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RESEARCH ARTICLE :

Bioefficacy of crude extracts from *Simarouba glauca* DC. against *Plutella xylostella* and *Helicoverpa armigera*

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SUMMARY : The present investigation was undertaken with the view of development of the new insecticidal biomolecule from *Simarouba glauca* DC. The crude methanolic refluxed extracts were screened (leaf, seed, root and bark) for insecticidal activity against *Plutella xylostella* and *Helicoverpa armigera* by leaf dip bioassay method. The extracts were found effective against *Plutella xylostella* showing highest 80 per cent mortality, whereas strong antifeedant activity was found against *Helicoverpa armigera*. The study revealed *Simarouba glauca* leaves and bark has very good potency against both insect pests assayed and can be exploited for management practices of agricultural pests. Further purification of extracts, characterization of active principal component and its conformation for bioactivity against wide range of agricultural pests will be helpful for identification of new source of biopesticide.

KEY WORDS :
Simarouba glauca, *Plutella xylostella*, *Helicoverpa armigera*, Insecticidal activity, Antifeedant activity, Plant extracts

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BACKGROUND AND OBJECTIVES

Simarouba glauca DC. (Simaroubaceae) commonly known as American bitter wood is having potent bitter principle molecule 'Quassin' (Joshi and Hiremath, 2000). In simaroubaceae various amoebicidal, anticancer, antiviral, antimicrobial and antimalerial activities were already reported by Anonymous (2004). Also the various plant extracts have been reported to have insecticidal activities on vectors of medical or veterinary interest or on agricultural and non agricultural pests. However, the botanical extracts have not been used due to overuse of synthetic pesticides (Ratandas and Wink, 2012). The use of botanical formulations for plant protection against insect pests has assumed greater importance as there is awareness all over the world due to ill effects of indiscriminate use of synthetic pesticides (Bami, 1997). However, the screening of plant extracts against insects are still continuing throughout the world to sort out the effective botanicals which are ecofriendly and can be used as economic biopesticides (Jeyasankar *et al.*, 2013). Therefore, the present study deals with screening of leaf, seed, root and

bark refluxed methanolic extracts of *Simarouba glauca* against *Plutella xylostella* and *Helicoverpa armigera*.

Objectives:

- To prepare the extracts from different plant parts of *Simarouba glauca* DC.

- To exploit the insecticidal potential of extracts from *Simarouba glauca* DC.

RESOURCES AND METHODS

Collection of plant material:

The plant material *viz.*, leaves (male and female plant individually), seed, root and bark of *Simarouba glauca* were collected from the field of Regional Station of National Bureau of Plant Genetic Resources located at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for extraction purpose.

Rearing of *Plutella xylostella*:

The larvae and pupae of *Plutella xylostella* were collected from cabbage and cauliflower field from outskirts of Akola for mass rearing. They were reared in the laboratory on the mustard seedlings upto F_4 generations for establishing homologous laboratory population. The rearing procedure described by Lu and Sun (1984) was followed to maintain the test culture of *Plutella xylostella*. Mustard seeds (Var. Pusa bold) were used for raising seedlings. The seeds were soaked in water for 12 hours, then treated with carbendazium 1 g/ lit to check the fungal contamination. The treated seeds were sown in plastic cups containing soil rite. The seedlings were placed in the mating chamber as a substrate for oviposition of *Plutella xylostella*. The moths (approximately 50 numbers) of *Plutella xylostella* were released in the mating chamber. Once in two days seedlings were replaced with another fresh set. The adults were provided with liquid adult diet (Moharil *et al.,* 2008). The eggs were hatched within l-2 days after oviposition and the neonates were allowed to feed on the same seedlings. After the consumption of the seedlings larvae were transferred to the fresh mustard seedlings. The rearing was done in rearing rack at temperature $27^0C \pm 1^0C$, relative humidity 75±1 per cent and photoperiod approximately 13:11 light: dark hours.

Rearing of *Helicoverpa armigera*:

Helicoverpa armigera larvae collected from field of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola were reared in the laboratory on semi-synthetic artificial diet (Moharil *et al*., 2008) under controlled conditions of temperature $25^0C \pm 2^0C$, 75±5 percent relative humidity and photo period of 13 hours light: 1l hours dark. Larvae allowed for pupating in trays; pupae were collected and disinfected with 0.02 per cent sodium hvpochloride solution. Disinfected pupae were segregated by sex determination and transferred to adult emergence chamber. The adults emerged were transferred into mating chamber by maintaining the male:female ratio 1:1 and provided with adult diet (Potdar, 2008). Two pairs of moths were released in each mating chamber. The small strips of cotton linear nappy were used as an ovipostional substrate for female moths. The strips of cotton cloth with eggs were kept in small incubation jars for hatching. The neonates emerged from the eggs were transferred on the freshly prepared semi-synthetic artificial diet. The 4^{th} instar larvae from F_3 generation were used for bioassay.

Preparation of leaf and bark samples for extraction:

For extraction purpose leaf (male and female individually) seed, root, and bark were dried in hot air oven at 40^0C till they become completely dried. The dried samples were powdered using mixer grinder which was ultimately used for further extraction using methanol.

Reflux extraction:

Soxhlet extraction was done with Universal Extraction System (Buchi). For this 10 gram of dried powder (leaf, seed, root and bark) was taken in glass thimble and extracted with methanol. The procedure was carried out for 10 cycles for each extract and the temperature of heating mantle was adjusted just below the boiling point of methanol. Most of the solvent from each extract was evaporated and extracts were dried at room temperature and weight of each extract was recorded (Harborne, 1973).

Bioassay:

Accurately weighed 10 mg of dried extract was mixed in 1 ml of Dimethyl Sulphoxide (DMSO) by vortexing to form clear solution of 1 per cent concentration. Similarly other required concentrations of the extracts were prepared for the final bioassay. All bioassays were carried out in triplicate.

Bioassay against *Plutella xylostella* and *Helicoverpa armigera*:

Cabbage and cotton leaves were used to conduct bioassays against *Plutella xylostella* and *Helicoverpa armigera* respectively. Cabbage and cotton leaves were first washed with distilled water containing 0.1 per cent Triton x 100 and dried for about 1 hour. Cabbage leaf discs cut with a scissor, while whole cotton leaves are taken for bioassay and then dipped in the test solution of various extracts prepared in DMSO to facilitate uniform treatment of active ingredient for about 10 second. The leaf discs were placed slanting for about 2 min over a blotting paper in a tray to drain excess solution at room temperature. Each leaf disc was kept in individual perti plate along with blotting paper at bottom and then 10 larvae starved for 8 hours (third instar for *Plutella xylostella* and forth instar for *Helicoverpa armigera*) were released on each leaf disc/leaf. The plates were observed for 72 hours for any insecticidal activity. The bioassay were conducted in environmentally controlled growth chamber at a temperature $27^0C \pm 1^0C$ with relative humidity 65±5 per cent, dark and light regime of 13:11 hours (Tabashnik *et al.*, 1987).

OBSERVATIONS AND ANALYSIS

The crude methanolic extracts of leaf and bark were evaluated for insecticidal activity against *Plutella xylostella* and *Helicoverpa armigera* using leaf dip bioassay method (Tabashnik *et al.*, 1987). The larval morality observed against *Plutella xylostella* was higher in case of bark (80%) extracts as compared to male (70%), female (60%) plants leaf, seeds (40%), root (20%) at concentration of 20% of crude extracts as given in Table 1. Fig. 1 illustrates the bioassay and the moribund insects died after 72 hours. The mortality was found proportional with the concentration of extract in all cases. When treated leaves are replaced with the fresh untreated leaves remaining larvae initiated feeding but their size and weight found reduced as compared to control.

Amongst all the extracts tested male leaf and bark

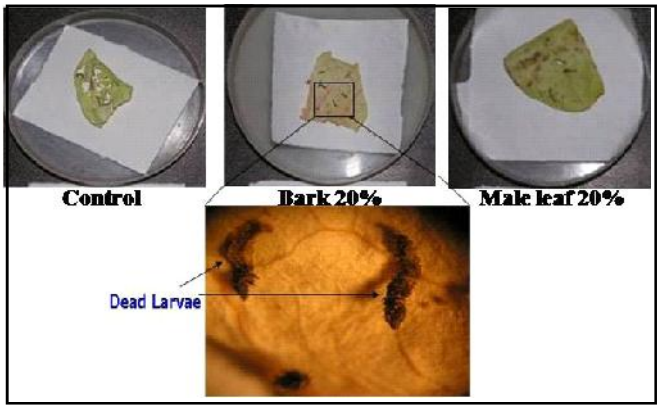

Fig 1: Bioassay of extracts from *S. glauca* against *Plutella xylostella*

Table 1: Screening of different extracts from *S. glauca* against *Plutella xylostella*

Sr. No.	Extract	Concentration (%)	Mortality (%)	Pupation (%)
1.	Male leaf	1	30	0
		10	60	0
		20	70	0
2.	Female leaf	1	20	0
		10	50	0
		20	60	0
3.	Seed	1	00	0
		10	30	0
		20	40	0
4.	Root	1	0	0
		10	10	0
		20	20	0
5.	Bark	1	20	0
		10	80	0
		20	80	0
6.	Control	DMSO	0	0

Table 2: Screening of different extracts from *S. glauca* against *Helicoverpa armigera*

Sr.No.	Extract	Concentration (%)	Mortality (%)	Feeding (%)
1.	Male leaf	5	0	20
		10	0	20
		15	0	12
		20	0	10
		25	0	5
2.	Bark	5	0	5
		10	0	2
		15	0	0
		20	0	0
		25	0	
3.	Control	DMSO	0	25

extract showed better insecticidal activity as compared to other extracts against *Plutella xylostella*. Therefore, only these extracts were used to test bioefficacy against *Helicoverpa armigera*. No any mortality was found in case of both extracts against *Helicoverpa armigera*. However, strong concentration dependant antifeedant activity was found for both extracts tested of which bark extract showed much prominent activity. This may be due to presence of quassinoids present in these extracts, which are the known active principals in Simaroubaceae family and reported to have insecticidal and cytotoxic action (Satpathi, 1984). As no mortality was observed, percent area of feeding was calculated by simple graphical method (Marshall, 1968). Male leaf extract resisted feeding upto maximum 95 per cent of leaf area at higher concentration (25%) while bark totally checked feeding (100%) at 20 per cent and 25 per cent concentration of extract (Table 1 and Fig. 2). This shows that male leaf and bark extracts from *Simarouba glauca* can be further studied for identification of potent molecule which can be exploited as a biopesticide.

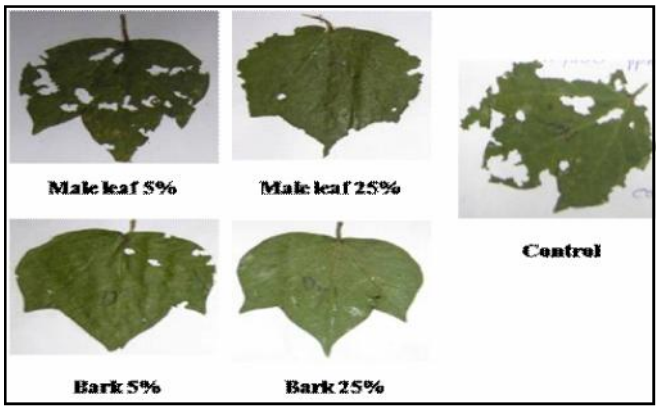

Fig 2: Feeding of *Helicoverpa armigera* on cotton leaves treated with extracts from *Simarouba glauca*

Conclusion:

Present study revealed potential insecticidal and antifeedant activity of extracts from different plant parts/ tissues of *Simarouba glauca* DC. Bark extract showed highest mortality (80%) and a strong antifeedant activity. Overall, the study revealed that *Simarouba glauca* leaves and bark has very good potency against both insect pests assayed and can be exploited for management practices of agricultural pests. Further purification of extracts, characterization of active principal component and its conformation for bioactivity against wide range of agricultural pests will be helpful for identification of new source of biopesticide.

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