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Efficacy of plant extracts of *Zanthoxylum armatum* as larvicidal agent against *Spodoptera litura* larvae

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Abstract
The need of plant based insecticides is increasing day by day because of resistance acquired by lepidopteran pests against synthetic pesticides. Considering this, larvicidal activity was tested with leaf extracts of *Zanthoxylum armatum* extracted with hexane and methanol against 3rd, 4th and 5th larval instars of *Spodoptera litura*. The plant extracts were prepared by maceration technique. The concentration of methanolic extract was 735.09g/L while that of hexane extract was 806.45g/L. Mortality rates were assessed on the basis of knock down toxicity i.e., KD_0, KD_{50} and KD_{100}. Results demonstrated that KD_{100} for 5th larval instars was 7±0.5 days for both hexane and methanol extracts of *Z. armatum*. Greater larvicidal effects against 4th larval instars were shown by methanolic extracts where KD_{50} was recorded to be only 3 days. The study reports that the leaf extracts from *Z. armatum* can be a potential source of bio- pesticide against *S. litura*.

Keywords: *Spodoptera litura*, *Zanthoxylum armatum*, knock down toxicity, larval mortality, bio- pesticides

Introduction
Spodoptera litura is a pest that has substantial economic impact and is found in Southeast Asia, Africa, America, India, Pakistan, China, Australia and Japan. The polyphagous pest, *S. litura* attack as a general feeder, consuming a variety of commercially significant crops including tomatoes, cotton, millets, maize, groundnuts, potatoes, soybeans, barley and many other horticulture crops in the globe, Ramzan *et al*. (2019) [10]. It is an important polyphagous pest infesting about 40 species of plants in India and causing heavy yield loss, Chari M.S. and Patel N.G. (1983)[4]. It may cause an economic loss ranged from 25.8 - 100% Dhir *et al*. (1992) [5]. Use of chemical insecticides to control *Spodoptera litura* has proved futile as it has developed resistance to several classes of insecticides, (Kranthi *et al*. (2002) [7].
Practice of alternate method such as use of botanicals can provide an environment friendly way of management of insect pests without contaminating the soil and protecting the harmful effects on non-target organisms, Liu S.Q.(2000) [8]. Because of cumulative problems generated by the usage of insecticides, more emphasis is being laid on botanical control of pests. Plants are rich sources of natural substances and have great potential to be formulated as botanical pesticides that can be utilized in the development of environmentally safe alternative methods for insect control in the place of synthetic insecticides Arnason G.S. (2017) [3]. Plants contain secondary metabolites that are deleterious to insect and other herbivores in diverse ways; through acute toxicity, enzyme inhibition and interference with the consumption and/or utilization of food, Arnason G.S. (2017) [3]. In India, several plant products have been screened and tested against these pests, Arivoli *et al*. (2013) [1].
However efforts are continuously being made around the world to screen different plant extracts to obtain eco friendly bio pesticides. So, the present study focuses on the insecticidal activity of plant extracts of *Z. armatum* against *Spodoptera litura* (Fab.)

Material and Methods
A laboratory culture of different larval instars of *S. litura* was maintained in the Department of Zoology, Government P.G. College, and Ranikhet. The eggs were collected from the nearby fields of cabbage, cauliflower and tomatoes. The larvae were maintained in individually in glass beakers (11 cm x 9 cm) covered with muslin cloth and tightened with rubber bands, under controlled laboratory conditions at 24 ± 1°C and 76 ± 5% relative humidity and were fed with fresh leaves of cabbage and pieces of tomatoes.

Collection of plant material
Leaves of *Zanthoxylum armatum* were collected from Someshwar region of Uttarakhand, to study their bioactivity against the larvae of *Spodoptera litura* (Fab.).

Preparation of plant extracts
To prepare the plant extract, the collected leaves were washed thoroughly and shade dried till complete evaporation of the moisture. After drying, the leaves were finely powdered using a grinder. Following the maceration technique, Handa *et al*. (2008) [6] 500gms. of this fine powder was then soaked in 1.5 ltrs. of hexane and 1.5 ltrs. of methanol separately for 72 hrs. During this period the mixture was stirred vigorously 2-3 times daily. After 72 hrs. The extracts were filtered using muslin cloth and then through filter paper. Following the protocol, 735.09 g/L of methanol extract and 806.45 g/L. hexane extract of *Z. armatum* was obtained
Both the extracts were stored separately in dark bottles for further use.

Bioassay
To conduct bioassay, fifteen larvae each of 3rd, 4th, and 5th larval instars were reared separately on hexane and methanol extracts of *Z. armatum*. For the study of knock down effect, beakers and food material to be given to the larvae under treated groups were sprayed with leaf extracts.

The timings of mortality of larvae under different instars were recorded.

For control group, fifteen larvae each of 3rd, 4th, and 5th larval instars were also reared separately.

Larvae of both the treated and control groups were provided with fresh food daily and the beakers were washed thoroughly to prevent fungal growth and other infections.

The efficacy of plant extracts as larvicidal agent was tested as their knock down effect (KD). Timings for KD_0 (for no larval mortality), KD_{50} (for half of the dead larval population), and KD_{100} (for complete larval mortality) were recorded for the larvae under treated group for both hexane and methanol extracts.

Statistical analysis

Standard error of mean and comparative regression relationship of mortality was applied to the data.

Result and discussions

The findings present in figure 1, 3 and 5 shows the result of knock down toxicity of plant extract for different larval instars. Under treated groups, complete larval mortality (KD_{100}), for the 5th larval instars was recorded to be 7 $\pm$ 0.5 days. KD_{100} for 4th larval instars were 6 $\pm$ 0.5 and 5 $\pm$ 0.57 days for hexane and methanol extracts respectively. KD_{100} for 3rd larval instars were 6 $\pm$ 0.55 and 6 days for hexane and methanol.

KD_{50} for the 5th larval instars was recorded to be 5 $\pm$ 0.57 days, for the 4th larval instars KD_{50} was 3 days for methanol extract and 4 $\pm$ 0.3 days for hexane extract and KD_{50} for 3rd larval instars was 4 $\pm$ 0.33 days

KD_0 was recorded to be 1 day for 3rd, 4th and 5th larval instars under treated groups.

A strong positive correlation was observed in all instars larvae of *Spodoptera litura*. In the 5th instar, both hexane and methanol extracts showed a similar strong correlation ($r = 0.981$, $r^2 = 0.964$). In the 4th and 3rd instars hexane extracts showed high correlation($r = 0.993$, $r^2 = 0.986$), while methanol extract exhibited a perfect correlation ($r = 1$, $r^2 = 1$) in the 4th instar and high correlation ($r = 0.993$, $r^2 = 0.986$). These findings are presented in Figures 2, 4 and 6.

Different larval instars under treated groups showed food deterrence, failure to moulting, several physical and physiological anomalies such as blister like swelling, transparent dermis, discolouring, slow and sluggish movement and ultimately their death, as shown in Fig.7 (a-f).

Another study conducted in India in 2022, reported that six crude extracts of herbaceous plants showed a definite level of toxicity against 3rd instar larvae of *S. Litura*, Singh D. and Bapatla K. G. (2022) [12]. They reported that among tested herbaceous plants *Cynodon dactylon* showed significant maximum larval mortality (75%) followed by *Phyllanthus niruri* (39%), *Cyperus rotundus* (36%), *Parthenium hysterophorus* (26%), *Boerhavia difusa* (22%) and *Euphorbia hirta* (22%) treatments, Singh D. and Bapatla K. G. (2022) [12] .

Ageratum conyzoides t concentration of 20% it can cause 100% mortality in *Spodoptera litura* with the period of mortality 26-60 minutes, Lumowa S.V.V(2011) [9]. *A. conyzoides* reduces total head protein of *Spodoptera litura* and can be used in pest control programme as botanical insecticide, Renuga F.B. and Sahayaraj K. (2009) [11].

Lantana camera shows antifeedant, oviposition deterrent, developmental and morphogenetic variations, toxic effects and ovicidal activity against larvae of *Spodoptera litura*. Arivoli S, Tennyson S. (2013) [2].

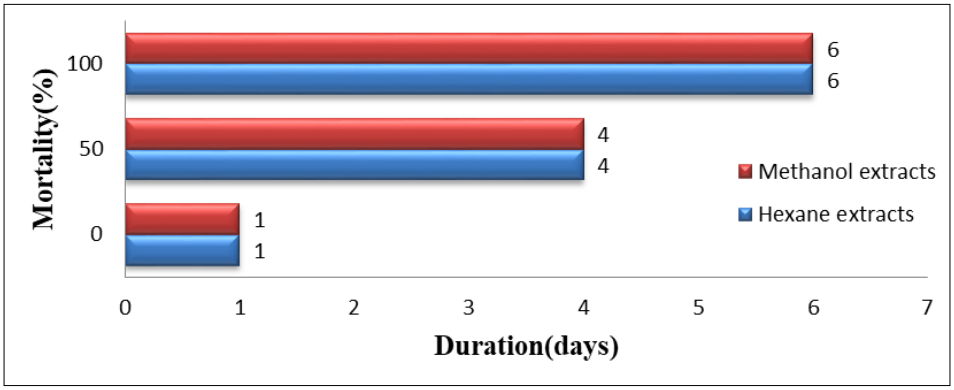

Fig 1: Comparative account of KD toxicity in 3rd instar of *Spodoptera litura*

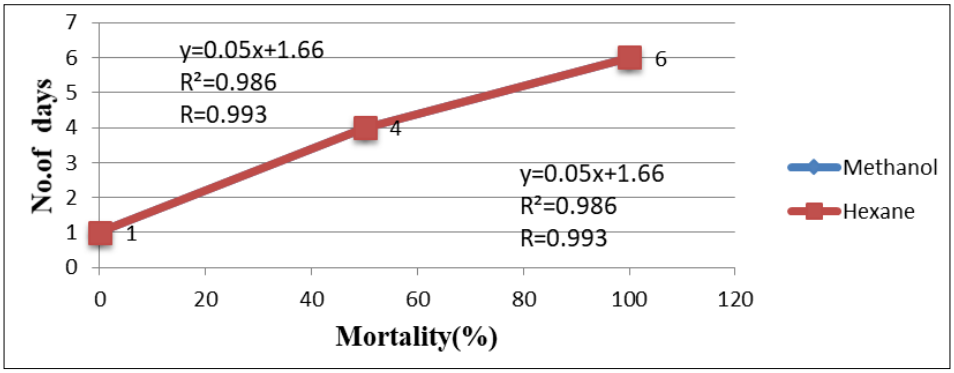

Fig 2: Comparative regression relationship of Mortality (%) with duration (days) in 3rd instar larvae of *Spodoptera litura* treated with Hexane and Methanol extract

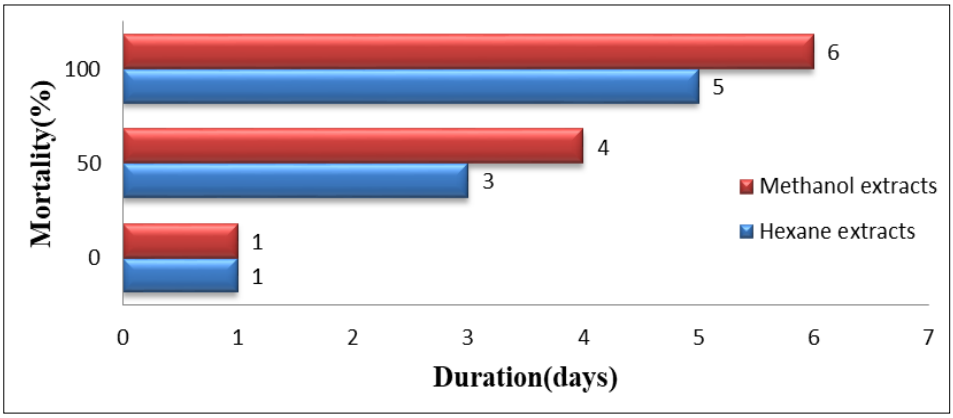

Fig 3: Comparative account of KD toxicity in 4th instar of *Spodoptera litura*

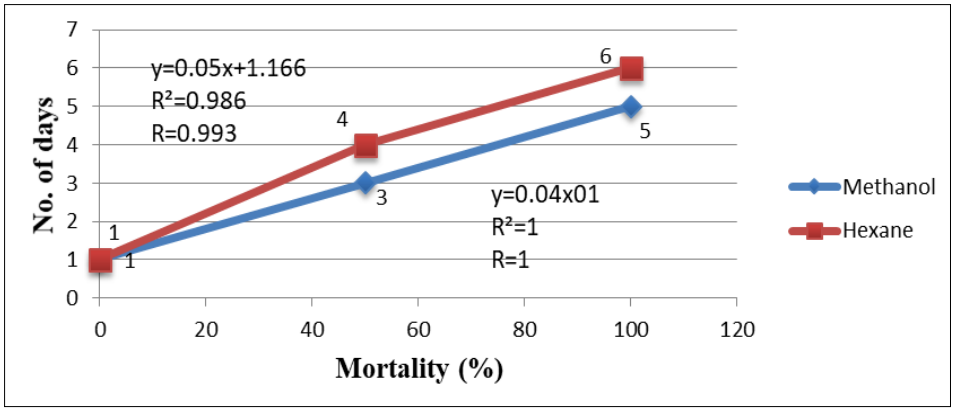

Fig 4: Comparative regression relationship of Mortality (%) with duration (days) in 4th instar larvae of *Spodoptera litura* treated with Hexane and Methanol extract

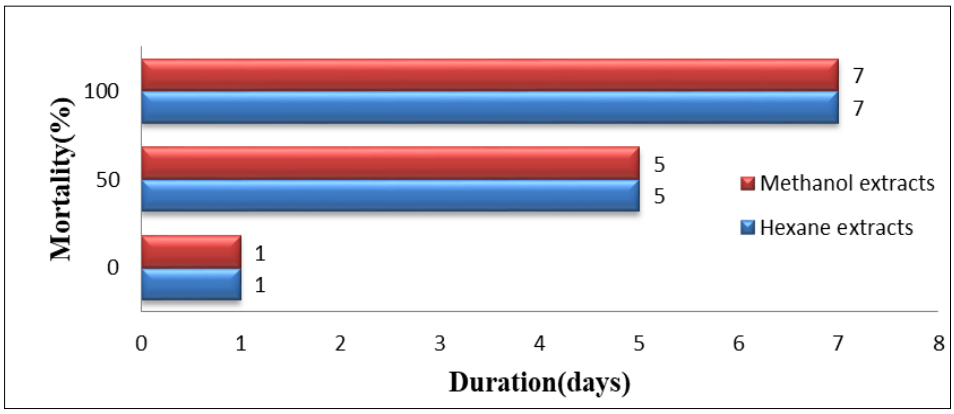

Fig.5: Comparative account of KD toxicity in 5th larval instar of *Spodoptera litura*

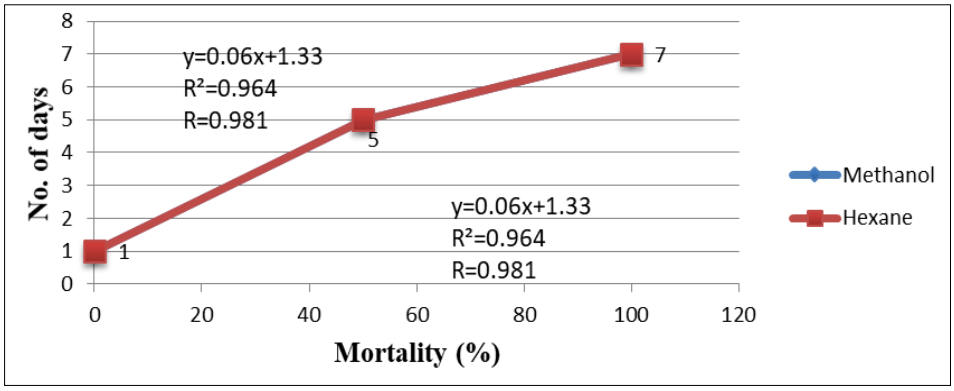

Fig.6: Comparative regression relationship of Mortality (%) with duration (days) in 5th instar larvae of *Spodoptera litura* treated with Hexane and Methanol extract

Fig 7(a): Abnormal body curling

Fig 7(b): Deformed larva with flattened and shrunken body

Fig 7(c): Larva with burn like skin patches

Fig 7(d): Swollen and distorted larva

Fig 7(e): Burn like damage at the anterior end

Fig 7(f): Desiccated larva with segmental wrinkling

Conclusion

Based on the findings of the current study, plant extracts of *Z. Armatum* can be further studied to develop it as a potent biopesticide, as it shows larvicidal properties. However, more research is needed to identify the active ingredients in the plant extracts in order to create plant-based biopesticides that are less harmful to the environment and safer for humans and beneficial organisms.

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