaf area consumption, % mortality of larvae in cages and under field conditions were analyzed by using one way ANOVA at 5% level of significance. Analyses of ‘T’ test and one way ANOVA were done by using SPSS 21.0 software for windows.

**Results**

**Determination of contact toxicity, oral toxicity and median lethal time of botanical extracts against *P. brassicae* (topical application method)**

Contact toxicity of n-hexane fractions of pericarp, seed and leaf was determined against *P. brassicae* caterpillars. Toxicity of pericarp extract was observed to be higher than leaf extract and seed extract (Table 1). In case of pericarp extract, the LC₅₀ was found to be 0.15% at 72 h; which is comparatively lesser than Azadirachtin 0.15 EC (commercial check). The relative toxicity of pericarp extract was 1.50, when Azadirachtin 0.15EC was considered as a unit (Table 1). Table 2 shows the time-mortality response of botanical extracts against *P. brassicae*. Median lethal time (LT₅₀) of pericarp and leaf extract was recorded to be 45.95 h and 50.80 h, respectively; whereas the LT₅₀ of Azadirachtin 0.15EC was observed to be 52.05 h. In comparison to the contact toxicity tests, the LC₅₀'s of *Z. armatum* extracts were found to be higher in case of oral toxicity tests. The LC₅₀ of pericarp extract was determined to be 5.13% at 72 h (Table 3).

**Sub-lethal effects of pericarp extract of *Z. armatum* against *P. brassicae***

Significant variation in larval period (t= 9.39, df=4, p≤0.01) and pupal duration (t= 6.94, df=4, p≤0.01, n=120) was observed in treated caterpillars. The larval and pupal duration was higher in treated larvae (28.83 ± 0.17 days and 14.17 ± 0.44 days, respectively) compared to untreated control (Fig. 1). Pupae formation was also considerably reduced (t= -32.14, df=4, p≤0.01, n=120) in treated caterpillars (53.50±0.70%) (Fig. 2) and amongst merely 77.67±1.45% could emerged as healthy adults (Fig. 2). Significant variation in leaf area consumption

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<th>Table 1 — Contact toxicity of <em>Z. armatum</em> extracts against <em>Pieris brassicae</em></th>
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<td>n-hexane (L) <em>Z. armatum</em></td>
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<td>Azadirachtin 0.15EC</td>
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Note: P: pericarp, S: Seed, L: Leaf.

*Relative Toxicity (RT) = LC₅₀ of Azadirachtin 0.15EC / LC₅₀ of the extract

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<th>Table 2 — Determination of lethal time of botanical extracts against <em>Pieris brassicae</em></th>
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Note: 1. P: pericarp, S: Seed, L: Leaf
2. Figures within parentheses are minimum and maximum values

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<th>Table 3 — Oral toxicity of <em>Z. armatum</em> extracts against <em>Pieris brassicae</em></th>
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Note: P: pericarp, S: Seed, L: Leaf
was also found in treated caterpillars. About 0.42% leaf area was found to be eaten by the caterpillars at higher concentration (0.7%) up to 48 h after treatment; whereas 96% food consumption was found in control (Fig. 3).

**Bio-efficacy of Z. armatum extracts against P. brassicae in cages (semi-field conditions)**

When *P. brassicae* females allowed to lay eggs on cabbage plants inside the cages, significant reduction in egg laying was observed in *Z. armatum* and Azadirachtin treated plants compared to control (Table 4). In terms of efficacy, pericarp extract of *Z. armatum* was found to be more effective, where 62.67% mortality of the caterpillars was observed, followed by leaf extract of *Z. armatum* (58.33%) (Table 4).

**Bio-efficacy of Z. armatum extracts against P. brassicae under field conditions**

Pericarp and leaf extract of *Z. armatum* were evaluated under field conditions at their LC50 and LC100 and compared with Azadirachtin 0.15 EC. Significant reduction in larval population was observed in both pericarp and leaf extract within 3 to 5 days of spraying (Table 5). About 67.92 and 62.92% reduction in pest population was recorded in pericarp extract (at LC100 and LC50, respectively); whereas leaf extract reduced 58.33 and 51.25% pest population over control at LC100 and LC50, respectively (Table 5).

**Discussion**

*Zanthoxylum* species are found in temperate and tropical areas across the World and commonly used in daily life as condiments and for therapeutic remedies. Several species of genus *Zanthoxylum* are reported to have significant repellent and larvicidal properties. Based on the literature, we used n-hexane fractions of seeds, leaf and pericarp of *Z. armatum* for the bioassay experiment; since hexane fraction of leaf and fruits contain large proportion of carbonyl compounds, Cinnamoyl amides and the monoterpene ketones. These compounds are reported to have larvicidal activities against insect-pests viz., *Keiferia lycopersicella* (Walsingham), *Spodoptera exigua* (Hubner), *Plutella xylostella*, *S. litura* and mosquito larvae. However, there was no literature yet available on the larvicidal activity *Z. armatum* against *P. brassicae*.

The pericarp and leaves of *Z. armatum* contains large proportions of carbonyl compounds and monoterpene ketones, and this could be the basic reason for higher toxicity of pericarp and leaf extract of *Z. armatum* in laboratory, cages and field conditions. Likewise other rutaceous crops, the aromatic compounds are generally stored in the pericarps of *Zanthoxylum* species. Larvicidal action and antifeedent effect of *Z. armatum* could be due to the presence of compounds like ‘2-undecanone’, ‘2-tridecanone’, ‘Cinnamoyl amides’ and ‘piperitone’. Several reports demonstrated the
antifeedent effect of Zanthoxylum species against insects, including *P. brassicae*.

In this study, the developmental time, pupation and adult emergence was affected when exposed to sub-lethal doses of pericarp extract. Alteration in physiological and biochemical functions, leading to starvation in larvae might be responsible for this. Larval feeding on limited diet can increase the larval and pupal period of insect. Paul and Solkhlet also found significant higher pupal period and larval mortality of *P. brassicae* due to *Z. armatum* extract. In present study, egg laying of the female butterfly was significantly reduced in plants treated with *Z. armatum* extracts. Before oviposition, the insect female internally weighs the various stimuli and inhibitory perceived through visual, chemical, and mechanical signals. Several reports indicated that *Pieris* butterfly’s flight and egg laying patterns can be affected by many reasons including plant chemistry. The strong aroma of the *Z. armatum* extracts might be responsible for avoidance of butterflies for oviposition on treated surfaces. Owing to the pest repellent properties, traditionally the tribal farmers of northeast India used to crush the *Jaiur-blai* (Zanthoxylum spp.) fruits on the body parts before working in the paddy field to get rid of worms, nematodes and external parasites etc. The foraging behavior of *P. brassicae* is also known to alter due to different host generated cues.

**Conclusion**

Overall study revealed that, pericarp and leaf extract of *Z. armatum* have ability to cause lethal and sub-lethal effects in brassica caterpillar pest, *P. brassicae* under laboratory and field conditions. Thus, the *Z. armatum* extract could be formulated and utilized on commercial scale for the eco-friendly management of cabbage butterflies.

**Acknowledgement**

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