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Antifeedant activity of some botanicals against *Spodoptera frugiperda* (JE Smith) under laboratory condition

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Abstract
The present experiment was conducted at toxicology laboratory of Entomology, Dr. PDKV, Akola, during kharif 2019-20, to find out the "Antifeedant activity of some botanical extracts against *Spodoptera frugiperda* under laboratory condition in Factorial Completely Randomized Design. The results revealed that amongst different botanical extracts 1% neem seed kernel extract followed by 1% lantana leaf extract and amongst different solvents used to extract the solvent ethyl acetate followed by hexane were observed with the highest antifeedant activity. In interaction study 1% neem seed kernel extract by using ethyl acetate recorded highest antifeedant activity against *S. frugiperda* in their third instar larval population and found at par with 1% neem seed kernel extract by using methanol and 1% lantana leaf extract by using ethyl acetate at 48 hours followed by 72 and 24 hours after treatment. A novel and cheap botanical extract against *S. frugiperda* is thus developed in this study.

Keywords: *Spodoptera frugiperda*, botanical extracts, different solvents, factorial completely randomized design, bioassay, antifeedant activity

Introduction
Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is native to tropical and subtropical regions of the America and is the key insect pest of maize in tropical regions. The occurrence of FAW was reported in Africa for the first time in late 2016 in West Africa (Goergen *et al.*, 2016; Abrahams *et al*., 2017) [7, 1]. Subsequently, FAW has confirmed in 44 African countries (Rwomushana *et al*., 2018) [14]. It consists of two genetically differentiated strains. They are commonly referred as the rice-strain (R-strain) and the corn-strain (C-strain) (Nagoshi and Meagher, 2004). FAW is a highly polyphagous insect pest that attacks more than 80 plant species, including maize, sorghum, millet, sugarcane, vegetable crops (Prasanna *et al*., 2018) [13] nevertheless, maize is the main crop affected by FAW in Africa. Given the importance of maize in Africa as a primary staple food crop, the recent invasion of FAW threatens the food security of millions of people in a region that will likely have an aggravated drought due to climate change in SSA (Rwomushana *et al.*, 2018; Prasanna *et al*., 2018) [14, 13]. According to recent estimates, in the absence of control methods, FAW has the potential to cause losses of 8.3 to 20.6 m tons of maize per annum, in just 12 maize producing countries. This represents a range of 21-53 per cent of the annual average production of the maize over a three year periods of these countries (Abrahams *et al.*, 2017) [1]. In India, *S. frugiperda* was reported in the month of May 2018 on maize for the first time from Karnataka (Sharanabasappa *et al.*, 2018a) [15] and molecular diversity studies of *S. frugiperda* from different states of India indicated prevalence of R Strain (Mahadeva Swamy *et al*., 2018) [10]. During September 2018, the infestation of FAW was first time recorded in Vidarbha from Malegaon Gond village of Nandura taluka of district Buldana, where the maize crop was severely infested and as a, the 8 acre maize crop was pulled out (Anonymous, 2018) [3]. In the same month, its feeding on two months old sugarcane crop, variety (Co86032) was noticed at Ghogaon village of Sangli district (Maharashtra). Other than sugarcane, it was also reported on maize, sorghum and sweet corn in different districts of Maharashtra. Infestation on sugarcane was less than 5 per cent in Sangli, Kolhapur and Pune districts (Ankush *et al.*, 2019) [2].
The larvae cause damage to the plant by consuming foliage, young larvae mainly feed on epidermal leaf tissue and also make holes in leaves, which is the typical damage symptom of FAW. Feeding on young plants through the whorl causes dead heart. In older plants, the larger larvae in the whorls can feed on maize cob or kernels, reduces yield and quality

(Abrahams *et al.*, 2017; Prasanna *et al.*, 2018) [1, 13]. Gravid female was observed laying eggs with the fecundity of 1064 eggs. Incubation, total larval and pupal period were observed to be from 2-3, 14-19 and 9-12 days, respectively (Sharanabasappa *et al*., 2018b) [16]. The total life-cycle of male and female was observed to be 32-43 and 34-46 days, respectively. The number of generations occurring in an area varies with the appearance of the dispersing adults.

Botanical extracts have long been proposed as attractive alternatives to synthetic insecticides for pest management. Botanical extracts are eco-friendly, economical, usually target-specific, and biodegradable. The greatest strength of botanical extracts is their specificity, as most are essentially nontoxic and non-pathogenic to animal and humans (Miresmailli and Isman, 2014) [12] and (Stevenson *et al.*, 2017) [20]. Various plant species shows insecticidal properties against FAW, for example extracts of neem, *Azadirachta indica* (A. Juss.) and tulsi, *Ocimum sanctum* Linn. (Silva *et al*., 2015) [18]. In addition to their medicinal uses these have shown biological activities against insects. The *Tagetes* genus (Asterales: Asteraceae) is an alternative candidate for the control of pests and diseases, because it contains secondary metabolites. As sources of pesticides, *Tagetes foetidissima* DC. and *Tagetes coronopifolia* wild are promising . *Lantana camara* plants reported to have insecticidal, antiovipositional, feeding deterrent, growth inhibition and toxic effects on pest insects in the land farm (Barreto *et al*., 2010) [4]. Thus, in the recent years, research on plant bio-insecticides for controlling the fall army worm has been renewed, and at present there is very little information available on antifeedant activity of botanical extracts against FAW. Taking into account the need, an experiment was framed to know the antifeedant activity of locally and cheaply available four botanical extracts against fall army worm *S. frugiperda.*

Materials And Methods

The present experiment was laid out in Factorial Completely Randomized Design during the year 2019-20 in toxicology laboratory of Entomology section, post graduate institute, Dr. Panjabrao Deshmukh Krishi Vidhyapeeth, Akola, with the objectives to know the effect of botanical extracts, effect of different solvents used for extraction and cumulative effect of botanical extracts using different solvents on antifeedant activity against third instar larvae of *S. frugiperda.* It consist of different botanicals (E_1- Neem Seed Kernel Extract, E_2 - Marigold Leaf Extract, E_3 - Lantana Leaf Extract and E_4 - Tulsi Leaf Extract) as main treatments (Factor A) and different solvents (S_1- Hexane, S_2- Diethyl ether, S_3- Dichloro methane, S_4- Ethyl acetate, S_5- Methanol) as sub treatments (Factor B).

For mass production of *S. frugiperda* larvae rearing technique prescribed by Sharanabasappa *et al*., (2018b) [16] were followed with proper laboratory conditions (26±2 ºC, 75±5% RH and photoperiod of 12:12 light and dark hours). The initial culture of *S. frugiperda* was initiated by collecting from untreated maize fields of Dr. PDKV, Akola campus. The larvae were reared on fresh maize leaf bits as larval food in plastic containers (21 cm dia. and 24 cm height) until pupation. The pupae formed were separated and kept for adult emergence. The newly emerged male and female moths were placed in domestically prepared oviposition cages for egg laying. They were provided with 10 per cent honey water solution on cotton swabs kept in petridish and placed at the bottom of cage to serve as food. The oviposition cages were covered with black cloth and tightened with rubber band to provide sufficient darkness required for mating and oviposition by the females. A piece of cotton cloth / wool cotton was kept in the oviposition chamber as egg laying substratum. The eggs laid on substratum were removed daily. The newly hatched larvae were reared in plastic boxes (21 cm diameter and 24 cm height) on maize leaf bits and periodically replaced with fresh cuts. The third instar larvae from the culture were used for bioassay studies.

For preparation of botanical extracts locally available botanicals viz., (Seeds of *Azadirachta indica*, leaves of *Tagetes erecta, Lantana camara* and *Ocimum sanctum*) were collected from Dr. PDKV, Akola campus. The leaves were washed to remove dust, shade dried in laboratory and were individually ground to a fine powder. Each powdered botanicals were sieved using a strainer. Each powdered botanicals 200g were separately soaked in 600ml of each of the solvents viz., hexane, diethyl ether, dichloro methane, ethyl acetate and methanol Chinnamani and Jeyasankar (2018) [5] and allowed to stand at room temperature for a period of 24 hours each and then strained through muslin cloth and filtered through Whatsman No. 1 filter paper. The filtered content was then subjected to rotary vaccum evaporator until solvents were completely evaporated to get the solidified crude extracts. The crude extracts thus obtained were stored in sterilized amber colour bottles maintained at 4 ºC in refrigerator. Standard one per cent (1%) (10,000 ppm) stock solution was prepared by dissolving 1000mg of crude extract in 100 ml of acetone and used for bioassay studies.

Leaf discs (4cm dia.) of maize (*Zea mays*) were used for bioassay tests, after washing with tap water. The leaf discs were sprayed with one per cent (1%) concentration of each botanical (crude) extracts of different solvents for twenty seconds, air dried at room temperature and kept in petri plates (9 cm dia.). The single pre-starved (24 h) larvae was placed on the treated leaf discs in petri dishes and allowed to feed for 24, 48 and 72 hours. For each treatment, ten replicates with one control (acetone) were also maintained. At the end of the experiment, the uneaten area of leaf discs in 24, 48 and 72 hours were measured by using leaf area meter or graph paper. The per cent antifeedant activity was calculated based on the formula of Singh and Pant (1980) [19] and the data was subjected to analysis of variance in factorial complete randomized design to study the effect of individual factors and its interaction. The data collected on antifeedant activity were subjected to the statistical analysis, for the test of significance after appropriate transformations.

$$\text{Per cent Antifeedant Activity} = \frac{\text{(Leaf disc consumed by the larvae in the control) - (Leaf disc consumed by the larvae in the treated)}}{\text{(Leaf disc consumed by the larvae in the control) + (Leaf disc consumed by the larvae in the treated)}} \text{ X } 100$$

Results and Discussion

The results in (Table1) indicated that among interaction effect highest per cent antifeedant activity against third instar larvae were recorded in 1% neem seed kernel extract by using ethyl acetate solvent at 48 hours followed by 72 and 24 hours of observation (75.17 to 60.95 per cent) with minimum leaf area consumed (0.45 to 0.5 cm^2) as compared to control (1.3 to 2.38 cm^2) and found at par with 1% neem seed kernel by using methanol solvent and 1% lantana leaf extract by using ethyl acetate solvent and lowest was recorded in 1% marigold leaf extract by using dichloro methane solvent (17.99 to 34.59 per cent). The highest per cent antifeedant activity among botanical extract was recorded in 1% neem seed kernel extract at 48 hours followed by 72 and 24 hours (69.23 to 56.43 per cent) followed by 1% lantana leaf extract and 1% tulsi leaf extract and lowest was recorded in 1% marigold leaf extract (44.28 to 48.84 per cent). Among solvents used for extraction the highest antifeedant activity was recorded in ethyl acetate at 48 hours followed by 72 and 24 hours of observation (66.70 to 55.73 per cent) followed by hexane, methanol and diethyl ether and lowest was recorded in dichloro methane (42.25 to 46.98 per cent).

The present results are in accordance with the findings of Joshi and Ramprasad (1975) [8] who showed that the neem seed kernel suspension in water at 3 per cent was found effective as an antifeedant against all the five instars of *S. litura*. The average leaf area consumed by larvae of each instar was significantly lower than that of control. Further, Kubo and Klocke (1982) [9] demonstrated that azadirachtin isolated from neem (*A. indica*) produced antifeeedant effect on *H. Zea* and *S. frugiperda*. Sharma *et al.*, (1983) [17] noticed antifeedant effect of neem extracts *viz*., solid fraction, petroleum ether extract, alcoholic extract both from shade dried neem seeds at 0.1 per cent conc. against third instar larvae of *Mythimna separata* (armyworm). EI-Sayed (1985) [6] found that the amount of untreated food consumed by the fourth instar larvae of *S. littoralis* was about 7.7 times that of food treated with one per cent suspension of neem seed. Mikolajczak and Reed (1987) [11] reported that feeding inhibition and mortality produced by hexane and ethanol extracts of seeds from 22 species of plants of family meliaceae were comparable to, and in some cases slightly higher than the effects of neem seed extracts when tested against larvae of *S. frugiperda.* The present findings are in line with the literature reviewed, however, as regards *S. frugiperda* and different dosages, days after exposure etc. cannot be compared due to limited literature available in this aspect.

Table 1: Effect of botanical extracts using different solvents on antifeedant activity against third instar larvae of *S. frugiperda* at 24, 48 and 72 hours after treatment.

Botanical extracts	24 HAT						48 HAT						72 HAT					
	Solvents					Mean Factor A	Solvents					Mean Factor A	Solvents					Mean Factor A
	Hexane S_1	Diethyl ether S_2	Dichloro methane S_3	Ethyl acetate S_4	Methanol S_5		Hexane S_1	Diethyl ether S_2	Dichloro methane S_3	Ethyl acetate S_4	Methanol S_5		Hexane S_1	Diethyl ether S_2	Dichloro methane S_3	Ethyl acetate S_4	Methanol S_5	
	Mean Interaction (A X B)						Mean Interaction (A X B)						Mean Interaction (A X B)					
E_1 (NSKE)	50.56 (45.34)	55.42 (48.14)	56.42 (48.72)	60.95 (51.37)	58.8 (50.10)	56.43 (48.73)	63.6 (53.15)	65.97 (54.40)	68.12 (55.67)	75.17 (60.38)	73.31 (58.94)	69.23 (56.51)	62 (52.03)	62.41 (52.35)	63.78 (53.07)	73.35 (59.00)	65.65 (54.37)	65.43 (54.16)
E_2 (MLE)	56.42 (48.72)	55.42 (48.14)	23.35 (28.63)	55.9 (48.44)	30.33 (33.24)	44.28 (41.43)	68.12 (55.67)	65.97 (54.40)	17.99 (24.72)	66.42 (54.79)	24.89 (29.69)	48.67 (43.85)	63.78 (53.07)	62.41 (52.35)	34.59 (35.94)	43.46 (41.19)	39.97 (39.04)	48.84 (44.32)
E_3 (LLE)	55.42 (48.14)	41.95 (43.95)	47.28 (43.95)	58.8 (50.10)	49.42 (44.64)	50.57 (46.16)	64.97 (53.93)	49.9 (44.96)	51.91 (46.09)	73.31 (58.94)	58.58 (49.98)	59.73 (50.78)	62.41 (52.35)	42.13 (40.36)	43.46 (41.19)	65.65 (54.37)	52.26 (46.31)	53.18 (46.91)
E_4 (TLE)	38.83 (38.32)	38.83 (37.64)	41.95 (43.95)	47.28 (43.95)	55.9 (48.44)	44.55 (42.46)	46.79 (43.10)	46.79 (43.10)	49.9 (44.96)	51.91 (46.09)	66.42 (54.79)	52.36 (46.41)	43.46 (41.19)	41.68 (40.10)	42.13 (40.36)	63.49 (52.99)	63.49 (52.99)	50.85 (45.53)
Mean Factor B	50.30 (45.13)	47.90 (44.47)	42.25 (41.31)	55.73 (48.46)	48.61 (44.11)		60.87 (51.46)	57.15 (49.22)	46.98 (42.86)	66.70 (55.05)	55.8 (48.35)		57.91 (49.66)	52.15 (46.29)	45.99 (42.64)	61.48 (51.89)	55.34 (48.18)	
	Factor (A)		Factor (B)		Factor (A X B)		Factor (A)		Factor (B)		Factor (A X B)		Factor (A)		Factor (B)		Factor (A X B)	
"F" Test	Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.		Sig.	
SE ± (m)	0.89		1		2		0.91		1.01		2.03		0.85		0.95		1.90	
CD at 5%	2.50		2.79		5.58		2.54		2.84		5.67		2.37		2.65		5.31	

Note: Figures in parentheses are corresponding angular transformation.
HAT-hours after treatments

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