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Research Article

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Biological and *in vitro* effects of Neem (*Azadirachta indica*) extract on second and third instar larvae of *Spodoptera litura*

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ABSTRACT

Spodoptera litura is a major threat to agricultural crops all over the world including Pakistan. It has developed resistance to several chemicals, especially synthetic insecticides. Plant based chemicals like extracts may serve as suitable and alternative biocontrol methods for pests in the future. The current study was conducted to determine the biology of *S. litura* on cabbage and the effects of two botanicals *i.e. Azadirachta indica* and *Eucalyptus globulus* against 2nd and 3rd instar larvae in controlled conditions. The incubation period of pest was 3-4 days. The average mean developmental period of 1st, 2nd, 3rd, 4th, and 5th larval instar was 2.44±0.71, 2.32±0.74, 3.43±0.68, 2.76±0.80, and 2.40±0.79 days, respectively. The pre-ovipositional, and ovipositional period of females was 2.04-3.58 and 2.89-5.71 days respectively. The total life cycle of pests was 30.83-38.79 days. Significantly higher mortality of 2nd and 3rd instar larvae was recorded in *A. indica*. The percentage of mortality increased with an increase in treatment time.

KEYWORDS: Spodoptera litura, Azadirachta indica, Euclyptus globulus, Bio-pesticides, Pakistan

INTRODUCTION

Armyworm, *Spodoptera litura* is the main destructive and polyphagous pest of several horticultural and agricultural crops around the globe, especially in Pakistan. The pest is commonly called cutworm and tobacco caterpillar. It has spread widely but mostly in tropical and subtropical areas of the globe (Ahmad *et al.*, 2013; Liu *et al.*, 2018; Lin *et al.*, 2019). This pest has invaded several agricultural crops (cotton, maize, okra, tomato, potato, onion, brinjal, millet, sorghum, maize, soya bean, sunflower), horticultural crops and ornamental crops etc. (Abdullah *et al.*, 2019). The severe attack of this pest causes 26-100% economic losses of various crops. The losses depend on the level of pest attack on crop stage and environmental conditions (Khan *et al.*, 2018; Ramzan *et al.*, 2019).

Different methods of pest management have been applied by scientists to minimize pest attacks on the crop and enhance crop production. Among the adopted methods, chemicals are the most widely used method against this pest in the globe. The excessive use of insecticides has caused environmental pollution, insect resistance to insecticides, and left toxic residual effects on the crops or fruits which become harmful to humans and animals (Shad *et al.*, 2012; Kumar *et al.*, 2013). The use of chemicals becomes harmful to biological fauna like pollinators, predators and parasitoids which is considered the most important strategy of integrated pest management (IPM).

To decrease the problems associated with the applications of insecticides, there is a need to develop effective and alternative approaches for the control of pest populations below the economic injury level and save the environment and human health from the harmful effects of insecticidal applications. It was reported by many researchers around the world that plant based chemicals are a rich source of toxic materials which kill the pest population (Qin *et al.*, 2010; Mathesius, 2018; Trivedi *et al.*, 2018). The plant based materials have various biological activities like lectins (de Lima Santos *et al.*, 2014; Ullah *et al.*, 2021) and proteins which act as insect repellent (Kumar *et al.*, 2012; Wu *et al.*, 2016). The biological parameters of pests were recorded in this study. To check the toxicity of plant based materials, plant extract has been tested against larvae of *S. litura in vitro* conditions in the current study.

MATERIAL AND METHODS

Survey, Collection and Rearing

Different unsprayed cabbage fields of nearby farmers were visited and egg batches were collected. The collected eggs were brought to the insect-rearing laboratory and eggs were shifted into Petri dishes with cabbage leaves. The first instar larvae were collected and shifted individually into plastic containers with washed and fresh cabbage leaves. The insect was reared till 2 generations, 2nd and 3rd generation instar larvae were used in the experiment. All biological parameters of the pest were recorded.

Plant Extract Preparation

To collect the plant materials, a research team visited the Neem (*Azadirachta indica*) plants planted alongside the university and the *Eucalyptus globulus* at the nearby university. The leaves with branches were brought to the laboratory for further processing. The leaves were separated from the branches and placed inside the shade to dry for 72 hours. The dried leaves were collected and grinded with the help of a grinder. The grinded material was soaked in 100 mL of water for 48 hours. After this, the extract was obtained by filtering the materials using filter paper consisting of cotton wool.

Treatments and Bioassay

There were three treatments including water as control with three replications. Cabbage leaves of 500 mg were dipped into each treatment and placed into filter paper under shade for absorbing extra water and drying purposes. Each treated leaf was placed into a Petri dish and five larvae per petri dish shifted which considered one replicate.

Data Recording and Analysis

Data were recorded on 24, 48 and 72 hours of applications. Collected data were statistically analyzed.

RESULTS AND DISCUSSION

Different management strategies have been tested by researchers and farmers to control insect pests either chewing or sucking in the globe. The production of crops can be enhanced by minimizing the pest population with the use of several chemical pesticides which play a major role in crop protection from several destructive pests. Among tested approaches, chemicals (insecticides) have been widely used throughout the world to control the pest population (Ramzan *et al.*, 2019). The excessive applications of such types of chemicals have caused severe harmful effects such as environmental pollution, insect resistance to insecticides and harmful effects on humans and animals by leaving residues inside fruits and foods. Biological fauna such as predators and pollinators can be badly affected by the use of chemicals (Murugasridevi *et al.*, 2017; Ramzan *et al.*, 2020b; Gorawade *et al.*, 2022).

To protect the environment, humans, animals and natural enemies of pests, there is a need to adopt an alternative strategy such as botanical based chemicals which are considered eco-friendly, and safer for biological fauna, humans and animals. The current study was conducted to determine the effects of two plant based chemicals against 2nd and 3rd larval instars of *S. litura* under laboratory conditions. The results of the current study will be helpful for future scientists in the applications of such chemicals against pests in laboratory and field conditions. Ramzan *et al.* (2020a) reported similar findings.

The incubation period of the pest was 3-4 days. The average mean developmental period of 1st, 2nd, 3rd, 4th, and 5th larval instar was 2.44±0.71, 2.32±0.74, 3.43±0.68, 2.76±0.80, and 2.40±0.79 days, respectively. The pre-ovipositional, and ovipositional period of females was 2.04-3.58 and 2.89-5.71 days respectively. A study was conducted in India to determine the comparative biology of various hosts (Narvekar *et al.*, 2018). They reported a minimum larval development period on the castor and a maximum (17.33 days) on tapioca. The pre-pupal and pupal periods were 2.00 and 7.33 days on the castor respectively. The total life cycle of pests from eggs to adult completion was 30.83-38.79 days on cabbage (Table 1). Many other researchers had reported similar results about the life cycle of *S. litura* on different host plants (Murtaza *et al.*, 2019). The detail of plant extracts tested against larvae was given in table 2.

After 24, 48 and 72 hours of post-treatment, 19.22, 33.00 and 45.56% mortality of 2nd instar larvae was recorded with

Table 1: Biological parameters of *Spodoptera litura* on cabbage leaves under laboratory conditions

Stage of insect	Mean±SE	Survival days
Eggs		
Incubation period	3±0	3-4
Larvae		
First instar	2.44±0.71	2-3
Second instar	2.32±0.74	2-3
Third instar	3.43±0.68	3-4
Fourth instar	2.76±0.80	2-3
Fifth instar	2.40±0.79	2-3
Total larval period	15.04±2.99	12-18
Pupae		
Pre-pupa	0.99±0.23	0.99-1.23
Pupa	6.99±0.84	6-7
Adult		
Male longevity	6.43±0.66	6-7
Female longevity	7.56±1.76	7-9
Ovipositional period		
Preoviposition	2.59±0.65	2.04-3.58
Oviposition	3.55±0.89	2.89-5.71
Fecundity (no. eggs)	756.81±20.51	747-921
Hatching percentage	87.87±5.78	86.64-90.72
Total life cycle (eggs-adults)	34.56±4.73	30.83-38.79

Table 2: Detail of plant based extracts tested against larvae in the current study

Common name	Scientific name	Order	Family	Plant part
Safeda tree	*Eucalyptus globulus*	Myrtales	Myrtaceae	Leaves
Neem	*Azadirachta indica*	Sapindales	Maliaceae	Leaves

Table 3: Mean percent mortality of 2nd instar larvae after 24, 48, and 72 h of post-treatment in controlled conditions

Botanicals	Mean percentage mortality		
	24 hour	48 hour	72 hour
Eucalyptus globulus	19.22±0.14b	33.00±0.09ef	45.56±4.99ef
Azadirachta indica	32.10±4.33a	43.18±2.00a	56.07±0.10a
Control	0.00±0.00ef	0.00±0.00ef	5.00±0.51ef

Table 4: Mean percent mortality of 3rd instar larvae after 24, 48, and 72 h of post-treatment in controlled conditions

Botanicals	Mean percentage mortality		
	24 hour	48 hour	72 hour
Eucalyptus globulus	15.33±0.10b	29.00±0.09ef	39.56±5.07ef
Azadirachta indica	30.18±5.21a	40.22±2.07a	51.09±0.14a
Control	0.00±0.00ef	0.00±0.01ef	5.00±0.47ef

the application of *E. globulus*, while 32.10, 43.18 and 56.07% through applications of *A. indica* (Table 3). The maximum mean percentage mortality (51.09%) of 3rd instar larvae was recorded with *A. indica* after 72 hours of treatment, while the minimum (15.33%) on 24 hours of *Eucalyptus globulus* treatment (Table 4). It was recorded that maximum mortality was caused by *A. indica* as compared to *E. globulus*. It was also observed that 2nd instar larvae were more susceptible to botanical extracts than 3rd instar larvae. The toxicity of botanicals increased with the increase in the time period of applications. The similar findings had been reported by early researchers in the world including Pakistan (Ali *et al.*, 2018; Ramzan *et al.*, 2021; Kaur *et al.*, 2022; Safder *et al.*, 2022).

CONCLUSION

The current data unquestionably demonstrates that the application of bio-pesticides to control pest populations was more promising, especially in their early stages, such as the first and second instar, as these give better results for newborns than later larval stages. Because such extracts contain numerous poisons, there is no evidence that insects have evolved a tolerance to *A. indica* derivatives. Therefore, it is likely that *A. indica* extracts can be utilized in integrated pest management systems indefinitely without causing resistance.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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