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IMPACT OF LEAF EXTRACT OF *WRIGHTIA TINCTORIA* ON THE GRAM POD BORER, *HELICOVERPA ARMIGERA* (HUB.)

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ABSTRACT
Laboratory evaluation of growth inhibitory and larvicidal activities of hexane, ethyl acetate and methanol extracts of leaves of *Wrightia tinctoria* on *Helicoverpa armigera* was studied. Second instar larvae were exposed to different concentration of solvent extracts ranging from 025-5.0% that were incorporated into an artificial diet. Methanol extracts *W.tinctoria* induced a significant decrease in larval survival at 10 days at 5% concentration and showed 100% larval mortality and gave the highest insecticidal activity when compared to other solvent extracts in which only 33.30 and 37% recorded for ethyl acetate and hexane extracts, respectively. At a concentration of 5%, hexane extracts showed high larval growth inhibition (8.5%) when compared other concentrations. None of the larvae showed pupation even at low concentrations of methanol extracts and larval period was extended upto 7 days when compared to control. At a concentration greater than 1.0%, the pupation was low and only 60 and 66.66 % pupation for hexane and ethyl acetate extracts of *W.tintoria*, respectively. Hexane and ethyl acetate extracts of *W.tinctoria* produced 52.38 and 100% malformed adults, respectively at 5% concentration. These results indicated that the solvent extracts from *W.tinctoria* affects the survival of *H. armigera*.

INTRODUCTION

Botanical pesticides are eco-friendly, economic, target-specific and biodegradable. In recent years, there has been a growing interest in the exploiting plant extracts for their insecticidal activities for different pests and to develop alternatives to conventional insecticides (Matharu and Mehta, 2016). The secondary metabolites play an important role in insecticidal, hormonal and antifeedant activities of many plant extracts and their bioactive compounds of many several insects have been demonstrated (Baskar and Ignacimuthu, 2012).

The indiscriminate use of insecticides against *H. armigera* leads to the development of resistance to all the major insecticide classes by this pest and it has become increasingly difficult to control its population in India (Indira, 2013). This has necessitated a search for more eco-friendly approaches for managing this pest. Plant based compounds have been isolated and extracted for their biological activities against pests and diseases and to find out new mode of action and to develop active agents based on natural plant products, efforts are being made to isolate, screen, and develop phytochemicals possessing pesticidal activity. These categories of pesticides are now known as biopesticides. Plant based pesticides are highly toxic to many insect species and more than 2000 plant species are known to possess some insecticidal activity (Selvam and Rama krishnana, 2014). For example methanol extract of *Terminalia arjuna* and *Trachyspermum roxburghianum* showed high per cent mortality of *Helicoverpa armigera* (Baskaran *et al*., 2016). Similarly, methanol extract of *Semicarpus anacardium* seeds showed pupicidal activity at 2% concentration whereas, hexane extract of *Strychnos nux-vomica* recorded deformed adults of *H. armigera* (Sivaraman *et al*., 2014).

Helicoverpa armigera (Hübner) (Lepidoptera; Noctuidae) is distributed to considerable geographical range from Europe, Africa, Asia and Australasia to the New World (Kriticos *et al*., 2015). This pest is polyphagous in nature and larval stage can survive and feed on a very wide range of host plant species and some of them are important agricultural crops like cotton, sorghum, groundnut, pigeonpea, chickpeas, tomato etc. Losses up to Rs.10, 000 million have been reported solely due to this pest in these crops (Indira, 2013; Parmar *et al*., 2015).

Pala indigo plant, *Wrightia tinctoria* belongs to the family Apocyanaceae and is distributed throughout the world and occurs abundantly in India. The various chemical constituents isolated from various parts of the plant are reported as 3,4-Seco-lup-20 (29)-en-3-oic acid, lupeol, stigmasterol and campetosterol, Indigotin, indirubin, tryptanthrin, isatin, anthranillate and rutin Triacontanol, Wrightial, cycloartenone, cycloeucalenol, â-amyrin, Alpha-Amyrin, and â-sitosterol, 14á-methylzymosterol. The Triterpinoids components of the leaves and pods of *Wrightia tinctoria* also isolated (Srivastava, 2014).Very few reports are available on insecticidal activity of leaves of *W. tinctoria* on mosquitoes (Sakthivadivel *et al*., (2014).

Several laboratory studies have been conducted on *Helicoverpa armigera* by using solvent extracts of different plant species and few studies are practically applicable to the field studies. Therefore the present laboratory study was aimed at assessing the growth inhibitory effect of solvent extracts from the leaves of *W. tinctoria* against *H. armigera*.

MATERIALS AND METHODS

Plant material

The leaves of *Wrightia tinctoria* were collected from Kewada Forest area of Southern Rajasthan India. The plant species was identified with the forest experts in the area, botany department and with the local people.

Extraction of crude extracts

The leaves were washed thoroughly and dried in the laboratory at room temperature for 15 days. The dried leaf material was pulverized into fine powder using a mixer-grinder. Five hundred gram powdered material was taken and soaked in n-hexane in a bottle for 72 h with occasional shaking for solvent crude extraction. The same procedure was repeated with other solvents *viz*., ethyl acetate and methanol sequentially and the extracts obtained with each solvent were filtered through Whatman filter paper No.1 and the respective solvents were evaporated (at 40ºC) with the help of Rotary vacuum evaporator (Heidolph, Germany) (Choudhary *et al*., 2014) The semi-solid substances were obtained and stored in refrigerator at 4ºC for further use.

Insect Culture of *Helicoverpa armigera*

The initial culture (adults and larvae) was collected from the chickpea fields of agricultural research station, borwat farm and maintained in the insect rearing laboratory under controlled conditions. The set of pupae were also procured from National Bureau of Agriculturally Important Insects, Bengaluru (NBAII-MP-NOC-02). A temperature regime of 25 + 1ºC and 60-70% RH were maintained in the laboratory. This culture room was backed up by a BOD incubator also. The artificial diet was prepared according to the method of Singh and Rembold (1992) with some modifications. The larvae were maintained in the artificial diet prepared from the following ingredients, chickpea seed flour (110 g); yeast powder (20 g); casein (10 g); methyl-p-hydroxy benzoate (2 g); sorbic acid (0.5 g); formaldehyde (1 ml); ascorbic acid (2.6 g); cholesterol (0.115 g); streptomycin sulphate (0.1 g); multivitamin mixture (1 g); vitamin E (0.6 g); bacto-agar (12 g); distilled water (720 ml).

Growth inhibitory activity of solvent crude extracts of *Wrightia tinctoria*

The procedure was done according to Sivaraman *et al*., 2014 with slight modifications. The stock solutions of respective solvents extracts were diluted further with water to obtain required concentration of 0.5, 1.0, 1.5 and 2.0% of test samples. The crude extracts of different solvents extracts of leaves of *Wrightia tinctoria* were incorporated as per above mentioned range of concentrations into the artificial diet of *H. armigera*. Control diet was prepared without the above said solvent extracts. Starved larvae of *H. armigera* (n = 24, 35-44 mg) were released into rearing trays containing control diet or solvent extracts containing diet. Larval weights were recorded at the same time alternate days, mean weight gain of each groups and percentage comparative growth in relation to control were calculated. Fresh diet was added as and when the larvae required or every alternate day. Date on pupal weight, malformed pupae and adults were recorded. Data were statistically analysed by using Student's t test. Significant differences between treatments were determined by Tukey's tests ($P < 0.05$).

RESULTS AND DISCUSSION

The effects of hexane, ethyl acetate and methanol solvent extracts on growth and development of *H. armigera* were evaluated initially at different concentration ranging from 0.25 to 5% and are shown in Table 1. Significant differences among the treated larvae were observed after exposure to different solvent extracts in the diet. Larval mortality, reduction in growth and development, % pupation and malformed adults were exhibited when early 2nd instar larvae of *H.armigera* exposed to different concentrations of solvent extracts of *W. tinctoria*.

The average weights of larvae fed on solvent extracts of *W.tinctoria* leaves after 10 days of incubation are shown in Table 1. Significant differences among fractions were observed. There was significant weight reduction in the surviving larvae when they were fed with different concentrations of solvent extracts of *W.tinctoria*. Despite continuous feeding with normal

Table 1: Insect growth regulatory activity of *Wrightia tinctoria* leaf extracts against *Helicoverpa armigera*

Plant	Solvents	larval weight (mg + SD)	%*	% larval mortality	% pupation	Malformed adults(%)
	Hexane					
Wrightia tinctoria	0.25	$258.96 + 29.50^{b}$	67.6	0	100	17.39
	0.5	$141.10 + 85.20^{c}$	36.8	25	70	21.73
	1.0	$86.00 + 70.20^{d}$	22.4	33.30	65	30.43
	5.0	$32.75 + 48.60^{e}$	8.5	37.0	60	52.38
Wrightia tinctoria	Ethyl acetate					
	0.25	$286.00 + 47.89^{b}$	74.7	0	100	66.66
	0.5	$245.00 + 119.60^{b}$	64.0	0	100	66.66
	1.0	$140.6 + 71.60^{c}$	36.7	25.00	70.83 (n = 07)	71.42
	5.0	$87.90 + 70.10^{c}$	22.9	33.30	66.66(n = 03)	100
Wrightia tinctoria	Methanol					
	0.25	$175.70 + 151.10^{b}$	45.9	25	75	60
	0.5	$91.80 + 104.36^{c}$	24.0	50	50	100
	1.0	$0 + 0.00^{d}$	0.0	100	0	-
	5.0	$0 + 0.00^{d}$	0.0	100	0	-
	Control	$383.10 + 51.60^{a}$	100	-	100	4.34

* % of weight with respect to control; Values represents mean + SD of five replications. Different alphabets in the column are statistically significant at $p < 0.05$. (ANOVA; Tukey's test)

diet, larvae on these treatments failed to recover to the normal weight. After 12 days of experiment, their maximum weights ranged from 32.75 mg + 48.60 mg to 286.00 mg + 47.89 mg which represented the weight % in different concentrations of solvent extracts from 8.5 to 74.7 as compared to the control larvae. In all the treatments the larvicidal activity was directly proportional to the concentration of the extract.

Larvicidal activity was found in *W.tinctoria* leaf extracts treatment. At higher concentrations of 5% methanol extracts *W.tinctoria* induced a significant decrease in larval survival at 10 days and showed 100% larval mortality and gave the highest insecticidal activity when compared to other solvent extracts in which only 33.30 and 37% recorded for ethyl acetate and hexane extracts, respectively. At a concentration of 5%, hexane extracts showed high larval growth inhibition (8.5%) when compared other concentrations. In a similar manner, the ethyl acetate and methanol extracts produced a decrease in the number of live larvae. Maximum larvicidal activity observed in the methanol extract of *Caesalpinia bonducella* extracts against *H.armigera* as observed by Muthusamy *et al* (2015). Similarly, hexane extract recorded maximum larvicidal activity of 85.78% whereas, ethyl acetate extract also had notable amount of larvicidal activity of 81.77% at 5% concentration. Hexane extract at 5 and 2.5% and ethyl acetate extract at 5% concentrations completely prevented the adult emergence of *H. armigera* (Chelliah Muthu *et al*., 2014). ,

Mishra *et al*. (2015) evaluated the effects of hexane and methanol stem extracts of *Thevetia neriifolia* on the food consumption and growth of *Helicoverpa armigera* early fourth instars. The higher efficiency of methanol extract of *T. Neriifolia* against early IV instars of *H. armigera* than the hexane extract as growth regulatory agent, the stem hexane extract causing only 28-66% growth reduction as compared to 46-83% reduced growth resulted by stem methanol extract.

At a concentration greater than 1.0%, the pupation was low and only 60 and 66.66 % pupation for hexane and ethyl acetate extracts of *W.tintoria*, respectively. Whereas, none of the larvae showed pupation even at low concentrations of methanol extracts and larval period was extended upto 7 days when compared to control. Hexane and ethyl acetate extracts of *W.tinctoria* produced 52.38 and 100% malformed adults, respectively at 5% concentration. Many sterols in the *W.tinctoria* may disrupt ecdysteroid metabolism in insects resulting in inhibition of emergence behaviour. Similarly, solvent extracts may interfere with the morphogenetic hormones of the insects which lead to the deformities at pupal and adult stages. These results are consistent with the earlier reports on various lepidopterans species Maximum insecticidal activity recorded in ethyl acetate extract of *Solanum pseudocapsicum* on *H.armigera* (Jeyasankar *et al*., 2012). Baskar *et al*. (2009) reported that hexane, chloroform and ethyl acetate extracts of *Atalantia monophylla* leaves at different concentrations showed that the highest larval mortality at hexane extracts. Sivaraman *et al* (2014) observed that *Strychnos nux-vomica* hexane and chloroform seed extracts 31.43% and 22.14% malformed adults, respectively. Ethyl acetate and methanol extracts of *Semicarpus anacardium* seed produced 16.67 and 20% deformed adults, respectively in *H.armigera*.

Plants are rich source of secondary metabolites like phenols, alkaloids, terpenoids, flavonoids etc., which play a defensive role against insects. Several authors reported the solvent extracts of different plant species against *H. armigera*. Jeyasankar *et al* (2012) who reported that the ethyl acetate extracts of *Solanum pseudocapsicum* exhibited highest insecticidal activity against *H. armigera*. Whereas, the present study showed highest larval activity in methanol extracts of *W.tinctoria*. This is possible due to the presence of secondary metabolites in this plant may inhibit the metabolic activities of the larvae and ultimately the larvae failed to feed and inhibit the moulting process and finally hinder the development and mortality occurs (Baskar *et al*. 2011). Jeong *et al*. (2001) reported the bioactivity of other species of the family Apocyanaceae against different insect species. Nathaniel *et al* (2007) observed that the stem bark and leaves of the *Alstonia boonei* contain insecticidal substances against *Sesamia calamistis*. Baskar *et al* (2015) recorded ethyl acetate extract of *Hygrophila schulii* at 5.0% concentration showed 68.66% larvicidal activity. It also showed 73.33% pupicidal activity and was statistically significant from other treatments. No pupicidal activity was observed in ethyl acetate extract of *Blumea mollis*. All concentrations of ethyl acetate extract of *H. schulii* showed promising biological activities which differed statistically from other treatments.

The present study confirms the presence of some secondary metabolites in the solvent extracts of *Wrightia tinctoria* leaves which could be used as an alternative to conventional synthetic pesticides. Further investigations are needed to explore and isolate the secondary metabolites from *W.tinctoria* for its effective use in the control of *H.armigera*.

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