Insecticidal Effects of Ethanolic Extract from *Verbascum cheiranthifolium* Boiss. Against Two Stored-Product Insect Pests Species

H. Khoshnoud and M. Khayamy
Department of Biology, Faculty of Science, Urmia University, Iran

Abstract: Ethanolic extract from flowers of *Verbascum cheiranthifolium* Boiss. (Scrophulariaceae) was examined for its effect on mortality and progeny production against adults of *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst.). The insecticide was applied at five dose rates, which 0.25, 0.5, 1.0, 2.0 and 3% (w/v). Adults of the above-mentioned species were exposed to the treated wheat at 25°C and 65% RH and mortality was assessed after 24 and 48 h, 7, 14 and 21 day of exposure. Then all adults were removed and the treated substrate remained at the same conditions for an additional 45 day after this interval, the commodity was checked for progeny production. For two species mortality increased with the increase of dose and exposure interval. Results indicate that *S. oryzae* was more sensitive than *T. castaneum*. In case of *S. oryzae* mortality was 100% after 21 day of exposure at the highest dose rate. For both species, complete suppression of the progeny production was observed in the treated wheat than in the untreated wheat even in the lowest dose rate. Therefore, this botanical material can be used for protection of stored Wheat from infestations of stored-product insect pests.

Key words: Insect pests, progeny production, mortality, *Verbascum cheiranthifolium*

INTRODUCTION

Recently, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides. Different types of plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as fumigants, repellents, anti-feedants, anti-coviposition and insect growth regulators (Rembold, 1994; Schmutterrer, 1995; Prakash and Rao, 1997; Isman, 2000; Weaver and Subramanyam, 2000).

Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Arman et al., 1989). Yang and Tang (1988) reviewed the plants used for pest insect control and found that there is a strong connection between medicinal and pesticidal plants.

*Tribolium castaneum* (Herbst.) and *Sitophilus oryzae* (L.) are considered as major pests of stored grain (Howe, 1965). Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to some serious problems including development of insect strains resistant to insecticides (Zettler and Cuperus, 1990; White, 1995; Riberio et al., 2003). Toxic residues on stored grains, toxicity to consumers and increasing costs of application.

Convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Toxic effects of plant products on some pests have been studied by Stevenson et al. (1993), Koul et al. (1996) Sahayaraj and Sekar (1996), Senthilkumar et al. (1997) and Sahayaraj and Paulraj (1998). Roy et al. (2005) established leaf extracts of *Shyalmmontra* (*Blumea lacer*) as botanical insecticides against lesser grain borer and rice weevil. Kaburu and Gichia (2001) revealed that the insecticidal and anti-feedant activity of extracts derived from different parts of the mangrove tree *Rhizophora mucronata* (Rhizophoraceae).

*Verbascum cheiranthifolium* boiss. (Scrophulariaceae) is locally used to kill fishes in northern and northwest regions of Iran. However, flowers of this plant have not been studied yet for insecticidal activity.

The aim of this study was to produce crude extract from *V. cheiranthifolium* for to determine insecticidal activity and effect on progeny production against of two stored-product beetle species.

MATERIALS AND METHODS

Plant extract and test insects: Crude extract of botanical was used. *Verbascum cheiranthifolium* boiss.
(Scrophulariaceae) collected at flowering stage from Urmia, Iran, in July, 2006. The identification of this plant was carried out according to flora of iranica (Rechinger, 1982). Flowers of this plant were separated and dried naturally on Laboratory benches at room temperature (23-24°C) for 10 days. The plant materials were powdered using an electric grinder. Hundred gram of the dried powders extracted with 70% ethanol.

The extract was concentrated using a rotary evaporator at a maximum temperature of 45°C and were then further dried in an oven at 40°C for 48 h and powdered again. The dried extract was then dissolved in distilled water to prepare solutions of different concentrations (0.25, 0.5, 1, 2 and 3% w/v).

Adults of S. oryzae and T. castaneum were used in the test. The S. oryzae adults were taken from a culture that was kept in the Laboratory on whole wheat at 27±1°C, 65±5% RH and continuous darkness.

The T. castaneum adults were taken from a culture kept on wheat flour plus 5% brewers yeast (by weight) at 28±1°C and 65±5% RH. All individuals used in the test 7-10 days old. Also, commodity was untreated, clean winter wheat (variety Zarrin) that obtained from Agricultural Research Center of west Azerbaijan, Urmia, Iran, was used in the tests.

**Bioassay:** All tests were conducted at 25°C, 65% RH and continuous darkness. Fixed quantity (1 kg) of diet (wheat) was then sprayed with 100 mL of each solution, 0.25, 0.5, 1, 2 and 3% (w/v). Also, there was 1 kg of wheat which was sprayed with water alone and served as control. From each combination, four samples, of 50 g each, were taken. Each sample was placed in a small glass pots (7 cm diameter and 8.5 cm height). Twenty five S. oryzae adults were introduced into each glass pots and then covered with nylon mesh secured with rubber bands. The pots were placed in incubators, at the conditions described above. Dead adults were counted 24 and 48 h, 7, 14 and 21 days later. The same procedure was repeated for T. castaneum and for two species repeated four times (Athanasiiou et al., 2005).

**Progeny production count:** After the 21 day mortality count, all adults (dead and alive) were removed and the glass pots were left in the incubators at the same conditions for an additional period of 45 day. Then, the glass pots were opened and the emerged individuals were counted. All the emerged S. oryzae individuals were adults, because the larvae of this species develop inside the grain kernels, whereas for T. castaneum apart from adults, immature also were recorded, given that this species is an external feeder (Athanasiiou et al., 2005).

**Data analysis:** Control mortality was corrected by using the formula Abbott (1925). The data were arcsine transformed before analysis. The mortality counts were analyzed separately for each species, by using the GLM procedure (SAS, 1996), with insect mortality as the response variable and dose rate and exposure interval as main effects. The dose required to kill 50% of the insect (LD$_{50}$) was estimated using probit analysis (SPSS, 1999). The Percentage of reduction in progeny production was determined by the $\left[\left(\frac{\text{No. Progeny in control}}{\text{No. Progeny in treatment}}\right) - 1\right] \times 100$ formula (Aldryhim, 1995).

**RESULTS**

**Mortality of S. oryzae adults:** All main effects as well as associated interactions were significant at the $p = 0.000$ level (dose: $F = 38.6; df = 4$; exposure: $F = 398.8; df = 4$; dose $\times$ exposure: $F = 8.37; df = 16$).

Mortality of the exposed S. oryzae adults increased with the increase of the exposure interval and dose rate (Fig. 1). On wheat treated with the lowest botanical dose rate almost 53% of the exposed adults were still alive at the 21 day exposure interval (Fig. 1), whereas after 21 day of exposure, adult mortality on diet treated with 3% (w/v) botanical dose rate reached 100% (Fig. 1).

Mortality of T. castaneum Adults. All main effects as well as associated interactions were significant at the $p = 0.000$ level (dose: $F = 83.5; df = 4$; exposure: $F = 136.5; df = 4$; dose $\times$ exposure: $F = 31.5; df = 16$).

As noted for S. oryzae, mortality increased with the increase of the exposure interval and dose rate (Fig. 1). In contrast with other species, mortality of T. castaneum adults at the low dose rates was negligible (Fig. 1); moreover, mortality at the highest dose rate was almost 64% after 21-d exposure time (Fig. 1).

Moreover, LD$_{50}$ value for S. oryzae lower than required value for T. castaneum (S. oryzae LD$_{50}$: 3.79, df: 3, chi square: 1.5 and T. castaneum. LD$_{50}$: 6.39, df: 3, chi square: 0.62) therefore, it is clear from the results that the botanical extract on S. oryzae was effective.

**Progeny production:** The application of this plant material significantly reduced progeny production. No progeny was found in wheat treated, therefore, for two species, complete suppression of the progeny production was observed in the treated commodity in comparison with the control, even in the lowest dose rate (100% reduction in progeny production for S. oryzae and T. castaneum at all dose rates).
Fig. 1: Mean mortality (±SE) of S. oryzae and T. castaneum adults exposed for 24 and 48 h, 7, 14 and 21 day in wheat treated with 0.25, 0.5, 1, 2 and 3% (w/v) botanical extract

DISCUSSION

The result of the current study suggest that this botanical extract is effective against S. oryzae and T. castaneum on wheat, but its effectiveness is highly determined by the characteristics of the dose rate and the exposure interval. Also, the same dose rate in not equally effective between the two species examined, except for effect on progeny production. Botanical extract is effective against S. oryzae than T. castaneum. Moreover, results show that for this species application rates and/or longer exposure intervals are needed to obtain a satisfactory level of mortality.

However, present results indicated that higher concentrations of this botanical extract for a relatively short period are much more effective than lower concentrations for a long period. In contrast is its ability to reduce progeny production in the treated grain. In our tests, progeny production of all species was inhibited completely in all the dose rates, indicating that even if oviposition occurred before death, the activity of botanical extract during the first molt of larvae was satisfactory.

Females of S. oryzae lay their eggs in the external part of the kernel (Brich, 1945; Golebiowska, 1969) and it is likely that newly hatched larvae are exposed to botanical before entering the kernel. But, in T. castaneum generally, F, numbers were extremely low, even in the control commodity; this finding could be due to the fact that T. castaneum cannot be developed easily in sound seeds (Aitken, 1975).

The finding of our study agree to earlier reports that indicated that most plant extracts though to have insecticidal properties and can control pests through affecting other biological activities (Berenbaum, 1989; Jacobson, 1989; Ascher, 1993; Schmutterer, 1995; Mostafa et al., 1996; Musabyimana et al., 2001).

From the progeny production of these insects, emergence of adults insects from all control samples indicated that tested insects were capable of effective oviposition and that prevention of progeny emergence was exclusively due to treatment. Thus extract of V. cheiranthifolium either suppressed oviposition or killed the larvae hatching from eggs laid in the medium culture. These results suggest that there may be different compounds in extracts possessing different bioactivities.
Similar observations on other plant extracts effect on several insects have been studied. For example, Sadek (2003) showed that the time of pupation of Spodoptera liturata (Boisduval) of larvae increased by the extract of Adhatoda vasica (Nees). Jeyabalans et al. (2003) have reported that extract of Pelargonium citrosum (Van Leeuwen), prolonged the duration of larval instars and the total developmental time of Anopheles stephensi (Liston). Zhong et al. (2001) have also highlighted that extract from Rhododendron molle (G. Dorn) flowers extend the duration of development of Pieris rapae L.

Present results have shown that V. cheiranthifolium Boiss. Posses high insecticidal activity on S. oryzae and T. castaneum. Abbasi et al. (2003) have found that same effect on desert locust Schistocerca gregaria (Forskal).

We can conclude that this study suggest that ethanolic extract of V. cheiranthifolium possesses toxic principles with significant insecticidal effect and could be a potential grain protectant against S. oryzae and T. castaneum.

Today, the environmental safety of an insecticide is considered to be of paramount importance. The world flora has a variety of plant species and in order to increase the number of plants used for pest control, more studies should be carried out. Thus, a variety of effective substance found in different plant species could be discovered. Consequently, substances alternative to many chemical pesticides, will pollute our natural sources and threaten our future, can be found. In addition, cheaper pesticides can be obtained and environmental pollution will gradually decrease.

REFERENCES


