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In vitro bioassay of certain botanical oils for their efficacy against maize fall army worm (J.E. Smith) *Spodoptera frugiperda* (Noctuidae: Lepidoptera)

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Abstract
Maize, *Zea mays* is one of the world's main agricultural crops and *Spodoptera frugiperda* (J. E. Smith) is the most devasting invasive pest. This study evaluates the effect of plant oils on the third instar larvae of *Spodoptera frugiperda*. The experimental design was completely randomized in a 6 x 3 factorial combination (clove oil 1%, lemon grass oil 1%, basil oil 1%, rosemary oil 1%) along with control consisting of acetone 100 per cent and an insecticide spinosad 0.03 per cent with three replicates per treatment. The bioassay has been conducted by allowing the larvae to fed on maize leaves treated with essential oils and a standard check. The mortality was observed after 12, 24 and 36 hrs of application. The leaf dipping method demonstrated that clove oil (1%) showed highest mean mortality of 77.77 per cent which is the most effective among the plant oils tested next to spinosad 45 SC (0.03%) which caused 97.77 per cent mortality. The results of this experiment suggested that, clove oil was the most promising essential oil to control *Spodoptera frugiperda* third instar larvae.

Keywords: Maize, *Spodoptera frugiperda*, essential oils, mortality

Introduction
Maize, *Zea mays* is the third most important cereal crop worldwide and having highest economic value in terms of production potential and nutritional value. It is one of the most consumed cereals by the mankind [12]. As per 2018-19 statistics, maize is the third largest food crop in India and its production was 27.23 MT. The *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is native to the tropical and sub-tropical regions of United States. It is considered as the most important maize pest in Brazil which is the third largest producer in the world next to USA and China [9]. Till 2015, this pest has not been reported in any other regions except America. In 2016, it was recorded in Africa causing serious damage in maize crop [8]. In late 2016 and 2017, it was recorded in Southern, Eastern and Northern parts of Africa [5]. In India, this pest was reported in maize for the first time in Chikkaballapur district of Karnataka during May 2018 [17]. The pest has also been reported in Bihar, Chattisgarh, Gujarat, Maharashtra, Odisha, Tamil Nadu, Telangana and West Bengal [9, 7].

The voracious fall armyworm *Spodoptera frugiperda* has marched across Africa in the last two years, causing millions of dollars loss to maize and other crops and threatening the food security and livelihoods of small holders and their families in nearly 40 African countries. The synthetic insecticides are normally preferred by the farmers to control fall army worm, which has the disadvantage of leaving residues in the food, environment and also creates resistance in pest populations. Hence, botanical pesticides have long been proposed as alternatives to synthetic insecticides for pest management [20]. They are eco-friendly, economical, target-specific, compatable with other low risk pesticides and easily biodegradable [13, 19, 11, 15]. The botanical oils are called as essences, volatile oils, etheric oils which have been identified in aromatic plants as a mixture of volatile components produced as secondary metabolites [1]. The highest plant families bearing essential oils are Apiaceae, Asteraceae, Combretaceae, Geraniaceae, Gramineae, Lamiaceae, Myrtaceae, Meliaceae, Piperaceae, Rutaceae, Verbenaceae and Zingiberaceae [3].

In this context, the present study aims to evaluate and compare the effect of the essential oils from clove *Syzygium aromaticum* (myrtaceae), basil *Ocimum sanctum* L. (Lamiaceae), lemon grass *Cymbopogan citratus* (poaceae), rosemary *Rosmarinus officinalis* (*lamiaceae*) on the

third instar larvae of *S. frugiperda*, along with positive standard check.

Materials and Methods

Experimental site

The study was conducted at the Centre of innovation laboratory, Department of Entomology, Agriculture College and Research Institute, Madurai.

Plant oils and insecticide

The tested essential plant oils were Clove, *Syzygium aromaticum* (Myrtaceae), Basil, *Ocimum sanctum*. L. (Lamiaceae), Lemon grass, *Cymbopogan citratus* (Poaceae), Rosemary *Rosmarinus officinalis* (Lamiaceae**)** on the third instar larvae of *S. frugiperda*, along with a recommended insecticide spinosad and control (Plate. 1)

Plate 1: Essential Oils Used in Bio- assay

Insect rearing and maintenance

Insect rearing was done by collecting *S. frugiperda* egg mass from the maize field in AC&RI Madurai. After the emergence of 1st instar larvae they were fed with fresh leaves of maize collected from the field and larvae were allowed to grow upto 2nd instar and later they were transferred to the multicavity trays to avoid cannibalism within the larvae.

Bioassay

Two hours starved third instar larvae were selected to determine the toxicity of the essential oils against *S. frugiperda*. Freshly collected young maize leaves were treated with oils by using leaf dipping method. The leaves were dipped in 1percent oils prepared by diluting with acetone as a solvent and a synthetic insecticide spinosad (0.03%) as a treated check and for the control the treatment material was substituted by acetone. Later the dipped leaves were air dried and placed in the petri plates with a wet filter paper and the larvae were released.

The three replications were maintained with 15 larvae per replication. A completely randomized design was followed. The caterpillars were allowed to expose essential oils, an insecticide and a control for 36 hours. Subsequently the caterpillar mortality rate was evaluated at 12, 24 and 36 hours after exposure. Insecticidal activity of essential oils and treated check was expressed as a percentage mortality of larvae.

Statistical analysis: The differences among the insecticidal activities of treatments tested were determined by the analysis of variance (ANOVA) test using AGRESS software. LSD tests were used for comparison of means.

Results and Discussion

Table 1: *In vitro* studies on the efficacy of certain plant derivatives against third instar larvae of *Spodoptera frugiperda* (J.E. Smith) (Noctuidae: Lepidoptera)

Treatments	Concentration	Mean Per cent Mortality			Grand Mean % mortality
		12 hrs	24 hrs	36 hrs	
T_1 - Clove Oil	1%	64.44 ± 8.01 (53.37)^b	82.22 ± 5.87 (65.78)^b	86.66 ± 3.84 (68.96)^b	77.77 (61.84)^b
T_2 - Lemon grass Oil	1%	31.10 ± 2.23 (33.86)^c	37.77 ± 2.22 (37.90)^d	51.11 ± 5.87 (45.63)^c	39.99 (39.20)^d
T_3 - Basil Oil	1%	35.55 ± 2.23 (36.58)^c	53.33 ± 5.02 (46.91)^c	79.99 ± 3.84 (63.63)^b	56.29 (48.59)^c
T_4 - Rosemary Oil	1%	15.55 ± 2.23 (23.12)^d	37.77 ± 2.22 (37.90)^d	57.77 ± 2.22 (49.47)^c	37.03 (37.46)^d
T_5 - Spinosad 45SC	0.03%	95.55 ± 2.23 (79.89)^a	97.77 ± 2.22 (84.75)^a	100 ± 0 (89.67)^a	97.77 (81.37)^a
T_6 -Untreated Check		0 (0.38)^e	0 (0.38)^e	0 (0.38)^d	0 (0)^e
SEd		4.24	4.04	3.27	3.28
CD (0.05)		9.24	8.81	7.13	7.16
CV (%)		13.69	10.85	7.58	8.91

Each value is the mean of three replications.
Figures in the parentheses are arcsine transformed values.
Mean values followed by the same alphabet in a column are not significantly different @ (P< 0.05)

Essential oils were toxic to *S. frugiperda* larvae at single concentration (1%) and the maximum mortality was recorded at 36 h. After 12 hours of treatment, spinosad 0.03 per cent caused highest mean mortality (79.89%) followed by clove oil (53.37%). Lemon grass oil (33.86%) and basil oil (36.58%) were statistically on par with each other. Rosemary oil showed the lowest mortality of 23.12 per cent (Table 1).

After 24 hours of treatment, spinosad 0.03 per cent caused highest mean mortality (84.75%) followed by clove oil (65.78%) and basil oil (46.91%). The minimum mortality percentage has been observed in lemon grass oil (37.90%) and rosemary oil (37.90%) and their results were statistically on par with each other (Table 1).

After 36 hours of treatment, highest mean mortality (89.67%)

was observed in spinosad 0.03 per cent followed by clove oil (68.96%) and basil oil (63.63%), they were statistically on par with each other. The minimum mortality percentage has been observed in lemon grass oil (45.63%) and rosemary oil (49.47%) and their results were statistically on par with each other (Table 1).

Among the essential oils tested, grand mean mortality showed that the clove oil caused highest mortality (77.77 per cent) next to positive standard check, spinosad 0.03 per cent (97.77 per cent mortality) followed by basil oil (56.29 per cent). The lemongrass oil (39.99%) and rosemary oil (37.03%) were caused lowest mortality compared to clove oil, basil oil and they were statistically on par with each other. No mortality has been observed in control (Fig. 1).

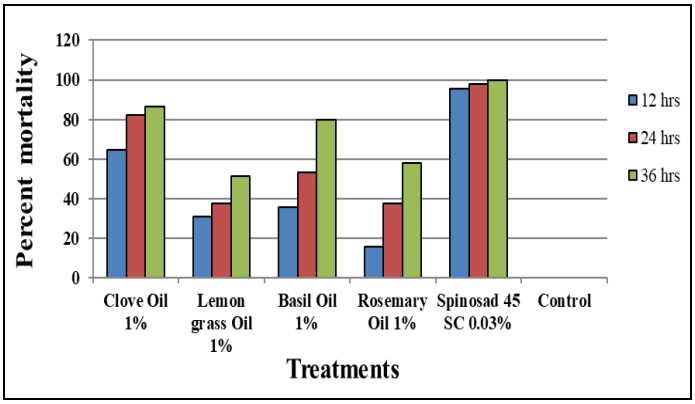

Fig 1: Bio-efficacy of certain oil formulations against Fall army worm, *Spodoptera*

***frugiperda* (J.E.Smith) (Noctuidae: Lepidoptera)**

Sousa *et al*. 2018 [18] concluded that the turmeric, clove and palmarosa plant oils posed inhibitory action on the feeding activity of first and second instar *S. frugiperda* caterpillars. Elumalai *et al.* 2010 [6] recorded the highest mortality of fourth instar *S. litura* larva treated with *Ocimum sp*. after 24 hrs of exposure. It is clearly evident that oils from camphor and clove were highly potent in their insecticidal activity against Bihar hairy caterpillar. Camphor oil was more effective at 2.0 and 2.5 µl caused 76 and 90 per cent mortality followed by clove oil caused 70 per cent terminal larval mortality at the highest dose of 2.5µl/ larva allowing 27 per cent of adult emergence [16]. The clove oil and its components had repellent and toxicity activity on the three important stored grain insect pest species, *R. dominica*, *S. oryzae* and *T. castaneum* [21]. Clove oil is generally more effective when delivered orally than as a contact insecticide to the noctuid caterpillars *Trichoplusia ni* and *Pseudaletia unipuncta*. The EC_{50} values for reduced growth of the two species of caterpillars were 400 ppm and 6,900 ppm, respectively [2]. Fourth instar larvae of *Spodoptera litura* when exposed to 1000 ppm concentration of *O. sanctum* oil caused 13.33 percent larval mortality [4]. Eugenol and its derivatives were the main constituents in the essential oil isolated from *Syzygium aromaticum*, it was reported as toxic to *Drosophila melanogaster* and *Spodoptera litura* [10]. Highest mortality (100%) was found in saunf and khas oil, followed by clove oil (93.33%) and lowest weight gain per larva 2 DAF (days after feeding) was seen in clove oil (-0.025g and -0.06g) at 1 and 2% [16].

Conclusion

The fall armyworm, *Spodoptera frugiperda* is a new invasive polyphagous pest in India. The tremendous use of synthetic insecticides would lead to the environmental contaminations, hence the study has been undertaken to control the pest with natural pesticides and thus we can harvest the residue free produces without polluting the ecosystem as a whole. In this study, clove oil was observed as a best treatment which contains major biologically active compounds namely Eugenol (60-90%), Eugenyl acetate (2-27%), β-caryophyllene (5-12%).

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