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Research Article
Effect of Seed Extracts as Insecticides Against Lepidopteran Pests on Cauliflower

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Abstract

Background and Objective: In recent years, the increasing information on ill effects of artificial prepared chemicals and synthetic insecticides on plants health has alarmed researchers to seek some alternative ways, which are eco-friendly. The objective of this study was to identify the best medicinal plants seed extracts which can inhibit the growth of *Trichoplusia ni* and *Pieris brassicae* (lepidoptera) larvae. **Materials and Methods:** The insecticidal activity experiments were evaluated on two lepidoptera larvae species (*Trichoplusia ni* and *Pieris brassicae*) by using four potential medicinal plants seed aqueous extracts (*Latana camara*, *Sapindus trifoliatus*, *Solanum trilobatum* and *Ceiba pentandra*) at 25-100 mg kg^{-1} concentrations. The data were analyzed using population standard deviation, calculated with online trial version (SPSS-IBM) product. **Results:** The medicinal plant seed aqueous extracts of *Sapindus trifoliatus* and *Solanum trilobatum* species showed rich amounts of phytochemicals like alkaloids, flavonoids and saponins showed best competence in controlling the insect larvae and caused delay in pupation at 100 mg kg^{-1} concentrations. **Conclusion:** The medicinal plant seed aqueous extract of *Sapindus trifoliatus* and *Solanum trilobatum* showed equal inhibition when compared to rosemary Oil. These plants species are suggesting its role as best bio-insecticides in the management of lepidoptera larvae pests.

Key words: Aqueous extracts, bio-insecticides, *Sapindus trifoliatus*, insect larvae, *Trichoplusia ni*, *Pieris brassicae*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The lepidopteran larvae, *Trichoplusia ni* and *Pieris brassicae* causes severe yield losses in cauliflower and became problem for the farmers to control. The insecticidal application overburdened the farmers. Cauliflower is generally infected by lepidopteran larvae which cause wide spread damage and thereby significantly dropping the marketable yield[1]. Synthetic chemical insecticides are widely used to control insect larvae of cauliflower, which may badly affect the health conditions of humans. So there is a urgent need to introduce and adopt environmentally safe, effective and eco-friendly measures to control these larvae[2]. With the introduction of synthetic insecticides, this crisis was resolved to greater extent. But the over usage of these artificial chemical insecticides will and ultimate in constrained spraying of them has necessitated for alternative in essence for concerns to the environment. Pollution of soil and ground water resources has resulted in barren lands. Synthetic insecticides residues also sometime increase problems in the food security among consumers and create trade obstacles for export vegetables. Hence an eco-friendly alternative approach is necessitated.

The bio-insecticides are very effective in controlling the larval population[3] and many other insect pests infesting vegetable crops[4-7]. The importance of botanical insecticides/ plant seed extracts is mainly recognized in the field of agriculture as they are safe, less hazardous and cheap. The plant extracts act as repellant, anti-feedant and its seed contains certain chemicals (phytochemicals), which inhibits the population of insect larvae. Over the past few decades, natural medicinal plant extracts and phytochemicals have been vigorously investigated to develop eco-friendly alternative ways against the usage of synthetic insecticides and pesticides[8]. Different species of medicinal plants are able to synthesis a wide range of bio-active phytochemicals[9-10] and many of them are promising novel sources of natural insecticides[11]. Phytochemicals found in many botanical insecticides have been the recent focus of many investigations[12-13]. Variety of plant extracts have been considered for us as insect antifeedants and repellents but few commercial successes have achieved for plant phytochemicals that can regulate arthropod activities[14]. In view of the above, the insecticidal and regulatory properties of plant extracts of four medicinal plants [Latana (*Latana camara*), Sapindus (*Sapindus trifoliatus*), Solanum (*Solanum trilobatum*) and *Ceiba pentandra*] on lepidopteran larvae causing severe damage to cabbage when taken-up and identified in the present study.

MATERIALS AND METHODS

The study was conducted in the Department of Botany and Microbiology lab, Acharya Nagarjuna University, Guntur District, Andhra Pradesh, India from December, 2016-March, 2017.

Chemicals: All the chemicals used in the experiment were of analytical grade and purchased from Sigma chemicals, India and rosemary Oil (commercial grade) was purchased from ITC Ltd (India).

Selection of the plant sources: The plant species selected for the present study are *Lantana camara*, *Sapindus trifoliatus*, *Solanum trilobatum* and *Ceiba pentandra.* The plant materials were identified and authenticated by the Department of Botany, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur District, Andhra Pradesh, India. The seeds collected from these plants were dried under shade for 20 days for this study.

Preparation of the seed extracts: The aqueous extracts were made by following the methods of Preethi *et al.*[15] with slight modifications. The extract was filtered through Whatman No. 1 filter paper and concentrated to dry under vacuum at 40°C using rotaevaporater. The extracted powder thus obtained was diluted with fixed concentration for the investigation. The extracted powder thus obtained was diluted with water at different concentrations (25, 50, 75 and 100 mg kg^{-1}) pure solvent for the investigation. Thus the obtained dried extracts were lyophilized, labelled and stored at 4°C in sterile bottles. Standard methods[16] were followed to detect various biologically active phytochemical constituents present in these extracts. Estimation of extractive value was done according to the method of Regina[17].

Plants used for the bioassay: Cabbage plants (*Brassica oleraceae*) were selected for the bioassay. The healthy plants were selected from the local crop fields. The leaves for investigation were collected from 5 weeks old cabbage plants.

Insects selected for the experiment: *Trichoplusia ni* (cabbage looper) and *Pieris brassicae* were selected for the experimental study to analyze the insecticidal effect of selected medicinal plant extracts. The insects are reared by following the method and procedures of Sharma and Gupta[18]. The first instar larvae of both the species are collected from the crop fields near the University Campus. The insect's larvae

are maintained at room temperature (25±2°C) at photoperiod of 16L: 8D, in 1 L glass jars and fed on fresh cabbage leaves for about a week.

Screening of medicinal plant extracts against selected insect larvae:

- Feeding deterrence bioassay and repellent action was performed by following the method of Akhtar *et al.*[19] with little modifications with different concentrations of seed extracts (25, 50, 75 and 100 mg kg^{-1}) and the rosemary oil was taken in the concentration of 300, 600, 900 and 1200 ppm as positive control. The experiment was terminated when approximately 50% of the control disc has been eaten (3-5 h). For each of the plant seed extracts, number (n) of larvae are used (n = 10)
- Mortality and toxicity study (spraying) on the larvae
- Pupation experiments were conducted by using the methods of Paul and Sohkhlet[8] with little modifications. Throughout the study the room temperature was maintained with photoperiod of 16L: 8D. Mortality was recorded after 48 h

Statistical analysis: Data were analyzed statistically using online trial version (SPSS-IBM product) and t-test was applied at significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

The plant species selected for the study are *Lantana camara*, *Sapindus trifoliatus*, *Solanum trilobatum* and *Ceiba pentandra*. These results show that the highest EV are obtained for *Sapindus trifoliatus* (18.39% w/w), moderate extractive values are recorded in the seeds of *Solanum trilobatum* (14.68% w/w) and the least values are observed in *Lantana camara* (11.56% w/w) and *Ceiba pentandra* (9.24).

Preliminary phytochemical analysis of plant seed extracts: Phytochemical studies regarding aqueous extracts in indigenous medicinal plants of Sapindus (*Sapindus trifoliatus*), Solanum (*Solanum trilobatum*), Latana (*Latana camara*) are scanty. The AQ seed extract of these plants secondary metabolites are shown in the Table 1.

In the second stage of this study the insecticidal activity of these medicinal plant extracts (*Lantana camara*, *Sapindus trifoliatus*, *Solanum trilobatum* Linn and *Ceiba pentandra* (L) with different concentration (25, 50, 75 and 100 mg kg^{-1}) on the two selected species of *Trichoplusia ni* and *Pieris brassicae* was investigated in two sets of individual experiments. In all the experiments rosemary oil which is commercially available was selected as a positive control at different concentration of 300, 600, 900 and 1200 ppm.

Experimental results on *Trichoplusia ni*

Feeding deterrence: The results clearly demonstrated that the *Sapindus trifoliatus* and *Solanum trilobatum* species plant seed extracts were more active than the positive control at highest concentration. The other two selected species (*Lantana camara* and *Ceiba pentandra* L.) reported low activity when compared to the positive control. The feeding deterrence index (FDI) values clearly demonstrated that the two plant species *Sapindus trifoliatus* (16.296) and *Solanum trilobatum* (19.225) has best anti-deterrence action against the test larvae, when compared to the positive control values (20.426). The other two species (*Lantana camara* and *Ceiba pentandra* (L)) has recorded the highest FDI values (33.173) and (40.056), respectively (Fig. 1). The results are in agreement with the study done by Akhtar *et al.*[19] on same species of *Trichoplusia ni* larvae with the plants extracts from *Melia volkensii*, *M. azedarach* (China-berry), *Trichilia americana*, *Origanum vulgare* and individual plant phytochemicals. These findings are also in consistency with the results of Huang and Renwick[20]. Many authors[21-22] also documented the role of plant seed extracts acts as good sources of antifeeding agents. The decrease in the feed deterrents was due to the presence of different types of phytochemicals (secondary metabolites, volatile oils, terpenoids, alkaloids and phenols)[16].

Repellent activity: The repellent action experiments revealed highest activity when the extracts are at 75 and 100 mg kg^{-1} concentrations respectively. All the larvae were strongly repelled at 100 mg kg^{-1} concentration of the plants extracts. Within 4 h of the experiment larvae were totally repelled from the treated leaves. Among the four medicinal plant seed extracts *Sapindus trifoliatus*, *Solanum trilobatum* Linn recorded the best as a good source of repellent when compare to the positive control (Fig. 2). Paul and Miranda[8] in their investigation on these lepidoptera larvae reported similar results with different plants extracts at different concentrations of 50-150 mg kg^{-1}. This may be due to the presence of many strong secondary metabolites in the plants extracts tested[22-24].

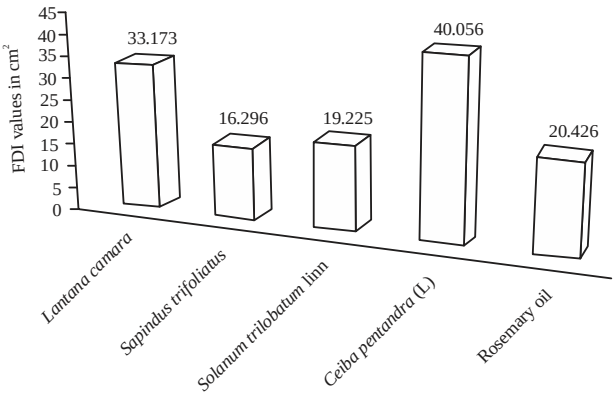

Fig. 1: FDI values when dipped with different concentration of the four plants seed extracts on *Trichoplusia ni* larvae

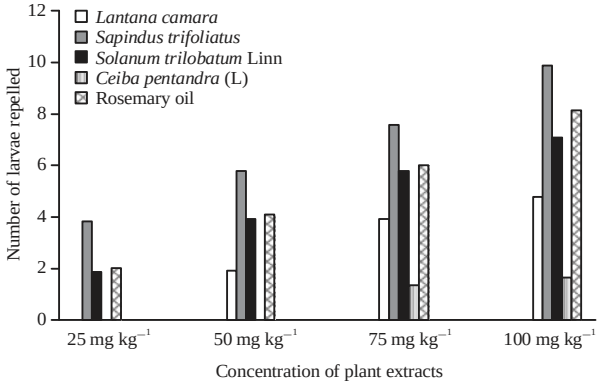

Fig. 2: Repellent activity of the four different plants seed extracts on *Trichoplusia ni* larvae

Table 1: Phytochemical analysis of plants seed aqueous extracts

Phytochemicals	Lantana	Sapindus	Solanum	Ceiba
Test for alkaloids				
Dragendorff's test	-	++	++	++
Meyer's test	-	++	++	++
Test for flavonoids				
Alkaline reagent test	++	-	++++	++
Lead acetate test	++	-	+++	+
Test for tannins				
Gelatin test	+++	++	++	++
Mitchell's test	++	+	++	+
Test for steroids and terpenoids				
Salkowski test	-	-	+	_
Libermann-Buchard test	++	+	+	+
Test for phenols				
Ellagic acid test	-	+++	-	+++
Test for quinones				
Alcoholic KOH test	+	-	-	_
NaOH test	+	-	-	_
Test for saponins				
Froth test	-	+++	+	-
Test for volatile oils				
Test I	+++	++	-	+
Test II	+++	++	-	+

+: Present, ++: Moderately present, +++: Highly present, ++++: Very highly present

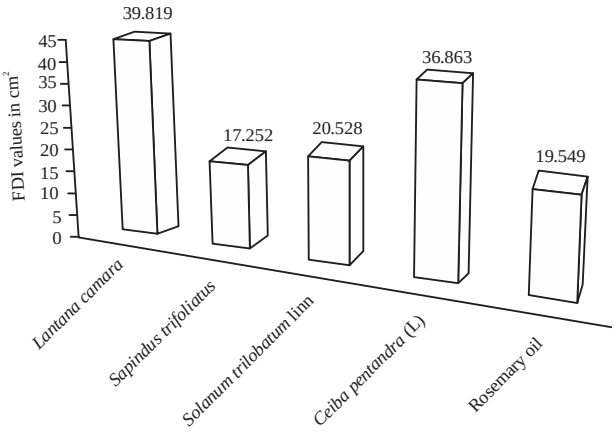

Fig. 3: FDI values when sprayed with different concentration of the four plant seed extracts on *Pieris brassicae* in larvae

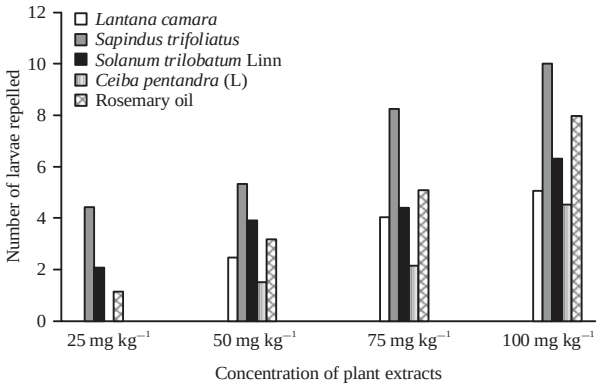

Fig. 4: Repellent activity of the four different plants seed extracts on *Pieris brassicae* larvae

Experimental results on *Pieris brassicae*

Feeding deterrence: The feeding deterrence index (FDI) values clearly demonstrated that the two plant species *S. trifoliatus* (16.296) and *S. trilobatum* (19.225) has best anti-deterrence action against the test larvae when compared to the control values (19.549). The other two species (*L. camara* and *C. pentandra*) has recorded the highest FDI values (33.173) and (40.056), respectively (Fig. 3). Leatemia and Isman[25] tested antifeedant activity of crude seed extracts of *Annona squamosa* against lepidopteran pests *Plutella xylostella* and *Trichoplusia ni* and reported that both the pests are susceptible to the aqueous seed extract. The FDI values given by Atwal and Pajni[26] were 0, 2, 24, 39, 63 at 2, 4, 6, 8 and 10% concentration of plant aqueous extract, respectively.

Repellent activity: The repellent action of the selected medicinal plant seed extracts against the test larvae (*Pieris brassicae*) was assessed by increasing the concentration of extracts on the cabbage leaf dips. All the larvae were strongly repelled at 100 mg kg^{-1} concentration of the plant extracts, within 4 h of the experiment larvae were totally repelled from the treated leaves. Among the four medicinal plant seed extracts *Sapindus trifoliatus*, *Solanum trilobatum* recorded as good source of repellent when compare to the positive control. The positive control also showed highest repellent activity of 8 larvae (Fig. 4). The other two medicinal plant seed extracts showed less repellent activity at 100 mg kg^{-1} concentration and at low concentration of (25 and 50 mg kg^{-1}) the plant *Ceiba pentandra* failed to show any repellent activity against the test larvae. This may be due to the presence of many strong secondary metabolites[22,24] in the plants extracts of *Sapindus trifoliatus* and *Solanum trilobatum*.

Mortality rate on both larvae: The mortality rate was calculated by direct spraying of the selected plant seed extracts at a concentration of 100 mg kg^{-1} on the freshly

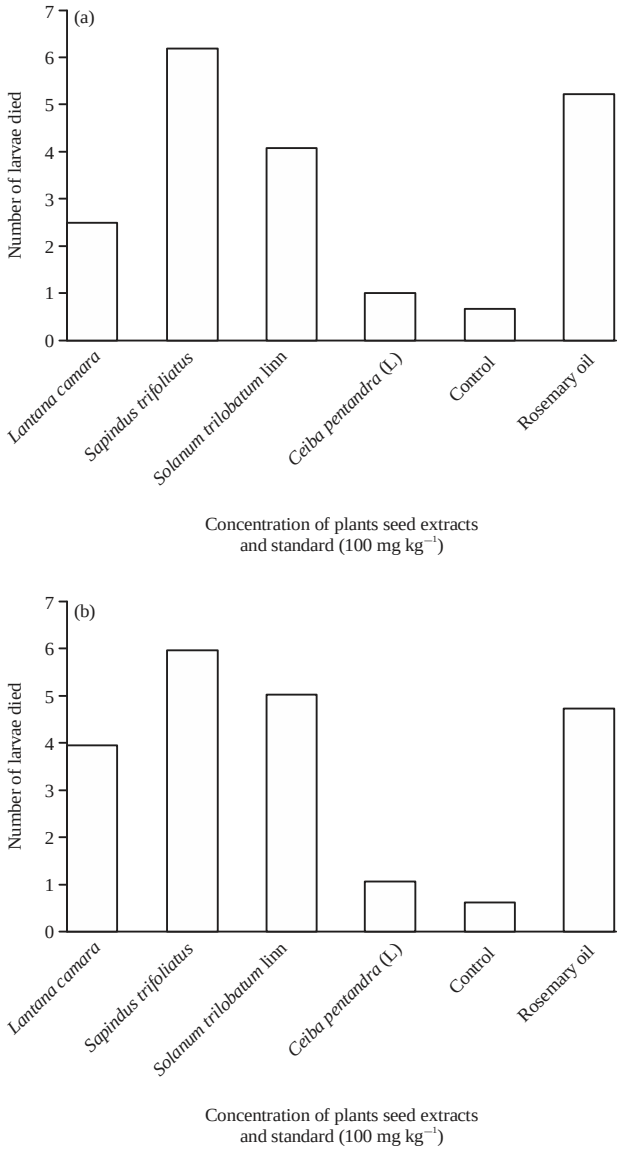

Fig. 5(a-b): Larval mortality of the four plants seed extract on (a) *Trichoplusia ni* and (b) *Pieris brassicae* larvae

moulted instar *Trichoplusia ni* larvae and *Pieris brassicae*. Survivals were determined after 48 h of feeding on the normal diet to observe the full extent of toxicity by these selected seed extracts. The medicinal plant seed extracts of *S. trifoliatus* recorded highest mortality rate by 60%, while rosemary oil (1200 ppm) and *S. trilobatum* at 100 mg kg^{-1} showed 50% mortality of the larvae in both the species tested (Fig. 5). Similar results were reported by Sharma and Gupta[18] and Paul and Sohkhlet[8]. Atwal and Pajni[26] reported 33.3 and 40% mortality against *P. brassicae* with alcohol extract (10 and 5%, respectively) of *M. azedarach*. The results finally conclude that the phytochemicals have strong inhibition mechanisms in all the physiological aspects of the larvae.

Insect growth rate (IGR) effects on both larvae: The IGR experimental results showed delay in the duration of pupation in both the larvae. In the control trays, pupation occurred on the 14th day (±2 days), where as in the test experimental trays, pupation occurred on the 18th day (±2 days). In the control tray the pupation was after 12 days whereas in the experimental setup it was delayed to 18-20 days. The late pupation was predominantly induced by the selected plants extracts (*Lantana camara*, *Sapindus trifoliatus*, *Solanum trilobatum* Linn and *Ceiba pentandra* at 100 mg kg^{-1} concentration comparing with that of positive control. The *Ceiba pentandra* plant seed extract failed in inducing the late pupation of the selected test organism larvae (*Pieris brassicae*) (Table 2). The results shown are in

Table 2: Occurrence of mortality and late pupation with 100 mg kg^{-1} concentrations of four different plants extracts on *Trichoplusia ni* and *Pieris brassicae* larvae

Plant extracts (100 mg kg^{-1})	Mortality (%)	Late pupation (%)
Lantana camara	40	60
Sapindus trifoliatus	60	40
Solanum trilobatum Linn	50	50
Ceiba pentandra (L)	10	100
Rosemary oil (1200 ppm)	50	50

agreement with the results provided by Paul and Sohkhlet[8]. Extracts are known to contain phytochemicals which have the potential to interfere with the physiology of the larvae by reducing their growth, fitness and pupation[9,21,27]. The same results from the both the species with minor differences showed that there was interrelation ship in the physiological and experimental conditions with the same seed extracts tested.

CONCLUSION

The statistical analysis of the experimental result shows considerable variations between the function and performance of both synthetic and bio-insecticides as compared to control. However, the bio-insecticides were eco-friendly and softer in their role on the beneficial than the artificial synthetic chemical compounds. This can influence the employment of these bio-insecticides in the integrated pest management and control of insects' pests in organic farming systems.

SIGNIFICANCE STATEMENT

The present study suggests the cheap and eco-friendly way of controlling the important pests of cauliflower by using plant seed extracts.

REFERENCES

1. Eswara, R. And S.G. Srinivasa, 2004. Management of shoot and fruit borer, *Leucinodes orbonalis* (Guen.) in brinjal using botanicals/oils. Pestology, 28: 50-52.
2. Gupta, G., 2013. Managing thrips on roses with combinations of neonicotinoide and biological insecticides. J. Agric. Urban Entomol., 29: 16-24.
3. Kumar, P. and D. Venkat, 2006. Bioefficacy of emamectin benzoate 5% sg (Proclaim) against diamond back moth in cabbage. Pestology, 30: 23-25.
4. Jeyarani, S. and J.S. Kennedy, 2004. Efficacy of certain biopesticides against the diamondback moth, *Plutella xylostella* (L.) in cauliflower. Indian J. Plant Protection, 32: 129-130.
5. Waghmare, U.M., D.M. Wadnerkar and P.R. Zanwar, 2006. Comparative efficacy of some bio-pesticides and endosulfan against cabbage aphid and diamondback moth. Pestology, 30: 33-35.
6. Hemchandra, O. and T.K. Singh, 2006. Evaluation of antifeedant properties of some plant extracts against diamondback moth, *Plutella xylostella* (Linn.). Pestology, 30: 36-39.
7. Shukla, A. and A. Kumar, 2004. Efficacy of some IPM modules against diamondback moth, *Plutella xylostella* Linn. infesting cabbage. J. Ent. Res., 30: 39-42.
8. Paul, D. and M.D. Sohkhlet, 2012. Anti-feedant, repellent and growth regulatory effects of four plant extracts on *Pieris brassicae* larvae (Lepidoptera: Pieridae). J. Entomol. Res., 36: 287-293.
9. Hasan, F. and M.S. Ansari, 2010. Effect of different cole crops on the biological parameters of *Pieris brassicae* (L.) (Lepidoptera: Pieridae) under laboratory conditions. J. Crop Sci. Biotechnol., 13: 195-202.
10. Gangula, S.R., H. Govada and M. Matta, 2013. Phytochemical screening and inhibitory effect of n-butanol, isopropanol and water extracts leaf extracts of *Sapindus saponaria* Vahl on selected pathogens. Adv. Applied Sci. Res., 4: 463-467.
11. Yesil Celiktas, O., M. Ganzera, I. Akgun, C. Sevimli, K.S. Korkmaz and E. Bedir, 2009. Determination of polyphenolic constituents and biological activities of bark extracts from different *Pinus* species. J. Sci. Food Agric., 89: 1339-1345.
12. Koul, O., S. Walia and G.S. Dhaliwal, 2008. Essential oils as green pesticides: Potential and constraints. Biopestic. Int., 4: 63-84.
13. Ntalli, N.G., U. Menkissoglu-Spiroudi, I.O. Giannakou and D.A. Prpphetou-Athanasiadou, 2009. Efficacy evaluation of a neem (*Azadirachta indica* A. Juss) formulation against root-knot nematodes *Meloidogyne incognita*. J. Crop. Prot., 28: 489-494.
14. Isman, M.B., 2006. Botanical insecticides, deterrents and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol., 51: 45-66.
15. Preethi, R., V.V. Devanathan and M. Loganathan, 2010. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. Adv. Biol. Res., 4: 122-125.
16. Samatha, T., P. Srinivas, R. Shyamsundarachary, M. Rajinikanth and N.R. Swamy, 2012. Phytochemical analysis of seeds, stem bark and root of an endangered medicinal forest tree *Oroxylum indicum* (L) Kurz. Int. J. Pharm Bio Sci., 3: 1063-1075.
17. Regina, S.N., 2003. Morphological and preliminary phytochemical studies of drug yielding herbs. Ph.D. Thesis, CSJM University, Kanpur.
18. Sharma, A. and R. Gupta, 2009. Biological activity of some plant extracts against *Pieris brassicae* (Linn.). J. Biopestic, 2: 26-31.

19. Akhtar, Y., M.B. Isman, P.M. Paduraru, S. Nagabandi, R. Nair and E. Plettner, 2007. Screening of dialkoxybenzenes and disubstituted cyclopentene derivatives against the cabbage looper, *Trichoplusia ni*, for the discovery of new feeding and oviposition deterrents. J. Agric. Food Chem., 55: 10323-10330.
20. Huang, X.P. and J.A.A. Renwick, 1995. Cross habituation to feeding deterrents and acceptance of a marginal host plant by *Pieris rapae* larvae. Entomol. Exp. Applic., 76: 295-302.
21. Hasan, F. and M.S. Ansari, 2011. Effect of different brassiceous host plants on the fitness of *Pieris brassicae* (Linn). J. Biopes, 2: 26-31.
22. Seffrin, R.D.C., I. Shikano, Y. Akhtar and M.B. Isman, 2010. Effects of crude seed extracts of *Annona atemoya* and *Annona squamosa* L. against the cabbage looper, *Trichoplusia ni* in the laboratory and greenhouse. Crop Prot., 29: 20-24.
23. Akhtar, Y. and M.B. Isman, 2004. Comparative growth inhibitory and antifeedant effects of plant extracts and pure allelochemicals on four phytophagous insect species. J. Applied Entomol., 128: 32-38.
24. Rathi, J.M. and S. Gopalakrishnan, 2010. Insecticidal activity of methanolic pooled fractions of *Lantana wightiana* Wall. J. Biopesticides, 3: 282-285.
25. Leatemia, J.A. and M.B. Isman, 2004. Toxicity and antifeedant activity of crude seed extracts of *Annona squamosa* (*Annonaceae*) against lepidopteran pests and natural enemies. Int. J. Trop. Insect Sci., 24: 150-158.
26. Atwal, A.S. and H.R. Pajni, 1964. Preliminary studies on the insecticidal properties of drupes of *Melia azedarach* against caterpillar of *Pieris brassicae* L. (Lepidoptera: Pieridae). Indian J. Entomol., 26: 221-227.
27. Lytan, D. and D.M. Firake, 2012. Effects of different host plants and rearing atmosphere on life cycle of large white cabbage butterfly, *Pieris brassicae* (Linnaeus). Arch. Phytopathol. Plant Prot., 45: 1819-1825.