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Effect of a selected rice bran extract and a chitin synthesis inhibitor on viability of eggs of the house fly *Musca domestica* Linnaeus.

Dalia, A. M. Salem[1]; Nadia Helmy[1]; Wafaa A. Radwan[1]; Reda F.A. Bakr[1&2]; Noha A. Guneidy[1] and Shimaa S. Mohammed.[1]
1-Department of Entomology, Faculty of Science, Ain Shams University, Cairo
2- Biology Department, Faculty of Science, King Khalid University, Abha, Saudi Arabia

ABSTRACT

The efficiency of chitin synthesis inhibitor (Lufenuron), ethanolic and acetonic extracts of rice bran (*Oryza sativa*) were evaluated against *Musca domestica* eggs by direct application using topical, dipping technique and by treating vitellogenic females by both compounds.

Treatment of females with different concentrations of both compounds significantly reduce egg hatchability and the effect is dose dependent. The inhibition of egg hatching increased with increase in concentration of the compound.

Direct application of both compounds to newly laid eggs (0-15 min) of *Musca domestica* proved to be more effective in reducing egg hatchability especially when using dipping technique. Percentage total inhibition reached 93.04±0.57 at 3 ppm for Lufenuron and 100% total inhibition at 200 ppm of acetonic extract of rice bran (*Oryza sativa*). This data confirm the efficiency of growth regulator derived from *Oryza sativa* straw and chitin synthesis inhibitor (Lufenuron). The results indicated that the acetonic extract of rice bran is significantly effective in preventing hatching than that of ethanolic extract. This is explained that the less polar the extract (acetonic) of rice bran, the more its effectiveness against eggs.

Key words: *Musca domestica*, chitin synthesis inhibitors, rice bran extract, viability of eggs.

INTRODUCTION

The common housefly, *Musca domestica Linnaeus,* 1958 (Diptera: Muscidae) is one of the dominant species found in human habitation, in tropical and subtropical regions (Slama *et al.,* 1964; Matsumura, 1975). It has gained importance as a serious public health hazard being a vector of some infectious diseases like eye inflammation, cholera, typhoid and dysenteries.

The extensive use of chemical pesticides or insecticides resulted in inducing resistance by insect pests beside contamination of human food, mammalian toxicity, reducing beneficial non-target biota and environmental pollution. These factors have created the need for environmentally safe and specific agents for pest control purposes.

The use of botanical insecticides is dating back at least 150 years and often much longer (Monin and Nair, 2002; Wood, 2003; Philogene *et al.,* 2005) probably with negative effects on the environment and pest resistance after application.

Over one thousand plant species contain bioactive substances many of these containing phytoecdysones, phytojuvenoids and antijuvenille hormones, which acts as IGRs (Marcard *et al.,* 1986; Neraliya and Srivastava, 1996). Botanical extracts, termed Insect Growth Regulators (IGRs), can have a pronounced effect on the development, growth, adult emergence, fecundity, fertility, and embryogenesis resulting in effective control (Shaalan *et al.,* 2005). The use of IGRs for housefly

control, as an alternative approach, leads to less contamination of environment and has a good lethal effect on the insect (Yu and Terriere, 1977; Doannia *et al.,* 1993).

Several hypotheses have been made to explain the mode of action of these IGRs including inhibition of chitin synthesis, increase in chitinase activity or inhibition of ecdysone metabolism (Meola and Mayer, 1980; Marks *et al.,* 1982; Doannia *et al.,* 1992).

Impairment of cuticle secretion in affected embryo may be the cause of the hatchability reduction that result from treatment with chitin synthesis inhibitors (CSI) (Grosscurt, 1978; Kostyukovsky and Trostanetsky, 2006).

Chitin synthesis inhibitors are benzoylphenylurea compounds discovered in the 1970s (Mian and Mula 1982) that interfere with insect development, disturbing the moult and resulting in deformations in the cuticle (Reynolds 1987).

The objective of this study is to evaluate the effect of selected rice bran extract (*oryza sativa*) and a chitin synthesis inhibitor (Lufenuron) on the viability of eggs of *Musca domestica* by using two methods:

a) Indirect application, by treating three days-old females with different doses of selected compounds.
b) Direct application on newly laid eggs either topically or by dipping technique.

MATERIALS AND METHODS

Insect rearing:

The housefly, *Musca domestica L.* was obtained from Institute of Medical Entomology, Ministry of Health, Dokki,Giza, Egypt. Both sexes were reared in wire cages with wooden frames (30 x 30 x 30 cm) at 27 ± 1 ºC, 60–70% RH, and constant light as described by Rockstein (1957) and Busvine (1962).

Tested compounds

- A chitin Synthesis inhibitor Lufenuron 10% EC) of chemical formula:
  N. [2, 5-dichloro-4-(1, 1, 2, 3, 3, 3- hexafluoroproxy)-phenyl/amino]-2,6-difluorobenzamide.
- Rice bran extract (*Oryza sativa*)

Extraction

Rice bran of *Oryza sativa* was extracted by different solvents (acetone and ethanol), each extracted solvent was evaporated till dryness (Bakr *et al.* (2010).

Application of the tested compounds:

Female treatment:

Three days-old females (vitellogenic female) were treated with serial appropriate concentration of Lufenuron (10%) and rice bran extract (*Oryza sativa*).

The adult females were fed for 24 h continuously on diet containing Lufenuron (10%) or rice bran extract (*Oryza sativa*) at appropriate concentrations. Then, they were fed on untreated diet.

Eggs were collected daily to follow their hatchability and to study the effect of the tested compounds on the fecundity and fertility

Egg treatment:

The effect of the tested compounds on embryonic development was studied by applying them directly to egg mass either by topical application or by dipping the egg mass in an appropriate solution of the compounds to be tested.

Newly laid eggs (0-15 minutes) postoviposition were treated topically by 0.1, 0.5, 1, 2 and 3 ppm for chitin synthesis inhibitor (Lufenuron) and 1, 10, 100 and 200 ppm for rice bran extract /30 eggs respectively.

In dipping technique, newly laid eggs(0-15minutes) post-oviposition were immersed in 100 ml water containing one of five different doses o.1, 0.5, 1, 2 and 3 ppm for chitin synthesis inhibitor and in 100 ml water containing one of four different doses (1,10, 100 and 200 ppm) for rice bran extract /30 eggs. The experiment was repeated 3 times (30x3). Eggs were dipped for 1 minute in each solution.

Evaluation of tested compounds:

The effect of tested compounds on the viability of the eggs, were determined by counting the number of unhatched eggs 24 hours after deposition or treatment (about 16 hours after the time they would normally hatch).

Statistical Analysis:

The obtained data were manipulated statistically with SPSS version 16. While probabilities (p) were carried out using STATISTICA version 6 and AVOVA, Duncan multiple range test ($p<0.01$). The level of significance was expressed as highly significant ($p\leq0.001$), significant ($p\leq0.05$) and non – significant ($p>0.05$).

RESULTS

1) Effect of different doses of Lufenuron on viability of eggs laid by treated *Musca domestica* females .

Musca domestica females at 3rd day of emergence were fed for 24hr on diet containing Lufenuron at appropriate concentrations (0.005, 0.01, 0.1 and 0.5 ppm). The eggs from normal and treated females were examined for their hatchability.

Data on Table (1) indicate that treatment of *Musca domestica* females with Lufenuron exhibit a significant effect on viability of eggs laid, where percentage of unhatched eggs increase significantly ($P< 0.01$) with increasing dose.

Table 1: Effect of different doses of lufenuron on viability of eggs laid by treated *Musca domestica* females at 3rd day of emergence.

Conc. (ppm)	%Total eggs laid	% Hatched eggs (% Fertility)	% Unhatched eggs
0.005	67.9±1.58^{b}	97.53±1.14^{a}	1.46±0.12^{cd}
0.01	58.76±1.02^{c}	97.1±0.97^{a}	2.6±0.15^{c}
0.1	43.43±1.48^{d}	88.3±1.21^{b}	13.03±0.4^{b}
0.5	37.7±1.68^{e}	79.63±0.47^{c}	20.9±0.54^{a}
Control	99.3±0.75^{a}	99.7+0.27^{a}	0.23±0.05^{d}

*Data are presented as mean±SE

*Means bearing different letters within column are significantly different ($P<0.01$) ANOVA, Duncan multiple range test.

The percentage of unhatched eggs was (1.46±0.12, 2.6±0.15,13.03±0.4 and 20.9±0.54) for (0.005, 0.01, 0.1. 0.5 ppm) Lufenuron concentration, respectively compared with 0.23±0.05 for the control (normal eggs). Whereas, the percentage of hatched eggs (fertility) decreases significantly ($P<0.01$) with increasing concentraion used. However, there is no significant effect on fertility for females fed on diet containing 0.005 and 0.01 ppm Lufenuron.

2) Effect of different doses of Ethanolic and Acetonic rice bran on viability of eggs laid by treated *Musca domestica* females .

Musca domestica females at 3rd day of emergence were fed for 24hr on diet containing appropriate concentration of ethanolic and acetonic extract of rice bran

(10, 50, 100 and 150 ppm). The eggs from normal and treated females were examined for their hatchability.

Data on Table (2) indicate that treatment of *Musca domestica* females with ethanolic and acetonic extract of rice bran exhibit a significant effect on viability of eggs laid by female,where the percentage of unhatched eggs increases significantly ($P< 0.01$) with increasing dose. The percentage of unhatched eggs was (0.49±0.08, 1.4±0.14, 5.76±0.11 and 10.16±0.9) for (10, 50, 100 and 150 ppm) of ethanolic extract of rice bran concentration, respectively compared with 0.2±0.05 for control. The acetonic extract showed higher effectiveness on percentage of unhatched eggs than that of ethanolic extract.It ranges from (0.93±0.21to 18.1±0.94) for (10, to 150 ppm) of acetonic extract concentration, respectively. Generally, the effect of plant extract (either ethanolic or acetonic) is the highest at 150ppm concentration.

Table 2: Effect of different doses of Ethanolic and Acetonic extract of rice bran on viability of eggs laid by treated *Musca domestica* females at 3rd day of emergence.

Conc. (ppm)	%Total eggs laid		% Hatched eggs (% Fertility)		% Unhatched eggs	
	Ethanolic	Acetonic	Ethanolic	Acetonic	Ethanolic	Acetonic
10	89.6±0.185^{b}	67.23±1.11^{b}	99.23±0.12^{a}	99±0.15^{ab}	0.49±0.08^{e}	0.93±0.21^{c}
50	79.23±0.38^{c}	56.9±0.94^{c}	98.36±0.187^{a}	97.93±0.21^{b}	1.4±0.14^{c}	2.23±0.38^{e}
100	74.1±0.44^{d}	48.16±0.91^{d}	94.5±0.25^{b}	89.16±0.42^{c}	5.76±0.11^{b}	11.76±0.61^{b}
150	66.96±1.47^{e}	35.5±1.24^{e}	89.76±0.88^{c}	83.16±0.41^{d}	10.16±0.9^{a}	18.1±0.94^{a}
Control	99.3±0.75^{a}	99.43±0.29^{a}	99.56±0.27^{a}	99.56±0.27^{a}	0.2±0.05^{d}	0.2±0.05^{d}

*Data are presented as mean±SE

*Means bearing different letters within column are significantly different ($P<0.01$) ANOVA, Duncan multiple range test.

The percentage of hatched eggs (fertility) decreases significantly ($P< 0.01$) with increasing dose (Table 2) However, there is no significant effect on percentage fertility for females fed 10 and 50 ppm of ethanolic and acetonic extract of rice bran.

The percentage of hatched eggs was (99.23±0.12, 98.36±0.18, 94.5±0.25 and 89.76±0.88) for (10, 50, 100 and 150 ppm) ethanolic extract of waste product concentration, respectively compared with 99.56±0.27 for control. The Acetonic extract showed higher effectiveness than that of ethanolic extract on percentage of hatched eggs (fertility). It ranges from (99±0.15to 83.16±0.41) for (10to 150 ppm) of acetonic extract of waste product concentration, respectively.

3) Effect of direct application of Lufenuron on viability of eggs:

3.1. Topical application:

In this experiment, newly laid eggs (0-15 min) postoviposition were treated topically with five different doses of Lufenuron (0.1, 0.5, 1, 2 and 3 ppm/egg mass). Thirty eggs were used for each dose and the experiment was repeated three times.

Results on Fig. (1) indicate that there is a significant effect of the compound on treated eggs ($p<0.05$). This effect was dose-dependent, where percentage of unhatched eggs increases with increasing dose. The effect is most pronounced in case of using 2 and 3 ppm where percentage of unhatched eggs were 43.4±4.6 and 70.1±0.12 respectively.

3.2. Dipping technique:

Newly laid eggs (0-15 min) were immersed in 100ml of distilled water containing one of five different doses (0.1, 0.5, 1, 2 and 3 ppm/30eggs) of Lufenuron.

Thirty eggs were used for each dose and the experiment was repeated three times. Eggs were dipped for 1 minute in each solution.

Results on and Fig. (1) showed that dipping the the eggs in 0.1, 0.5, 1, 2 and 3 ppm Lufenuron solution has a highly significant effect ($P \leq 0.01$) on eggs,increased with increasing dose; where percentage of unhatched eggs were16.7±6.3, 26.7±6.5, 33.3±5.7, 45.1±5.7 and 74.1±0.57 for Lufenuron concentrations (0.1,0.5,1,2 and 3 ppm), respectively compared with 3.3±0.57 for the control.

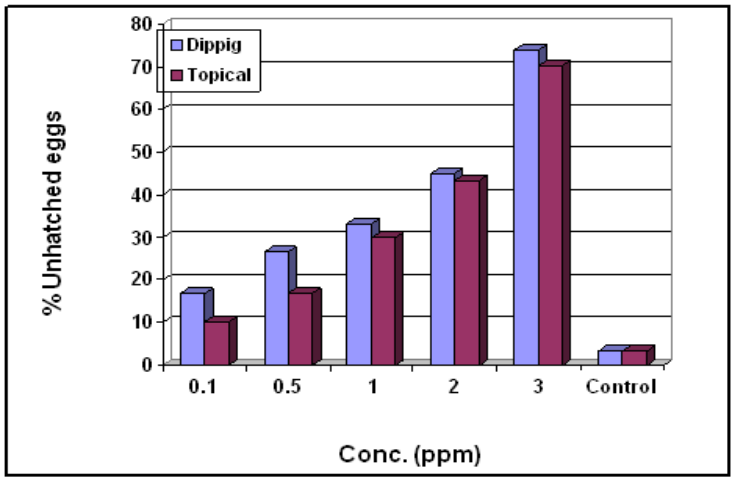

Fig. 1: Hatchability of *Musca domestica* eggs treated topically and by dipping technique with different doses of Lufenuron at (0-15) min after deposition.

It is of interest to mention that a number of hatched eggs were abnormal , therefore we estimate % total inhibition by adding % unhatched to % of abnormal hatched.

4) Effect of direct application of Ethanolic and Acetonic rice bran extract on viability of eggs:

4.1. Topical application:

In this experiment,newly laid eggs (0-15 min) were treated topically with four different doses of ethanolic and acetonic extract (1, 10, 100 and 200 ppm/egg mass). Thirty eggs were used for each dose and the experiment was repeated three times.

Results on Fig. (2) indicate that ethanolic extract has a significant effect on treated eggs ($P<0.05$) and the effect was dose-dependent increased with increasing concentration, where percentage of unhatched eggs were (20±5.77, 30±5.76, 39.8±5.76 and 46.6±5.19) for concentration (1, 10, 100 and 200 ppm), respectively compared with 3.3±0.57 for the control.

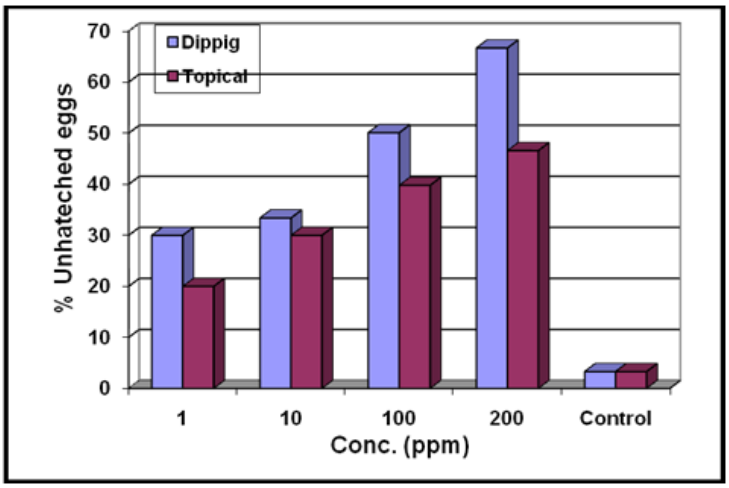

Fig. 2: Hatchability of *Musca domestica* eggs treated topically and by dipping technique with different doses of Ethanolic extract of rice bran (*Oryza sativa*) at (0-15) min after deposition.

Results on and Fig. (3) indicate that acetonic extract has a highly significant effect on treated eggs (P≤0.01), where percentage of unhatched eggs were (31±5.77, 40±5.77, 43.4±5.19, and 87.7±1.27) for concentration (1, 10, 100 and 200 ppm), respectively compared with 3.3±0.57 for the control.

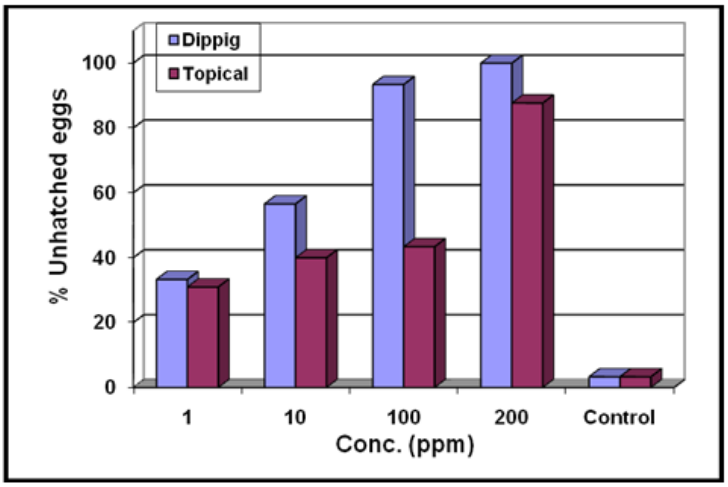

Fig. 3: Hatchability of *Musca domestica* eggs treated topically and by dipping technique with different doses of Acetonic extract of rice bran (*Oryza sativa*) at (0-15) min after deposition.

4.2. Dipping technique:

Newly laid eggs (0-15 min) were immersed in 100ml of distilled water containing one of four different doses (1,10, 100 and 200 ppm/30eggs) of ethanolic and acetonic rice bran extract.

Thirty eggs were used for each dose and the experiment was repeated three times. Eggs were dipped for 1 minute in each solution.

Results from and Fig. (2) showed that dipping the the eggs in solution containning 1,10, 100 and 200 ppm of ethanolic rice bran extract has a highly significant effect (P≤0.01) on eggs; the effect was dose – dependent .Percentage of unhatched eggs were (30±5.7, 33.4±6.3, 50.1±6.5 and 66.7±6.3) for concentration (1, 10, 100 and 200 ppm), respectively compared with 3.3±0.57 for the control.

Results on and Fig. (3) showed that dipping the eggs in solution containning different concentrations of acetonic waste extract has a highly significant effect (P≤0.01) on eggs. The percentage of unhatched eggs were (33.3±6.3, 56.6±5.77, 93.4±0.57 and 100) for concentration (1,10, 100 and 200 ppm), respectively compared with 3.3±0.57 for the control.

From this result,it is clear that acetonic extract showed higher effectiveness on reducing hatching (viability) of eggs than that of ethanolic extract. Generally, the effect of rice bran extracts (either ethanolic or acetonic) is the highest at 200ppm concentration.

DISCUSSION

1)Effect of different doses of Lufenuron and a rice bran extract on viability of eggs laid by treated *Musca domestica* females.

The present study revealed that feeding vitellogenic females(3rd day of emeregence) of *Musca domestica* on different concentrations of the chitin synthesis inhibitor (Lufenuron) and two extracts (ethanolic and acetonic) of a waste product (*Oryza sativa*) reduce the viability of eggs that was manifested as an impairment of egg hatchability. *Musca domestica* oogenesis is classified into three stages

previtellogenic, vitellogenic stage (at 3rd day after emerege) and ovulation stage (Just before egg laying) (Shanbaky *et al*. 1993). Vitellogenic females (3-days) of *Musca domestica* are the most sensitive to the effect of different insect growth regulator (Siriwattanarungsee *et al*. 2008, Shanbaky *et al*. 1993).

Reduction of female fertility after feeding on chitin synthesis inhibitor has been also reported for different insect species; Colorado potato beetles *Leptinotarsa decemlineata* fertility and fecundity were reduced after feeding on plant treated with Novaluron (Alyokhin *et al*. 2008). Fecundity and egg viability of *Coptotermes formasonus* was lowered after treatment with Lufenuron (Rojas and Morales-Ramos, 2004). Reduction of egg hatch has been reported in *Blattella germanica* after feeding on bait containing 0.25% Diflubenzuron (Koehler and Patterson, 1989), *Ctenocephalides felis* after adult feeding on 0.125 and 1 ppm of Lufenuron *(Meola et al.* 1999).

As early as (1976) Grosscurt recorded that topical application of adult *Musca domestica* with Diflubenzuron inhibit egg hatching.

Wolfenbarger and Nemec (1991) also reported that egg hatch was highly reduced in *A. grandis grandis* after females being treated with Diflubenzuron or Penfluron.

According to Wilson and Cryan (1997) Lufenuron has a dramatic effect on the viability of eggs oviposited by females *Drosophila melanogaster* transferred to lufenuron food. This effect of Lufenuron on egg fertility is reversible after transfer of the adult to regular food for nearly a week.

Failure of hatching was explained by Wilson and Cryan (1997) that the complete embryos were unable to perforate the surrounding membrane, probably due to a weakened chitinous mouth hook assembly that was insufficiently rigid to effect hatching.

On the other hand, Hami *et al*. (2004) explained the effect of Flucycloxuron on viability of eggs of *Tenebrio molitor* as it reduces the thickness of chorion from freshly laid eggs.

Findings of the present study indicate that feeding vitellogenic *Musca domestica* females on different concentrations of ethanolic and acetonic extract of rice bran, *Oryza sativa* has a significant effect on viability of laid eggs. Similar results were recorded, using different plant extracts for different insect species; Fertility of *Locusta migratoria* were reduced after treatment of newly emerged females with methanolic extract of leaves and stems of *Haplophyllum tuberculatum* (Acheuk *et al*. 2012). The number of eggs laid per female and egg viability of *Spodoptera littoralis* was reduced after treatment with 2% suspension of ground seed of *A. indica* (El-sayed, 1982).

Mostafa (1993) also reported the reduction of Fertility of *Trogoderma granarium* after treatment with three plant extracts. Complete inhibition of hatchability was achieved by the action of *Piper nigrum* extract.

2) Effect of direct application of Lufenuron and rice bran extract on viability of eggs by topical and dipping techniques:

From the present study,topical application of different doses of Lufenuron, and ethanolic and acetonic extracts of *rice bran, Oryza sativa*, have a significant effect by inhibiting the development of *Musca domestica* eggs treated (0-15min) postovipotion.

This is also the case with several insect growth regulators; Direct application of Dimilin, Methoprene and hydroprene on the eggs of *Musca domestica* caused a decrease in the rate of hatching (Lineva and Chunina, 1979). Exposing the eggs of *culex quinquefasciation* to Sir 8514 also decrease viability of eggs; younger embryos (0.5-4.5 hours) were the most sensitive (Miwra & Takahasli 1979). Similar

observations occurred with Marchiondo *et al*.(1990), Adham and Shoukry (1984) and Saenz *et al*. (2006), Sharaby (1988) found that spraying 2% concentration of essential oil and lemon grass *Cymbopogen citratiis* on spodoptera eggs caused inhibition of hatching. The topical application of adult females *Boophilus microplus* with *Melia azedarach* extract caused a partilliy or totally inhibition of egg productions and embryogenesis (Borges *et al*. 2003).This was also observed with Savolainen *et al*. (1995), Gajmer *et al*. (2002) and Cabral *et al*. (2007).

In the present study, immersion of newly deposited eggs (0-15 min) of *Musca domestica* in Lufenuron, ethalonic and acetonic extracts of rice bran, *Oryza sativa* solution of different concentrations for 1 min have a highly significant effect in reducing egg viability. Similary, dipping of *phytoseilus persimilis* eggs in 100ppm of Kenoprene, Methoprene and Hydroprene inhibit egg hatching by 100% (Mandanlar and Kismali,1994). Dipping technique proved also to be effective in treatment of *Acheta domestica* eggs with Dimataf and Diflubenzuion that show a good ovicidal effect on treated eggs (Matolin and Chwakova, 1983), and the same was observed in treatment of *leptinotarsa decemlineata* eggs dipped in Novaluron solution (Alyokin *et al.,* 2008).Sharma and Bhargaua (2001) recorded ovicidal effect of some growth disrupting compound present in neem, undi (*Calophyllum inophyllum*), karanj (*Pongania glabra*) lemon grass oil (0.25, 0.5, 1, 2, 3 and 5 %) were applied on eggs of *Cocyra cephalonica* by dipping method. Dipping of eggs of *Myllocerus undecimpustulatus* in 1% concentration of neem extract and undi extract resulted in 100% and 94.66% egg mortality (Agarwal, 1990). Similar results were observed with Dwivedi and Kumar (1999), Dwivedi and Gang (2000) and Kumar and Jain (2004).

It is observed also that the acetonic extract of waste product seemed to be significantly effective in preventing hatching than that of ethanolic extract. This indicates that the less polar the extract (acetonic) of rice bran, the more its effectiveness against eggs (Borges *et al*. 2003).

Results of the present work suggest that topical application seem not to permit enough amount of the tested compounds to penetrate through the chorion of the egg; where the compounds could have been evaporated and the time for penetration may be short. However, dipping technique appears to allow a better chance and enough time for the tested compound in the solvent to penetrate and pass into the contents of the egg. Egg immersion in the tested compounds enabled penetration to occur through the whole surface of the egg (including the micropyle) during the time of immersion (1min) and afterwards.

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ARABIC SUMMARY

تأثير مستخلص نخالة الأرز وإحدى مثبطات الكيتين على حيوية البيض فى الذبابة المنزلية

داليا عبد البديع محمد سالم[1] – نادية حلمى أحمد[1] – وفاء أحمد رضوان[1] – رضا فضيل على بكر[1-2]
نها عونى جنيدى [1] – شيماء صلاح محمد[1]
1- قسم علم الحشرات – كلية العلوم – جامعة عين شمس- القاهرة – مصر
2- قسم الأحياء – كلية العلوم – جامعة الملك خالد – أبها – السعودية

شملت الدراسة تقييم فعالية مثبط الكيتين ومستخلص الأسيتون والإيثانولى لنخالة الأرز باستخدام (الأسيتون والإيثانول) على بيض الذبابة المنزلية باستخدام طريقة الملامسة والغمس وكذلك بمعاملة الأناث فى مرحلة تكوين المح باستخدام المركبين وقد وجد أن معاملة الأناث بتركيزات مختلفة للمركبات نتج عنه تأثير معنوى على (حيوية) البيض وذلك بخفض نسبة الفقس ويرتبط التاثير بالجرعة المستخدمة ويتزداد نسبة التثبط فى فقس البيض مع زيادة تركيز المركب كذلك فإن التطبيق المباشر للمركبين على البيض حديث الوضع (صفر- 15 دقيقة) للذبابة المنزلية أثبت أنه أكثر فعالية فى تثبط عملية فقس البيض خاصة عند استخدام طريقة الغمس. وقد وجد أن النسبة المئوية لتثبط الفقس تصل الى 0.57 ± 93.04 عند 3 جزء فى المليون لمركب ليوفينورون، 100% لتثبط الفقس عند 200 جزء فى المليون لمستخلص الأسيتون لنخالة الأرز. هذه النتائج تؤكد فعالية المركبات المستخلصة من نخالة الأرز وكذلك مثبطات الكيتين (ليوفينورون).

وقد أظهرت النتائج أن مستخلص الأستيون نخالة الأرز له تأثير معنوى فى تنثبيط عملية الفقس بأعلى من مستخلص الأيثانول نخالة الأرز.

وهذا يمكن تفسيره بأنه كلما كان المركب الأقل قطبية (الأسيتون) لنخالة الأرز كان تأثيره أعلى على البيض.