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Study on larvicidal activity of weed extracts against *Spodoptera litura*

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Abstract

Larvicidal activity of various concentrations of crude aqueous leaf extracts of *E. triplinerve* were studied against *S. litura* following the treatment on fourth instar larvae. The results of the total percent mortality (from fourth instar to adult) demonstrated that the mortality rate progressively increased (41.42, 58.57, 69.80, 84.49, 86.77, 90.55, 91.30, 90.54) as the extract concentrations increased (1, 2, 3, 5, 7.5, 12.5, 15, 17.5%). The maximum of 92 % total mortality was observed at 15 % concentration of the extract. It was significant to note that the percent mortality observed from 5 % onwards was more than 85 %. Results of adult emergence inhibition indicated that the EI_{50} of *E triplinerv*e was more effective at 4.07 %. Interestingly the calculated EI_{90} value was 14.10 %. This is an ideal eco-friendly approach for the control of agricultural pest, *S. litura*.

Key words

Eupatorium triplinerve, Larvicidal activity, *Spodoptera litura,* Weed extracts, Mortality

Introduction

India is basically an agro-based country where more than 80% of Indian population depends on agriculture. Insects are known to cause significant damage to crops and affect agricultural productivity. *Spodoptera litura* is an important polyphagous pest in India, China and Japan. It is serious pest of various economically important crops such as cotton, groundnut, chilli, tobacco, caster, bendy and pulses etc. (Armes *et al*., 1997; Niranjankumar and Regupathy, 2001). It was found to cause 26 - 100 % yield loss in ground nut (Dhir *et al*., 1992). Loss of major crops due to insect pest varies between 10 and 30% (Ferry *et al.,* 2004). The use of chemical insecticides has been a fundamental tool for pest control, but leads to serious consequences such as intoxication of people and animals, contamination of water, air and soil, residues on food, high persistence in the environment, resistance in pests, and impact on beneficial insects, among other effects (Rodríguez *et al.,* 2003; Regnault-Roger *et al.,* 2004). However, harmful effects and persistent nature of the chemical pesticides demand for eco-friendly alternatives. Therefore, researchers world over are engaged in a mission to hunt for novel phytochemicals that could potentially be used in the management of insect-pests. Plant derived pesticides are eco-friendly, non-toxic to non target organisms, non-persistent in nature, besides they do not promote drug resistance (Liu *et al*., 2000).

Plants are rich sources of natural substances that can be utilized in the development of environmentally safe methods for insect control (Sadek, 2003). Numerous plant species have been identified as possessing pesticidal properties and have shown potential as alternative to chemical pesticides (Singh, 2000; Sahayaraj *et al.,* 2003; Kaushik and Kathuria, 2004).

The plant secondary metabolites that show feeding deterrent or toxic effect to insects in laboratory have been 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

chemicals may produce toxic effects when ingested by insects, antifeeding activity may determine the extent of insect herbivory. Several papers have been published on the entomotoxic properties of crude extracts from different plant species (Sadek, 1997, 2003; Rodriguez-Saona and Trumble, 1999; Ciccia *et al*., 2000; Tapondiou *et al*., 2005; Ulrichs *et al*., 2008; Baskar *et al*., 2009).

The review of literature indicates that exploration of the use of *Eupatorium triplinerve* Vahl. weed plant extracts have not been done to the greater extent. Only few workers have tried to explore the insecticidal activity of this plant. Insecticidal activities of alcoholic extracts of *C. inerme*, *V. negundo*, *E. triplinerve* on the larvae of *Achaea janata* L. showed insecticidal activity and *E. triplinerve* plant posses moult inhibition, ovicidal, repellant ant antifeedant activity (Yenkanchi *et al*., 2010). In this view, effect of leaf aqueous extracts of *Eupatorium triplinerve* have been evaluated for their efficacy in the management of the insect pest *Spodoptera litura*.

Materials and Methods

Test organism : Egg masses of *Spodoptera litura* collected from the Department of Entomology, College of Agriculture, University of Agricultural Sciences, Dharwad, Karnataka (India), were surface sterilized with 0.02% sodium hypochlorite solution, dried and allowed to hatch. After hatching, the neonate larvae were reared on normal diet i.e. castor leaf (*Ricinus communis* L.) in a controlled environmental chamber (27±1°C, 65-70% RH 16:8 L/D photoperiod). The larvae were set in petri dishes lined with filter paper in the base, wet with distilled water, and were fed with fresh castor leaves until they developed into fourth and fifth instars. The laboratory reared fourth instar larvae were used for study to minimize handling effects for the present investigation.

Aqueous extract of weed : Leaves of *Eupatorium triplinerve*. **(**Family**:** Compositae) weed were collected from College of Agriculture, University of Agricultural Sciences, Dharwad, in poly bags and brought to laboratory.The fresh leaves were washed thoroughly with distilled water and dried on blotting paper. 250 gm of dried leaves were autoclaved under 15 lbs pressure for 15 min, homogenized and squeezed through cheese cloth and filtered through filter paper. The volume of the filtrate was made up to 500 ml. The extract was considered as 50 % stock solution and used for bioassay studies. This stock solution was further diluted with water to obtain the 1, 2, 3, 5, 7.5, 12.5, 15 and 17.5 % extract solutions.

Evaluation of insecticide efficacy: Crude aqueous extract of *E.triplinerve* were sprayed on the fourth instar larvae of *S.litura* with the help of a glass automiser. Two ml of extract was sprayed on 20 larvae (T1-T8) and untreated (T9-T10) checked larvae were sprayed with 2 ml of distilled water alone. The treatment was replicated thrice. The sprayed fourth instar tobacco caterpillar larvae were transferred to transparent plastic containers of 4x2.5 cm size capped with perforated plastic lids and fed with sufficient quantity of castor leaves. The experiment was conducted at 27(±1) ºC, 65-70% relative humidity and long-day photoperiod (16L:8D). Observations were recorded at 24 hrs of intervals.

Observations recorded : Mortality was recorded at 24 hrs of intervals. Larval, pupal, adult mortality and deformities in the surviving treated individuals were carefully observed and recorded. The results obtained were subjected for statistical analysis (ANOVA) using a factorial completely randomized design. Percent mortality observed in the control groups if any were corrected by using Abbott's formula (Abbott, 1925).

Adult emergence inhibition effect: At the end of 24 hrs of observation period**,** the impact was expressed as emergence inhibition (EC %) based on the number of larvae that did not develop successfully into viable adults. The experiments were stopped when all the larvae or pupae in the controls died or emerged as adults. Emergence inhibition (EI) was calculated using the Finney's formula (Finney, 1971).

Results and Discussion

The data revealed that leaf extract of *E. triplinerve* tested did not produce any mortality during fourth instar (Table 1). Whereas 2 % concentration produced as high as 28.33% mortality during the fifth instar. The subsequent concentrations of 3 and 7.5% extract did not reveal any mortality in fifth instar, while 12.5% concentration again affected 5% mortality in fifth instar. The larvae which successfully moulted to sixth instar showed highly variable percent mortality against various concentrations of crude extract tested for. For example, 1% concentration produced only 3.3% while 2 and 3% extract produced as much as 3 fold increase (10 to 11%) during the sixth instar stage. Surprisingly, the subsequent concentrations, from 5 to 15 % had no effect during the sixth instar. On the other hand, the 17.5 % concentration again demonstrated 5% mortality during sixth instar.

The results summarized in Table 2 clearly demonstrated that the percent mortality during the pupal stage was significantly higher at all levels of treatments except 2 % treatment. The results of the total % mortality (from fourth instar to adult) revealed that the mortality rate progressively increased as the extract concentrations increased (Table 1). The maximum of 92 % total mortality was observed at 15 % concentration of the extract. It was significant to note that the percent mortality observed from

Table 1 : Larvicidal activity of crude aqueous leaf extract of *Eupatorium triplinerve* on the development profile of tobacco caterpillar, *Spodoptera litura* following the treatment on fourth instar larvae

Treatment (%)	Percent mortality in Vth instar	Percent mortality in VIth instar	Percent mortality in prepupae	Percent deformity in pupae	Percent deformed adults	Percent total mortality
$T_{1\ (1)}$	0	3.33±1.66	31.66±13.01	13.33±4.40	0	48.33±10.13(41.52)
$T_{2\ (2)}$	28.33±7.26	11.66±6.00	5.00±2.88	6.66±1.66	11.66±7.26	63.31±4.40(58.47)
$T_{3\ (3)}$	0	10.00±0	36.668.81	20.00±2.88	6.66±4.40	73.32±1.66(69.80)
$T_{4\ (5)}$	0	0	46.66±13.64	28.33±4.40	11.66±4.40	86.65±10.92(84.49)
$T_{5\ (7.5)}$	0	0	33.33±4.40	31.66±8.33	23.33±3.33	88.32±9.27(86.77)
$T_{6\ (12.5)}$	5.0±2.88	1.66±1.66	48.33±13.64	26.66±8.81	10.0±1.66	91.65±1.66(90.55)
$T_{7\ (15)}$	0	0	46.66±8.81	20.00±1.66	25.66±4.40	92.32±9.27(91.30)
$T_{8\ (17.5)}$	1.66±1.66	5.0±2.88	43.33±4.40	28.33±8.33	13.33±7.26	91.65±9.27(90.54)
T_9 Distilled water control	0	1.66±1.66	6.66±1.66	3.33±1.66	0	11.65±1.66
T_{10} Absolute control	0	0	0	0	0	0

Values are mean of replicates ±S.E.; Level of significance P<0.001; No mortality at IVth instar; Values in parentheses represent the corrected percent mortality

5 % onwards was more than 85 %. The present experiment was also extended to determine the adult EI of the larvae treated with crude aqueous extracts of *E.triplinerv*e (Table 3). The results revealed that the EI_{50} of *E. triplinerv*e was more effective at 4.07 %. Interestingly, the calculated EI_{90} value was 14.10 %.

Prohibitive expense to meet the challenges of increasing resistance in insects, resurgence of pests and escalating environmental pollution caused by synthetic pesticides call for the discovery of less-expensive, non-hazardous alternatives in the management of insect-pests. Naturally occurring insecticides may play a more prominent role in pest control programmes in future (Mordue and Blackwell, 1993). Plants produce a wide spectrum of allelochemicals, however, many of such chemicals have not been explored for their physiological significance **(**Deota and Upadhyay, 2005**).** These phytochemicals specifically inhibit growth, morphogenesis, metamorphosis and reproduction (Ahmad, 2007). Koul *et al*. (2000) administered phytochemical extracts orally through food to determine the toxicity or efficacy of plant materials for antifeedancy, inhibition of growth or emergence as adults. However, an understanding of structure-activity relationship and knowledge on the mode of action is required for large-scale production**.**

S.litura is polyphagous insect pest of several important commercial food plants cotton, tomato, maize, sunflower, groundnut etc. (Sharma and Seth, 2005). Growth disruptive activity of crude aqueous extracts of *E.triplinerve* against fourth instar larvae of *S.litura* was very evident

Table 2 : Effect of crude aqueous leaf extract of *Eupatorium triplinerve* on percent total larval and pupal mortality on tobacco caterpillar, *Spodoptera litura* following the treatment on fourth instar larvae

Treatment (%)	#Total larval mortality (%)	#Total pupal mortality (%)
$T_{1\ (1)}$	3.33	45
$T_{2\ (2)}$	40	11
$T_{3\ (3)}$	10	56
$T_{4\ (5)}$	0	75
$T_{5\ (7.5)}$	0	64
$T_{6\ (12.5)}$	6.66	75
$T_{7\ (15)}$	0	66
$T_{8\ (17.5)}$	6.66	72
T_9 Distilled water control	1.66	10
T_{10} Absolute control	0	0

Results are corrected to round figures

Table 3 : Emergence inhibition of *Spodoptera litura* following the treatment of crude aqueous extracts of *Eupatorium triplinerve*

Adult emergence inhibition	*Eupatorium triplinerve*
EI_{50}	4.07(1.39 to 6.63)
EI_{90}	14.10(8.34 to 81.16)

Values in parentheses indicate the confidential limits at 95%

from the present investigation. Larval population was significantly reduced. The EI_{50} value of 4.07 % was observed in crude extract of *E.triplinerve* which showed significant reduced larval population. On the basis of the % larval mortality results it may be argued that the lesser effect of the crude extract during larval stage may be due to the development of requisite biochemical strategies against the various biochemical components present in the crude extracts tested in this study. This is in accordance with the findings of Pavela (2004) who obtained EI_{50} value at 3.74% concentration with leaf extract of *Melissa officinalis* on *S. littoralis*. The adult emergence inhibition activity of *C.inerme* is also comparable to different species of plant extract in different families (Muthukrishnan *et al*.,1999; Pushpalatha and Muthukrishnan, 1999).

The toxic effect of the crude extracts during the pupal stage was significantly higher. This is in accordance with the findings of Pavela (2004) on *S. littoralis,* who observed pupal mortality of 40.2 and 40.8% at 10 and 5% concentrations, respectively in the crude extract of *Origanum benedictus*. It may also be noted that the death during the prepupal stage was higher than that of pupae. The prepupal stage is not a distinct stage from that of last instar (in this case sixth instar). During this stage they do not feed and the body shrinks in size and they are in quiescent state. The abnormalities in the metamorphosis might be due to imbalance in hormones (Karmegam *et al*., 1997). However, those survived and entered into pupal stage were deformed and could not emerge as normal adults, if emerged the adults were also deformed. Murugan *et al.* (1998) followed no-choice method in which the test insect was provided with treated leaf disc. Usually larger doses of plant extracts inflict mortality either by inhibiting feeding or reducing digestibility or inhibiting growth. Smaller doses of extracts may not be adequate for killing the insects, however it may sometimes induce malformation (Ahmad, 2007).

In conclusion, the study reveals that crude aqueous extract of *E.triplinerve* can potentially be used as eco friendly biopesticide to control the devastating damage caused by fourth instar larva of *S.litura*. Application of crude aqueous extract is an inexpensive and effective technique, and its easy adaptability will give additional advantages leading to acceptances of this technology by farmers.

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