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Full Length Research Paper

Toxicological effects of neem (*Azadirachta indica)*, Kanair (*Nerium oleander*) and spinosad (Tracer 240 SC) on the red flour beetle (*Tribolium castaneum)* (Herbst.)

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The persistence and insecticidal activity of a commercial biological insecticide Spinosad, based on fermentation product of Actinomycetales bacterium, *Saccharopolyspora spinosa* (Actinomycetales: Actinomycetaceae) and two plant extracts, namely Neem (*Azadirchta indica*) and Kanair (*Nerium oleander*) were evaluated against *Tribolium castaneum* (Hbst) on stored wheat grains. Five concentrations, namely 0.5, 1.0, 1.5, 2.0 and 2.5% of each insecticide were used at different exposure times, that is, 24, 48, 72 and 168 h. Filter paper dip method was used. Results revealed that Spinosad (Tracer 240 SC) was the best against target pest, with maximum mortality, that is, 55% at 2.5% dose in 168 h exposure time and minimum 16.66% with 0.5% concentration at 24 h exposure time. Neem showed 45% mortality at 168 h exposure time with 2.5% concentration and 16.67% at 0.5% dose at 24 h exposure time followed by Kanair with 38% mortality at maximum application rate and exposure time, that is, 2.5% and 168 h and minimum 15% at minimum application rate, that is, 0.5% at 24 h exposure time. LC_{50} values were also calculated using Probit analysis technique. LC_{50} values of Neem, Knair and Spinosad after 24, 48, 72 and 168 h interval were determined. LT_{50} values of these bioinsecticides interval were also calculated at 2.5% concentration. Results revealed that bio-pesticides are better ways to manage Red flour beetle infestation in stored wheat grains.

Key words: *Tribolium castaneum*, Spinosad, *Azadirchta indica, Nerium oleander*, efficacy, LC_{50} and LT_{50}.

INTRODUCTION

Stored grain loss in weight and quality of products due to insects is a serious problem world wide. It is estimated that stored grain loss of over 10% occur each year due to insect pests among the stored houses throughout the world and tropics, in particular, *Tribolium castaneum* is a major secondary pest of processed or damaged grains (Danahaye et al., 2007). The specie has gained economic importance because infested products may contain insect fragment, benzoquinones and cast skin in addition to individuals of each life stage. Being polyphagous and cosmopolitan, number of insecticides has been used for successful control of pest (Islam and Talukdar, 2005). But the use of chemicals against insect pests of stored grains has become ineffective due to the development of resistance, in different strains of *T. castaneum* (Guedes et al., 1996; 1997).

Throughout the world, the new trend is use of biopesticides for insect pest control in storage of cereals (Rizvi et al., 2001). Environmentalists across the world are proclaiming less use of persistent insecticides. The deleterious effect of plant extracts and pure compounds

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on insects can be manifested in several manners including toxicity, mortality, antifeedant, growth inhibitor, suppression of reproductive behavior and reduction of fecundity and fertility (Rogger, 2004; Jabilou et al., 2006 and Kamali, 2009). Many indigenous plants have been used against insect pest of stored grains and other crops in Pakistan. Over 120 plants and plant products can be used for the control of stored grain insect pests (Dales, 1996; Imtiaz et al., 1999).

Various scientists across the world reported that plant derived materials do not cause resistance in insects, have broad spectrum activity, safe to natural enemies compatible with bio-control agents for IPM and are non toxic to environment. They are generally known to be cheaper and environmentally friendly. This study therefore has the following objectives:

1. To evaluate the percent mortality of *Azadirachta indica*, *Nerium oleander* and *Saccharopolyspora spinosa* against *T. castaneum* after 24, 48, 72 and 168 hours interval.
2. To determine lethal time responses of *A*. *indica*, *N. oleander* and S. *spinosa* against *T. castaneum*
3. To determine lethal concentration of *A. indica, Nerium oleander* and Spinosad against *T. castaneum.*

MATERIALS AND METHODS

Collection of insects

Heterogeneous sample (different age) of adults *T. castaneum* was collected for rearing in laboratory, from various godowns of Punjab Food Department located in Faisalabad district of Pakistan. Samples were brought to stored grain lab in Department of Entomology, University of Agriculture Faisalabad.

Rearing of insects

The insect culture was maintained in sterilized jars in incubator at 30±2°C and 60±5% R.H to get the homogenous insect population. The culture medium was the whole meal flour sterilized at 60°C for 60-90 minutes. Each jar was filled with 200 gm flour and 30 beetles were added to each jar. Beetles were left in the culture medium for about 3 days for oviposition and feeding, then removed with the help of sieves and fine camel hair brushes and were added to another set of sterilized jars filled with 200 gm flour for continuation of culture. The flour containing eggs was used as culture medium for obtaining adult beetles of same age (Saleem, 1990).

Preparation of plant extracts

Bioassays were carried out at 27-30°C and 70-75% relative humidity. 1 kg fresh leaves of Kanair and Neem were grinded after shade drying. Extraction of leaves was done by the following method: 50 gm of ground sample was taken in a flask and 50 ml ethanol was added as a solvent. Mouth of each flask was closed with cotton plug and aluminum foil.

The flasks were placed in Rotary shaker at 32°C and 120 rpm for 24 h. After this extract was be filtered with the help of filter paper (Hassan et al., 2005).

Preparation of solution

The plant extracts and commercial formulation of Spinosad (Tracer 240 SC) were used in the form of solutions of 0.5, 1.0, 1.5, 2.0 and 2.5% obtained by mixing it in water by Charles formulae (Beer, 1840).

Bioassay

Filter paper dip method was used. Petri dishes were used as exposure chambers. A filter paper disc of 5 cm diameter was placed in each Petri dish. Ethanolic extracts of Neem, Kanair and Spinosad were used. The required concentration of botanical extract and insecticide were sprayed thoroughly on the filter papers placed in the Petri dishes by using syringe. The Petri dishes were left exposed to open air for 2-3 min so that acetone and water used to make different concentrations of plant extracts and spinosad may evaporate. Twenty (20) adults of *T. castaneum* were put in each Petri dish on the filter paper sprayed with different concentrations of extracts and insecticide, and the Petri dishes were covered with the lid so that adults may not run out of the dishes (Hassan et al., 2005).

Data collection and statistical analysis

For direct toxicity tests, insect mortalities were recorded at 24, 48, 72 and 168 h after interval. Data were corrected by Abott formulae, 1925 and analyzed statistically using ANOVA 2 and MSTAT-C. The mean values were adjusted using Duncan Multiple Range test (Duncan, 1951). Median lethal doses were calculated using probit analysis with log10 transformation of concentration of extracts and insecticides. Results were expressed in ppm. Data for lethal time were corrected using Abott formulae and analyzed using the method of Finney (1971).

RESULTS

Effect of neem (*Azadirachta indica*)

Neem and neem based products are often used against different insect pests of field crops and stored grains. In the present project, neem was found to be comparatively better than Kanair. Different mortality data were recorded at different exposure time and concentration. Table 1a demonstrated that maximum mortality (45.63%) was found at exposure time with maximum dose of 2.5% and minimum control (16.88%) was at 24 h with 0.5% concentration. In all treatments, 2.5% concentration was at par than 2.0% at different LSD.

The results described that prolonged exposure time is most effective to control Red flour beetle in stored wheat. Percent mean mortality (45.63%) at exposure time of 168 h at dose of 0.5% was greater than the mortality found at minimum exposure time (24 h) with maximum dose (2.5%), that is, 22.500%.

LC_{50} values of these bio-pesticides were determined against red flour beetle. LC_{50} value of neem was 6273 after 24 h, 4822 after 48 h, 566 after 72 h and 79.785 after 168 h. LT_{50} value of Neem was 190 h. Slope of regression line in median lethal doses and median lethal

Table 1a. Comparison of mean values of data regarding the interaction between concentration of Neem and exposure time on percent mortality of *Tribolium castaneum* (Herbst).

Concentration	Exposure time (h)			
	24	48	72	168
0.5%	16.875^{b}	25.000^{d}	27.500^{c}	32.500^{b}
1.0%	18.125^{b}	26.250^{c}	30.00^{bc}	35.625^{b}
1.5%	18.125^{b}	28.750^{bc}	31.250^{b}	36.875^{a}
2.0%	23.750^{a}	29.375^{b}	32.500^{b}	37.500^{a}
2.5%	22.500^{a}	30.000^{a}	35.625^{a}	45.625^{a}
Level of significance	2.830	1.530	1.836	2.380

Table 1b. Comparison of mean values of data regarding the interaction between concentration of Kanair and exposure time on percent mortality of *Tribolium* (Herbst).

Concentration	Exposure time(h)			
	24	48	72	168
0.5%	15.625^{c}	18.125^{a}	21.875^{b}	28.750^{d}
1.0%	16.875^{bc}	19.375^{ab}	22.500^{ab}	30.625^{cd}
1.5%	17.500^{bc}	21.875^{ab}	23.750^{ab}	33.125^{bc}
2.0%	18.125^{ab}	21.875^{bc}	25.000^{ab}	35.000^{ab}
2.5%	20.000^{a}	22.500^{c}	25.625^{a}	38.125^{a}
Level of significance	1.798	3.151	2.927	2.863

Table 1c. Comparison of mean values of data regarding the interaction between concentration of Spinosad and exposure time on percent mortality of *Tribolium castaneum* (Herbst).

Concentration	Exposure time (h)			
	24	48	72	168
0.5%	16.875^{b}	21.875^{c}	28.750^{d}	40.000^{d}
1.0%	17.500^{b}	22.500^{bc}	31.250^{cd}	45.000^{c}
1.5%	20.625^{a}	23.750^{abc}	33.125^{bc}	46.250^{bc}
2.0%	21.875^{a}	25.000^{ab}	35.000^{b}	48.125^{b}
2.5%	23.125^{a}	26.250^{a}	42.500^{a}	53.750^{a}
LSD at 0.05	2.311	2.351	2.270	2.053

time were below 2.0, which is indicative of homogeneity of field strains (Tables 2 and 3). Figures 2 and 3 provide graphical illustration of toxicity of insecticides against red flour beetle.

Effect of kanair (*Nerium oleander*)

Ethanolic extract of Kanair was found least effective against *Tribolium* sp. in comparison with Neem extract and Spinosad. Mortality data were recorded at different exposure times and concentrations. Table 1b depicted maximum percent mean mortality (38.13%) at 168 h exposure time with maximum dose of 2.5% and minimum (15.63%) at 24 h with 0.5% dose. Similar to Neem, prolonged exposure time is most effective to control target pest in Knair. Percent mean mortality (38.13%) wasfound at exposure time of 168 h with the dose of 0.5% is greater than the mortality found at minimum exposure time (24 h) with maximum dose (2.5%), that is, 20% (Table 1b; Figure 1b).Median lethal doses of Knair was determined after 24, 48, 72 and 168 h intervals. The results revealed that LC_{50} values of Knair was 76222.156 to 203.700 g/L. LT_{50} values of knair was 262 h at 2.5% concentration. The results revealed that Knair is least effective against T. *castaneum* than Neam and Spinosad

Effect of spinosad (Tracer 240 SC)

Results revealed that maximum mortality, that is, 53.75% was found at 168 h with 2.5% concentration and

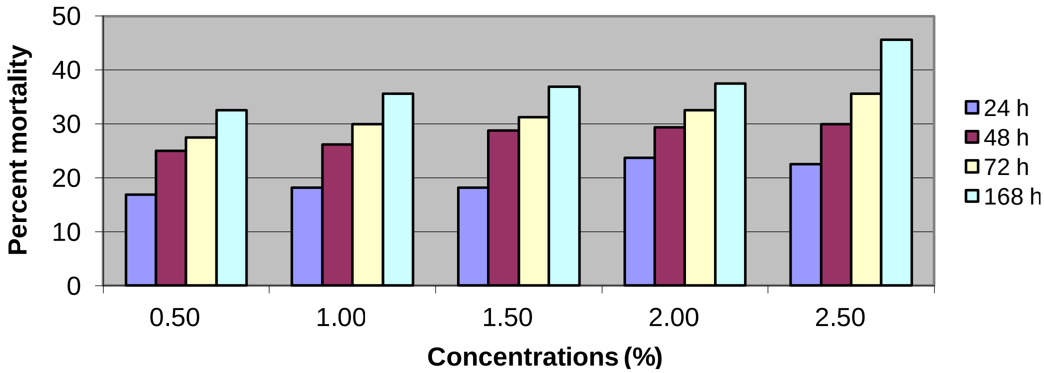

Figure 1a. Comparison of mean values of data regarding the interaction between concentration of Neem and exposure time on percent mortality of *Tribolium castaneum* (Herbst).

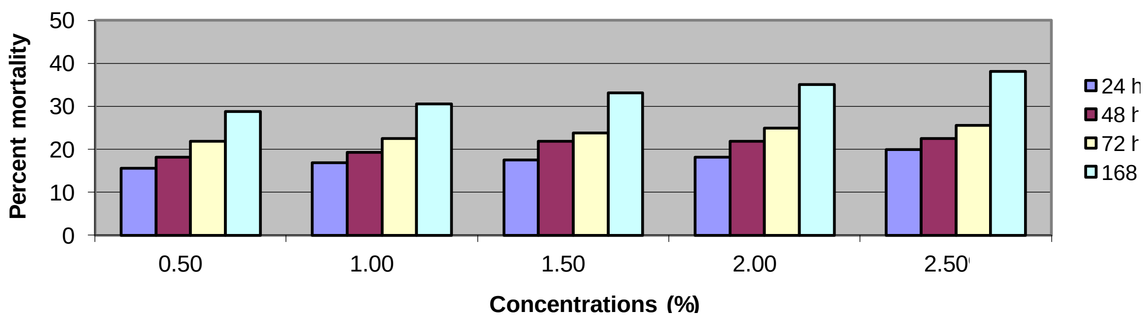

Figure 1b. Comparison of mean values of data regarding the interaction between concentration of Kanair and exposure time on percent mortality of *Tribolium* (Herbst).

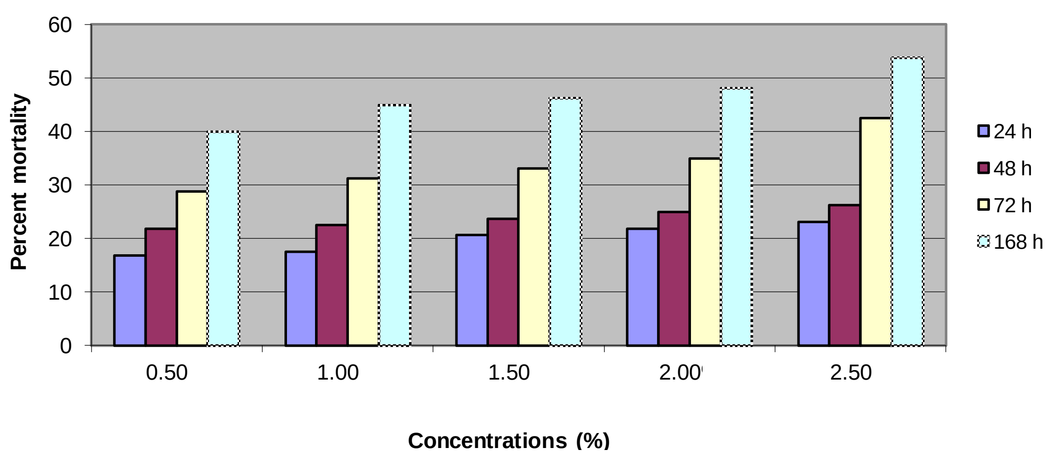

Figure 1c. Comparison of mean values of data regarding the interaction between concentration of Spinosad and exposure time on percent mortality of *Tribolium castaneum* (Herbst).

minimum (16.87%) at 24 h interval with 0.5% concentration. Median lethal dose values were determined after different time intervals. LC_{50} values of this pesticide were in the range between 5727.269 to 19.357 g/L. LT_{50} value of this bio-pesticide was 108 h. In all values slope of regression line was below 2.0, which is indicative of homogeneity of reared culture.

DISCUSSION

Neem is most commonly used botanical in Asia. Results

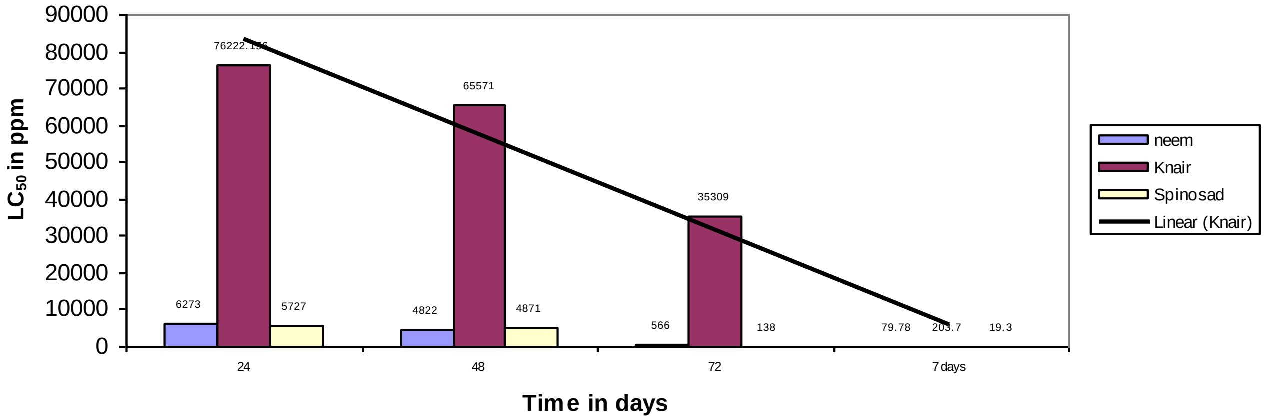

Figure 2. Toxicity of Bio-pesticides against red flour beetle *T. castaneum* Herbst.

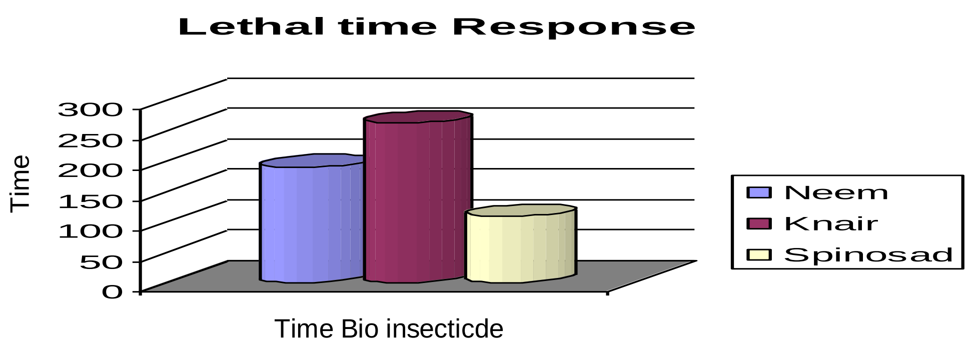

Figure 3. Lethal time response of different bio-pesticides against red flour beetle.

of the present studies suggested that neem controlled upto 45.63% red flour beetle population after 168 h interval. Islam and Talukdar (2005) reported that 100 µg/insect controlled Red flour beetle population upto 53.13% after 72 h interval. Whereas the results of the present studies depict that 2.5% neem extract can control upto 45.63%. Reduction in mortality may be due to field strains exposure level in different countries. The result of the present study is similar to Ahmed and Koppel (1988), Ahmad et al. (1999) and Akhter et al. (1993) who used neem as insecticide for control of insects. The results of the present study reveal that neem can be good alternative to Knair and Insecticides in Asia; however, different formulation of insecticides needs to be addressed. The results of present studies were similar to Islam and Talukdar (2005) who reported that gradual decrease in LD_{50} value was with time on red flour beetle.

Knair is also commonly used botanical in Asia. Results of this study suggest that knair can control red flour beetle population upto 38.125%. Anwar et al. (2005) conducted similar experiment with bagging material and determined the efficacy of knair. Results revealed that 5% concentration provided 75% mortality after 30 days. This study revealed that at 2.5%, 38.33% insect died after 168 h at 2.5% concentration, which is similar to that of Anwar et al. (2005) and Jbilou (2006).

Commercial formulation of Spinosad was used for comparative efficacy trials. Spinosad was found to be the best insecticide among all, and this is in line with that of Bonjour et al. (2008) and Yousafnezhad (2009) who reported that spinosad provided long term control on *Rhyzopertha dominica and T. castaneum* population up to 96 weeks. This might be due to the fact that inhibition of C.E (Carboxylesterase) activity and Glucoamylase activity, which play significant role in resistance to other chemicals (Hussain et al., 2009). Similar results were also reported by Bonjour et al. (2008) on stored grain wheat. Huang et al. (2004) reported that field strains of red flour beetle have developed 20-7.5X resistance than laboratory strains.

Daglish and Nayak (2006) reported that spinosad can control *R. dominica* for 9 months at 0.5 mg/kg. Huang et al. (2004) reported that *T. castaneum* is the least susceptible species to Spinosad than stored grain beetles. Athanassiou (2008) reported that 1 ppm spinosad can cause mortality of upto 6.3%, while our results indicate that 50% mortality occurs at dose after 168 h. Thus this study's results are in line with that of Athanassiou (2008) who further reported that *T. castaneum* is not very susceptible to spinosad and survival can be high at high dose rate and increased exposure time. Collins (1986) reported that data correlating the number of genetic factors with enzyme system included total esterase, carboxylestrase, mixed function oxidase and Glutathion S transferase. Single genes may be involved in resistance to a wide range of insecticides. Rajendran and Preshanthi (1998) evaluated that resistance in stored products insects is a relationship, such as single gene detoxifying enzyme and polygenes multiple target site/ enzyme system. The study's results were similar to Fang and Subramaneum (2008) evaluation that spinosad caused 39% mortality in *R. dominica* population at 0.1 mg/kg dose after 14 days interval.

Results of the present study revealed that neem can be good alternative to knair and conventional insecticides under conditions. Keeping in view the aforementioned results is suggested to give more attention to develop commercial botanical insecticide, which will not only control the insect pests of stored products, but are also cheap, easy to use, and safe for health and environment.

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