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Antifeedant Activity of *Azadirachta indica* against *Spodoptera frugiperda* (J. E. Smith)

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***Abstract*:** In the dual choice test, feeding deterrence was recorded in the second instar larval stage of *Spodoptera frugiperda* when treated with the sub-lethal concentrations (LC_{30} and LC_{50}) of the neem-based formulations i.e., commercial neem formulation (0.15%), neem oil and neem seed kernel extract (NSKE).). The highest antifeedant activity was in NSKE (43.53 and 72.06 per cent) in comparison to the commercial neem formulation (27.37 and 58.97 per cent) and neem oil (17.51 and 50.59 per cent). The relative consumption index (RCI) was also high in case NSKE (0.808) followed by commercial neem formulations and neem oil. The higher antifeedant activity and RCI indicates high level of feeding deterrent.

Keywords: Antifeedant, Neem based formulations, Relative consumption index, *Spodoptera frugiperda*

Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera; Noctuidae) is polyphagous, noxious insect-pest native to tropical and subtropical regions of the Americas. Although it is a key pest of maize crop, but also infest around 80 plant species including rice, sorghum, cotton, alfalfa, millet, peanut and other cultivated and wild plant species (Belay et al., 2012, Prasanna et al., 2018, Kammo et al., 2019, Sisay et al., 2019). There are two major strains of the fall armyworm i.e. the maize strain and rice strain (+6). Among these two, maize strain is more prevalent and feeds on maize stem and leaves (Bhusal and Chapagain 2020). Larval stage of this insect-pest is the voracious feeder which causes a huge damage to the host crop. This pest has extensive defoliation capacity making it one of the economically interesting pest (Barbosa et al., 2018).)

Fall armyworm is a migrating pest which causes significantly high damage in host crop and can fly up to 1600 Km in time period of 30 hours (Bhusal and Chapagain 2020). It has migrated to continents other than Americas, being detected first in Central and Western Africa (Benin, Nigeria, Sao Tome and Principe, and Togo) in 2016 and reported further in Southern Africa (except Lesotho), Madagascar and Seychelles (Goergen et al., 2016, FAO 2018, Bhusal and Chapagain 2020). In 2018, fall army worm was reported in almost all Sub-Saharan African countries, except Djibouti, Eritrea and Lesotho (FAO 2018). It is distributed in 40 sub- Saharan African countries. The first incidence of FAW was reported in July 2018 in India from Karnataka (Saranbassappa et al., 2018) and thereafter reported from states Bihar, Chhattisgarh, Gujarat, Maharashtra, Andhra Pradesh, Odisha, Tamil Nadu and West Bengal (Deole and Paul 2018, CABI 2019, Bhusal and Chapagain 2020).The irrational use of synthetic insecticides to control insect pests has often resulted in problems like chemical residues in food, biological disequilibrium, intoxication to non target organisms, development of insect resistance (Gutierrez-Moreno et al., 2019) The search for alternative methods includes the use of natural products that are both effective and less environmentally aggressive, such as plant extracts (Roel and Vendramim 2006).

Azadirachta indica A. Juss. (Meliaceae), commonly known as neem or Indian lilac, is indigenous plant of the Indian subcontinent and Southeast Asia which is known for thousand years for antiseptical properties (Roel et al., 2010, Campos et al., 2016, Duarte et al., 2019). Every plant part i.e. root bark, fruits, twigs, seeds, flowers and stem produces more than 100 active biological compounds, mainly, terpenoids (Copping and Duke 2007, Shannag et al., 2015, Campos et al., 2016) which have repellent, insecticidal and antifeedant properties for control of many insect-pests (Kubo and Klocke 1982, Kumar and Poehling 2007, Shannag et al., 2015, Duarte et al., 2019). Azadirachtin, a steroid-like tetranortriterpenoid is the primary active compound responsible for wide range of bioactivity on many insect-pests (Mordue (Luntz) 2004, Mordue (Luntz) 2005, França et al., 2017). Azadirachtin also affects both juvenile and ecdysteroid hormone levels by inhibiting release of morphogenetic peptides from the brain which results in a blockage of ecdysteroids synthesis and a regulation of the release of juvenile hormone in insects (Mordue (Luntz) and Nisbet 2000, Garcia et al., 2006, Abdullah and Subramanian 2008, Morgan 2009). The present study was conducted to determine the antifeedant activity of *A. indica* formulations against *S. frugiperda.*

MATERIAL AND METHODS

The *S. frugiperda* population was collected in perforated polythene bags from maize fields of Punjab Agricultural University, Ludhiana, Punjab during 2020-21 and brought to the laboratory. The culture was maintained on the maize leaves in incubator at the temperature and relative humidity of 26±1oC and 65±5 per cent respectively. The larvae were kept in small plastic vials (2 x 2.5 cm). Leaves were changed daily. The larvae developed to the pupal stage and then the pupae were separated in battery jars. The sand layer in jar up to 5 cm for pupation after six instar larvae. The separate glass jars covered with muslin cloth were used to transfer the collected pupae and these jars secured with rubber bands till the adult emergence stage. The emerging adult were sexed and transferred

Table 1. Antifeedant effect and relative consumption index of neem based formulation on the *S. frugiperda*

Formulation	Concentration (%)	Antifeedant activity (%)	Relative consumption index (RCI)
Commercial neem (0.15%)	LC_{30} (0.000169)	27.37	0.173
	LC_{50} (0.000288)	58.97	0.605
Neem oil	LC_{30} (0.045)	17.51	0.084
	LC_{50} (0.238)	50.59	0.371
NSKE	LC_{30} (0.695)	43.53	0.449
	LC_{50} (1.348)	72.06	0.808

into hollow glass cylinders which were placed on top of pots containing maize plants. Cotton swab dipped in a 10 per cent solution of honey was hung from the top of the muslin cloth covering the mouth of the glass cylinder. The adults were sexed based on differences in the size and shape of the wings of male and female moths. The female moths are slightly smaller than males and the forewings have fawn-colored spots towards the center and white patches at the apical margin in adult male moths, but without spot and brown forewings in adult female moths. Adults, after mating, lay eggs on the maize leaves. The oval/round and dirty white eggs were laid in clusters and covered with hairs. To facilitate regular oviposition the maize leaves with eggs was removed on daily basis and replaced with the fresh one. All laboratory glassware used in the experiments was carefully cleaned using detergent, disinfected with 2% formalin, and subsequently dried in an oven at 30°C for 8 hours to prevent any microbial contamination in the insect cultures. The neem-based treatments were commercial neem formulation (Neem Kavach), neem oil, and 5% neem seed kernel extract (NSKE).

Determination of antifeedant effects of *A. indica* on *S. frugiperda:* The feeding performance of test larvae was evaluated by employing a 'dual choice' test. Maize leaf portion was cut into two halves. One portion was treated by dipping in test formulations (LC_{30} and LC_{50}) and the second portion was dipped in distilled water which was served as control. After drying on paper towels both portions were placed in Petri plate (9 cm diameter). Ten test larvae were released in the center of the plate and feeding exposure of 24 hours was given. There were five replications for each treatment. Per cent antifeedant activity and relative consumption index (RCI) was calculated by using the following formula is given by Saleh et al. (1986) and Sandhu and Singh (2000), respectively.

$$\text{Antifeedent activity (\%)} = \left(1 - \frac{\text{(Per cent treated area consumed)}}{\text{(Per cent control area consumed)}} \times 100\right)$$

$$RCI = \frac{C - T}{C + T}$$

Where, C and T refer to percent leaf eaten in control and treated leaf portions.

Statistical analysis: The data were analyzed using the statistical software package CPCS1 and SPSS 16.0 statistical software.

RESULTS AND DISCUSSION

In the dual choice test, feeding deterrence on the second instar larvae of *S. frugiperda* was observed with the sub-lethal concentrations (LC_{30} and LC_{50}) of the neem based formulations, i.e., commercial neem formulation (0.15%), neem oil and NSKE were observed for antifeedant effects. The maximum antifeedant activity was reported at the concentration LC_{50} of the NSKE (72.06 per cent) as a comparison to the other LC_{50} concentrations of commercial neem formulation (0.15%) and neem oil, i.e. 58.97 and 50.59 per cent, respectively on larvae of the *S. frugiperda*. Similarly, the highest antifeedant effect wan in LC_{30} concentration NSKE (43.53 per cent) followed by commercial neem formulations (0.15%) and neem oil. However, the LC_{30} and LC_{50} values of NSKE showed a more pronounced effect on *S. frugiperda* larvae compared to the commercial neem formulation (0.15%) and neem oil derived from *A. indica*. The higher antifeedant activity indicates the higher feeding deterrence. The sub-lethal concentration LC50 significantly increased the antifeedant activity in comparison to LC30 in three different neem based formulations (Table 1). Three relative consumption indices (RCIs) were significantly highest in NSKE (0.808), followed by commercial neem formulations (0.15%) and neem oil). Similarly, the leaves treated with the LC30 concentration had the highest relative consumption index of NSKE as compared to other LC30 concentrations of commercial neem formulations (0.15%) and neem oil (Table 1). The sub-lethal concentration LC50 significantly increased the relative consumption index in comparison to LC30 in three different neem based formulations.

Ghoneim and Hamadah (2017) also observed highest antifeedant activity of Nimbecidine (0.03% Azadirachtin) against the second instar larval stage of *Spodoptera littoralis*. Wondafrash et al., (2012) concluded that neem seed extract had higher antifeedant action than neem leaf extract, against *Helicoverpa armigera* larvae.. In *Spodoptera eridania* (second instar), short-term feeding (2 days) on food treated with Azatrol, a commercial neem-based azadirachtin formulation, resulted in a reduced relative consumption rate (Shannag et al., 2015). Prasoona et al. (2022) reported strong antifeedant activity of neem seed kernel extract against third instar larvae of *S. frugiperda*.

CONCLUSION

Neem seed kernel extract demonstrated the most potent antifeedant effect among all neem-based formulations, although each formulation exhibited some degree of antifeedant activity. The proven safety of these neem formulations for non-target organisms and mammals, along with their deterrent effects on *S. frugiperda*, makes them promising candidates for field trials in the pest's management.

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