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Dodonaea angustifolia – a potential biopesticide against *Helicoverpa armigera*

Dodonaea, a common hop bush and a perennial shrub belonging to Sapindaceae is known for its folk remedies. It is distributed from the coast to 2000 m elevated terrain. It is the most aggressive colonizer on disturbed ground, even in rocky gravel or limestone. *D. angustifolia* L.f., predominantly present in India, has a wide range of therapeutic applications since ancient times against pneumonia and other pulmonary diseases including tuberculosis. A decoction of the plant or the wood is used as a purgative in fever and the young twigs are used as tonic[1].

Tribals of Kolli Hills, Tamil Nadu, use *Dodonaea* as green manure for rice crop along with other plants and have found that the crops show resistance to many pests. Besides, it is observed that *Dodonaea* by itself is free from any pests with the presence of an array of secondary metabolites like catechol, tannins, quinines, saponins, flavones, alkaloids, terpenoids, resins, diterpenoids, phenols, coumarins and essential oil[2].

Helicoverpa armigera, a lepidopteran, commonly known as American boll worm, afflicts nearly 67 cultivated species; the most affected ones are cotton, bhendi (okra), gram species, sunflower and tomato. Of the various strategies adopted to control this pest, no single treatment was successful. Perhaps, repeated application of synthetic pesticides developed resistance in these pests. Loss of control due to the development of multi-resistant strains has been reported in many crops[3].

The environmental hazards posed by synthetic pesticides provide an impetus for investigations into some ecofriendly and biorational alternatives. A critical literature survey reveals that *Dodonaea* has not been studied for its pesticidal character, except against cotton leaf worm, *Spodoptera littoralis*[4]. Hence, we aimed to explore the possible biopesticidal activity of this plant to combat the devastating pest *H. armigera*.

The leaves of *D. angustifolia* from Kolli Hills, Tamil Nadu were collected, shade-dried and powdered. Then they were extracted successively using non-polar to polar solvents, viz. hexane, petroleum ether, chloroform and acetone. Aqueous extraction was also done. The plant material was soaked in each solvent for 24 h at 30 ± 2°C, filtered, and to the residue the same solvent was added. This procedure was repeated thrice to obtain maximum extractables. All the filtrates were pooled and evaporated under vacuum in a rotary evaporator[5]. The crude extracts were weighed to measure the yield and then used in desired concentrations for bioassay.

H. armigera larvae collected from redgram field were reared in semi-synthetic diet[6]. Each extract at 10% conc was fed to the adult moths and the longevity, fecundity and hatchability were checked. Solvent control (10%) and 10% sugar solution (normal control) were also maintained. Five pairs of adults were released into the mud pot and kept in the dark. Adult feed was changed daily and the whole set-up was maintained at 22 ± 2°C with 70–75% RH. Triplicates were maintained for each treatment and the data were analysed statistically using Agres package.

Crude concentrate (40 g) of hexane extract was dissolved in hexane (30 ml) and fractionated through a silica-gel column, using solvent combinations of hexane/ethyl acetate, benzene/acetone and petroleum ether/acetone. Totally, 12 fractions were obtained. The eluted fractions were tested against adult moths as mentioned earlier, with their respective controls.

The powdered material of hexane extract (10 g) was eluted in a silica gel column (5 cm × 50 cm), with different solvent combinations, further purified by preparative TLC (on silica gel 60 F_{254} gel-coated glass sheets). The purified fraction was subjected to reversed-phase HPLC (on bondapak column with flow rate of 1.5 ml/min and pressure up to 300 psi) using MeOH-H_2O (9 : 1) as elutent to obtain the pure sample. The single fraction which eluted at 2.9 min was evaporated to dryness and subjected to FTIR (Fourier Transform Infrared). Infra-red spectral data were measured on Perkin-Elmer 1600 series FTIR Spectrometer (Nujol, KBr disks). To determine the molecular weight, the sample was subjected to EIMS (Electro Impact Mass Spectrometry) and ESIMS (Electro

Spray Ionization quadrupole Mass Spectrometry) Finnigan LCQ MS Detector. Source conditions were set as follows: Voltage 5 kV, nitrogen sheath gas pressure 60 psi, heated capillary temperature 200°C, full scan 50 to 2000 m/z. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker 300 MHz instrument using TMS (tetra methyl silane) as the internal standard. Using the spectral data of FTIR, EIMS, ESIMS, ^{1}H-NMR and ^{13}C-NMR, the compound responsible for insecticidal activity was identified.

Among the various extracts tested, petroleum ether and chloroform extracts significantly reduced the adult longevity for three days and adversely affected the reproductive potential, compared to their respective solvents. Despite induction of fecundity by all the solvents (except for hexane), no hatchability was observed (Table 1). However, when treated with plant extracts, except for hexane extract, all other treatments showed reduction in fecundity/hatchability. This might be due to impairment of gonotrophic cycle of adults by the secondary metabolites[7] present in the members of Sapindaceae[8].

It is observed that the hexane extract alone stimulated the fecundity of adults (970 eggs), higher than that of the normal control (901). However, the per cent hatchability was low (Table 1). Similar results have been reported with wild tomato against *Helicoverpa zea*. A compound (+)-(*E*)-endo-beta-bergamoten-12-oic acid, a sesquiterpene acid is responsible for this activity[9].

Among the four extracts, the hexane extract stimulated egg-laying compared to control. However, the hatchability, growth and development of the resultant progenies were adversely affected. The crude hexane extract was fractionated into 12 fractions and tested against the adult moths for their longevity, fecundity and egg hatchability along with their respective controls. The results are represented in the Figure 1 *a–c*.

When the 12 fractions of crude hexane extract were tested against the adult moths, T12 drastically reduced egg laying to two numbers in nine days of longevity, which was followed by fractions T2 and T11 with 8 and 13 eggs from 11 and 10 days of longevity respectively. In contrast, T9 and T6 (100% acetone and 100% hexane) stimulated the fecundity with 262 eggs in 13 days of longevity in the former and 133 eggs in 14 days of longevity with the latter.

Table 1. Bioefficacy of *D. angustifolia* in combating *H. armigera*

Treatment		Character		
		Adult longevity (days)	Egg (numbers)	Hatchability (%)
Dodonaea extract with	Water*	8 (10)	395 (901)	27 (86)
	Hexane	10 (5)	970 (17)	38 (0)
	Petroleum ether	3 (14)	0 (1219)	0 (0)
	Chloroform	3 (13)	0 (740)	0 (0)
	Acetone	8 (13)	236 (1510)	36 (0)
CD ($P = 0.05$)		3.1	228	6.3

Mean of triplicate with five pairs each; Values in parentheses indicate its respective solvent control; *Control is honey.

Table 2. Bioefficacy of secondary metabolites of hexane extract of *D. angustifolia* in combating *H. armigera*

Treatment (fraction)	Character		
	Adult longevity (days)	Egg (numbers)	Hatchability (%)
T13	11 (8)	26 (11)	0 (1)
T14	14 (9)	38 (38)	25 (20)
T15	10 (6)	35 (157)	0 (2)
T16	10 (6)	8 (8)	0 (0)
T17	8 (6)	0 (30)	0 (8)
T18	10 (7)	112 (261)	12 (19)
T19	9 (5)	323 (76)	20 (10)
Control	16	515	80
CD ($P = 0.05$)	8	301.5	35.4

Mean of triplicate with five pairs each; Values in parentheses indicate its respective solvent control. T13, 80 : 20 ethyl acetate: hexane; T14, 50 : 50 benzene: acetone; T15, 20 : 80 benzene: acetone; T16, 100 hexane; T17, 100 petroleum ether; T18, 80 : 20 petroleum ether: acetone; T19, 100 acetone.

Though fraction T9 recorded maximum eggs compared to other fractions, the per cent hatchability was only 4.93. While most of the fractions recorded zero hatchability, fractions T8 and T7 showed 33.89 and 22.64% hatchability.

As the hexane fraction (100%) T6 stimulated the fecundity and totally arrested the hatchability, it was further fractionated to seven samples labelled T 13 to T 19. Similar analyses were done as earlier and the results are depicted in Table 2.

Among the secondary metabolites tested, T17 (100% petroleum ether fraction) recorded zero egg-laying followed by eight eggs in T16 (100% hexane fraction) in their total longevity period of 10 and 8 days respectively. Thus, 100% hexane fraction was further purified and the active principle involved in the insecticidal activity was identified.

The preparative TLC (on silica 60 F_{254} gel-coated glass sheets) revealed a single spot under UV light (254 nm) and also in daylight, when sprayed with anisaldehyde/sulphuric acid, vanillin/sulphuric acid. The main peak of the retention time, 2.9 min, was collected and subjected to an UV detector setting at 254 nm in HPLC with reference. The infrared spectrum showed 1736 cm^{-1} (conjugated lactone carbonyl), 3416 cm^{-1} (hydroxyl group) and 1645 cm^{-1} (double bond in the pentane ring). EIMS and ESIMS showed the molecular ion $[M]^+$ 576 and the fragment ions $[M\text{-}CH_3]^+$ 561 and $[M\text{-}COCH_3]^+$ 533.

The molecular structure of the constituent responsible for insecticidal activity, using the data of ^{1}H-NMR (Figure 2, with chemical shifts in terms of ppm) and ^{13}C-NMR was identified as cardiac glycoside, oleandrin ($C_{32}H_{48}0_9$). The data obtained were similar to those reported by Wang *et al.*[10]. Earlier work had specifically shown that coumarins and triterpenes from different plants act as good insect repellents[7,11]. Oleandrin is reported in *D. angustifolia* and due to the presence of the insecticidal property and its wide distribution, it could be used as a botanical pesticide after exploring its toxicity and field trials.

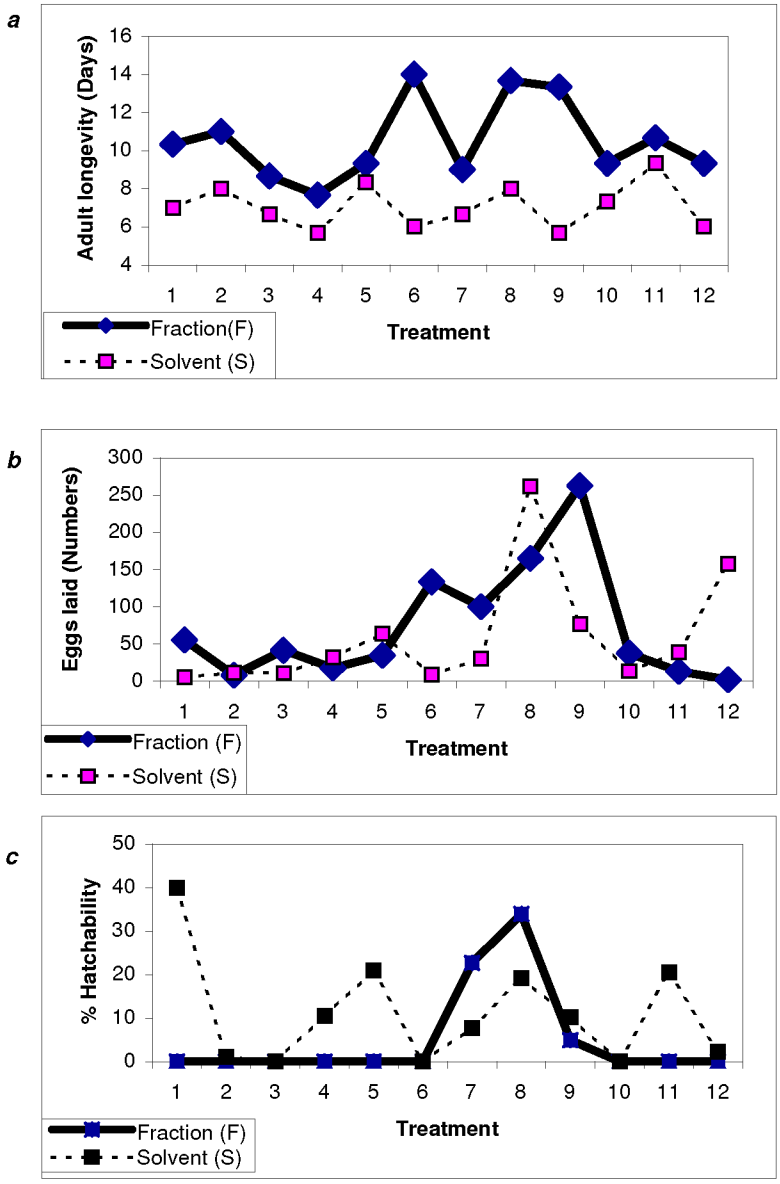

Figure 1 ***a–c.*** Bioefficacy of fractions of hexane extract of *D. angustifolia* in combating *H. armigera*. Mean of triplicate with five pairs each. T1, 100 EA; T2, 80 : 20 EA: hexane; T3, 60 : 40 EA: hexane; T4, 40 : 60 EA: hexane; T5, 20 : 80 EA: hexane; T6, 100 hexane; T7, 100 PE; T8, 80 : 20 PE: acetone; T9, 100 acetone; T10, 40 : 60 PE: acetone; T11, 50 : 50 benzene: acetone; T12, 20 : 80 benzene: acetone; EA, Ethyl acetate; PE, Petroleum ether.

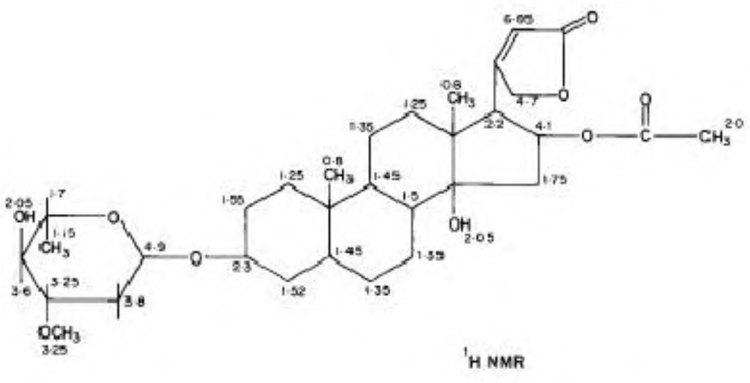

Figure 2. Structure of oleandrin (^{1}H NMR).

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