THE EFFECT OF REGULATING COMPOUNDS ON THE GROWTH OF *TRIBOLIUM CONFUSUM* DU VAL (COLEOPTERA: TENEBRIONIDAE)

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ABSTRACT: Confused flour beetle, *Tribolium confusum* du Val (Col.: Tenebrionidae) is one of the most important stored product pests of flour in Iran. In this research the effects of insect growth regulators (IGRs): teflubenzuron, hexaflumuron and pyriproxyfen on oviposition rate, emergence of adults, repellency effects of 17 days-larvae and adults of confused flour beetle have been studied. The results showed that, the highest and lowest of inhibitory reproduction were observed in teflubenzuron (88.5%) and pyriproxyfen (77.4%), respectively. All IGRs significantly were reduced the oviposition rate of *T. confusum*.The maximum and minimum of inhibitory emergences of adults were observed in Teflubenzuron (86.26%) and pyriproxyfen (75.73%), respectively. Results showed that, reproduction and adult emergences inhibitories were directly correlated with the IGRs concentrations. Repellency effects of IGRs on adults indicated that, the highest and lowest were recorded for teflubenzuron (48.12%) and pyriproxyfen (39.37%). This effect's experiment on 17-days old larvae showed that, maximum and minimum repellency were in teflubenzuron (47.5%) and pyriproxyfen (36.87%), respectively. These results suggested that IGRs could be used as safe insecticides to control of confused flour beetle.

KEY WORDS: insect growth regulator, teflubenzuron, hexaflumuron, pyriproxyfen, oviposition rate, emergence of adults, repellency of adults and larvae.

In Iran, an average of 10 to 20 percent of agricultural products, stored up in warehouses is wasted by pests (Moharamipour et al., 2003). *Tribolium confusum* is considered as one of the most important storage pests and can cause serious damage to store up products. This pest is more damaging in areas with hot and semisoft climates (Hollingsworth et al., 2002; Songa Reno, 1998). These insects not only cause a lot of damages while feeding, but also infest stored products with their larva layers and excrements and consequently lower the quality of stored products greatly. Adult insects and larva also feed on broken up grains (Bagheri Zenouz, 1995). At present, one of the most common methods of controlling stored up products is using the fumigant compounds such as methylbromide and phosphin. These two pesticides are very toxic for humans and have very severe side effects, therefore they are not used much anymore and are becoming limited (Bell & Wilson, 1995; Daglish & Collins, 1999). Furthermore it has been reported from many countries that the stored up products pests have become resistant against phosphine poison (Marouf et al., 2008). Presently extensive research is being done to find new compounds which cause less damage to the environment. Insect growth regulators like teflubenzuron, hexaflumuron (chitinase inhibitor) and pyriproxyfen (semi juvenile hormone) are among such compounds, which are less poisonous for human and other vertebrates. These compounds are cheap and

can be easily produced on an industrial scale. In addition to these, low resistance of insects to such products and their complete degridation in short time are the most specific characters, (Mc Gregor and Kramer, 1977). Insect growth regulators are suitable for controlling stored up pests (Loschiavo, 1976). Furthermore application of diflobenzuron, chitin inhibitor, can cause 100% decrease in the first generation of adult *Tribuliom confusum* (Mc Gregor & Kramer, 1977). Since Mrize (1986) has shown that using doses higher than 600 ppm of R-20458 compounds in diet of *Sitophilus oryzae* cause infertility of *S. oryzae* species and stop the generation of F1.

In this research, the effect of the two chitin inhibitor, including, teflubenzuron and hexaflumuron and also pyriproxyfen semi juvenile hormone on oviposition and adult emergence and repelling effect in the larva stage of *Tribolium confusum* was studied to find the best compound affecting *Tribolium confusum*.

MATERIALS AND METHODS

In this study the effect of teflubenzuron, pyriproxyfen and hexaflumuron compounds were tested on 15 day larva and adult *Tribolium confusum* insects at agricultural college of Islamic Azad University–Arak branch in 2009-2010.

Rearing of *Tribolium confusum*

An experiment was done by Arak university entomology group at its laboratory to test the *Tribolium confusum*'s population. Mass rearing of this insect was maintained at the growth chamber set at 27 ± 2 °C and 75 ± 5% relative humidity on foodstuff in dark condition containing flour mix with yeast (17:1, w/w) in cylindrical container with height of 18 centimeter and diameter of 8 centimeters.

The rate of inhibitory reproduction (IR)

To examine the effect of toxins on inhibitory reproduction of adults, on seven day adult insects were tested. First, paper filters with concentration of 4, 8, 16, 32 and 64 ppm were floated in compounds (with acetone solvent) and then were transferred to testing containers of 18 centimeter height and with diameter of 8 centimeter. After 30 minutes, to evaporate the acetone with the help on an aspirator, 20 seven day adult insects were released to the containers with define volume, having five grams of wheat flour and yeast (17:1), after 5 days oviposition on flours, the insects were softly eliminated from the containers. The containers were kept in testing conditions of (27 ± 2 °C and 75 ± 5% RH), and the larvae were counted after 20 days.The inhibitory rate was calculated by the following formula (Nazem Rafie et al., 2003).

$$\mathbf{IR}=\frac{CK-T}{CK}\times 100$$

CK: The number of larvae in observer
T: The number of larvae in under care

Effect of the compounds on emergence of adults

7day adult insects used to measure the emergence rate. In this experiment, 5 different concentrations of compounds including 1, 2, 4, 8 and 16 ppm were used. Also in checking the experiment, only acetone solvent was used. One milliliter of each concentration was sprayed on 5 grams of flour and mixed with flour. 20, 7day adult insect were selected and transferred to dishes containing flour and

permitted to feed and lay eggs. After 4 days adults were removed from dishes and were kept in 27 ± 2 °C and 75 ± 5 % RH in incubator. After 40 days the number of adult insects in each dish was counted.

Repellency rate

In this experiment Wattman forty paper filters with the diameter of 8 cm was cut into half from the middle. One half of the paper was floated in specific concentration of insecticide compound (containing three concentration of 65, 250, and 1000 ppm), and the other half was floated only in acetone solvent. After 20 minutes when paper filters were completely dried, paper filter halves which were treated with solvent and compounds were stick together. 20, seven day adult were selected and placed in the center of the paper filter. This experiment was also carried out with 20 and 17 day larvae in a separate experiment; after 24 hours, adult and 17 day larvae of *T.confusum* were counted on each half of paper filters. Repellency percentage of different concentration compounds were calculated using Talukder & Howse formula, 1993 and 1995. (Nazem Rafie et al., 2003).

$$PR=2(C-50)$$

C: Percentage of insects on half of the paper observer filter
PR: Repellency Percentage

Analytical analysis

Each of the experiments executed and repeated under completely randomized design in 4 replications. The data statistics were derived by SAS software and the average of experimental treatments were compared by with Duncan's range test were at a 5 % probability level.

RESULTS

The effect of growth regulating compounds on inhibitory reproduction rate of *T. confusum*

The results of the analysis proved that there is a meaningful relation between studied concentration and inhibitory reproduction. According to the results obtained, teflubenzuron and pyriproxyfen compounds had respectively the most and the least effect on the inhibitory reproduction of Adult *Tribolium confusum* in such a way that with the concentration of 65% ppm the inhibitory reproduction rate is recorded to be 100% and 93.28 % respectively. Based on the results, in 65% ppm and 32% ppm concentrations the teflubenzuron compound, and in the concentration of 65 ppm the hexaflumuron compound had the 100 % inhibitory reproduction.

According to the data obtained the least amount of inhibitory reproduction is from pyriproxyfen compound showing 58.95% inhibitory reproduction at concentration of 4 ppm .It was also observed that teflubenzuron compound did not make a significant difference at concentration ranges of 16, 32 and 56 ppm. Also, at concentrations of 32 and 65 pyriproxyfen did not show any significant difference either. The experiment showed that there weren't any significant differences between 8 ppm Teflubenzuron, 8ppm Hexaflumuron and 16 ppm pyriproxyfen compounds. The obtained data also showed that there was not any significant difference between 4 ppm compounds tested.

The effect of growth regulatory compounds on inhibitory emergence of adult of *T. confusum*

Analysis results showed that the highest inhibitory rate on *T. confusum* was caused by teflubenzuron compound, so that this compound at 16 ppm concentration made the inhibitory emergence rate of adult insects increase to 98.66 percent and the lowest inhibitory rate related to pyriproxyfen compound, so that this compound at 16 ppm increased the inhibitory rate to 93.3 percent. According to data obtained, the inhibitory rate of 16 ppm hexaflumuron was 96 % which showed a significant difference compared to 1, 2 and 4 ppm. Results showed that the inhibitory rates of teflubenzuron, hexaflumorun and pyriproxyfen compounds at 16 ppm were 98.66 and 93.33 %, respectively, which did not show a significant difference. The inhibitory rate of 4 ppm teflubenzuron was 90% which did not have a significant difference compared to 8 ppm teflubenzuron. Also the inhibitory rate of this compound at 2 ppm was found 80% which did not show any significant difference compared to 4 ppm hexaflumorun and pyriproxyfen compound with inhibitory rate of 84 and 81.33 %, respectively. At 1 ppm concentration hexaflumorun and pyriproxyfen inhibitory emergence rates were 50.66 and 46 percent respectively which showed that there wasn't any significant difference between these two compounds at this density.

The comparison percentage of repellency effect of compounds on 7 day adult of *T. confusum*

The results of the repellency test of compounds on adult *T.confusum* insects showed that teflubenzuron compound had the highest repellency percentage, compared to hexaflumuron and pyriproxyfen compounds .Furthermore the pyriproxyfen compound showed the lowest repellency percentage. The variance analysis results showed that the teflubenzuron repellency percentage shows a significant difference compared to the other two compounds but there wasn't any significant difference between hexaflumuron and pyriproxyfen compounds, so have been put in the teflubenzuron compound showed 95 recent repelling at 1000 ppm (Fig. 1). Pyriproxyfen compound showed 35% repellency at 65 ppm was the lowest rate. In a comparative study, at 250 ppm, teflubenzuron compound had the highest effect on 7 day adult *T. confusum* insects compared to hexaflumuron and pyriproxyfen compounds, with the rate 65%. Also at this concentration hexaflumuron and pyriproxyfen compounds, both showed 52.5% repellency, which overall all three compounds didn't show a significant difference at this concentration. Variance analysis of repellence percentage of compounds showed that compound mutual effect concentrations are significant. Results showed that teflubenzuron compound at 1000 ppm have a higher repellency compared to other compounds tested, but at 1000 ppm, hexaflumuron and pyriproxyfen compounds didn't show a significant difference at level of 5 percent. Also at 65 ppm hexaflumuron, teflubenzuron and pyriproxyfen at level of 5% didn't show any repellency percentage.

Comparison of repellency percentage compounds on old *Tribolium confusum* larvae

The result of this repellency percentage test on *T. confusum* larvae showed that Teflubenzuron compound had the highest and pyriproxyfen compound had the lowest repellency percentage. According to statistical analysis results, there was no significant difference between hexaflumuron and pyriproxyfen compounds at 5 percent level and they were placed in one group. Variance percentage analysis of repellency percentage of compounds being studied showed

that the mutual effects of compounds and concentration were significant. This study showed that teflubenzuron compound at 1000 ppm had the highest repellency percentage compared to other who compounds and increased repellency rate to 98.5 % and this compound at this concentration showed a significant difference in repellency; this repellency percentage was higher than those in hexaflumuron and pyriproxyfen compounds, which shows its higher strength in repellency. At 250 ppm concentration teflubenzuron compound with 67.5% repellency did not show significant difference compared to the repellency percentage of hexaflumuron and pyriproxyfen compounds at 1000 ppm concentration regarding the *T. confusum* larvae. At 1000, 250 and 65 ppm concentration of hexaflumuron and pyriproxyfen there was no significant difference in their repellency percentage (Fig. 2). Also hexaflumuron, teflubenzuron and pyriproxyfen compounds being tested did not show a significant difference at 65 ppm.

DISCUSSION

According to the studies on inhibitory reproduction, it was observed that as the concentration increases, the inhibitory reproduction increases the oviposition as well, this is in agreement with previous results obtained (Loschiavo, 1976), pointing out that with the increasing the concentration of inhibitory growth compounds being used, prevents adult oviposition. Also according to the studies done, it was observed that inhibitory compounds for chitin synthesis in high concentrations, stop oviposition hundred percent in the performed experiments. These results is also in agreement with obtained results (Nawrot et al., 1987) showed that using teflubenzuron decreases *T. confusum* oviposition hundred percent. According to these studies insect growth inhibitory compounds play a role in oviposition inhibitory which agrees with the results obtained by Leschiavo in 1976. Results relate to the inhibitory effect of anti-chitin synthesis compound is more than that of be a Babu et al. (1991), which reported that triflumuoron and flucydoxuroun compounds at 20 ppm concentration reduce *Callosobruchus chinensis* oviposition at the rate of 75 and 80% respectively, While teflubenzuron and hexaflumuron inhibitory oviposition at 16 ppm concentration where 93.93% and 86.86% respectively. The results of this experiment also indicated that anti-chitin synthesis compounds have a high effect on inhibitory of the oviposition compared to synthetic juvenile hormone compounds which is in agreement with the results obtained (Eisa et al., 1986) pointing out the high effect of inhibitory chitin synthesis toxins in *T. castaneum* . Results from this study, agree with the effects of triflumuron on *T. castaneum* oviposition rate (Eisa et al., 1984). In these experiments mutual effect and concentration where not found significant which leads to the conclusion that regardless of the kind of the compound, concentration increase, leads to higher percentage of inhibitory rate .Results are obtained from the effect of inhibitory corrupt compounds on inhibitory growth rate of adult insects show that teflubenzuron and pyriproxyfen have the highest and lowest effect on emergence rates of adult *T. cofusum* insect respectively, so that at 16 ppm they show 96.66% and 93.33% inhibitory rate, respectively. Methoprene is highly effective in reducing adult *Plodia interpunctella* emergence after larval exposure (Jenson et al., 2009).

Also similar experiment by Mulla and Main (1982) showed that using triflumuron compound at five ppm concentration cause 92.5% reduction of the first generation for *T. castaneum*, for *Oryzaephilus surinamensis* 97.5% and for *R. dominica* it was 100%. This research showed that in low concentrations of

teflubenzuron the inhibitory emergence rates of adult insect was more than that of RH-5849 and triflumuron. Since Ammar (1988) has shown that chlorfluazuron, flufenozuron and 473 compounds with concentration of 0.5-25%, on adult *Sitophylus oryzae*, caused 91% - 98% reduction of the first generation within eight months after. Also five month after treatment, the reduction of first-generation of *S. oryzae* was 76/54 – 100%, while this research showed that the two anti-chitin synthesis, Teflubenzuron and Hexaflumuron compound and Pyriproxyfen, synthetic juvenile hormone, will cause a 93%- 98% reduction after four and five months, respectively. The results of the experiments performed, showed that increasing the concentration of growth inhibitory compounds in the insect diets, decreases the emergence rates of healthy insects, in such a way that the reduction trend continued up to 2 ppm concentration. In Teflubenzuron compound concentrations higher than 16 ppm and in Hexaflumuron compounds higher than 32 ppm no adult emerged. Since Eisa et al. (1986) has shown that chlorofluazuron and flufenoxuron compounds at densities of 1 and 10 ppm where tested on *T. castaneum* and no adults emerge. Pyriproxyfen compound at densities higher than 8 ppm a percentage of adult emerged with the incomplete formation. At densities from 2 to 16 ppm, besides the emergence of complete insects which appear to have no unnatural characteristics in their limbs, a percentage emerged as unnatural insects as well. In these insects unnatural characteristics were seen more in the front wings, in such a way that we are sure to deform and twisted in odd figures (asymmetry on left and right wings) were observed. Furthermore, sometime the formations were so severe that insects could not even walk. Sometimes the wings and feet were deformed in such an extent that the emergence of the insect out of its pupa is almost impossible half way through which causes the insect to die inside. Also, the internal and external organs of existing pupa, in a specific concentrations, have been deformed in such a way that the larvae dies.For example the similarity of pupa and larva's head. The general conclusion of the discussion clarifies the point that anti-chitin synthesis teflubenzuron and hexaflumuron compounds are more effective than pyriproxyfen synthetic juvenile hormone compounds on emergence of adults and also along with the increase in diet concentration compounds, which larva and pupa periods go through it, the rate of complete emergence would reduce to that extent that it higher concentrations than 16 ppm there is no adult emergence which can be due to prolonged larval growth as well as interference with metamorphosis or the death of pupa.

In this research the effect of insects growth preventing compounds on adult insect exit rate was studied and it was shown that teflubenzuron had the highest repelling rate in such a way that in 1000 ppm this rate was recorded to be 98.7% and 97.4% on adult *Tribolium confusum* larvae and adult insect, respectively .In a similar experiment by Liu & Ho (1999) the *Evodia rutaecrpa* herbal oil had the highest repelling factor with 88.5 %. In this experiment the lowest percentage was for pyriproxyfen in such a way that in 65 ppm concentration this rate was 47.9 % and 48.5 % for *Tribolium confusum* larvae and adult insects respectively. The difference between these repelling compounds can be due to the fact that the fumigant activity of pyriproxyfen is much less than teflubenzuron compound and as a consequence its repelling property is low as well or the teflubenzuron compound odor is more repelling in comparison to that of the pyriproxyfen. The other reason that can be accounted for teflubenzuron more repelling property is that probably the effective compound can strongly repel the insect. This is in agreement with the results obtained (Xie et al., 1996), pointed out that *Aphanamixis polystachya* could strongly repel *T. castaneum*. The repellency

effect of *Melia toosendan* which can strongly repel adult *T. castaneum* could be attributed to its major constituent, tousendanin (Nazem Rafie *et al*., 2003). Studies conducted to test the repellency of pea, *Pisum sativum* (L.), products against *Tribolium castaneum* (Herbst.), *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) showed that paddy grains treated with 1% concentration were repellent to adults. *Tribolium castaneum* was repelled most quickly followed by *S. oryzae* and *R. dominica* (Pretheep Kumar et al., 2004).

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Table 1. Shows the effect of regulating compounds with different concentrations on the inhibitory reproduction of adult *T.confusium* under the laboratory simulated conditions.

concentration	Teflubenzuron	Hexaflumuron	Pyriproxyfen
4 ppm	ef 58/2±67.91	f 2.58±64.92	f 3.41±58.95
8 ppm	cd 4.66±79.10	ed 2.58±76.11	ef 5.92±68.65
16 ppm	ab 7.75±95.52	ab 5.17±90.29	cd 17.09±79.10
32 ppm	a 0.00±100	ab 6.46±94.20	cb 4.66±87.31
65 ppm	a 0.00±100	a 0.00±100	ab 5.92±93.28

1- Means ± SE
2- Means within column followed by the same letter not found significant (P<0.05, DMRT)

Table 2. The effect of growth regulatory compounds with various concentrations on inhibitory emergence of adult of *T .confusum* in laboratory conditions.

Concentration	Teflubenzuron	Hexaflumuron	Pyriproxyfen
1 ppm	e 5.77±66.66	f 9.01±50.66	f 4.00±46.4
2 ppm	d 4.00±80	e 1.15± 70.66	e 2.00±68
4 ppm	bc 0.00±90	cd 3.46±84	d 1.15±81.32
8 ppm	ab 2±96	b 3.05±90.66	bc 2.00±90
16 ppm	a 2.3±98.66	ab 2.00±96	ab 1.15±93.33

1- Means ± SE
2- Means within column followed by the same letter not found significant (P<0.05, DMRT)

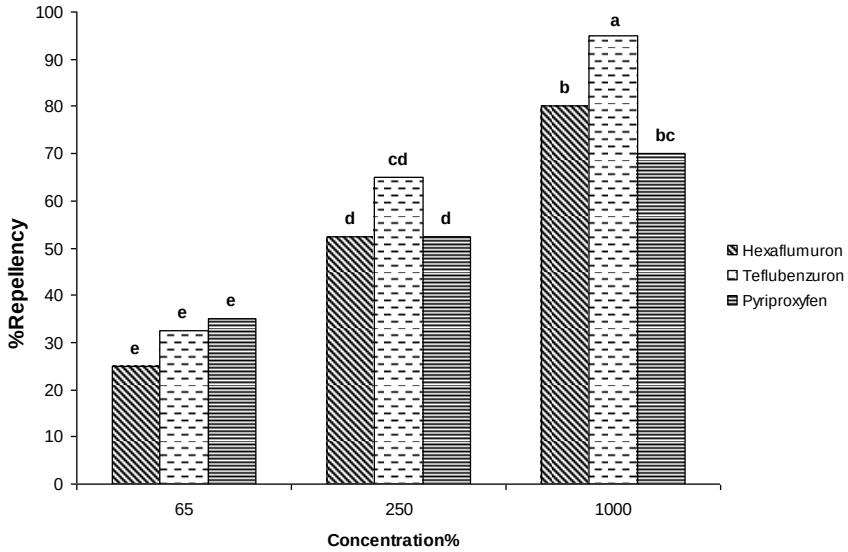

Figure 1. Repellency rate of 7 day adult *T.confusum* insects by different concentrations of Hexaflumuron, Teflubenzuron and Pyriproxyfen compound

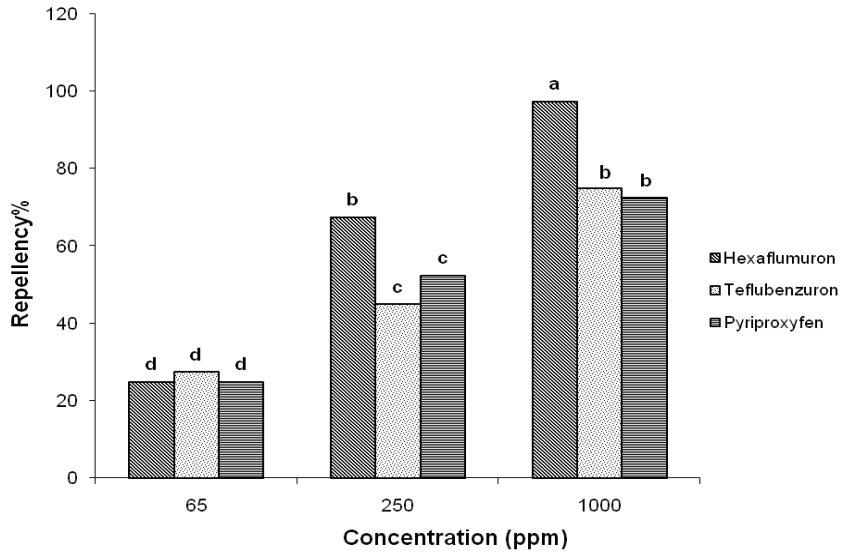

Figure 2. Old *Tribolium confusum* larvae repellency rate in various concentrations of Hexaflumuron, Teflubenzuron and Pyriproxyfen compounds