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SCREENING FOR ANTIFEEDANT ACTIVITY OF *GYMNEMA SYLVESTRE* LEAF EXTRACTS AGAINST *SPODOPTERA LITURA* F. (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT
Antifeedant activity of *Gymnema sylvestre* leaf extracts with different solvents viz., hexane, methanol, chloroform, ethyl acetate, butanol and acetone precipitated were evaluated against third instar larvae of *Spodoptera litura* through choice as well as no choice methods. Antifeedant activity of various extracts was compared based on their AI_{50} values. It was observed that saponins mixtures showed lowest AI_{50} value (0.047%) followed by azadirachtin (0.086%) through no-choice test method observed after 24 hours. When it was tested with choice method, saponins mixture also showed lowest AI_{50} as 0.036% after 24 hours of treatment. After 48 hours of observation through no-choice and choice method precipitated saponin mixtures of *G. sylvestre* showed antifeedancy within the fiducial limit of azadirachtin. In the present study precipitated mixture of saponins extracted from *G. sylvestre* showed very good antifeedant activity, which could be used for the ecofriendly management of lepidopteran insect pests.

INTRODUCTION

Tobacco caterpillar, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) is a serious and economically important agricultural pest on over 150 plant species in different parts of the world including India, Japan, China, and other countries of Southeast Asia due to its high reproductive capacity, migration ability over long distances, polyphagous in nature and voracious feeder (Wightman and Amin, 1988; Rao *et al.,* 1989; Gahukar, 1992; Mallikarjuna *et al.,* 2004; Sintim *et al.,* 2009). In India, *S. litura* is serious under open field conditions as well as under polyhouse conditions and badly affecting the production of cotton, mungbean, soya bean, cabbage, cauliflower, tomato, cucumber, rose, sweet pepper, groundnut, castor and millets (Maree *et al.,* 1999; Mallikarjuna *et al.,* 2004; Sood, 2010). The young caterpillars of *S. litura* feed voraciously on leaves, defoliating the plants making insecticidal application mandatory for the cultivation of crops.

The insect pest management strategies were dominated by synthetic insecticides in the last seven decades. This has envenomed the surroundings as well as non-target organisms, led to environmental pollution, destruction of the natural enemy complex and development of resistance against toxicants in over 500 species of insects and mites (Thomas, 1999; Bhandari *et al.,* 2009; *Khanna et al.,* 2011). Whereas, *S. litura* is one of the first insect pests of agricultural importance in India had developed resistance to synthetic insecticides and defied synthetic insecticide based control strategies and developed resistance against a wide range of insecticides (Ramakrishnan *et al.,* 1984; Armes *et al.,* 1997; Kranthi *et al.,* 2002; Mallikarjuna *et al.,* 2004; Ahmad *et al.,* 2007; Huang and Han, 2007). Thus, there is a need to look for safer eco-friendly alternatives in view of the pressing need to protect the environment, natural enemies and human health.

Natural products of plant origin with insecticidal properties have been tried as an indigenous method for the control of a variety of insect pests in the recent past. The use of plant extracts for insect control has several appealing features as these are generally more biodegradable, less hazardous and a rich storehouse of chemicals of diverse biological activities. Moreover, herbal sources give a lead for discovering new insecticides (Isman, 2006; Khanna *et al.,* 2011).

Gudmar (Periploca of woods), *Gymnema sylvestre* (Retz) Schult is a perennial herb which belongs to family Asclepiadaceae, found in several parts of India. Its leaf extracts is used in traditional medicine to cure a variety of human illnesses viz. hypoglycemic action, hepatosplenomegaly, halminthiasis, dyspepsia, cardiopathy diuretic, anti-diabetic, anti-inflammatory and antimicrobial activities, especially antifungal has been demonstrated (Sastri, 1956; Lalitha and Venkataraman, 1991; Satdive *et al.,* 2003). The active constituent from its leaves has already been isolated and established as saponins (oleanane and dammarene type triterpenoid), phytosterols, phenols, flavonoids and tannins

Table 1: Comparative antifeedant activity (AI_{50}) of various extracts through no-choice test method (After 24 hours)

Extracts	Heterogeneity Ç²	*df*	Regression equation	b ± SE	AI_{50}	Fiducial limits (%) minimum	maximum
GS1	5.217	4	4.841 + 0.590x	0.590 ± 0.091	1.862	0.600	5.774
GS2	3.241	4	5.409 + 0.559x	0.559 ± 0.066	0.185	0.101	0.339
GS3	4.243	4	4.005 + 0.729x	0.729 ± 0.082	1.347	0.723	2.509
GS4	9.481	4	4.879 + 0.536x	0.536 ± 0.074	1.677	0.698	4.024
GS5	1.879	4	5.528 + 0.530x	0.530 ± 0.064	0.101	0.058	0.176
GS6	1.636	4	5.648 + 0.594x	0.065 ± 0.065	0.081	0.051	0.130
GS7	7.935	4	5.234 + 0.736x	0.736 ± 0.083	0.481	0.299	0.772
GS8	3.427	4	5.409 + 0.712x	0.712 ± 0.073	0.266	0.157	0.452
GS9	1.295	4	5.675 + 0.599x	0.599 ± 0.065	0.075	0.047	0.119
GS10	3.871	4	4.995 + 0.587x	0.587 ± 0.063	1.021	0.522	1.996
Aza	1.705	3	6.033 + 0.971x	0.971 ± 0.107	0.086	0.063	0.119

Table 2: Comparative antifeedant activity (AI_{50}) of various extracts through no-choice test method after 48 hours

Extracts	Heterogeneity Ç²	*df*	Regression equation	b ± SE	AI_{50}	Fiducial limits (%) minimum	maximum
GS1	2.733	4	5.179 + 0.401x	0.401 ± 0.065	0.357	0.167	0.762
GS2	0.511	4	5.298 + 0.406x	0.406 ± 0.062	0.185	0.234	0.422
GS3	6.417	4	4.965 + 0.518x	0.518 ± 0.071	1.166	0.517	2.631
GS4	9.007	4	5.002 + 0.532x	0.532 ± 0.071	0.990	0.464	2.114
GS5	3.373	4	5.672 + 0.571x	0.571 ± 0.064	0.066	0.042	0.116
GS6	3.406	4	5.547 + 0.664x	0.664 ± 0.069	0.150	0.092	0.245
GS7	1.879	4	5.254 + 0.652x	0.652 ± 0.074	0.407	0.446	0.859
GS8	3.321	4	5.769 + 0.766x	0.766 ± 0.071	0.099	0.067	0.146
GS9	1.232	4	5.712 + 0.523x	0.523 ± 0.062	0.043	0.027	0.071
GS10	2.621	4	5.083 + 0.585x	0.585 ± 0.059	0.722	0.391	1.331
Aza	7.026	3	6.247 + 1.003x	1.003 ± 0.105	0.057	0.043	0.076

Table 3: Comparative antifeedant activity (AI_{50}) of various extracts through Choice test method after 24 hours

Extracts	heterogeneity ÷²	*df*	Regression equation	b ± SE	AI_{50}	Fiducial limits (%) minimium	maximum
GS1	4	2.472	4.956 + 0.703x	0.703 ± 0.091	1.153	0.628	2.111
GS2	4	1.459	5.621 + 0.497x	0.497 ± 0.069	0.056	0.034	0.097
GS3	4	3.871	4.967 + 0.641x	0.641 ± 0.091	1.124	0.584	2.161
GS4	4	1.456	5.121 + 0.497x	0.497 ± 0.084	0.571	0.296	1.097
GS5	4	Less than 20% antifeedancy observed at 2% dose					
GS6	4	4.701	5.848 + 0.591x	0.591 ± 0.071	0.037	0.024	0.056
GS7	4	3.138	5.649 + 0.914x	0.914 ± 0.099	0.194	0.125	0.302
GS8	4	1.849	5.658 + 0.623x	0.623 ± 0.073	0.088	0.054	0.143
GS9	4	2.266	5.729 + 0.051x	0.051 ± 0.068	0.036	0.022	0.056
GS10	4	3.855	5.083 + 0.726x	0.726 ± 0.081	0.769	0.411	1.439
Aza	3	0.084	5.640 + 0.627x	0.627 ± 0.098	0.095	0.057	0.157

(Subbarao and Joseph, 1971; Gooper, 1887; Khanna *et al.,* 2011). Saponins are a class of natural products that are surface-active sterol or triterpene glycosides. Quantitative analysis results suggested that saponin (5%) was present in a high concentration followed by tannins (1.0%) (Khanna *et al.,* 2011). Though, *G. Sylvestre* is known to contain triterpenic saponin in their leaves but information is limited on their pesticidal properties. The insecticidal effects of this medicinal herb, *G. sylvestre* has been demonstrated on *Tribolium castaneum* Herbst, *Anopheles subpictus* Grassi and *Culex quinquefasciatus* Say (Tandon and Sirohi, 2010; Ahalya and Mikunthan, 2011; Khanna *et al.,* 2011). Saponins possess a diversity of properties and a potential agent to manage pests having economic importance in agriculture have just commenced recently (Saha *et al.,* 2010). The objective of the investigation was to evaluate the feeding detergency activity of *G. Sylvestre* leaves extracts on *S. litura,* one of the most destructive insect pests of agricultural crops.

MATERIALS AND METHODS

Preparation of *G. sylvestre* leaf extracts

G. sylvestre shade dried leaves powder (3.9 kg) was extracted sequentially with different solvent combinations. The detailed extraction procedure is presented in Fig 1. All the filtrates were concentrated under *vacuo* in a rotary evaporator (Hiedolph, Germany) at 45ºC. A total of ten extracts of *G. sylvestre* leaves were prepared with different solvents and extracted product name coded GS1 to GS10. The methanol extract (GS2) was partitioned between water and n-butanol to remove water

Table 4: Comparative antifeedant activity (AI_{50}) of various extracts through Choice test method after 48 hours

Extracts	heterogeneity $\div^2$	*df*	Regression equation	b ± SE	AI_{50}	Fiducial limits (%) minimum	maximum
GS1	4	3.268	5.138 + 0.734x	0.734 ± 0.091	0.649	0.406	1.037
GS2	4	2.501	5.562 + 0.562x	0.562 ± 0.072	0.100	0.057	0.175
GS3	4	3.154	5.097 + 0.754x	0.754 ± 0.093	0.742	0.459	1.201
GS4	4	0.926	5.240 + 0.539x	0.539 ± 0.083	0.358	0.213	0.602
GS5	Less than 20% antifeedancy observed at 2% dose						
GS6	4	2.815	5.843 + 0.625x	0.625 ± 0.071	0.045	0.029	0.068
GS7	4	6.987	5.241 + 0.817x	0.817 ± 0.817	0.507	0.344	0.747
GS8	4	2.082	5.807 + 0.681x	0.681 ± 0.074	0.065	0.043	0.099
GS9	4	6.424	5.746 + 0.055x	0.055 ± 0.069	0.045	0.028	0.072
GS10	5	5.281	5.163 + 0.662x	0.662 ± 0.074	0.568	0.308	1.046
Aza	3	2.644	5.742 + 0.473x	0.473 ± 0.095	0.027	0.020	0.056

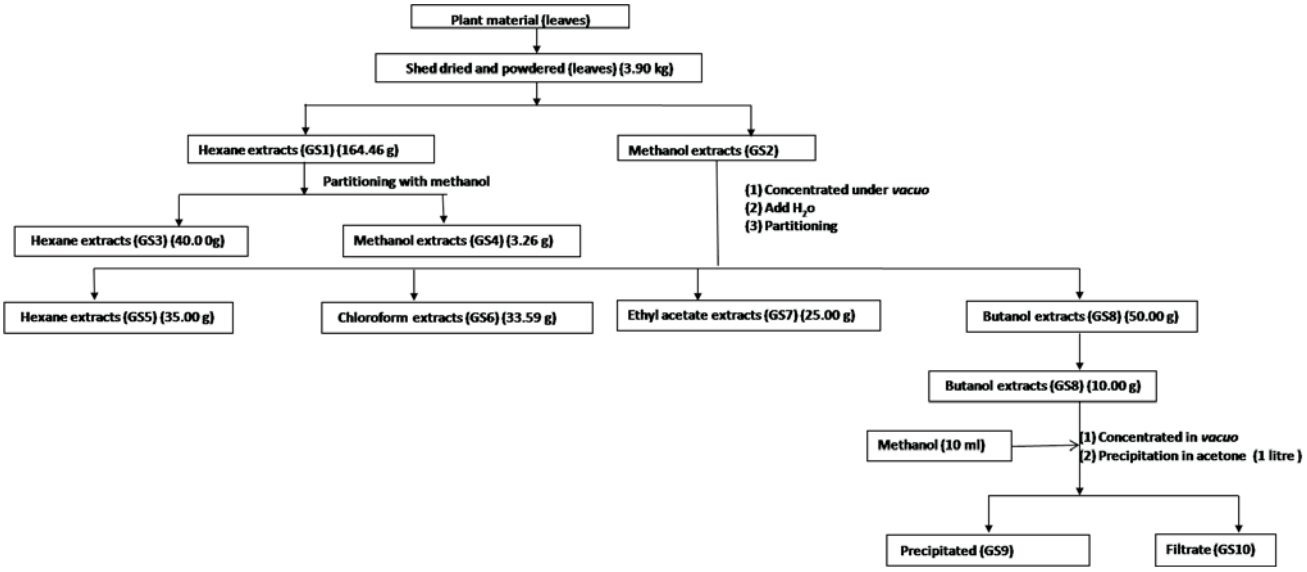

Figure 1: Schematic diagram for different solvents extraction from *Gymnema sylvestre* leaves

soluble free sugars. Butanol concentrate (10 g) was dissolved in minimum amount of methanol (10 mL) and precipitated in large excess (1 litre) of acetone with stirring on a magnetic stirrer to obtain a precipitated mixture of saponins (GS 9) (Saha *et al.,* 2010). The precipitated solid saponin mixture was filtered through sintered funnel and the remaining filtrate (acetone solution) was concentrated under *vacuo* to obtain GS10 fraction. Technical azadirachtin (50%) which was procured from division of agricultural chemicals, IARI, New Delhi, taken as a positive control and standard check in the present experiment.

Preparation of stock solutions

Stock solutions of the concentrated extracts were prepared in the carrier solvents. Further dilution was done in emulsified water by maintaining emulsifier (Tween-80) level at 0.5 % to yield various concentrations. Final concentrations for bioassay against *S. litura* were fixed after preliminary screening of extracts from 0.001 to 1.0% concentrations.

Rearing of test insects

A susceptible nucleus culture of *S. litura* was maintained on artificial diet (Temp., 25 ± 1ºC; RH, 60 ± 5 % and light: dark phase, 16:8 hour) in the Division of Entomology, Indian Agriculture Research Institute, New Delhi. The composition of agar based meriodic artificial diet was prepared according to the Gupta *et al.* (2005) for mass rearing of *S. litura* was used in the study. Neonate larvae were transferred to fresh castor leaves thoroughly washed with sterile distilled water (SDW). Five-day old larvae were transferred to plastic boxes (30cm x 20cm x 7cm) containing pieces of artificial diet in the group of two larvae. Boxes were cleaned daily and larvae were fed with fresh diet. Last instar larvae (non feeding wandering stage) were transferred to boxes containing saw dust for pupation. Pupae were collected after four to five days and disinfected with 0.02% sodium hypochlorite and kept in insect rearing cages (dimension) for emergence of adults and provided with cotton swabs soaked in 20 % honey solution and SDW after adult's emergence. Castor leaves with water dipped petiole were kept in cages for egg lying whenever needed.

All the jars and cages used for rearing were disinfected periodically with Protasan DS® (Qualigens). This enabled to maintain a disease-free and healthy stock culture for further experiments.

Antifeedant activity of different extracts through choice and no-choice method

The antifeedant activity of different leaf extracts of *G. sylvestre* were assessed against 3rd instar (7 day old) larvae of *S. litura* on castor leaves by choice and no-choice bioassay methods with azadirachtin 50% as a positive control. The observations were recorded at 24 and 48 hours.

Castor leaf discs (9 cm diameter) were dipped thoroughly in each of the concentration and air dried. Moist filter paper discs were placed in glass Petri plates (9 cm diameter) on which a single treated leaf disc was kept. Single pre-starved (3-4 hours), 7-day old larvae of *S. litura* was released into each Petri plate. Ten replicates were kept for each concentration. Leaf discs treated with solvent emulsified water served as control. The unfed area in each treatment was measured using a leaf area meter (Licor-3100) after 24 hours. Similarly, another set of experiment was kept and leaf area was measured after 48 hours.

For the choice method experiment conducted in the same way as it was in no-choice method. But in this method, in each Petri dish, along with a treated leaf disc, one untreated leaf disc of same size was kept. The area left over by larva was measured after 24 as well as 48 hours in both the leaf discs.

Statistical analyses and presentation of data

No-choice method

Per cent feeding was tabulated for treatments and control. Mean per cent antifeedancy was calculated for each concentration using the formula of Singh and Pant (1980).

$$\text{Per cent feeding} = \frac{\text{Initial area given for feeding - area left over after feeding}}{\text{Initial area given for feeding}} X100$$

$$\text{Per cent antifeedancy} = \frac{\text{\% protection in treatment - \% protection in control}}{\text{100 - \% protection in control}} X\ 100$$

Choice method

Deterrence activity was calculated by the formula given by Isman *et al.* (1990).

$$\text{Per cent feeding deterrence} = \frac{C - T}{C + T} X\ 100$$

C = area consumed in control

T = area consumed in treatment

All the data were subjected to analysis of variance (ANOVA), after transformation of data, and Finney (1971) method was used to determine AI_{50}, data were subjected to probit analysis by using a basic LD_{50} program version by Trevors (1986).

RESULTS

Based on antifeedancy at individual doses, lethal concentration of antifeedant activity (AI_{50}) of solvent crude extracts and fractions and azadirachtin(20%) was calculated. Antifeedant activity of various extracts was compared based on their AI_{50} values. Higher antifeedant index normally indicated decreased rate of feeding.

Antifeedant activity by no-choice method

G. sylvestre leaf extract GS9 was the most effective against 3rd instar larvae of *S. litura* and showed lowest AI_{50} value (0.075%) followed by GS6 (0.081%) and azadirachtin (0.086%) after 24 hours of treatment. The order of antifeedancy of different extracts was GS1 < GS4 < GS3 < GS10 < GS10 < GS7 < GS8 < GS2< GS5 < Aza < GS6 < GS9 (Table 1). Extract GS9 showed better antifeedant activity compared to azadirachtin. Whereas, the lowest antifeedant activity was observed with GS1 which showed 19.50 times higher AI_{50} value as compare to azadirachtin followed by GS4 after 24 hours of treatment.

Similar pattern of AI_{50} was observed after 48 hours with no-choice test method except the few extracts. After 48 hours, mixtures of saponins (GS9) showed lowest AI_{50} (0.043%) which is significantly comparable to technical azadirachtin and GS3 showed highest AI_{50} value (1.166%) (Table 2). Moreover, extract GS5 and GS8 also showed good antifeedant activity as their AI_{50} values being 0.066 and 0.099%, respectively coming in the same range of fiducial limits. Whereas, antifeedant activity of GS3 was 20.45 times less effective than azadirachtin.

Antifeedant activity by choice method

Antifeedant activity of various extracts were studied through choice method, extract GS9 showed lowest AI_{50} as 0.036% followed by GS6 (AI_{50} 0.037%), GS2 (AI_{50} 0.056%) and GS8 (AI_{50} 0.088%) after 24 hours (Table 3). These extracts showed higher antifeedant activity compare to azadirachtin. Lowest antifeedant activity was observed with GS1 extract. Antifeedant activity of GS1 and GS3 were 12.14 and 11.83 times less than azadirachtin, respectively. The order of antifeedant activity of extracts was GS5 < GS1 < GS3 < GS10 < GS4< GS7 < Aza < GS8 < GS2 < GS6 < GS9. Whereas, after 48 hours, the azadirachtin gave maximum antifeedant activity against *S. litura* followed by GS9 and GS6 extracts of *G. sylvestre*. However, GS3 (AI_{50} 0.742%) showed lowest antifeedant activity followed by GS1 (AI_{50} 0.649%) and GS10 (AI_{50} 0.568%) (Table 4).

DISCUSSION

In the present study, acetone precipitated butanol extract (saponin mixtures) of *G. sylvestre* (GS9) was found most effective among different solvent extracts used against 3rd instar larvae of *S. litura* in no choice as well as choice methods of evaluation after 24 hours of treatment. However, it (GS9) found at par with azadirachtin giving almost same antifeedant activity after 48 hours of treatment. The good antifeedant activity of acetone precipitated butanol extract of *G. sylvestre* (GS9) attributed to affinity of saponin compound with butanol in the extract (Saha *et al.,* 2010). So, the present study indicated that GS9 extract may have mixture of triterpene saponins (Kanetkar *et al.,* 2007) giving good antifeedant activities. Investigations concluded that saponins act as feeding deterrents to insects which fed on saponin containing food (Taylor *et al.,* 2004; Shinoda *et al.,* 2002). Food consumption was reduced in our experiments also due to antifeedant activity. According to Ishaaya (1986) saponins slow down the passage of food through insect gut. Perhaps they reduced the digestibility of food by inhibiting the secretion of digestive enzyme (Ishaaya and Birk, 1965; Golawska *et al.,* 2006) or an obstruction of

alimentary content in the gut would limit or inhibit food intake. Whereas Sridhar *et al*. (2001) studied the effect of different solvent extracts of *G. sylvestre* for antifeedant activity against tobacco caterpillar, *S. litura*. They observed that among the various extracts, acetone extract of *G. sylvestre* showed best antifeedant activity.

Present results showing antifeedant activity of various extract of *G. sylvestre* was in agreement with the work of Sridhar *et al*. (2001) who studied the antifeedant activity of 26 medicinal plants against *S. litura*. They observed that among the various plants studied treatment with *G. sylvestre* extract gave lowest mean larval weight (0.057gm) at 2% concentration compare to 1.421 gm in control.

Present result are supported by the work of Seenivasan *et al*. (2003) who studied the efficacy of leaf extract of *G. sylvestre* against *P. xylostella* and found effective in controlling *P. xylostella* larvae by recording mortality and feeding ratio up to 30.75% and 13.6, respectively. Deterrent effect of gymnemic acid from *G. sylvestre* was also observed by Granich *et al*. (1974). They explained that gymnemic acid are known to distort the taste of amino acids (Meiselman and Halpern, 1970) which may be one of the reason for deterrent activity of gymnemic acid extract from *G. sylvestre* against *S. litura*. In conclusion, mixture of saponins (GS9) offers potential antifeedant activity against *S. litura*. This could be used for the development of new botanical pesticide formulations for the control of this serious lepidopteran pest. Further studies are in progress to characterise the individual saponins and evaluate of insect growth regulatory activities.

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