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RESEARCH ARTICLE

ANTIFEEDANT ACTIVITY OF VOLATILE OIL OF *TAGETES PATULA* AGAINST ARMYWORM, *SPODOPTERA LITURA* (FAB.) (LEPIDOPTERA: NOCTUIDAE)

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ABSTRACT

The present investigation revealed that the volatile oil obtained from *Tagetes patula* contained 10 compounds and they were tested against the fourth instar larvae of *Spodoptera litura* for their antifeedant activity by leaf disc bioassay. Among the compounds tested Terpinolene was the most effective feeding deterrent agent against *Spodoptera litura* in the laboratory condition. The mean area fed 100 ppm / cm^2 and 500 ppm/cm^2. The all compounds tested the antifeedant activity in both concentration appreciable value were recorded.

INTRODUCTION

Spodoptera litura (Lepidoptera: Noctuidae) is a major polyphagous pest, infects more than 180 plant species which includes various economically important crops such as cotton, groundnut, chilly, tobacco, caster, bendy and pulses etc. (Dhir *et al.,* 1992: Armes *et al.* 1997; Niranjankumar and Regupathy, 2001). Plant and insects have co-evolved over million of year, plant have accumulated specific secondary metabolites to counteract insect damage (Kanniyan, 2002). Worldwide attention now focuses towards alternative method of pest control, which is derived from naturally available resources. The practice of using plant derivatives or botanical insecticides as we now know them, in agriculture dates back at least two millennia in ancient China, Egypt, Greece, and India (Ware, 1883; Thacker, 2002). Natural chemical of plant origin, namely alkoloids (Bentley *et al.* 1984), terpenoids (Mordue & Blackwell 1993) and steroids (Bergamasco and Horn 1983) have been shown to have diverse biological effects on insects numerous plant species have been identified as possessing pesticidal properties and have shown potential as alternative to chemical pesticides (Ahmad *et al*. 1984; Singh 2000; Kaushik and Kathuria 2004). However, synthetic Insecticides led to numerous problems unforeseen at the time of their introduction: acute and chronic poisoning of applicators, form workers, consumer, fish, birds and other will life animals etc. (Wattanachari and Tintanon, 1999; National Research council, 2000; Rohani *et al*., 2001). The plant secondary metabolities that show feeding deterrent or toxic effect to insects in laboratory biology and botanical insecticides have been the subject of several recent volumes (Dev and Koul, 1997; Koul and Dhaliwal, 2001; Raghault-Roger *et al*., 2005; Elumalai *et al*., 2008; Pugazhvendan *et al.*, 2009). Plant essential oil have been subjected as alternative sources for insect control, because some selective, biodegradable to non-toxic products, and have few effects on non-target organism and the environment (Singh and Upadhyay, 1993; Isman, 2006; Pavela, 2007). Essential oils have also been documented to exhibits acute toxic effects against insects. Several experiments have been conducted on the Insecticidal properties of essential oil against various mosquitoes (Shalaby *et al*; 1998; Zaridah *et al* 2006; Knio *et al*., 2008). Plant of the family Myrtaceae, Owing to presence of essential oil and Tannins are subjected to great interest in this context (CSIR 1981; Roger and Hamraoui, 1995; Dales, 1996). Biological activities of plants belonging to the myrtacea against stored grain insets are well documented (Tunc *et al*. 2000; Lee Byungtlo *et al* 2001; Sharma *et al*; 2001). A comparison of the biological activity of commercially produce essential oils is therefore highly value in the narrow selection of suitable plant species development of suitable cultivation technology, extraction and subsequent formation of plant insecticides.

MATERIALS AND METHOD

Essential Oil

The fresh leaves of *Tagetes Patula* were collected from Ooty. Uthagamandalam Dist, Tamil Nadu, India and the essential oil was obtained by steam distillation method.In

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each case 20g of plant material was distiller in 300 ml of distiller water in a 500ml flask for 60 min the oil sample was store at 4°C until the commencement of the experimentation.

Test organism, *Spodoptera litura*

Third instar larvae of *spodoptera litura* were collected from Pitchavaram area and the stock culture was maintained on castor leaves, (*Ricinus Communis*) under cultivated conditions in a BOD incubator maintained at 27±1°C, 65-70% RH and 14:10 L/D photoperiod, the larvae were provided with fresh castor leaves during their development. During the last instar 15-20 larvae were transferred to the glass Jars and observed daily for moth emergence freshly emerged adult were kept separately and mated on the third day of emergence in a adult emergence cage (20x20x20 cm). Adult were fed on 10% honey solution and filter paper strips were provided for egg laying. Egg hatched in 3-5 days and neonates were reared on tender castor leaves. From this F1 generation third instar larvae of equal size and length were chosen for the present experiment.

(RI 1248, P0.4), Piperitone (RI 1458, P 6.1), Piperitenone (RI 1352, P 4.9), Geranyl acetate (RI 1458, P 0.4), Z-nerolidol (RI 1551, P 0.1), Spathulenol (RI 1578, P 0.5), Hepatadecane (RI 1702, P 0.1).

LEAF – DISC BIOASSAY

This boassay was designed to check the antifeedant activity of essential oil compounds. *S. litura* fourth instar larvae were starved for 4 hours prior to commencement of the experiments. Larvae were allowed to feed on 12.5 cm^2 castor leaf-disc dipped for 5 sec water based test solution, in a non – choice bioassay five replicates, with tolerance per replicate for each treatment after 4 hours. Leaves treated with distilled water was used as a reference control. The leaf area consumes by the larvae was recorded using the method of Lewis and Emden (1986).

DATA ANALYSIS

Antifeedant activity of compounds from essential oil (*T.patula*) against *spodoptera litura* fed on *Ricinus communis* was calculated for each insect as

$$AF = (1 - T/C) \times 100$$

Table 1. Antifeedant activity of ten compound from *tagetes patula* against the larvae of *spodoptera litura* fed on castor leaves

Compounds	Mean leaf area fed (cm^2)[a]		AF (%)[b]	Mean leaf area fed (cm^2)		AF %
	Control	1mg /cm^2		Control	5mg /cm^2	
Azadirachtin	0.00 ± 0.63	0.30±0.03^{de}	72	0.85±0.05	0.05±0.03^{c}	90
Myrcene	53.1±5.50	20.3±4.30^{c}	60	53.2±2.86	13.0±2.17^{d}	77
Limonene	0.74±0.02	0.34±6.02^{e}	67	0.88±0.04	0.13±0.03^{d}	87
Terpinolene	0.86±0.01	0.32±6.03^{de}	68	0.89±0.04	0.07±0.04^{cd}	89
Carvone	0.91±0.01	0.39±0.04^{ef}	60	0.91±0.13	0.19±0.04^{de}	81
Piperitone	53.0±1.97	19.8±2.28^{cd}	63	53.6±4.76	7.2±4.78^{cd}	87
Piperitenone	53.2±1.94	20.5±2.67^{d}	57	0.86±0.04	0.17±0.06^{de}	83
Geranyl acetate	52.6±1.34	21.6±3.05^{d}	55	53.5±2.38	13.6±2.61^{d}	75
Z-nerolidol	0.80±0.05	0.40±0.03^{c}	56	0.86±0.02	0.28±0.01^{d}	74
Spathulenol	0.82±0.03	0.43±0.02^{f}	53	53.4±2.63	13.3±3.33^{d}	76
Hepatadecane	0.88±0.01	0.37±0.04^{ef}	61	0.99±0.17	0.21±0.04^{c}	80

Values are mean ± S.E. (n=5); within each coloumn, data followed by the same letter (c-f) are not significant different; * P < 0.05, ANOVA.

Chemical analysis

GC-MS analyses were performed on a finnigan GCQ instrument with a zebron 2B-5 column (phenomenex, USA), 30mx0.25mm x 0.25µm, using the following temperature program; Initial temperature 60°C min^{-1} to 275°C and hold 5 min at this temperature. Temperature of the transfer line was 275°C, in source was 200°C. Linear velocity of the carrier gas (helium) was 40 cms^{-1} fall scan spectra in a range of relative mass m/z 50-450 Da was obtained.

TEST MATERIALS

The ten compounds were isolated from the *Tagetes patula* essential oil. Those compound were, Myrcene Retention indices (RI) 991, Percentage (P) 0.3, Limonene (RI 1031, P. 13.6), Terpinolene (RI 1057, P11.2), carvone

Where C denotes consumption of the control disc, and T the consumption of the treated disc. In order to calculate the concentration where 50% Antifeedant activity occurs probit analysis was performed using statistical software (SPSS version 9.0, 1999). The values obtained from the experiment were transformed using angular transformation and subject to analysis of variance (ANOVA).

RESULT AND DISCUSSION

Table 1. Clearly shown the antifeedant activities of ten compounds from *Tagetes Patula* against the fourth instar Larvae of *S. litura* fed on *Ricinus Communis* leaves. Though compounds are known to posses antifeedant activity against a variety of agricultural pests the result of the present study against *S. litura* was concentration

dependant. Terpinolene was the most effective 1mg/cm^2 (68%) and 5 mg/ cm^2 (89%), as it showed appreciable antifeedant activity of 1mg/cm^2 and 5mg /cm^2. *T. patula* oil is a complex mixture of several compounds. A total of 52 compounds constituting 77.5% of oil from herbage were identified. Synergistic data obtained from the larvae used for the antifeedant assay showed certain remarkable abnormalities like, larval-pupal intermediate, abnormal wing pattern in adult moths. The abnormalities in the metamorphosis might be due to imbalance in hormones (Karmegam *et al*., 1997) prolonged larval and pupal periods while using plant extracts have been reported by Saxena and Saxena (1992) and Daniel *et al*., (1995). Similar type of study had been already reported by several workers earlier (Wells,1993; Singh *et al* 2001). The dose will have to be standardized, depending on the geographical location and the pest insect species, but these results suggest that the use of botanical pesticide may help in reducing the environmental ill effects otherwise caused by the synthetic pesticides.

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