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ANTI-INSECT ACTIVITY OF PLANT EXTRACT FROM *Datura stramonium* L. AGAINST *Spodoptera litura* FAB. (NOCTUIDAE: LEPIDOPTERA)

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Abstract

In the present study, the *Datura stramonium* extract was tested against *Spodoptera litura* and the results anti-insect properties of various accessions of *D. stramonium* showed different degrees of anti-insect activities, when extracted and bio-assayed. When comparing the extracts obtained by following Soxhlet, Cold and aqueous extraction methods, among the bioassay methods experimented, Poison food bioassay (Leaf disc bioassay). Soxhlet extracts possess highest proportion of insecticidal properties and shown its supremacy in causing mortality over that of the extract and aqueous extracts of *D. stramonium* plant parts. Fruit extract causes highest mortality than other plant parts tested S. *litura.*

Key words: Wind *Datura stramonium*, *Spodoptera litura*, Fruit extract and Anti-insect activities.

1. Introduction

Quandaries such as health hazards and harming to beneficial organism *viz.*, natural enemies and pollinators are also experienced because of the extensive use of broad spectrum synthetic chemical pesticides. However, insecticides have serious drawbacks such as pest resurgence and resistance, lethal effects on non-target organisms, the risk of user's contamination, food residues, and environmental pollution. Further, botanical insecticides manifest their effect on insects in several ways including mortality, antifeedancy, growth inhibition, suppression of reproductive behaviour and reduction of fecundity and fertility, there by the chance of resistance development by insect pests are reduced or prolonged. By and large the risks associated with the use of synthetic insecticides and benefits of botanicals have renewed the interest in insecticides of natural origin especially botanicals, which have evolved astonishingly diverse array of natural chemical constituents (Yadav *et al.*, 2010). *Datura* plant rich in secondary metabolites which have antibacterial activity by different mechanisms (Saadabi *et al*., 2006). *Datura stramonium* (Family: Solanaceae), it is indigenous to Caspian region and in United States, South America, France, Germany, Hungary and Middle East. The main active constituents of plant are atropine and scopolamine. Dry leaves consists 0.25 % of alkaloids of *stramonium*, it was used as an aphrodisiac, medicinal, psychotropic, sacred and anti spasmodic (Kulkarni, 2005). *S. litura* was a poly - phagous pest throughout tropical and subtropical Asia (Garad *et al.*, 1984). It is one of the most economically important insect pests of 51 countries including India, Japan, China, and other countries of Southeast Asia. In India, it feeds on 74 species of cultivated crops and few wild plants (Ranga Rao *et al.*, 2008). Moths are strong flier and disperses to a long distances. Management of this pest using synthetic chemicals has failed

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because tobacco cutworm has developed resistance against most of the insecticides in the market (Mushtaq Ahmad *et al.*, 2007).

2. Materials and Methods

Mass rearing of test insects

Tobacco caterpillar, *Spodoptera litura* Fabricius (Noctuidae: Lepidoptera), a polyphagous pest reared on Bengal gram flour based semi synthetic diet (PDBC, 1998) under the temperature of 25 ± 2 °C and 70 to 80 per cent relative humidity continuously throughout the study period in our laboratory, was utilized in the antifeedant bioassays as test insect.

Preparation of plant materials

Fresh *D. stramonium* L. plant parts *viz.*, leaves, stem, flower and fruits were collected and allowed to shade dry to prevent the loss of active principle from the plant parts during drying. Shade drying process was continued for more than one month, until the plant materials were dried enough to be powdered. The dried plant materials were taken individually and ground in to powder with the help of Willey mill. Whole plant powder was prepared by mixing leaf, flower, stem, and root powder in equal proportions.

Extraction of *Datura stramonium* L.

Powdered form of *D. stramonium* plants were extracted using various solvents such as n-hexane, benzene, ethyl acetate, acetone and combinations of solvents at different ratio *viz.*, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50) and ethyl acetate and acetone (50:50). Two methods such as Soxhlet extraction method and Cold extraction method (room temperature extraction method) had employed in extracting berries by using solvents. In addition, aqueous extract was obtained using HPLC water.

Soxhlet extraction method

The powdered form of *D. stramonium* plants were made into small packets with Whatman No. 40, filter paper separately for leaf, flower, stem, root and whole plant and extracted with n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50), ethyl acetate and acetone (50:50) (HPLC grades) for 72 hrs in a Soxhlet apparatus individually. Then, extracts were passed through anhydrous sodium hydroxide to absorb water particles and dried under reduced pressure using vacuum pump. Finally, the extracts were labeled and stored in a deep freezer (-10 ºC).

Screening against *S. litura*

Two different types of bioassays such as poison food bioassay and topical bioassay were followed in this screening. Fourth instar of *S. litura* was obtained from our culture and utilized in the bioassay.

Poison food bioassay (Leaf disc bioassay)

Castor leaf discs of 2 cm diameter were cut and the respective extract was smeared on the both the adaxial and abaxial surfaces of the leaf disc @100 µl/side using a blunt glass rod. Untreated leaf discs served as absolute control. Treated leaf discs were air dried and placed in petriplates. Three larvae of fourth instars of *S. litura* were introduced separately into each petriplate and covered. All the petriplates were kept under controlled conditions of 25 ± 2 ºC temperature and 70 ± 5 % relative humidity. Leaves were collected from the container when the control leaf disc was completely fed and the leaf area unfed was measured in each treatment by using leaf area meter (Elico model). Per cent leaf area protection over control was computed and the antifeedancy was rated as per the formula and scale given below. The larvae were supplied with untreated leaves and reared till the life cycle ends. Observations were also made on mortality and malformations in any of its life stages.

Per cent leaf area protection over control	Scale	Rating
> 80	++++	Strong antifeedancy
50 – 80	+++	Medium antifeedancy
20 – 50	++	Weak antifeedancy
< 20	+	Insignificant antifeedancy

Table – 1: Efficacy of fruit extracts (Soxhlet method) of *D. stramonium*(L.) plants on fourth instar of *S. litura* – Poison food bioassay

T. No	Treatment	*Per cent mortality at 1% concentration				
		Leaf extract	**Flower extract**	**Stem extract**	**Fruit extract**	**Whole plant extract**
1.	n-hexane extract	6.66 (8.85)^{bc}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
2.	Benzene extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
3.	Ethyl acetate extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
4.	Acetone extract	86.66 (72.28)^{a}	86.66 72.28)^{a}	93.33 (81.14)^{a}	93.33 (81.14)^{a}	93.33 (81.14)^{a}
5.	n-hexane and benzene (50:50) extract	6.66 (12.28)^{bc}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}
6.	Benzene and ethyl acetate (50:50) extract	3.33 (6.14)^{c}	0.00 (0.00)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}
7.	Ethyl acetate and acetone (50:50) extract	16.66 (23.85)^{b}	20.0 (26.57)^{b}	13.33 (21.14)^{b}	10.00 (18.43)^{b}	13.33 (21.14)^{b}
8.	Untreated check	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
#9-15.	Solvent controls	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
CD (0.05)		17.11	13.50	14.21	13.86	12.98

*Mean of three replications

Values in parentheses one arc sine transformed values

Values with different alphabets differ significantly

It includes solvent controls such as (T9-T15) n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50) and ethyl acetate and acetone (50:50)

Table – 2: Efficacy of fruit extracts (Cold extract method) of *D. stramonium* L. plants on fourth instar of *S. litura* – Poison food bioassay

T. No	Treatment	*Per cent mortality at 1% concentration				
		Leaf extract	Flower extract	Stem extract	Fruit extract	Whole plant extract
1.	n-hexane extract	0.00 (0.00)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
2.	Benzene extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
3.	Ethyl acetate extract	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
4.	Acetone extract	93.33 (81.14)^{a}	100 (90.0)^{a}	100 (90.00)^{a}	93.33 (81.43)^{a}	93.33 (81.14)^{a}
5.	n-hexane and benzene (50:50) extract	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}
6.	Benzene and ethyl acetate (50:50) extract	3.33(6.14)^{c}	3.33 (6.14)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	3.33 (6.14)^{c}
7.	Ethyl acetate and acetone (50:50) extract	20.10 (26.07)^{b}	16.66 (25.85)^{b}	10.00 (18.43)^{b}	10.00 (18.43)^{b}	16.66 (23.85)^{b}
8.	Untreated check	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
#9-15.	Solvent controls	0.00(0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}
CD (0.05)		17.11	0.00 (0.00)^{c}	3.33 (6.14)^{c}	0.00 (0.00)^{c}	0.00 (0.00)^{c}

*Mean of three replications

Values in parentheses one arc sine transformed values

Values with different alphabets differ significantly

It includes solvent controls such as (T9-T15) n-hexane, benzene, ethyl acetate, acetone, n-hexane and benzene (50:50), benzene and ethyl acetate (50:50) and ethyl acetate and acetone (50:50)

Table - 3: Efficacy of fruit extracts (aqueous method) of *D. stramonium* plant on fourth instar of *S. litura* – Poison food bioassay

T. No	Treatment	*Per cent mortality at 1% concentration
1.	Leaf extract	100.00 (90.00)[a]
2.	Flower extract	93.33 (81.14)[a]
3.	Stem extract	93.33 (81.14)[a]
4.	Fruit extract	100.00 (90.00)[a]
5.	Whole plant extract	93.33 (81.14)[a]
6.	Untreated check	0.00 (0.00)[c]
CD (0.05)		14.71

*Mean of three replications
Values in parentheses one arc sine transformed values
Values with different alphabets differ significantly

Statistical analysis

Allthe percentage data were subjected to arc - sine transformation and whole numbers was log transformed. Lethal concentrations were worked out by using Probit analysis. Analysis was done with ANOVA and the means were compared by following Duncan's multiple range test (DMRT) at p = 0.05 (Gomez and Gomez, 1984).

3. Results

Screening against *S. litura*

Poison food bioassay (Leaf disc bioassay)

Soxhlet extract

Acetone extracts of *D. stramonium* L. plants stem extract 3, fruit extract - 4 and whole plant extract -5 recorded 93.33 % mortality against fourth instar *S. litura* at 1 % concentration. This was followed by acetone extracts of accessions Leaf extract - 1 and Flower - 2, where 86.66 % mortality was observed. Among the combinations of solvents used for extraction, ethyl acetate and acetone combination at 50:50 showed better performance than control. The mortality range falls between 10 to 20 % and 3.33 to 10.00 in Plants respectively in the above combination. Untreated check also recorded no mortality in any of the replications was observed (Table - 1). In all the solvent, control zero per cent mortality was noted.

Cold extract

The accessions extracted by following cold solvent method showed a different result in exerting mortality. Acetone extracts (T4) recorded the maximum per cent mortality among all the treatments and within the treatment the accessions flower dust extract - 2 and Stem - 3 reported cent percentage morality. The other plants extract such as leaf - 1, fruit - 4 and whole plant - 5 recorded 93.33 % mortality. Other solvents used in extraction of fruits did not record considerable mortality (Table - 2).

Aqueous extract

Regarding the aqueous extract of *D. stramonium* L. plants 1, 4 and 6 reported 100 per cent mortality against *S. litura* and the accessions 2, 3 and 5 recorded 93.33 % mortality. There was no mortality in untreated check (Table - 3).

4. Discussion

Perusal of data on bioassays against larvae of *S. litura* indicated that Fruit extract showed good insecticidal property. All the three plant materials exerted more than 86 % mortality and even few registered 100 % mortality. Leaf extract and stem extract recorded low insecticidal properties (46 % to 66 %) when compared with fruit and whole plant. When comparing the extracts obtained by following Soxhlet, Cold and aqueous extraction methods, the results conveyed that cold extraction method was superior to soxhlet method and equal to aqueous extraction method. It was well established fact that different climatic, soil and seasonal conditions influence the quantity of the secondary metabolites of plants as shown by Menut *et al.* (1993). Similarly, rhizomes of *Acorus calamus* L., which was widely distributed in Asia, North America and Europe, contain β-a sarone a toxic and sterilizing chemical. However, β- a sarone content of plants growing in Asia was higher (70 – 96 %) in contrast to the plants growing in North America and Europe (15 %) (Schmidt and Streloke, 1994). Arivudainambi (2001) found that the anti-insect properties of stilt root extracts of *Rhizophora* spp. was not static

and were greatly influenced by seasonal conditions. The stilt root collected during summer caused higher anti-insect properties than winter collected. Our findings in relation to extraction procedure is in accordance with the statement of Jaglan *et al.* (1997) who reported that *Azadirachta indica, A. juss, Melia azedirach, A. Juss* and *Lantana camara* extracted by following cold extract method was effective than Soxhlet method.

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