EFFECT OF FIVE DIFFERENT TYPE PESTICIDES ON THE SUNN PEST, *EURYGASTER INTEGRICEPS*

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ABSTRACT: The Sunn pest, *Eurygaster integriceps*, a key constraint on increasing wheat production in the wide area, causes severe damage to the vegetative growth stage of wheat in the early season. In this study, the effects of selected biopesticides and insect growth regulators were investigated to open a new gate for pesticide application against this major pest of wheat fiels. Five concentrations of different chemicals were used for toxicity and evaluation of LC_{50} values along with a control treated with acetone. Comparison of LC_{50} values showed that *A. annua* extract, *B. bassiana* secondary metabolites and Buprofezin were more effective than pyriproxyfen and metoxyphenozide because of lower amount of LC_{50}. Comparison of mortality percentage of this chemicals demonstrated the significant differences and the most percentage (100%) was observed in *A. annua* extract, *B. bassiana* secondary metabolites and Buprofezin treatments. Elucidation of the mode of action of chemicals is of practical importance for insect control because it may give useful information on the appropriate formulation types. This study has further confirmed the insecticidal potential of metabolic compound produced by *A. annua*, *B. bassiana*, pyriproxyfen, metoxyphenozide and buprefezin. In the future we can expect additional development and more advanced final adjustment of the insecticides and application techniques and obtaining new knowledge about physical-chemical properties that determine their destiny in the environment and in biological systems.

KEY WORDS: *Eurygaster integriceps, Artemisia annua*, Secondary metabolites, insect growth regulators.

A key constraint on increasing wheat production in the wide area of the Near and Middle East, Eastern and Southern Europe and North Africa is the Sunn pest (*Eurygaster integriceps* Puton) (Hemiptera: Scutelleridae), which causes severe damage to the vegetative growth stage of wheat in the early season. The pest also feeds on wheat grains in the late growth stage; damaged grains greatly reduce the baking quality of dough. The most important times in the life cycle of *E. integriceps* are (i) the period of late nymphal development and (ii) the intense feeding of newly emerged adults. Nymphs in the early instars do not feed intensively. After the third instar, feeding intensifies and the damage to crops becomes obvious. The emerged adults start intense feeding on wheat grains (Paulian and Popov, 1980; Popov et al., 1996). During feeding, the Sunn pest, with its piercing–sucking mouthparts, injects saliva from salivary gland complexes into the grains to liquefy the food. The liquefied food is ingested and further digested inside the gut. The enzymes that are injected into the grain during feeding degrade gluten proteins and cause rapid relaxation of dough, which results in the production of bread with poor volume and texture (Boyd et al. 2002).

The control of *E. integriceps* was based on intensive usage of two organophosphorus insecticides, fenthione and fenitrothine, by air plaines. Although, biological control by *Trissolcus* species was effective, farmers have not been interested in it. Today worldwide concerns about our fragile ecological balance are making people re-think the use of synthesized pesticides. Chemical pesticides are generally effective against a wide range of insects, have long halflives and often are found in streams and lakes as pollutants from ground run-off. Hence, agrochemical research has resulted in the discovery of novel insecticides that act on selective biochemical sites present in specific insect groups. This has led to an increase in efforts to find and develop natural pesticides that are species specific and efficient such as pathogens, insect growth regulators (IGRs) and botanical insecticides and antifeedants.

The genus *Artemisia* is a member of a large plant family Asteracea (Compositae) encompassing more than 300 different species of this diverse genus. The species *A. annua* known as sweet worm wood grows wild in Europe and America and is planted widely in China, Turkey, Vietnam, Afghanistan and Australia (Bhakuni et al., 2001). The plant also grows wild in the northern parts of Iran around paddy fields. Several isolated compounds from this species have been shown antimalarial, antibacterial, antiinflamatory, plant growth regulatory and cytotoxicity (antitumor) activities (Bhakuni et al., 2001). Although many studies have reported insecticidal effects of plant extracts (Isman, 2006) including growth retardation and arrest of ovarian development their mode of action has not been elucidated (Akhtar and Isman, 2004).

Well-known entomopathogenic fungi include; *Beauveria bassiana*، *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* are used for pest control (Feng et al., 1990; Wraight et al., 1998). *B. bassiana,* was used for many sucking pests and showed satisfactory results. Wraight et al. (1998) reviewing entomopathogenics of *P. fumosoroseus, P. farinosous* and *B. bassiana* isolates on silver leaf whitefly showed that all of them have pathogenicity on this pest. Hatting et al. (2004) showed that *B. bassiana* could control up to 65% of *Duraphis noxia* in field condition. Talaei (2002), tested *B. bassiana* on *Eurygaster integriceps* and showed that were highly effective especially on the nymphal instars and adults. Many fungi have been found to display antagonistic or parasitic activity against plant pests and diseases. This activity is normally mediated via metabolites secreted in the environment (Wood and Way, 1989). Fungi are, therefore, considered to be a potentially rich source of bioactive molecules for exploitation as agrochemicals or pharmaceutical drugs (Rodgers, 1989).

The insect growth regulators are advantageous because they do not persist long in the environment due to their rapid biodegradation and exhibit low toxicity. The development of resistance to these substances has not been proved as yet and their effectiveness in practical applications has been considered sufficient (Dhadialla et al., 1998; 2005). Insect growth regulators (IGRs) such as chitin inhibitors, juvenile hormone analogues (JHA) and ecdysteroid agonists, affect on the hormonal regulation of moulting and development processes and disrupt the regular physiological processes of insects (Ishaaya, 1990; Dhadialla et al., 1998; 2005; Palli and Retnakaran, 2001).

The aim of the present study was to validate the effects of selected biopesticides and insect growth regulators under laboratory conditions which using of *A.annua* extract and *B.bassiana* secondary metabolites against *E. integriceps* is made for the first time because the majority of works were done on lepidopterous insects. The results can serve as a basis for the development of optimum and reliable technological procedures, which will improve their prospects and use in the control of sunn pests within an integrated *E. integriceps* protection programme.

MATERIAL AND METHODS

Insects rearing

The insects were collected from the Karadj wheat farm in Tehran Province, Iran, and maintained on wheat plants in the laboratory at 27±2° C under a 14 h light:10h dark (LD 14:10) photoperiod. Voucher specimens are kept in the Entomological Laboratory, Plant Protection Department, Tehran University.

Methanolic extract from leaves of A. annua

Leaves of A. annua were collected in June around paddy fields in Rasht, Guilan province of Iran. Leaves were washed with distilled water and dried at room temperature in the shade. Methanolic extraction was carried out according to the procedure described by Moharamipour et al. (2003). Briefly, 30 g of dried leaves were stirred with 300 ml of 85% methanol in a flask for 1 h. The methanolic solution was incubated for 48 h at 4 °C and then stirred for additional hour and then filtered through Whatman No.4 filter paper. The solvent was removed by vacuum in a rotary evaporator and the dark green residue was dissolved in 10 ml acetone and used as a starting stock solution. Further dilutions with either acetone or distilled water were used to prepare different concentrations.

Beauvaria bassiana toxin production

Conidia were harvested from 14-day-old sporulating cultures of *Beauvaria bassiana* by scraping the surface with a spatula suspending the conidia in sterile 0.03% v/v aqueous Tween 80 (BDH) and diluting to 10^8 conidia per ml. of the conidial suspension, 1 ml was used to inoculate 250 ml Erlenmeyer flasks containing 100 ml of Czapek Dox (Oxoid) liquid medium supplemented with 0.5% w/v Bactopetone (Oxoid). Flasks were incubated at 23°C ina cooled orbital incubator at 10000 *r/min* for 12 days. The cultures were harvested by filtering the mycelium through four layers of cheesecloth. The culture filtrate was filtered through a Buchner funnel lined with Whatman No.1 filter paper to ensure removal of conidia and hyphal debris. Culture filtrates were extracted as described by Bandani et al. (1999). This briefly entailed extraction of culture filtrate with Chloroform, filtration of the solvent phase through Whatman No. 1 (Phase separator) filter paper to remove any aqueous residue then removal of solvent on a rotary evaporator. The residue was dissolved in acetone, filtered through a cotton plug, and concentrated under a stream of dry nitrogen at 40 °C. The residue was weight and stored at 4 °C.

Bioassay

Five concentrations of *Artemisia annua* extract, *Beauvaria bassiana* secondary metabolites, Pyriproxyfen, Metoxyphenozide and Buprofezin were used for toxicity and evaluation of LC_{50} values along with a control treated with acetone. In each experiment 30 insects were tested with 5 replicates for each concentration. Insects were treated topically with 2 µl of each concentration on the third thoracic sternum of adults using a microapplicator. Mortality was recorded at 24 and the LC50 was calculated using Polo-Pc software (1987).

RESULTS

The LC values, confidence limit (95%) and regression slope at 24 h exposure to plant extract are shown in Tables 1, 2 and Figure 1. The LC_{10}, LC_{30}, LC_{50} and LD_{90} for plant extract, fungus secondary metabolites, Pyriproxyfen, Metoxyphenozide and Buprofezin were 10, 15, 25, 80; 20.38, 31.75, 43.16, 91.43;

2031, 4584, 8056, 31094; 4256, 6831, 9479, 21108; 631.45, 1508, 2775, 12045, respectively. Comparison of LC_{50} values showed that *A. annua* extract, *B.bassiana* secondary metabolites and Buprofezin were more effective than pyriproxyfen and metoxyphenozide because of lower amount of LC_{50} (Table 1).

Comparison of mortality percentage of this chemicals demonstrated the significant differences and the most percentage (100%) was observed in *A. annua* extract, *B. bassiana* secondary metabolites and Buprofezin treatments (Figure 1). The toxic effect, however, increased with increase in the concentration of extract and duration of exposure to the treated concentrations (Figure 1).

DISCUSSION

Secondary organic compounds synthesized by plants have an important role in protecting plants against insect pests. These compounds affect insects by being toxic causing a delay in larval growth and can act as antifeedant (Isman, 2006). Our results show for the first time, that the methanolic extract of *A. annua* has insecticidal effects on *E. integriceps*. The insecticidal characteristic of *A. annua* extract on elm leaf beetle was topically applied to adults and our results show that they were susceptible to the leaf extract. Jalali et al. (2005) reported that adults were more sensitive than larvae against several plant extracts. Tripathi et al. (2000) showed that adults of *Tribolium castaneum* were more susceptible to cineole which had been extracted from *A. annua*. Shekari et al. (2008) demonstrated that adults of elm leaf beetle were more susceptible to the leaf extract of than larvae *A. annua*. The ethanol extracts from *A. hippocastanum*, A. glutiosa, *H. androsaemum*, and *A. absinthum* have been shown to possess insecticidal and insect repellent components and these protect the Pistacia tree from insect damage (Erturk, 2006). 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 substances found in different plant species could be discovered.

This study shows for the first time that *B. bassiana* secondary metabolites at relatively low concentrations have insecticidal properties against treated insects. Presumably the epicuticular waxes may assist the uptake of the toxin through the host cuticle as LC_{50} values were significantly lower in all individuals. All the larvae of both insect species died at 24 h at high dose suggesting that these insects absorb the toxins through the cuticle. The toxic effect however increased with increase in the concentration of extract and duration of exposure to the treated concentrations (Figure 1). These observations are in agreement with the report that fungi used in the biological control of pests have to be able to elaborate metabolites harmful to the pests (Zahner et al., 1983). Bandani et al. (1999) showed that efrapeptins extracted from fungus *Tolypocladium* sp. has a significant insecticidal, antifeedant and repellency effects on larvae of *Galleria melonella* Essien (2004) showed that the *Aspergillus niger* extract exhibited a moderate toxicity or low killing effect on *Chrysomya chloropyga* larvae. Although the exact models of toxicity was not determined in the present study, earlier investigations have shown that fungi, particularly the *Beauvaria* spp posses the ability to elaborate harmful metabolites which can induce acute and chronic toxicological effects on insects. The killing effect of *B. bassiana* on *E. integriceps* larvae may be attributed to the mould ability to elaborate toxic chemicals such as oxalic acid and oxalate associated with the related species *B. bassiana*.

With regard to marked differences in the development of insects and vertebrates, the growth and development regulators (IGRs) fulfill, to a considerable degree, the requirements of high selectivity and low toxicity (Dhadialla et al., 1998; 2005). Pyriproxyfen, metoxyphenozide and buprofezin induce changes associated with larvae shedding, from the earliest developmental stages. Larvae cannot shed their old skin and move to the following developmental stage. Those of them that survive have an abnormal shape and some produce deformed stages. In a study, Fenoxycarb, a juvenile hormone analog, was tested in the laboratory at three concentrations for toxicity to eggs, three larval instars and pupae of *Chrysoperla rufilabris* (Burmeister). Significant effects of fenoxycarb on all immature stages of *C. rufilabris* were found and the degree of effects depends on the stages treated and the concentrations used (Liu and Chen, 2001). Kocisova et al. (2005) showed that diflubenzuron and cyromazine strongly affect the development of housefly larvae from the earliest stages. Pyriproxyfen has shown long-term effectiveness against *Ae. aegypti and Ae. albopictus***.** With pyriproxyfen, mortality can occur in larvae soon after application, but with time, mortality is more often observed during adult emergence (Vythilingam et al., 2005). The regulators of growth and development of insects can be used successfully in the control of flies provided that we can affect the population of the target species in the susceptible stage. Another precondition of sufficiently high effectiveness is the synchronous occurrence of the susceptible insect stage in a time-acceptable interval (from the point of view of the persistence of the active ingredient in the environment). On this assumption, the biorational insecticides can successfully reduce the pest population while other components of insect entomocoenosis that are not in the susceptible stage at the time of intervention remain unaffected. However, the insect regulators usable in practice are not so far as selective on the level of organisms as many authors have assumed (Slama, 1999).

Today, the environmental safety of an insecticide is considered of paramount importance. An insecticide does not have to cause high mortality to target organisms in order to be acceptable. Antifeedant and growth inhibiting activity can therefore be incorporated into other insect control techniques in the strategy of integrated pest management (IPM). Elucidation of the mode of action of chemicals is of practical importance for insect control because it may give useful information on the appropriate formulation types. This study has further confirmed the insecticidal potential of metabolic compound produced by *A. annua*, *B. bassiana*, pyriproxyfen, metoxyphenozide and buprefezin. However the accessibility for large scale use is hindered by lack of detailed information on the chemical stability, photo stability, phytotoxicity and non-target of the active compounds in the metabolites. To enhance our knowledge of these factors routine analytical studies on the active properties and their specific toxicity are necessary. In the future we can expect additional development and more advanced final adjustment of the insecticides and application techniques and obtaining new knowledge about physical-chemical properties that determine their destiny in the environment and in biological systems. It should be stressed that there are no safe insecticides; there are only safe methods of their use.

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Table 1. Toxicity of *Artemisia annua* extract, *Beauvaria bassiana* secondary metabolites on *Eurygaster integriceps*.

Different dosages[1]	*Artemisia annua* extract	*Beauvaria bassiana* secondary metabolites
LC_{10}	10	20.38
95% Confidence interval	5.11-16.83	9.86-28.2
LC_{30}	15	31.75
95% Confidence interval	10.23-29.63	20.51-40.4
LC_{50}	25	43.16
95% Confidence interval	20.98-39.43	32.6-53.32
LC_{90}	80	91.43
95% Confidence interval	68.54-120.06	70.88-153.2
Slope±SE	2.161±0.315	3.932±0.564
X^2 (df)	5.369	4.75
p-Value	6.85	6.97

[1]. Concentration in percent

Table 2. Toxicity of Pyriproxyfen, Metoxyphenozide and Buprofezin on *Eurygaster integriceps*.

Different dosages[1]	Pyriproxyfen	Metoxyphenozide	Buprofezin
LC_{10}	2031.6	4256.92	631.45
95% Confidence interval	1124-2904	2871-5391	213-1112
LC_{30}	4584.73	6831.33	1508
95% Confidence interval	3292-8541	5394-8003	771-2274
LC_{50}	8056	9479.2	2775.86
95% Confidence interval	6362-1031	8105-10838	1744-4019
LC_{90}	31946	21108	12045
95% Confidence interval	21821-60449	17476-28524	7602-27297
Slope±SE	2.142±0.372	3.68±0.625	1.624±0.545
X^2 (df)	2.1157	5.9	3.04
p-Value	0.1948	1.553	0.2952

[1]. Concentration in ppm

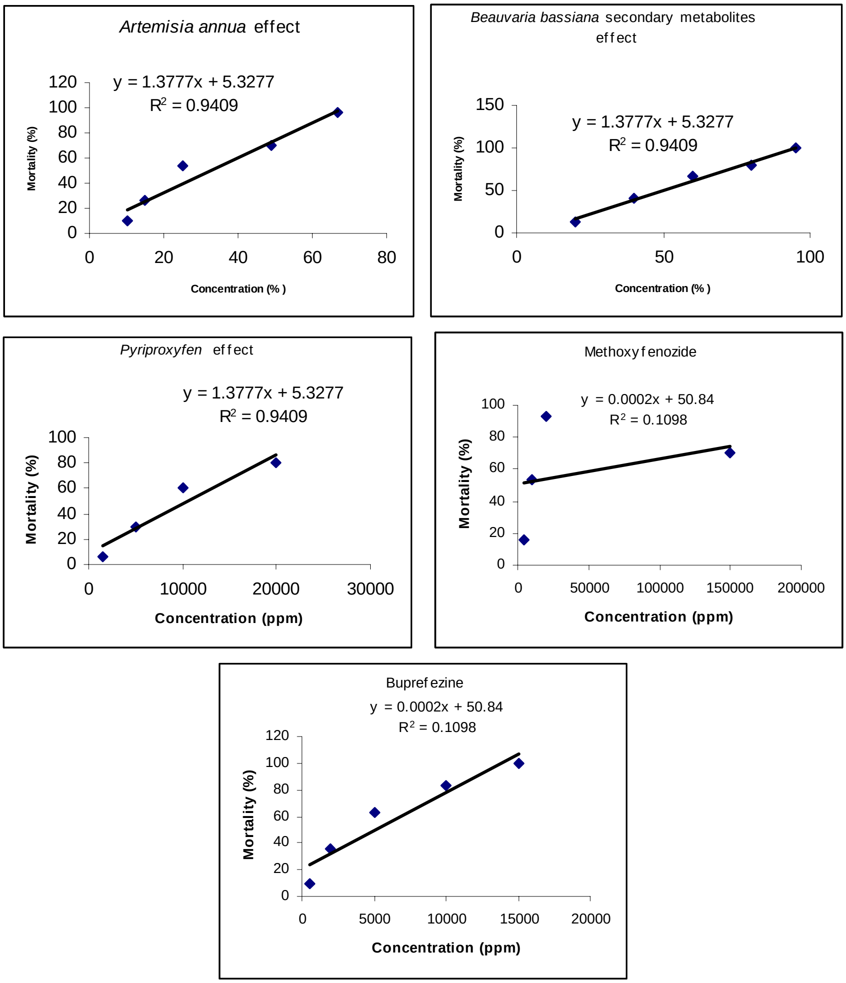

Figure 1. Mortality percentage of different biopesticides on adults of *Eurygaster integriceps*.