ANTIFEEDANT ACTIVITIES OF SOLVENT EXTRACT OF *PIPER NIGRUM CORNS* AGAINST THE COTTON PEST *HELICOVERPA ARMIGERA*

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ABSTRACT

Growing awareness of environmental hazards from synthetic pesticides has attracted the attention of the researchers. In recent years attention is being diverted towards use of botanicals as key component of Integrated Pest Management (IPM) as alternatives to synthetic pesticides because they are biodegradable and having low mammalian toxicity. In the present study different solvent extracts (Petroleum ether and Methanol) obtained from corns of Piper nigrum were screened for antifeedant activity against the cotton pest Helicoverpa armigera. The two different extracts were tested for antifeedant activity against third instar larvae of H. armigera at four different concentrations (250, 500, 750 and 1000 ppm). The methanolic extracts of corns of P. nigrum exhibited significant activity at 1000 ppm concentration. The methanol extracts showed spots of 72.38 ± 7.4 from the larvae feeding followed by 40.37 ± 4.56 in petroleum ether extracts respectively which are significantly less than the number of spots produced in control (94.24 ± 2.00 and 83.33 ± 2.10 respectively). All the activities are dose dependent. The mean results were statistically significant at 1% level ($p<0.01$) for three replications.

Keyword: *Piper nigrum, Corns, Antifeedant, Helicoverpa armigera, Solvent extracts*

1. INTRODUCTION

In a tropical country like India, owing to climatic conditions and its particular environment, agriculture is suffering from severe losses due to pests. Considering the agro-ecosystems with an increase in population and dwindling land resources there is worldwide demand for natural insecticides to increase the agriculture production. Due to these problems, a search is going on to discover new, less damaging pest management tools [1]. Chemical pesticides have been used for several decades in controlling pests as they have a quick knock down effect. However, their indiscriminate use resulted in several problems such as resistance to pesticides, resurgence of pests, elimination of natural enemies, toxic residues in food, water, air and soil which affected human health and disrupt the ecosystem, leading to the threat that their continued use may further harm the environment. Under such alarming situations, plants and plant derived products offered a tremendous advantage over synthetic pesticides in use as control agents for the pests of agriculture, veterinary and public health since plant kingdom is the most efficient producer of chemical compounds, synthesizing many products that are used in defense against insect [2]. However, the screening of plant extracts against insects is still continuing throughout the world to find out different kinds of effects of botanicals to obtain an ecofriendly and economical biopesticide. The cotton pest *Helicoverpa armigera* (Hub.) has gained increased attention in many parts of the world.

H. armigera is a highly polyphagous species which primarily attacks ornamental plants and flowers. Other economically important hosts are cotton, tobacco, tomato, potato, maize, soya bean, lucerne, phaseolus and other

Leguminosae, as well as a number of fruits (Prunus, Citrus) and forest trees. Larvae cause severe damage, particularly to reproductive organs, although vegetative organs may also be attacked. The first *H. armigera* damage was noticed in Montenegro on tomato fruits in 1999. In the following years, symptoms of attack were sporadically found in pepper fruits. Since 2008, the damage caused by this pest has often been detected on tomato and pepper fruits, indicating that the cotton bollworm *H. armigera* could become a serious pest on these crops and a potential threat for other host crop plants [3].

In the group of medicinal plants, the *Piper nigrum* possess excellent medicinal properties due to the presence of enormous phytochemicals. The piperine is an alkaloid, majorly found in *P. nigrum*, which belongs to the Piperaceae family that is massively cultivated at India and Sri Lanka [4,5]. *P. nigrum* is a valuable medicinal plant. It is one of the most commonly used spices and considered as "The King of spices'' among various spices. Black pepper is grown in many tropical regions like Brazil, Indonesia and India. *P. nigrum* is commonly known as Kali Mirch in Urdu and Hindi, Pippali in Sanskrit, Milagu in Tamil and Peppercorn, White pepper, Green pepper, Black pepper, Madagascar pepper in English [6,7].

P. nigrum corn is a widely used spice throughout the world. It has been extensively used in improving appetite, enhancing the digestion, subsidizing the sore throat, cold, breathing and heart problem, to cure colic, diabetes, anemia and piles by mixing with other constituents [8]. Black pepper corn contains various alkaloids, among which piperine act as a central nervous system depressant. Some research suggests that the piperine have potential in the treatment of vitiligo, as it helps increase pigmentation in the skin. In addition to black pepper corn, the other part, especially, leaf has certain phytochemical compounds that have been extracted with different solvents and their antimicrobial activities were analyzed [9]. Very few reports were present pertaining to the antifeedant activity of peppercorn extracts against *H. armigera* [10]. Therefore, the present study deals with screening of various solvents extracts for their antifeedant activity against *H. armigera*.

2. MATERIALS AND METHODS

2.1 Collection of plant materials

The corns of *P. nigrum* were collected in and around, Kerala, India.

2.2 Extraction

The pepper corns were thoroughly washed with tap water and shade dried under room temperature (27.0± 20C and 75 ± 5% RH) at Department of Zoology, Nirmala College for Women, Coimbatore. After complete drying the pepper corns were powdered using electric blender and sieved through a kitchen strainer. 500g of pepper corns powder was extracted by soxhlet extraction methods with petroleum ether and methanol.

2.3 Rearing of Test Insects

Different larval stage of *H. armigera* was collected from Tomato field, Chittur, Palakkad Dist. Kerala, India. Larvae were reared in laboratory conditions (27.0˚C ± 2˚C; 70% RH) throughout the study period at Department of Zoology, Nirmala College for Women, Coimbatore, Tamil Nadu, India. Generally, healthy and uniform sized third instar larvae of *H. armigera* were used for the experiments and the cultures were maintained throughout the study period.

2.4 Antifeedant activity

Antifeedant activity of crude extracts was studied using leaf disc no choice method [11]. Required concentration of crude extracts (5%) was prepared by dissolving in respective solvents. Fresh cotton leaf (for *H. armigera*) discs of 3 cm diameter were punched using a cork borer and dipped in 250, 500, 750, 1000 ppm and air dried for 5 minutes. After air drying, treated leaf discs were kept inside the Petri dishes (15mm × 90 mm diameter) separately containing wet filter paper to avoid drying of the leaf disc and single 4 hrs pre starved third instar larva of *H. armigera* was introduced on each treated leaf disc. One treatment with respective solvent alone was used as control. Three replications were maintained for each treatment. A progressive consumption of leaf area by the larva in 24 hrs period was recorded in control and treatments using a leaf area meter (systronics 211). Leaf area consumed in plant extract and treatments was corrected from the control. The percentage of antifeedant index was calculated using the formula of Ben Jannet *et al* [12].

$$AFI = \frac{C - T}{C + T} \times 100$$

Where AFI = Antifeedant Index;
C = Area protected in control leaf disc;
T = Area protected in treated leaf disc.

2.5 Phytochemical analysis

Petroleum ether and methanol extracts of *P. nigrum* corns were subjected to phytochemical screening using standard protocols

3. RESULTS AND DISCUSSION

Antifeedant activity of methanol extracts of *P. nigrum* corn tested against third instar larvae of *H. armigera* and the results pertaining to different concentrations are presented in the Chart 1. Different concentration showed varying range of antifeedant activity. It was noted that the antifeedant activity of methanol extracts tested against the third instar larvae of *H. armigera* was found to be statistically significant than the petroleum ether extracts. At 1000 ppm concentration, methanol extracts showed 72.38 ± 7.41 area of leaf protection from the larvae feeding followed by 40.37 ± 4.56 in petroleum ether extracts respectively. Preliminary phytochemical analysis of petroleum ether and methanol extracts of *P. nigrum* corn showed the presence of a variety of plant secondary metabolites as it is evidenced from the Table 1. Among the two extracts methanol extracts of *P. nigrum* corn showed presence of majority of secondary metabolites such as Alkaloids, Terpenoids, Phenols, Tannins, Saponins, Flavonoids, Quinones, Steroids and Phlobotanin.

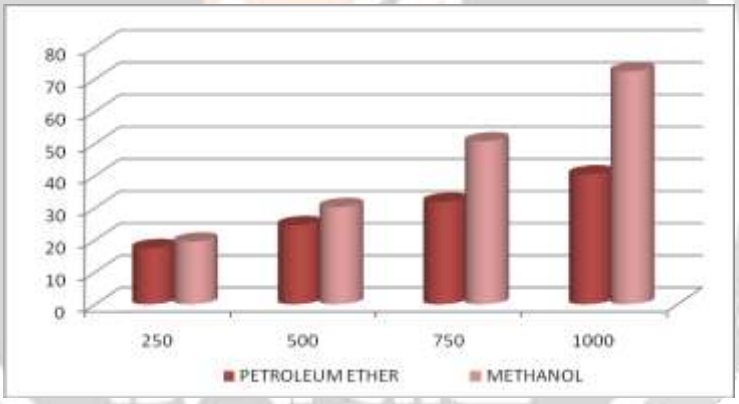

Chart 1: Antifeedant activity of solvent extracts of *Piper nigrum* against third instar larvae of *Helicoverpa armigera*

Table 1: Qualitative phytochemical screening of *Piper nigrum* corns extracts

Sl. No.	Secondary Metabolites	Tests Performed	Petroleum Ether	Methanol
1	Alkaloids	Mayer's	+	+
2	Terpenoids	Copper acetates	-	+
3	Phenols	Ferric chloride	-	+
4	Coumarin	NH_4OH	+	-
5	Tannins	Ferric chloride	+	+
6	Saponins	Foam	-	+
7	Flavonoids	Lead acetate	+	+
8	Quinones	Sodium hydroxide	-	+
9	Steroids	Liebermann burchard	+	+
10	Phlobotanin	HCl	-	+
11	Giycosides	Fehlings	-	-

'+' Presence '-' Absence

Several authors have reported that plant extracts possess antifeedant, insecticidal, oviposition deterrent, ovicidal and growth inhibition activities against Lepidopteran pests [13]. In the present study, it was observed methanol extract at 1000 ppm concentration reduced the feeding rate of *H. armigera*. Jeyasankar *et al* [14] has reported that the possible insecticidal property in the selected plant may arrest the various metabolic activities of the larvae during the development and ultimately the larvae failed to moult and finally died. Earlier studies pertaining to *Couroupita guianensis* leaf extracts showed antifeedant, larvicidal, and ovicidal activities against *H. armigera* [15].

In another study, Jeyasankar *et al* [11] had reported that among six different indigenous plants screened for insecticidal activity against fourth instar larvae of *Henosepilachna vigintioctopunctata*, ethyl acetate extracts of *Achyranthes aspera* showed higher percentage of larval mortality against fourth instar larvae of *H. vigintioctopunctata*. Due to the toxic effect of plant extracts, maximum number of treated larvae died in spite of less food consumption. Leatemia and Isman [16] reported that high concentrations of extracts caused high mortality of larvae even though only very small portions of the leaf discs were consumed.

In case of phytochemical analysis, reducing sugars, flavonoids and traces of alkaloids were found to be present in *Psidium gujauva* according to the previous investigations [17] while in present investigation, reducing sugars, terpenoids, alkaloids, flavonoids and phlobatannins all were present in it and alkaloids were more in concentration as compared to other phytochemicals. From previous research work it was reported that in the leaves of *Morus nigra* [18], phytoconstituents i.e., alkaloid contents were found to be present [19]. It was reported that the ethanolic/aqueous extract of *Momordica charantia* contained alkaloids, flavonoids and phlobatannins [20]. In concordance to this in the present investigations phlobatannins were found to be present in methanol extracts of *P. nigrum* corns.

In an investigation carried out by Swapana *et al* [21] flavonoids were found to be present in *Morus alba* and it have excellent antioxidant activities and have important bioactive components. While our studies also showed the same results that, flavonoids were detected in both methanol and petroleum ether extracts of *P. nigrum* corns. From previous studies of Adeoti and Oyedapo [22] it is confirmed that flavonoids and reducing sugars were present in the methanolic extract of *Fagonia cretica* while terpenoids were present in the n-hexane extract of the *F. cretica*. In research studies carried out by Dongdong *et al* [23] flavonoids were found to be present in *Morus alba*. In the present research, it is observed that flavonoids and terpenoids were found to be present in their extracts. The rich phytochemical source might be the reason for the antifeedant activity of *P. nigrum* corns against the selected pest.

4. CONCLUSION

Medicinal plants play a vital role in preventing various diseases. The antifeedant, antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs for treatment of various diseases. Thus we hope that the important phytochemical properties identified by our study in the *P. nigrum* corns will be helpful in the copping different diseases. The results obtained from the present investigation clearly suggested that further studies on isolation and identification of the active antifeedant principle present in the selected plant product will emerge as an alternative method or tool for the control of *H. armigera*.

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