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RESEARCH ARTICLE

Influence of partially purified fractions of acetone extract of Acacia nilotica (L.) on development of Spodoptera litura (Fab.)

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Abstract

Environment and human health are being adversely affected because of the extensive use of chemical pesticides in the fields. These pesticides enter in the food chain resulting in their bioaccumulation at different trophic levels. Consequently biopesticides are being explored as new safer alternatives. Plant's secondary metabolites such as phenolics play an important role in their defense against various insect pests. Phenolics in plants act as deterrent and feeding barriers to different insect pests. Their accumulation in different plant parts resist herbivory. In the present study, two partitioned fractions *viz.* ethyl acetate and water fraction, of acetone bark extract of *Acacia nilotica* (L.) were tested for their influence on the development of second instar larvae of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). Both fractions showed a toxic influence on larvae of *S. litura* but the effect was markedly greater with ethyl acetate fraction. The toxic effects were manifested in the form of increased larval mortality, reduced pupal weight, decreased adult emergence and prolongation in larval, pupal and total development period. Several deformities were observed in pupae and adults of *S. litura*. The findings indicated the anti insect potential of *A. nilotica* against *S. litura*.

INTRODUCTION

The deleterious influence of pesticides on human health and environment has necessitated that alternatives be explored which are safe and environment friendly. The excessive use of pesticides has resulted in accumulation of pesticide residues in the food and fodder besides exercising deleterious influence on the beneficial organisms. In the last few years, there has been resurgence in interest in exploring plant derived allelochemicals as pest control agents. As these compounds occur naturally in plants, they are degradable and safe for humans and other animals. Some of them have been reported to have repellent, deterrent, anti-ovipositional and growth inhibitory effect on insects (Jacobson and Crosby 1971; Maramorsch, 1991; Champagne et al., 1989). Plant phenolics are one of the major allelochemicals which have been extensively explored for their medicinal and anti-inflammatory properties. But only limited reports are available regarding their effect on insect pests where they have been implicated in the development of host plant resistance to insects by acting as toxic natural bioinsecticides (Leszczynski et al., 1985, 1996).

Acacia nilotica (L.), commonly called babul, is an important medicinal plant found all over India. It is rich in phenolic compounds (Verma et al., 2012) and has been reported to possess significant antimicrobial activity (Banso, 2009; Naqvi et al., 2011; Nagumanthri et al., 2012) antioxidant, anticancer and antimutagenic properties (Singh and Arora, 2007; Kalaivani and Mathew, 2010; Gowri et al., 2011); phytotoxic activity (Mehmood et al., 2011), antihelminthic, antiplatelet aggregatory and vasoconstrictor activity (Verma et al., 2012). However its anti- insect activity has not been reported.

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae) is a polyphagous pest, with a wider geographic range throughout Asia, from North Africa to Japan, Australia and New Zealand (Feakin, 1973). It has been reported to attack over 112 cultivated plant species and about 60 species are from India (Garad et al., 1984). In India, it has become particularly notorious in most tobacco growing regions. In the last three and half decades, its host range had been extended to other crops such as cotton, mungbean, soyabean, cabbage and leafy vegetables, including groundnut (Maree et al., 1999). Therefore the present study was aimed at evaluating the influence of partially purified fractions of acetone bark extract of *A. nilotica* on development of *S. litura*.

Materials and Methods:

The bark of *A. nilotica* was washed and grinded. Extraction was done with the soxhlet apparatus employing six different solvents in the increasing order of their polarity, viz. hexane, chloroform, ethyl acetate, acetone, methanol and distilled water. In the bioassay studies conducted with different extracts against second instar larvae of *S. litura*, the acetone extract which showed the lowest LC_{50} value was further subjected to partial purification. Two partitioned fractions viz. ethyl acetate and water fraction obtained from acetone bark extract using separating funnel were further evaluated for their insecticidal potential against second instar larvae of *S. litura*. The second instar larvae were fed on artificial diet (Koul et. al. 1997) supplemented with different concentrations (5ppm, 25ppm, 125ppm, and 625ppm) of two partitioned fractions along with control. The insecticidal potential of these two partitioned fractions against *S. litura* was determined by examining larval mortality, pupal weight, adult emergence, larval period, pupal period and total development period. The observations were made daily.

Statistical analysis:

The data was subjected to one-way ANNOVA and LC_{50} was calculated by probit analysis (SPSS-16). The means were compared by the Tukey's honestly difference test ($P \geq 0.05$) according to Assistat (7.7).

Results and Discussion:

The bioassays conducted with crude extracts of *A. nilotica* against second instar larvae of *S. litura* showed that the LC_{50} value of 310.573ppm was lowest for the acetone crude extract (Table 1). Partial purification of the acetone bark extract yielded two partitioned fractions *viz*. ethyl acetate and water fraction. Significant adverse effect of both the partitioned fractions was observed on the survival and development of second instar larvae of *S. litura*. The larval mortality increased significantly with increase in concentration in both the fractions but the increase in mortality was more with ethyl acetate fraction (Table 2). The pupal weight too decreased with treatment. The decrease was considerably greater in the ethyl acetate fraction where 49.40% reduction in pupal weight was observed as compared to only 40.10% reduction in pupal weight by water fraction (Table 2). Both the partitioned fractions inhibited the adult emergence in a dose dependent manner (Table 2). In the larvae fed artificial diet containing the ethyl acetate fraction the adult emergence was inhibited by 42.48% whereas in the larvae fed water fraction the adult emergence was inhibited by only 33.91%. The larval period, pupal period and total development period were found to be prolonged with both the fractions but the delay was more with ethyl acetate fraction than with water fraction (Table 3). The larval period was delayed by 7.93 days with ethyl acetate fraction whereas with water fraction, a delay of only 5.03 days was observed. The total development period too was delayed by 15.32 days with ethyl acetate fraction as compared to a delay of 11.79 days observed with water fraction. LC_{50} value of ethyl acetate fraction (217.115ppm) was lower when compared with LC_{50} of water fraction (462.924ppm) (Table 4). Abnormalities in pupae and adults (Figure 1), in the form of blackened and bulged out head pupae, partially emerged adults with deformed wings, also showed the adverse effects of the partitioned fractions on *S. litura*.

Among all the extracts, crude as well as partitioned, the ethyl acetate partitioned fraction had the lowest LC_{50} value clearly indicating that it was more effective than the other extracts. These findings are in agreement with the work of Deepa and Reemadevi (2011) who had also reported that the ethyl acetate leaf extract of *A. concinna* was most effective against larvae of teak defoliator, *Hyblaea puera* (Cramer) causing 100% mortality at the lowest concentration. Toxic effects of leaf extract of *A. Arabica* have also been reported against *S. litura* (Rajguru and Sharma, 2012). The bark of *A. nilotica* contains several polyphenols (Verma et al., 2012). Phenolic compounds upon ingestion by insects can induce a negative response because of their antinutritional or toxic effects (Hagerman and Butler, 1991). Thus the delayed development as well as decrease in pupal weight, adult emergence and increase in larval mortality of *S. litura* could be correlated to higher levels of phenolic compounds in the ethyl acetate fraction of the acetone extract from bark of *A. nilotica*.

Table 1: LC_{50}, Regression Line Equation and **R-Values** of crude extracts of *A. nilotica* tested against second instar larvae of *S. litura*

Extracts	LC_{50} (ppm)	Regression Line Equation (y=mx+c)	R - Value
HE	7448.597	7.8091x –10.665	0.9022
CE	5837.367	8.3817x - 9.336	0.9445
EAE	709.031	12.667x – 14.891	0.9695
AE	310.573	14.763x – 16.672	0.9853
ME	403.441	13.428x – 13.109	0.9781
WE	786.086	12.381x – 14.447	0.9611

HE- Hexane Extract, **CE**- Chloroform Extract, **EAE**- Ethyl Acetate Extract, **AE**- Acetone Extract, **ME**- Methanol Extract, **WE**- Water Extract

Table 2: Larval mortality (in percentage), **Pupal weight (in mg)** and **Adult emergence (in percentage)** in *S. litura* when second instar larvae were fed on different concentrations of two partitioned fractions of acetone crude extract of *A. nilotica*

Concentrations	Larval Mortality (%) Mean±S.E.		Pupal Weight (mg) Mean±S.E.		Adult Emergence (%) Mean±S.E.	
	E-AE	**W-AE**	**E-AE**	**W-AE**	**E-AE**	**W-AE**
Control	6.67 ± 4.22^{a}	6.67 ± 4.22^{a}	262.92 ± 8.68^{a}	261.72 ± 5.27^{a}	96.67 ± 3.33^{a}	96.67 ± 3.33^{a}
5ppm	13.33 ± 4.22^{ab}	10.00 ± 4.47^{a}	172.46 ± 3.35^{b}	194.84 ± 1.70^{b}	85.00 ± 4.83^{ab}	92.50 ± 4.79^{a}
25ppm	26.67 ± 4.22^{b}	20.00 ± 0.00^{ab}	170.33 ± 3.35^{b}	183.14 ± 6.27^{b}	79.17 ± 7.68^{ab}	83.33 ± 5.27^{ab}
125ppm	46.67 ± 4.22^{c}	30.00 ± 4.47^{b}	155.75 ± 3.79^{b}	178.83 ± 1.39^{b}	63.89 ± 9.04^{ab}	77.78 ± 7.95^{ab}
625ppm	60.00 ± 5.16^{c}	56.67 ± 6.15^{c}	133.04 ± 3.70^{c}	156.77 ± 2.45^{c}	55.6 ± 15.9^{b}	63.89 ± 7.95^{b}
F - Value	25.68**	21.10**	97.43**	101.39**	3.16*	4.44**

**Significant at 1%, *Significant at 5%, Mean followed by the same letter within the columns are not significantly different according to Tukey test at $P \geq 0.05$

E-AE- Ethyl Acetate Fraction of Acetone Extract, **W-AE**- Water Fraction of Acetone Extract

Table 3: Larval period (in days), **Pupal period** (in days) and **Total development period** (in days) of *S. litura* when second instar larvae were fed on different concentrations of two partitioned fractions of acetone crude extract of *A. nilotica*

Concentrations	Larval Period (days) Mean±S.E.		Pupal Period (days) Mean±S.E.		Total Development Period (days) Mean±S.E.	
	E-AE	**W-AE**	**E-AE**	**W-AE**	**E-AE**	**W-AE**
Control	14.33 ± 0.27^{a}	14.30 ± 0.28^{a}	7.81 ± 0.17^{a}	7.71 ± 0.15^{a}	22.08 ± 0.35^{a}	22.04 ± 0.33^{a}
5ppm	16.01 ± 0.25^{d}	16.03 ± 0.20^{c}	9.10 ± 0.22^{c}	8.31 ± 0.16^{a}	24.83 ± 0.45^{d}	24.39 ± 0.18^{d}
25ppm	18.12 ± 0.19^{c}	17.38 ± 0.26^{b}	9.11 ± 0.43^{c}	8.90 ± 0.37^{a}	27.11 ± 0.52^{c}	26.19 ± 0.33^{c}
125ppm	21.25 ± 0.08^{b}	17.98 ± 0.36^{b}	13.92 ± 0.24^{b}	10.83 ± 0.14^{b}	34.92 ± 0.20^{b}	28.72 ± 0.38^{b}
625ppm	22.28 ± 0.27^{e}	19.33 ± 0.36^{d}	15.40 ± 0.20^{d}	14.17 ± 0.65^{c}	37.40 ± 0.20^{e}	33.83 ± 0.42^{e}
F - Value	229.48**	41.86**	158.48**	54.28**	325.56**	178.75**

**Significant at 1%, *Significant at 5%, Mean followed by the same letter within the columns are not significantly different according to Tukey test at $P \geq 0.05$

E-AE- Ethyl Acetate Fraction of Acetone Extract, **W-AE**- Water Fraction of Acetone Extract

Table 4: LC_{50}, Regression Line Equation and **R-Values** of two partially purified fractions of acetone extract of *A. nilotica* when tested against second instar larvae of *S. litura*

Partially purified Fractions of acetone extract	LC_{50} (ppm)	Regression Line Equation (y=mx+c)	R - Value
E – AE	217.115	14x-11.332	0.9877
W-AE	462.924	12x-11.332	0.9447

E-AE- Ethyl Acetate Fraction of Acetone Extract, **W-AE**- Water Fraction of Acetone Extract

Figure 1: Aberrations in pupa and adult of *S. litura*

Conclusion:

The findings of present work showed that *A. nilotica* can be a potential source of bioactive compounds for the management of *S. litura*.

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