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RESEARCH ARTICLE

PESTICIDAL ACTIVITY OF *LEUCAS ASPERA* (WILLD.) LINK ON SUGARCANE SHOOT BORER *CHILOINFUSCATELLUS*

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ABSTRACT

Leucasaspera is a short green and highly branched herb belongs to family labiatae and order tubiflorae. In India, *Chiloinfus catellus* is a major pest of sugarcane fields. It is very much essential to find out controlling approaches for this pest for greater manufacture of sugarcane. The present study indented to use the herb *Leucasaspera* to control the major sugar cane pest yellow stem borer i.e. *Chiloinfuscatellus*. Quantitative analysis of ethanol extract of *Leucasaspera* revealed the presence of tannin, flavonoids, protein, saponins, steroids, alkaloids and carbohydrates. The percentage of larval mortality was higher in ethanol extracts than the water extract. Results clearly indicates the lethality of the extracts on soft bodied third instar larva. *Leucasaspera* have the larvicidal and pupicidal activity due to presence of phytochemical compounds like Limonene and β-caryophyllene. They are also responsible for ecdysal failure and mortality. Percentage of adult emergence of *C. infescatellus* in ethanol extracts of *L. aspera* are clearly proved that *L. aspera* devastatingly destroys the life stages of the pest *C. infescatellus.* The results clearly proved that ethanol extracts of*L. aspera* devastatingly destroys the life stages of the pest *C. infescatellus.* However, water extract is not much effective when compared to ethanol extracts. Which literally stated that water is not suitable solvent to extract the chemical constituents of the plant *L. aspera*. Thus the current study clearly demonstrate the role of local herb on control of a pest of native crop. However, field observations suggested usage of chemical and other modern farming techniques reduce the diversity of native herb like *L. aspera* which are potential alternative botanical pesticides. The study explore the value of the herb and suggest the farmers who are intended to follow the modern chemical based techniques to protective potential natural resources.

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INTRODUCTION

Development of alternative drug from the natural plants is the trendiest idea around the world. As a result, various traditional herbal medicines are redeployed and got much attention from the modern technically sound human community (Zakia *et al.,* 2022). The renovating connection between man and nature ultimately plays a vital role in the progressive human culture. Due to their adaptation for the wide range of habitats, diversity of plants and insects have extensive range of distribution on desert, aquatic medium, tropical forests and arctic regions. As a consequence plants and insects roughly make up the half of the identified biota of the earth. Plants and insects have thorough going interactions through protection, pollination, seed dispersal, food supply and nest construction (Calatayud *et al.,* 2018). In Tamil Nadu wide range of ecosystems including marine coastal, inland water bodies and terrestrial evergreen forests are support the most unique and stunning quantity of biodiversity. Existence of around six thousand floral species in Tamilnadu made it to attain the first rank among Indian states (ENVIS 2006). *Leucasaspera* is a short green and highly branched herb belongs to family labiatae and order tubiflorae. They have up to 1.5 cm length, thick, sub-sessile leaves with short petiole and linear narrow oval shape. Flowers are bright white, sessile and dense in terminal or axillary whorls with modified green leaf bract. Bracts are with cilia and hairs. Varied tubular calyx is around 12 mm long. The flowers are tube curved with contracted just above nutlets, membranous lower half, ribbed upper half, small mouth with oblique and not villus. Smooth fruit nutlets are around 3 mm long, quadri-lateral, brown with angular inner face and round outer face. In South East Asian countries like India and Sri Lanka, the Yellow stem borer i.e. *chiloinfu scatellus* is a major pest of sugarcane fields. The insect belongs to family crambidae and order lepidoptera. They can cause serious damage in nursery stage also. Heavy infestation of the pest may leads to more than 50 percentage damage. They are mostly monophagous pest of monocot plants. They are very active during March to November and pass the winter as fully grown larva. The pest is very injurious to the young plants. They tunnels the sugarcane stalks and inhibit the nutrient flow. The larva also moves upward and downward, which leads to cause dead heart by damaging the central part of the stem. Sugarcane (*Saccharumofficinarum* L.) is one of the most important economic crop of the world. Around 80 percent of the global sugar production is depends on sugarcane. The crop cultivated in tropical, sub-tropical and semi-tropical regions. Sugarcane is raw material for the production of sugar, alcohol, etc.

In India, Sugarcane industry is the second largest agro based industry with annual production of 305.25 million tonnes. Thus the crop is one of the important cash crop India. Dhanraj and Dharne (2013) reported that around forty five million farmers are depends on the sugarcane crops and associated industries.This cash crop is faces serious damage by various insect pests of our tropical region. They are prone to different types of insect pests belongs to orders including Lepidoptera, Coleoptera, Hemiptera, etc. Dense plant distribution for long period of time in the agro-ecosystem of the sugarcane filed supports wide range of pests which also favors to complete whole life of insect pests (Chaudhary 2008). All stages and all the parts of the sugarcane are damaged by insect pests like stem borers, leaf borers and root borers. In India, the borers *Chiloinfu scatellus* (early shoot borer), *Scirpophaganivella* (top borer), *Emmaloceradepressella* (root borer) and *Chiloauricilius* (stalk borer)are major pests of sugarcane fields. The early shoot borer *C. infuscatellus* forms 'dead hearts' and also attacks the sugarcane plants in caterpillar stage which leads to almost twenty percent of damages in young shoots usually during the period of April and June (Dhaliwal and Atwal 2004). Sugarcane fields of Tamilnadu is much affected by the pest and resulted in heavy loss. Thus, it is very much essential to find out controlling approaches for this pest for greater manufacture of sugarcane. Biological characteristics of the pest is needed to know the feeding pattern and period of changed progressive stages and behaviour which in turn help in its management. The knowledge on lifecycle of the pest is a condition example to work out its control processes.

Planting of resistant varieties, removal of leaf tips with egg masses of yellow stem borer, hand picking of adult moths, plantation with proper space arrangement, harvest of crop up to ground level and catching the adults through light traps are common control measures for the stem borer. In addition, pheromone control, chemical control and integrated pest control measures are applied. However, still the pest causes great amount of damage in the sugarcane fields. Therefore, the present study indented to use the local herb *Leucasaspera*to control the major sugar cane pest yellow stem borer i.e. *Chiloinfuscatellus*.

MATERIALS AND METHODS

Leucasaspera plants for the present study are collected from farms of Guruvarediyur Village, Erode District. Extraction of the plant materials is followed by the standard methods of Chessbrough (2000). Preliminary phytochemical screening was carried out following standard procedures by Harborne (1973), Kokate (1994) and Sani*et al.,* (2007). The eggs and larvae were collected from the Sugarcane field at Guruvarediyur, Anthiyur Taulk, Erode District. The collected eggs and larvae of *Chiloinfu scatellus* were cultivated and maintained in laboratory conditions (28˚C). The larvae were cultivated in glass containers and provided with fresh sugarcane leaves. The emerged larvae were also fed with sugarcane leaves and it maintained up to pupation. In the present experiment, III, IV, V instar larvae and pupae were used. Enough number of particular instars and pupae were took for the experiments. A leaf disc analyze method was trailed to evaluate the larval mortality of *C.infuscatellus*. *C. infuscatellus* freshly laid eggs were creamy white and transparent yellowish in colour. Inside of the egg, larval head is clearly visible. Eggs are laid in masses at the lower surface of sugarcane leaves. Eggs of *C.infuscatellus* is hatched after the incubation period of 4 to 6 days. The life cycle of *C. infuscatellus* includes five instars due to four molting. The larva comes out of the egg is very smaller and grey in colour with dark black colour head and scattered dark dots in body. The first larval period extends up to two to three days. Straw colour with mild stripe markings are the features of second and third instar larvae which ranges from three to four days and three to five days respectively. The length of the third instar larva (i.e. nearly 11.5 mm) is distinct to the second instar larva. The fourth instar larva is dirty white in colour with dark brown head and violet stripes on the body, which lives up to four to six days. The last and fifth instar larva is whitish in colour with brown head and violet stripes. It ranges from 6 to 7 days.

The pupal stage extended up to 5 or 6 days. The pupa was elongated, slender and transparent yellowish brown in colour. Later it changes into dark brown in colour. Adults emerged from the pupa during early morning hours. In general, the *C.infuscatellus* moths are yellowish brown colour. Presence of dark markings on the outer edge of forewings and greyish white hind wings are features of male moths. While, female moths have forewings without any markings, hind wings are greyish white in colour. Total life span of *C.infuscatellus* is varied from 30 to 45 days. Based on the seasonal factors and the host plant genome the life cycle of the pest is varied. Percentage of oviposition deterrence, ovicidal activity, antifeedant activity, larvicidal activity and pupicidal activity was calculated using the formula of Abbott (1925).

RESULTS

In the present study, qualitative tests were conducted for water, 50% and 100% extracts of *L.aspera*. Qualitative study revealed that the presence of protein, phenols, alkaloids and carbohydrates in water extracts. However, 50% and 100% of ethanol consists tannins, flavonoids, protein, saponins, phenols, steroids, alkaloids and carbohydrates. Based on qualitative analysis, extracts selected for further analysis. In the present quantitative analysis, ethanol extract of *L.aspera* contains 0.3 % tannin, 0.4 % flavonoids, 6.38 % protein, 0.86 % saponins, 0.91 % steroids, 8.0 % alkaloids and 11.92 % carbohydrates were quantified. Presence of flavonoids content in *L.aspera* is 100 mg / ml for aqueous extract and 185 mg / ml for ethanol extract. Phenolic compounds are estimated by using regression equation of calibration curve at 765 nm. The results are expressed with the unit of Gallic Acid Equivalents (GAE). In the present experiment, total phenolic compounds are 94 mg/GAE/ml and 172 mg/GAE/ml for aqueous and ethanol extracts respectively. Ethanol extracts of *L. aspera* were applied on thin layer chromatography to separate the compounds. The Retention Factor (RF) value was measured as 0.75, which clearly indicate the presence of alkaloids and saponins.

PESTICIDAL ACTIVITIES OF *LEUCAS ASPERA*

Hatchability of Egg: In the present experiment on pesticide activity of *L. aspera*, 50 % ethonal showed highest percentage of unhatched eggs at 24 hrs. However, water extracts showed higher percentage of hatchability at 48 hrs (table 1).

Larval Mortality: 100 % percentage of larval mortality was recorded for 3rd in star larva in 50 % and 100 % ethanol. While, 4th and 5th larval instars were showed highest larval mortality in 100 % ethanol only. Similarly, antifeedant effect of 3rd, 4th and 5th were recorded high in 100% ethanol (table 1). Results of statistical analysis (one way ANOVA) presented in table 2.Mean values (three replicas) within the same row sharing the same superscript are not Significant different at 5% level.

Pupal Mortality: Pupicidal effect of the plant extract was observed high in 100 % ethanol. However, 50 % ethanol extract also caused serious damage to the papal population (graph 1).

Survival and Adult Emergence: Egg hatchability, survival and adult emergence of the *C.infuscatellus* was high in water extract, however 100 % ethanol caused the lethal effects. While, 50 % ethanol extract exhibited moderate impact only (graph 2).

DISCUSSION

Pesticidal activity of *Leucasaspera* plant extracts on different life stages of mosquitoes were reported by various studies (Kovendan *et al.,* 2012, Elumalai *et al.,* 2015). However, the present study indented to explore pesticidal activity of *L.aspera* against *C. infescatellus* i.e yellow stem borer of sugarcane. Results of the present study clearly demonstrated that 50% ethanol extract of *L. aspera*as an effective composition to destroy the eggs of *C. infescatellus.*

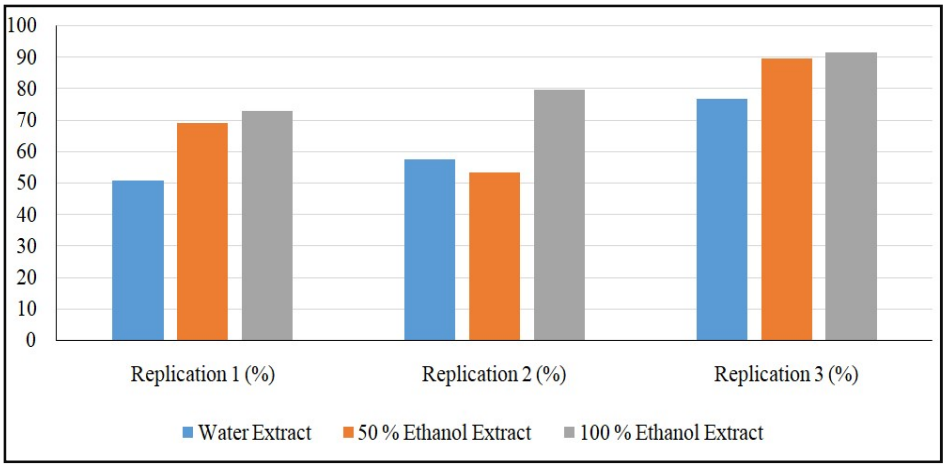
Table 1: Effect of *Leucasaspera* extracts on egg hatchability, larva stages of *C. infescatellus*

Concentrations	Percentage of Unhatched Eggs (Age of Eggs)		Percentage of Larval Mortality			Percentage of Antifeedant Effect		
	24 Hrs	48 Hrs	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar
Water Extract	23.3	24	87.3	57.7	62.33	43	74.33	63.66
50 % Ethanol	42.7	22	100	79.7	76	48.3	82.33	84.33
100% Ethanol	33.3	11	100	81.3	81.66	52.7	86.33	89.66

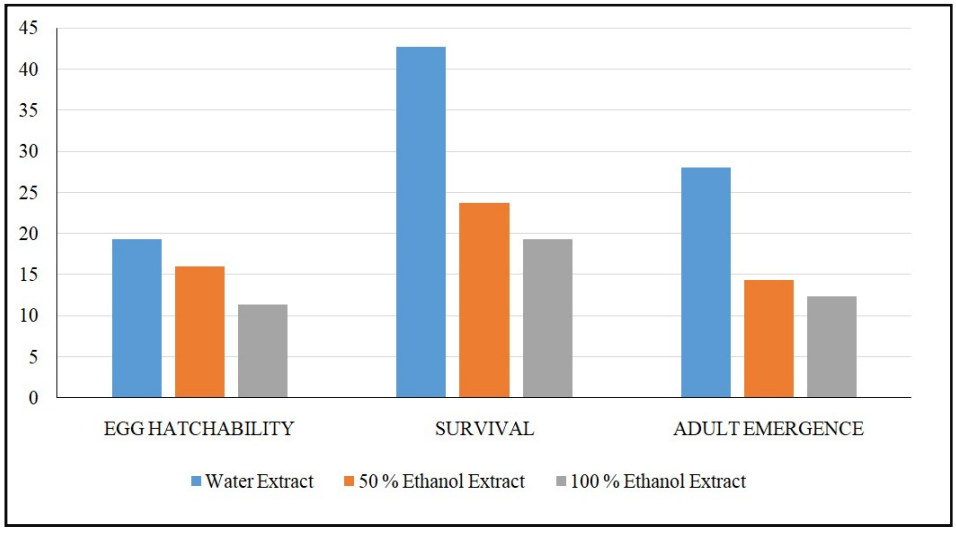
Table 2. Effect of *Leucasaspera* extracts on egg hatchability, larva stages of *C. infescatellus*

Concentrations	Percentage of Unhatched Eggs (Age of Eggs)		Percentage of Larval Mortality			Percentage of Antifeedant Effect		
	24 Hrs	48 Hrs	3rd Instar	4th Instar	5th Instar	3rd Instar	4th Instar	5th Instar
Water Extract	23.33^{c}	24.33^{a}	86.53^{b}	57.63^{c}	62.14^{c}	43.00^{c}	74.54^{c}	63.34^{c}
50 % Ethanol	42.46^{a}	21.66^{b}	100^{a}	79.13^{b}	76.00^{b}	48.36^{b}	82.59^{b}	84.53^{b}
100% Ethanol	33.73^{b}	11.33^{c}	100^{a}	81.56^{a}	81.52^{a}	52.90^{a}	86.44^{a}	89.55^{a}

Mean (n = 3), Mean values within the same row sharing the same superscript are not Significant different ($p>0.05$)



Graph 1. Pupicidal effect of *Leucasaspera*on *Chiloinfuscatellus*



Graph 2. Egg hatchability, Survival rate and adult emergence of *C. infuscatellus*

Similarly, 50% and 100% ethanol cause 100% mortality at tiny soft bodied third instar larval stage. In extension, pure ethanol and 50% ethanol extracts also instigated more than 75% mortality on fourth and fifth instars of *C. infescatellus.* Even water extract also showed more than 50 % larval mortality on fourth and fifth instar larva. Pupal stage of *C. infescatellus* is extended upto one week. Pupicidal activity of pure ethanol extract was varied from 72 % to 91 %, which clearly indicated the actual potential of *L. aspera* against the pest. *L.aspera* have the larvicidal and pupicidal activity due to presence of phytochemical compounds like Limonene and β-caryophyllene. They are also responsible for ecdysal failure and mortality (Prasad and Kumar 2012). In the present study, egg hatchability of C. *infescatellus* on pure ethanol extract was very least of 11 %. Water extract also showed 19 % of hatchability only. In general, less than 25 % of egg hatchability of eggs of a pest insect due the plant extracts revealed that particular plant species can be opted for further studies on pesticidal activity. Survival of the pest *C. infescatellus* life stages is very least of 19 % in pure ethanol extract. Similarly, 50 % ethanol extract also have minimum survival of 23 % only. While, results clearly revealed that water extract showed highest percentage of 43 %

of survival, which clearly demonstrates that water is not good solvent to be used for exploring the pesticidal activity of *L. aspera* against *C. infescatellus*. Percentage of adult emergence of *C.infescatellus* in 100 % and 50 % ethanol extracts of *L. aspera*are 12 % and 14 % respectively. The results clearly proved that *L.aspera* devastatingly destroys the life stages of the pest *C.infescatellus.* However, water extract is not much effective when compared to ethanol extracts. Which literally stated that water is not suitable solvent to extract the chemical constituents of the plant *L. aspera*. Thus the current study clearly demonstrate the role of local herb on control of a pest of native crop. However, field observations suggested usage of herbicides and weedicides and invasion of alien plants (Prakash *et al.,* 2022) reduce the native herbs which are potential alternative botanical pesticides.

Key Points

- In the present quantitative analysis, ethanol extract of *L. aspera* contains 0.3 % tannin, 0.4 % flavonoids, 6.38 % protein, 0.86 % saponins, 0.91 % steroids, 8.0 % alkaloids and 11.92 % carbohydrates were quantified.
- Presence of flavonoids content in *L. aspera* is 100 mg / ml for aqueous extract and 185 mg / ml for ethanol extract.
- Results of the present study clearly demonstrated that 50% ethanol extract of *L. aspera*as an effective composition to destroy the eggs of *C. infescatellus.*
- *L. aspera* have the larvicidal and pupicidal activity due to presence of phytochemical compounds like Limonene and β-caryophyllene. They are also responsible for ecdysal failure and mortality.
- Percentage of adult emergence of *C. infescatellus* in 100 % and 50 % ethanol extracts of *L. aspera*are 12 % and 14 % respectively. The results clearly proved that *L. aspera* devastatingly destroys the life stages of the pest *C. infescatellus.*

Ethical Issues: No ethical issue in performing this study as CT is routinely done in Radiology

Department Conflict of interest: Nil

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