ABSTRACT

Title of thesis: Bioefficacy and Residue Study of Lufenuron on Tribolium castaneum (Herbst) (Coleoptera : Tenebrionidae)

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INTRODUCTION

A new approach to insect control is the use of substances that adversely affect insect growth and development. These substances are classified as ‘insect hormone mimics’ or ‘insect growth regulators’ (IGRs) owing to their effects on certain physiological regulatory processes essential to the normal development of insects or their progeny. They are quite selective in their mode of action and potentially act on target species. An IGR does not necessarily have to be toxic to its target, but may lead instead to various abnormalities that impair insect survival (Siddall, 1976). Interestingly, most of the IGRs that have shown effectiveness against insect pests cause the rapid death of the insect through failure of a key regulatory process to operate or function. IGRs generally control insects either through regulation of metamorphosis or interference with reproduction (Riddiford and Truman, 1978). Compounds developed to disrupt metamorphosis ensure that no reproductive adults are formed. Those that specifically interfere with reproduction may include the development of adults with certain morphogenetic abnormalities that reduce their reproductive potential. Adults may be
sterile or possess abnormally developed genitalia, which hinders the mating process or the capacity to produce fertile offspring.

Since the target sites of common insecticides on insects and mammals are known to be similar, it is desirable to develop insecticides whose primary target site does not exist in mammals for selective toxicity. IGRs may belong to this type of (selective) insecticides and can be grouped according to their mode of action, which are as follows: chitin synthesis inhibitors (i.e. of cuticle formation) and substances that interfere with the action of insect hormones (i.e. JHs, ecdysteroids).

In addition to their short environmental persistence and low toxicity to vertebrates IGRs have several characteristics that make them potentially successful alternative to conventional insecticides. The discovery of benzoylphenylureas during the early 70’s was an important step towards the development of new group of insecticides, which exert their action on the insect integument. Subsequently several compounds of this type have been evaluated for their insecticidal action against a wide range of insect pests.

Lufenuron (Match®) is an acylurea insect growth inhibitor that interferes with chitin biosynthesis. Like other benzoylphenylureas it acts mainly by ingestion (Anonymous 1997). Buholzer et al. (1992) observed that lufenuron ingested pest larvae ceased feeding, stopped growing and finally died. Similar exposure did not affect adults. Lufenuron was found to be more selective control product than traditional organophosphate sprays (Whiting et al. 2000). Further, it has been reported that lufenuron is suitable for integrated pest management (IPM) programs because of its long residual action and safety to adult beneficial insects, mites and spiders (Anonymous 1998). Lufenuron has been reported to be effective against number of serious pests of
fruit crops such as *Epiphyas postvittana* the light brown apple moth (Whiting *et al.* 2000). The effect of lufenuron on potato tuber moth, *Phthorimaea operculella* (Zeller) eggs was studied by Emmanuel *et al.* (2000). Lufenuron was found to be effective against many pests of horticulture (Buholzer *et al.* 1992). However sublethal effects of lufenuron on stored product pests have not been reported so far. Similarly, residual life of lufenuron in wheat flour has not been reported so far with respect to its bioefficacy and concentration.

The present endeavor was to study and investigate the effect of sublethal doses (LC$_{10}$, LC$_{20}$ and LC$_{40}$) of lufenuron on the various stages in the life cycle of *Tribolium castaneum* with respect to growth, development and adult reproductive end points. Along with the above mentioned effect of lufenuron on the life cycle of *T. castaneum*, field simulated experiments were carried out to assess the feasibility of its use in IPM program, hence residue analysis studies by GC-MS technique was carried out. These studies describe the development of a new sensitive GC-MS method for analysis of trace levels of lufenuron in the wheat flour. The aim of the residue analysis study was to evaluate the effect of lufenuron treated and milled wheat on the survival of *Tribolium castaneum* and simultaneous determination of residual concentration of lufenuron in wheat flour as a function of time.

The thesis has been divided into two aspects. A) Biological studies on the effects of sub-lethal concentrations of lufenuron on *T. castaneum* & B) Time dependant residue analysis of lufenuron in wheat flour by GC-MS along with bio-efficacy studies.
Materials

Insect: Red flour beetle, *Tribolium castaneum* (Herbst), a stored grain pest.

Source: From stock culture at N. C. L. Entomology laboratory, Pune.

Insect diet: Wheat flour, 5% Brewer's Yeast i.e. 100gm wheat flour and 5gm Brewer's yeast.

Culture: Culture of *T. castaneum* was maintained on a diet at a temperature of 30 ± 1°C with 60% relative humidity in a Remi's® cooling incubator.

Insect growth regulator: Lufenuron (MATCH®)

Formulation: 5% E.C. (Emulsified concentrate).

CGA Number: 184699

Source: SYNGENTA INDIA Ltd., Mumbai, India.

Use type: Insecticide

Chemical class: Benzoylphenylurea.

Mode of action: Molt inhibitor.

IUPAC Name: N\{2,5-dichloro-4-(1,1,2,3,3,3-hexafluoropropoxyl)phenylamino\}carboryl\}-2,6-difluoro-benzamide (CA)

Chemical Structure:
A) Biological studies

METHODOLOGY (using wheat flour)

A stock culture of *T. castaneum* was maintained on a diet containing wheat flour and 5% Brewer's yeast, at 30 ± 1°C and 60 % relative humidity. Eggs were collected by sieving, (sieve number 40) diet infested with adults. Newly emerged adults were obtained by collecting pupae and monitoring them for adult emergence. A stock solution of lufenuron (2.5µl in 50 ml) acetone was prepared. Different amounts of lufenuron from stock solution were thoroughly incorporated into the diet. The treated flour was kept at room temperature for twenty four hours, for complete evaporation of the solvent before use in the experiments. Determination of LC$_{50}$ through diet was carried out by releasing two day old larvae of *T. castaneum* in diet treated with various concentrations of lufenuron. Acetone treated diet was used as control. The control and experimental batches were kept in a cooling incubator at 30°C before and after the treatment. For each concentration tested sets of five replicates of twenty larvae each were taken. The mortality count was taken after seven days. Subsequently, the sub-lethal doses (LC$_{10}$, LC$_{20}$ and LC$_{40}$) were deduced by extrapolation from the regression line obtained by probit analysis.

1. Effect on growth and development:

Effect of sub-lethal concentrations (LC$_{10}$, LC$_{20}$ and LC$_{40}$) of lufenuron through diet on survival and metamorphosis of the larvae was examined by releasing 2 days old larvae (20 nos. per concentration) in the treated diet. Acetone mixed diet was used as control. The experiment was replicated five times. After 24 hours, the larvae were transferred to normal diet. On seventh day after the start of the experiment, the larvae...
were weighed ten at a time and their survival was recorded. Once pupation had begun in any treatment, observations were made every day for adult emergence. Percentage pupation, time taken for pupation, percent adult emergence and time taken for adult emergence were recorded. Regression analysis was performed to determine dose dependent effects.

2. Effect on fecundity and fertility of adults:

To study the effect of sub-lethal concentrations, LC_{10}, LC_{20} and LC_{40} of lufenuron (as obtained for two day old larvae) through diet, on adult’s reproductive potential, newly formed pupae were isolated and sexed (Sokoloff, 2001). Both male and female pupae have a pointed structure at the posterior end of their abdomen, these are urogomphi. Just above this structure ventrally are present genital papillae. The female papillae, which are much larger than those of male, are two finger-like structures anterior to the pointed urogomphi. The male papillae are enough smaller that they look like just fingertips rather than fingers. Two days after adult emergence a pair of male and female was kept in separate vials containing diet treated with sub-lethal concentrations of lufenuron for 48h. Then they were transferred to normal diet for mating and egg laying. Acetone treated diet was used as control. The crosses were performed as follows:

Untreated females × untreated males
Treated males × untreated females
Treated females × untreated males
Treated males × treated females
All experiments were replicated five times. Eggs were collected after 48h to record fecundity, fertility and survival of larvae. Data was analyzed by one way ANOVA.

3. Effect on hatching of eggs:

Effect of sub-lethal concentrations (LC$_{10}$, LC$_{20}$, LC$_{40}$) as obtained for two day old larvae) of lufenuron through diet on the hatchability of eggs was determined by placing ten eggs in treated diet and recording hatching of eggs every day, till hatching in the control was completed. Acetone treated diet was used as control All experiments were replicated five times. Data was analyzed by one way ANOVA.

4. Effect of topical application of lufenuron on pupae:

Newly formed pupae were obtained by collecting last instar larvae and monitoring them for pupal formation. Effect of various lufenuron concentration on newly formed and three day old pupae was monitored by topically applying 0.005, 0.0005, 0.00025, 0.00016, 0.000125, and 0.0001 µg /µl per pupae in acetone with a Hamilton micro-syringe on the ventral surface of pupae. Control pupae were similarly treated with acetone. The dispensing volume was always 1µl. Each experiment was replicated five times with ten pupae per replicate per concentration and monitored for % mortality, time taken for adult emergence, % abnormal adults such as pupal-adult intermediates and % normal adults

5. Effect of sublethal concentrations of lufenuron on female reproductive system:

To study the effect of sub-lethal concentrations, LC$_{10}$, LC$_{20}$ and LC$_{40}$ of lufenuron (as obtained for two day old larvae) through diet, on adult’s reproductive system, the newly formed pupae were isolated and sexed (Sokoloff, 2001). Two days after adult emergence a pair of male and female was kept in separate vials containing diet treated
with sub-lethal concentrations of lufenuron for 48h. Acetone treated diet was used as control. Then they were transferred to normal diet for mating and egg laying. After three days of feeding adult females were dissected and the female reproductive system was isolated and fixed in Bouin’s fluid. The reproductive systems were photographed by using a Olympus Stereo Zoom Microscope (Model:SZX) with micro-photographic attachment at constant magnification.

6. Field simulated experiments

a) Treatment of sacks and effect on egg laying:

Small gunny bags made out of jute measuring 4”x 6” were treated with sublethal concentrations of lufenuron (LC10, LC20 and LC40). Acetone treated bags were used as control. Twenty five grams of wheat (variety Lok1) was stored in each bag along with two day old five males and five females per concentration for 10 days. The experiment was replicated three times. The After ten days microscopic examination of wheat was carried out to see the effect of treated sacs on egg laying.

b) Treatment of wheat and effect on egg laying:

Different sublethal concentrations of lufenuron (LC10, LC20 and LC40) were used to treat the wheat. Acetone treated wheat was used as control. 25 grams of wheat was used for each experiment. For complete evaporation of acetone, treated wheat was dried for 24hrs before introducing two day old five male and five female adults. Each experiment was carried out three times. Infested wheat was stored for 10 days. After ten days microscopic examination of wheat was carried out to see the effect of treated wheat on egg laying.
c) Treated and crushed wheat and effect on egg laying:

Wheat was treated similarly as mentioned above. After complete evaporation of acetone i.e. after 24 hrs it was crushed by using a Waring blender. Crushed wheat was stored in a beaker along with five male and five female insects for 10 days. Acetone treated crushed wheat served as the control. The experiment was replicated three times. After ten days microscopic examination of crushed wheat was carried out to observe the effect on egg laying.

d) Treated and milled wheat effect on larval survival:

Wheat was treated with different sublethal concentrations as mentioned above and kept for 24h after which it was milled. Milled wheat was fed to two day old larvae, ten per replicate for 24h. After which the larvae were transferred to normal diet and observed for survival. Acetone treated milled wheat served as control. The experiment was replicated three times.

e) Effect of treated wheat on the reproductive potential of adults:

Wheat was treated with different sublethal concentrations as mentioned above and kept for 24h after which it was milled. A pair of male and female (two day old) was introduced for two days. Then they were transferred to normal diet for egg laying. Eggs were collected after every 48 hrs. The hatching percentage and % survival was recorded and compared with the control. The experiment was replicated four times.
B) Residue analysis of lufenuron by GC-MS

METHODOLOGY

Preparation of Lufenuron Standards for GC-MS analysis:

MATERIALS AND METHODS:

1. Chemicals and biological materials:

Lufenuron (99% technical) and EC 5% formulation (Match 5®) was obtained from Syngenta India Ltd., Mumbai, India, as a kind gift. Ethyl acetate (99%) and anhydrous sodium sulfate (99%) were obtained from Qualigens, India and Primary Secondary Amine (Bondesil-PSA), 40 μm, from Varian, USA. All the reagents and solvents were HPLC grade or more and filtered through 0.45 μm Nylon filter paper before use. Wheat used was of Indian Lok I variety.

2. Extraction and Clean up Lufenuron from wheat flour:

Wheat flour (25 g) was weighed in centrifugation bottle and 30 g sodium sulfate was added in it and extracted with 50 ml of ethyl acetate. The mixture was homogenized (Heidolf, Germany) at 15,000 rpm for 3 min. Subsequently centrifuged for 5 min at 5000 rpm at 4°C. Two ml extract supernatant was removed in eppendorff tube pre-filled with 50 mg PSA and centrifuged at 12,000 rpm for 5 min at 4°C. Supernatant thus obtained was filled in the vial for GC-MS analysis.

3. Preparation of Lufenuron Standards for GC-MS analysis:

Lufenuron (99%), was weighed accurately using an analytical electronic balance (Mettler, AX-205) in 10 ml volumetric flask and dissolved in ethyl acetate to make 1000 ppm stock solution. Working standard solutions were prepared by serial dilution with ethyl acetate to make desired concentration range of lufenuron. All solutions were kept at
\(-20^\circ C\) and fresh working standards of 100 to 500 ng/ml were prepared each time from 1000 ppm stock solution to investigate assay linearity for GC-MS.

4. **Gas Chromatography-Mass Spectrometry:**

GC-MS system consisted of Thermo Finnigan Trace GC Ultra with TriPlus autosampler equipped with PolarisQ Ion Trap MS/MS detector (Thermo Electron Corporation, Italy) controlled by Xcalibur software. The following optimized conditions were used: The injections of 15 \(\mu\)L were done by using Triplus autosampler with 4 pre-injection washes and 4 post injection washes with make up solvent. The samples were injected using a programmable temperature vaporization injector, large volume (PTVI-LV) mode with base temp. \(60^\circ C\), split flow 100ml/min., splitless time 1 min., solvent valve temp \(100^\circ C\), surge pressure 3 kPa, inject pressure 70 kPa, inject time 0.1 min., vent flow 30 ml/min, evaporation pressure 140 kPa, evaporation rate 14.5deg/sec, evaporation temp \(85^\circ C\), evaporation time 1 min, transfer pressure 210 kPa, transfer rate 14.5deg/sec, transfer temp. 280 \(^\circ C\), transfer time 3 min, clean rate 14.5deg/sec, clean temp 285\(^\circ C\), clean time 10 min, clean flow 20 ml/min. The chromatographic separation of Lufenuron was performed on Mega 5 MS column (Mega Capillary Columns Laboratory, Italy.) with 30 m length x 0.32 mm internal diameter with 0.5 \(\mu\) film thickness. Carrier gas was Helium with 99.9% purity with flow rate of 1ml min\(^{-1}\). Oven was programmed with 50\(^\circ\)C initially, ramped (1) at 15\(^\circ\)C/min to 130\(^\circ\)C held for 0 min, further ramped(2) at 30\(^\circ\)C/min to 182\(^\circ\)C-0 min. and then ramped (3) at 0.3 \(^\circ\)C/min to 184 \(^\circ\)C and held for 1 min. (Total run time 14.76 min). Electron Impact ionization was achieved with 70eV ionization energy with source temperature 230\(^\circ\)C and auxiliary temp. 285\(^\circ\)C. Positive mode full Scan was performed with mass range \(m/z\) 50-450 with the solvent delay of 6 min.
5. **Residual life of lufenuron in wheat flour:**

Lufenuron residue in the treated and milled wheat was examined simultaneously at an interval of 15 days initially and 30 days subsequently for a period of 90 days by using GC-MS.

6. **The effect of residual lufenuron on larval survival:**

Determination of LC$_{50}$ of lufenuron was carried out by feeding two day old *T. castaneum* larvae with wheat flour treated with various concentrations of lufenuron. LC$_{50}$ x 5 was the dose of lufenuron selected for treatment of wheat, which was thoroughly incorporated into wheat using water as carrier solvent. (final conc. of lufenuron in treated wheat was 0.0875 ppm). The treated wheat was kept for 24 hrs at 30°C for complete evaporation of the solvent before milling. To study the residual action of lufenuron on the survival of *T. castaneum* larvae, twenty larvae (two days old) were periodically released on 5 gm of treated and milled wheat at every ten days interval for 90 days. All the experiments were carried in triplicates at 30 ± 1°C and average 60% relative humidity.

Lufenuron (99%), was weighed accurately using an analytical electronic balance (Mettler, AX-205) in 10 ml volumetric flask and dissolved in ethyl acetate to make 1000 ppm stock solution. Working standard solutions were prepared by serial dilution with ethyl acetate to make desired concentration range of lufenuron. All solutions were kept at -20°C and fresh working standards of 100 to 500 ng/ml were prepared each time from 1000 ppm stock solution to investigate assay linearity for GC-MS.

**Residue analysis of Lufenuron:**

It was carried out with treated and milled wheat by developing a Gas Chromatography-Mass Spectrometry (GC-MS) technique and monitoring its bio-efficacy
was carried out against *Tribolium castaneum*. Lufenuron residue in the treated and milled wheat was examined simultaneously at an interval of 15 days initially and 30 days subsequently for a period of 90 days by using GC-MS consisting of Thermo Finnigan Trace GC Ultra with TriPlus autosampler equipped with PolarisQ Ion Trap MS/MS detector (Thermo Electron Corporation, Italy) controlled by Xcalibur software. The following optimized conditions were used: The injections of 15 μL were done by using Triplus autosampler with 4 pre-injection washes and 4 post injection washes with make up solvent. The samples were injected using a programmable temperature vaporization injector, large volume (PTVI-LV) mode with base temp. 60 °C, split flow 100ml/min., splitless time 1 min., solvent valve temp 100 °C, surge pressure 3 kPa, inject pressure 70 kPa, inject time 0.1 min., vent flow 30 ml/min, evaporation pressure 140 kPa, evaporation rate 14.5deg/sec, evaporation temp 85°C, evaporation time 1 min, transfer pressure 210 kPa, transfer rate 14.5deg/sec, transfer temp. 280 °C, transfer time 3 min, clean rate 14.5deg/sec, clean temp 285°C, clean time 10 min, clean flow 20 ml/min. The chromatographic separation of Lufenuron was performed on Mega 5 MS column (Mega Capillary Columns Laboratory, Italy.) with 30 m length x 0.32 mm internal diameter with 0.5 μ film thickness. Carrier gas was helium with 99.9% purity with flow rate of 1ml min⁻¹. Oven was programmed with 50°C initially, ramped (1) at 15°C/min to 130°C held for 0 min, further ramped(2) at 30°C/min to 182°C-0 min. and then ramped (3) at 0.3 °C/min to 184 °C and held for 1 min. (Total run time 14.76 min). Electron Impact ionization was achieved with 70eV ionization energy with source temperature 230°C and auxiliary temp. 285°C. Positive mode full Scan was performed with mass range m/z 50-450 with the solvent delay of 6 minutes.
RESULTS AND DISCUSSION:
A) Biological studies

Sub-lethal concentrations of lufenuron for 2-day old larvae of *T. castaneum* deduced from the regression equation \( Y = 3.563X - 7.4876 \) and were as follows: \( LC_{50} = 0.0175 \text{ppm} \); \( LC_{40} = 0.0137 \text{ppm} \); \( LC_{20} = 0.00937 \text{ppm} \); \( LC_{10} = 0.006879 \text{ppm} \).

1. Effect on growth and development:

Sublethal dose of lufenuron (\( LC_{10}, LC_{20} \) and \( LC_{40} \)) incorporated in the diet and fed for 24 hrs to two day old larvae of *T. castaneum* were shown to affect growth, development and fertility. Growth retardation was reflected by lower larval weight and delay in pupation and adult emergence. At sublethal dose pupae and adult developed from treated larvae failed to shed their cuticle, which lead to death or deformity in them. Along with the above effects abnormal stages like pupal-adult intermediate were also formed.

2. Effect on adult fecundity and fertility:

Fecundity of adults was not affected when fed on lufenuron treated wheat flour. However, % hatching i.e. (fertility) and survival of larvae was affected. The percentage of hatching in the control was always > 95% while that in treated sample it was inversely proportional to the lufenuron concentration in the diet.

3. Effect on hatching of eggs:

When eggs were kept in lufenuron treated diet their hatching percentage was not affected in contrast to that observed in case of flufenoxuron on *T. castaneum* by Salokhe et al. (2003). This is due to the fact that lufenuron has no contact action (Anonymous, 1997) unlike flufenoxuron.
4. Effect of topical application on pupae:

Topical application of lufenuron on newly formed and three day old pupae was examined. Newly formed pupae (24hrs.old) when treated topically with various concentrations of lufenuron 0.005, 0.0005, 0.00025, 0.00016, 0.000125, and 0.0001 µgms / pupa the emerging adults had morphological deformities such as reduced wings and they were unable to free themselves from the pupal cuticle. The inability of the adults to free themselves from pupal skin could be due to the weakening of elytra by the residue of lufenuron. Lufenuron treatment of newly formed pupae also resulted in the higher % of mortality and formation of pupal-adult intermediates. However three days old pupae showed higher % of adult emergence and small % of abnormal adults. It was observed that pupal period is prolonged and it was more in freshly formed pupae than that of old pupae. The morphological deformities were also less evident in three days old pupae compared to one day old pupae.

5. Effect on female reproductive system:

Effect of dietary treatment of sub-lethal concentrations of lufenuron i.e. LC_{10}, LC_{20} and LC_{40} on newly emerged adults of *T. castaneum* female insect was studied by dissecting them under binocular microscope. Female reproductive system was isolated and fixed in Bouin's fluid. It was observed that female reproductive system was not affected by the dietary treatment of sub-lethal dose of lufenuron when compared with the control.
6. Field simulated experiments:

a) Treatment of sacks:

When *T. castaneum* adults were released in gunny bags treated with lufenuron (@ LC$_{10}$, LC$_{20}$ and LC$_{40}$ concs.) egg laying was not observed similar to that in untreated sacks. This might be due to the normal behavior of laying eggs in crushed wheat or wheat flour. These studies were carried out to see whether lufenuron has any contact action on *T. castaneum* adults. These insects when transferred to normal diet after 10 days, were found to start egg laying and their fecundity, fertility and survival was similar to the control insects.

b) Treatment of wheat:

When *T. castaneum* adults were released in untreated wheat and in wheat treated with different sub-lethal lufenuron concentrations i.e. LC$_{10}$, LC$_{20}$, and LC$_{40}$, it was observed that they did not lay eggs in treated and untreated wheat. Insects from treated and untreated wheat, when transferred to normal wheat flour, started laying eggs. This clearly indicates that exposure to treated wheat has no effect on reproductive behavior of *T. castaneum*.

c) Treated and crushed wheat:

Crushing of wheat after treatment with lufenuron (LC$_{10}$, LC$_{20}$ and LC$_{40}$) had no effect on fecundity of *T castaneum*. However % hatching and % survival was less than that of the control. These results are similar to the results obtained for wheat flour treated with different sub-lethal concentrations of lufenuron.
d) Treated and milled wheat

i) Effect on larval survival:

Two day old larvae of *T. castaneum* when fed with wheat flour of treated wheat (LC_{10}, LC_{20}, and LC_{40}) showed high percentage of mortality as compared to control. At all concentrations the larvae exhibited typical symptoms of benzoylphenylurea poisoning such as black shriveled body, failure to shed old cuticle.

ii) Effect on reproductive potential of adults:

Fecundity of *T. castaneum* adults fed on flour of treated (with sub-lethal concentrations LC_{10}, LC_{20}, LC_{40} as obtained for larvae) wheat was not significantly different from that of the adults fed on the normal diet. However, percentage hatching (fertility) of eggs laid by treated adults reduced with increase in the concentrations of lufenuron for first two days. It was resulted in large mortality of F1 generations. Few larvae became black and shriveled, failed to shed the old cuticle and died while hatching after 2-3 days.

B) Residue analysis:

Pesticide residue study by GC-MS technique:

Monitoring of residues in food is mandatory for proper assessment of human exposure to pesticides in foods. In practice it is important to decide the concentration of pesticide in the food item to control the pest, keeping in mind the (Maximum Residue Limit) MRL value set by regulatory authorities. Attempts have been made for analysis of benzoylphenylureas residues in fruits, vegetables like tomato by using Liquid Chromatography-Mass Spectrometry (LC-MS) (Galera et al., 2001; Valenzuela, 2000; Sannino, 2005). UV determination has also been used for detection of residues in apple
and pear pulps for baby food in some published reports which lack sufficient
discrimination of signal of pesticides form matrix noise. (Bicchi, 1996; Balinova, 1998;
Tsiropoulos, 1999). However, most of these methods do not directly appear to be
applicable to the analysis of lufenuron in a complex matrix like wheat flour. To
overcome this problem we developed a method for extraction and clean up as well as
analysis of lufenuron from wheat flour by Gas Chromatography-Mass Spectrometry (GC-
MS). We studied the time dependant residual concentration of lufenuron by GC-MS and
its effect on the mortality of Tribolium castaneum larvae.

Since whole wheat (2 kg) was treated and milled, there is a possibility of uneven
dispersal of the EC formulation of lufenuron. As a result the recovery is neither uniform
nor in descending order. This is reflected by the GC-MS recovery data, however it gives
100% mortality on 15th day of exposure indicating the residue amount of lufenuron,
although not uniformly present in the sample, was sufficient to bring about mortality for
three months. This indicates that very low concentrations of lufenuron (in present study it
is 88 ppb), is sufficient to control T. castaneum larvae in wheat flour, which is much
lower than the MRL value of lufenuron set for vegetables (approx. 500 ppb) (López-
López, 2004; Kramer, 1979)

CONCLUSION:

Sub lethal doses of lufenuron (LC10, LC20 and LC40) incorporated in the diet and
fed for 24 hrs to two day old larvae of T. castaneum were shown to affect growth,
development and fertility. At sub lethal dose pupae and adult developed from treated
larvae failed to shed their cuticle, which lead to death or deformity in them. Along with
the above effect abnormal stages like pupal-adult intermediate were also formed in LC$_{20}$ and LC$_{40}$ concentrations.

Fecundity and hatchability of eggs in case of adults developed from treated larvae was not significantly different from that of control. Also, when eggs were kept in lufenuron treated diet their hatching percentage was not affected.

Female reproductive system was not affected by dietary treatment of lufenuron.

When males treated with sublethal dose of lufenuron were crossed with untreated females, hatching percentage of their eggs and survival was similar to that of control. Adults emerging from lufenuron treated pupae had morphological deformities such as reduced and folded wings and they were unable to free themselves from pupal cuticle.

Newly emerged pupae were more sensitive to topical treatment of lufenuron than the three days old ones.

Treatment of jute bags and wheat grains had no effect on adult reproductive potential/behavior.

Findings from this study shows that lufenuron has a strong larvicidal, pupicidal and transovarial ovicidal property in T. castaneum and this chemical could be valuable in reducing damage caused by red flour beetle to stored grains products in IPM program. Present study provides the methodology for lufenuron residue analysis in wheat flour and opens up a new vista for its possible application for protecting stored product commodities against the red flour beetle, T. castaneum, since the MRL values are very much within the prescribed limits for other food products. These findings indicate that lufenuron can possibly be used in the management of this stored grain pest. Further
research, under actual storage conditions would be needed to verify these findings for application purposes.

References:


