PHYSIOLOGICAL AND BIOCHEMICAL EFFECT OF PYRIPROXYFEN ON INDIAN MEAL MOTH *PLODIA INTERPUNCTELLA* (HÜBNER) (LEPIDOPTERA: PYRALIDAE)

Aida Ghasemi, Jalal Jalali Sendi, Mohammad Ghadamyari*

Department of Plant Protection, University of Guilan, Rasht, Iran

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Abstract: Insect growth regulators generally have a selective effect on the target insects and have practically no apparent side effect on non-target organisms especially vertebrates. Hence, insect growth regulators could be a suitable choice to control pests in stored products. Ten-day-old larvae of Indian meal moth *Plodia interpunctella* (Hübner) were expressed to the juvenile hormone analogue pyriproxyfen in order to have an effect on growth, metamorphosis, reproduction, lipid and protein contents of ovaries. The larvae were treated by 0.02, 0.04, 0.08, 0.16, and 0.3 ppm of JHA in an artificial diet where controls received acetone alone. The results indicated significant differences in duration of growth, mean longevity of hatched adults, percentage of emerged normal adults, abnormal pupae, hatched larvae and mean oviposition ratein addition to the lipid and protein of ovaries compared to the controls. An inhibition concentration of fifty (IF_{50}) for prevention of emerging adults was recorded 0.134 ppm. Pyriproxyfen caused significant defects in the legs and wings of some adults and sever morphological changes in the ovaries of emerged adults. The results showed that pyriproxyfen may be applied as an insecticide to decrease the damage caused by Indian meal moth on stored products. Pyriproxyfen can be used with low side effects to humans.

Key words: pyriproxyfen, oviposition, longevity, protein, lipid, Indian meal moth

INTRODUCTION

Indian meal moth *Plodia interpunctella* (Hübner) is a cosmopolitan pest of raw stored commodities and of packaged and processed food (Cox and Bell 1991). In recent years, there has been great concern over the toxicity of pesticides on non-target organisms and the environment. Due to such a concern, the use of more specific chemicals on target pests has grown (Paoletti and Pimental 2000). Ordinarily, the control measures in stores are based on fumigation with chemicals like hydrogen phosphate. Residues and insect resistance are reasons for potentially limiting the use of fumigation with chemicals in the near future (World Metrological Organisation 1994). Nowadays, alternative methods are being appreciated. One of the alternatives may be the inclusion of insect growth regulators (IGRs). These compounds are highly effective against various insects attacking stored products and other pests that have become resistant to organic insecticides. Meanwhile, all these compounds are less toxic to mammals and non target organisms because of their non-toxic effect and their quick disintegrating abilities (Carter 1975; Staal 1975; Zurfleuh 1976; Oberlander *et al*. 1978, 1979; Ishaaya *et al*. 1987; Ishaaya and Horowitz 1998; Kostyukovsky *et al*. 2000).

Synthetic IGRs and hormone analogues imitate natural hormones and the physiological processes of insects and are classified as juvenile hormone analogues, ecdysteroid hormones or as ecdysis inhibitors (Mondal and Parween 2000). These compounds are aimed at the physiology and normal growth of the target pests in the juvenile stages. The compounds are meant to reduce adult emergence (Pedigo 2002; Arthur 2003) so that their effect may be more prevalent in the next generation and their sublethal effect may be more pronounced (Rumpf *et al*. 1998).

IGRs block embryogenesis of insects (Retnakaran 1970; Abdallah *et al*. 1975), reduce egg production in emerging adults (Metwally *et al*. 1972) and cause severe morphological disorders (Williams and Amos 1974; Arias and Mulla 1975). Although the prime target site of IGRs (like JH) are endocrine systems there are reports of many physiological and biochemical changes in metabolic pathway caused by these compounds (Kim and Kim 2002; Leonardi *et al*. 2001).

The juvenile hormone analogue, methoprene, has been reported to affect the growth of ovaries and cause a significant reduction of oocytes when using a topical application (Maiza *et al*. 2004) on newly emerged adults of *Blatta germanica* L. (Dictyoptera: Blatellidae). Egg production in *Rhyzopertha dominica* Fabricius (Coleoptera: Bostrichidae) was significantly reduced by treatment with methoprene and it simultaneously affected egg

*Corresponding address:
jjalali@guilan.ac.ir; j.j.sendi@gmail.com

hatchability (Chanbang *et al*. 2008). Progeny survival was significantly reduced with methoprene in *Onthophagus taurus* Schreber (Coleoptera: Scarabaeidae) (Nino *et al*. 2009). Another JHA, hydroprene, is considered to be an alternative to conventional insecticides because of its specific activity against immature insect stages, low persistence in the environment, and nontoxic effect on mammals (Mohandass *et al*. 2006).

Pyriproxyfen is a pyridine-based juvenile hormone agonist that competes for juvenile hormone binding site receptors in insects, mimicking the action of juvenile hormone and thus maintaining an immature state (Sullivan and Goh 2008). This compound has a relatively low toxicity for mammals and was first registered in Japan in 1991 for controlling public health pests (Miyamoto *et al*. 1993). The suppression of embryogenesis and adult formation in *Bemisia tabaci* Gennadius (Heteroptera: Aleyrodidae) (Ishaaya and Horowitz 1992) and *Trialeurodes vaporariorum* Westwood (Heteroptera: Aleyrodidae) (Ishaaya and Horowitz 1995) was also attributed to pyriproxyfen.

Insect growth regulators generally have a selective effect on the target insects and have almost no apparent side effect on non target organisms especially vertebrates. Hence, they could be a suitable choice to control pests on stored products because of their non or low toxicity to humans. The present study is an extension of previous studies which dealt with the effect of IGRs on stored product insects. Particular attention as paid to sublethal effects aimed at reproduction and its biochemistry.

MATERIALS AND METHODS

P. interpunctella (Hübner) was collected from infested rice in Rasht, Guilan province, in the northern region of Iran. The insects were reared in rectangular plastic jars (7.5x145x18.5 cm), the lids were cut (2x2 cm) and replaced by mesh cloth for aeration. The insect were reared in a controlled room (26±1°C, 14 : 10 LD and 65±5% RH) on an artificial diet (800 gm wheat, 160 gm yeast, 200 cc glycerol and 200 cc natural honey) (Oberlander *et al*. 1978). A group of 10-day-old larvae were used for this study.

Pyriproxyfen (Admiral 10EC CNCCC.JS China) was diluted in acetone and used in 0.02, 0.04, 0.08, 0.16 and 0.3 ppm concentrations in the diet and a control with acetone was used for comparison in the diet. This experiment was replicated 8 times in a controlled room which was meant to provide as similar as possible the natural rearing conditions. In every experimental unit, 30 gm of treated food with 10, ten-day-old larvae were used.

The percentage of adult emergence, and the duration of growth from the onset of the experiment till adult emergence, the adult longevity, the inhibition concentration (IC_{50}), the percentage of abnormal pupae, the oviposition rate and the hatchability percentage were recorded. When an adult emerged from different concentrations, the ovaries were removed from the cold-anesthetized insect under a dissecting microscope. The ovaries were homogenized in 1 ml of Tris 50 mM buffered at pH 7.

Protein content was quantified according to the Bradford method (1976) using BSA as a standard. The absorbance was read at 630 nm in an eliza reader (Awareness Technology INC USA). Three replicates were run for this analysis.

Lipid was extracted using the van Handel method (1965). Each dissected ovary was homogenized in 1 ml of chloroform/methanol (1 : 1 v/v). The homogenate was centrifuged at 10 000 rpm for 10 min at 4°C. The aqueous part was discarded (repeated twice). The lipid extract was evaporated to dryness. The sample was digested with 1 ml of sulphuric acid at 100°C for 10 min. The tube was cooled and 5 ml of sulphosphovanillin reagent (orthophosphoric acid/0.6% aqueous vanillin solution 4 : 1) was added to the mixture. After 40 min, the absorbance was read at 530 nm and lipid level was calculated by a standard curve using cholesterol palmitate.

The comparisons of means were performed based on Tukey's test, in 5 percent estimation with SAS software (SAS 1997). IC_{50} was calculated by Polo-PC software (LeOra software 1987).

RESULTS AND DISCUSSION

The effect of pyriproxyfen on the developmental and biochemical parameters of *P. interpunctella* (Hübner) larvae are depicted in tables 1, 2, and 3. Based on these results, by increasing concentrations of pyriperoxyfen, the duration of the larval period till the emergence of adults were significantly increased compared to the controls ($p < 0.0001$). Increasing the concentrations, resulted in decreased adult longevity and significant reduction in mean eggs laid by adults compared to the controls ($p < 0.0001$) (Table 1). Development and reproduction in insects is affected by a number of hormones, including juvenile hormones (JHs). Williams (1967) proposed that timely application of JHs could be employed to control insects because of their ability to disrupt normal physiological functions. Insect growth regulators (IGRs) are synthetic mimics and analogues which mimic naturally occurring hormones and physiological processes of insects. Insect growth regulators are generally classified as JH analogues (JHAs), ecdysone agonists, or molt inhibitors (Mondal and Perween 2001). These synthetic mimics affect the normal development of immature insects (Mohandass *et al*. 2006). In the present investigation, pyriproxyfen, a juvenile hormone analogue, showed the potential for extending life stages which corresponds to the results of Edwards *et al*. (1995), Kellouche and Soltani (2006) and Sashindran *et al*. (2007). The prolongation of larval or pupal duration may be due to the persistence of JH in the hemolymph where it is only in the absence of JH that ecdyson could be activated and lead to the formation of the next stage (Kuwano *et al*. 2008). The prolongation or shortening of stage duration with IGRs, except chitin synthesis inhibitors, is due to the interference of these compounds on an endocrine source or inhibition of the release site of the prothoracicotropic hormone (PTTH) (Schmutter 1989; Subramanyam *et al*. 1989). *In vitro* and *in vivo* studies clearly showed that pyriproxyfen remarkably caused an inhibition of the ecdysone production thus interfering with normal development in *Tenebrio molitor* (Aribi *et al*. 2006).

There was no egg hatchability in all treatments compared to the control. Increasing the concentrations re-

Table 1. Effects of pyriproxyfen on growth, longevity, fecundity and fertility of *P. interpunctella* (Hübner)

Treatment [ppm]	Growth duration	Adult longevity [days]	Fecundity	Fertility
Control	13.97±0.10 f	8.81±0.11 a	294.7±2.38 a	98.67±0.33
0.02	16.23±0.10 e	7.3±0.11 b	261.5±2.38 b	0.00
0.04	18.18±0.10 e	7.2±0.11 b	107.9±2.38 c	0.00
0.08	19.21±0.10 c	6.17±0.11 c	101.2±2.38 c	0.00
0.16	22.28±0.10 b	5.18±0.11 d	73.4±2.38 d	0.00
0.3	25.63±0.10 a	3.57±0.11 e	47.8±2.38 e	0.00

Means within column followed by different letters indicate significant differences ($p < 0.0001$)

Table 2. Effect of pyriproxyfen on the percent of adult emergence and on the percentage of abnormal pupae after treatment of 10-day-old larvae of *P. interpunctella* (Hübner)

Treatment [ppm]	Total percentage of emerged adults	Percentage of abnormal adults	Percentage of abnormal pupae
Control	96.25±2.32 a	0.0±2.85 c	0.0±2.20 d
0.02	93.75±2.32 a	6.25±2.85 bc	6.25±2.20 d
0.04	71.25±2.32 b	10±2.85 b	28.75±2.20 c
0.08	67.5±2.32 b	12.5±2.85 a	32.5±2.20 c
0.16	50±2.32 c	20±2.85 a	50±2.32 c
0.3	20±2.32 d	10±2.85 b	80±2.20 a

Means within column followed by different letters indicate significant differences ($p < 0.0001$)

Table 3. Effect of pyriproxyfen on lipid and protein content of the ovaries in adults emerging from 10-day-old treated larvae of *P. interpunctella* (Hübner)

Treatment [ppm]	Total protein [mg/ovary]	Total lipid [mg/ovary]
Control	4.48±0.07 a	371.45±14.5 a
0.02	3.08±0.07 b	330.93±14.5 a
0.04	1.96±0.07 c	96.33±14.5 b
0.08	1.84±0.07 c	71.68±14.5 b
0.16	1.28±0.07 d	5.67±14.5 c
0.3	1.2±0.07 d	–

Means within column followed by different letters indicate significant differences ($p < 0.0001$)

Table 4. IC_{50} values and confidence limit (95%) and slope of pyriproxyfen treatment of 10-day-old larvae

Compound	No. of insects	Slope ±SE	GL 95%	IC_{50} [ppm]
Pyriperoxyfen	480	1.0.22	0.067–0.305	0.134

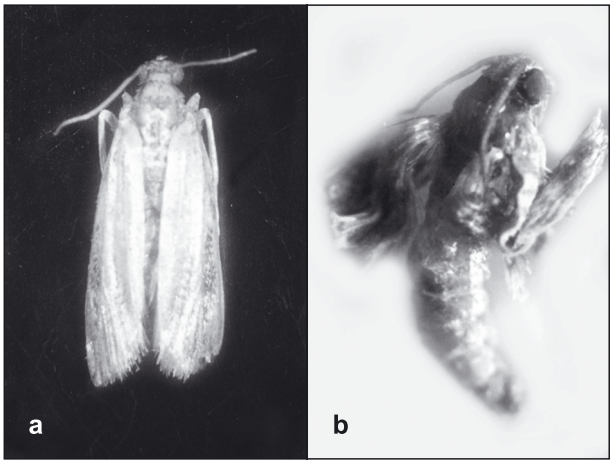

Fig. 1. Normal adult (a) and abnormal adult with twisted wings (b) (450 X)

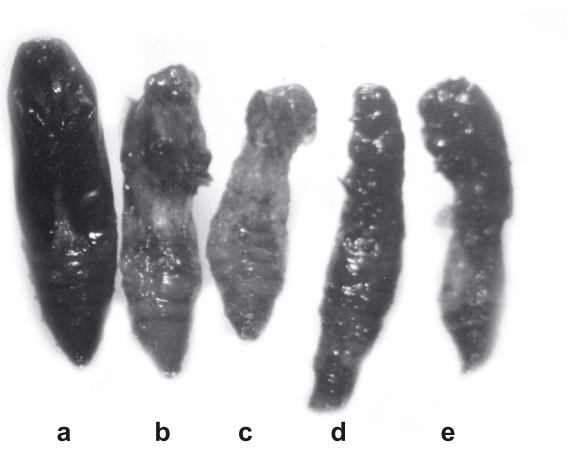

Fig. 2. Normal pupa (a) and different morphological abnormalities in treated larvae (b–e)

sulted in low emergence of normal adults so that some adultoids were emerged from treated larvae which was more pronounced in 0.16 ppm concentration. These adultoids showed abnormal legs and twisted wings thus, the adults were unable to move their legs and wings (Fig. 1). The current study demonstrated that the longevity of the adults emerging from treated larvae was significantly reduced compared to the control. The result corresponded to those reported for *Lipaphis orsini* (Liu and Chen 2001), *Thrips tabaci* (Thysanoptera: Thripidae) (Liu 2003) and *Bicyclus anyana* (Steigenga *et al*. 2006). The percent of emerged adults from the larval treatment was reduced which was similar to the results of Richardson and Lagos (2007) on *Aphis glycines* (Heteroptera: Aphididae) and Arthur (2004) on *Rhyzopertha dominica* (Coleoptera: Bostrichidae). Pyriproxyfen treatment made significant changes in external morphology of resultant pupae and emerged adults which is similar to the results of Hussein *et al*. (2005) and Arthur (2004) on *Tribolium castaneum* and *T. confusum* and Aribi *et al*. (2006) on *Tenebrio molitor.* In the pupal period, JH is not produced by the insects and only ecdyson is produced (Chapman 1998). Therefore, by the addition of external JHA during the larval period the insect is unable to exude excess JH. For this reason, the presence of JHA may lead to inhibition of ecdysone production therefore leading to discrepancy in later development as was shown in *T. molitor* (Aribi *et al*. 2006).

The inhibition concentration was calculated to be 0.134 which could inhibit 50 percent of adult emergence (Table 4). Treated larvae led to pupa like pupoids or larval like larvoids and the maximum effect was observed in the 0.3 ppm treatment (80±2.20 percent) (Fig. 2).

When dissecting the adultoids a significant reduction in ovaries was observed (Fig. 3a–f). Total protein and lipids showed significant reduction compared to the controls in various treatments so that their amounts decreased between 4–300-fold compared with the control (Table 3). The result corresponded to the results obtained by Perween and Miyata (2000) on *Spodoptera litura*, Kelluche and Soltani (2006) on *Callosobruchus maculatus*, Hami *et al*. (2004) on *Tenebrio molitor*. Shekari *et al*. (2008) found similar results after treatment of *Xanthogaleruca luteola* larvae with a plant extract. One of the main reasons for reduction in ovarian size in treated insects by JH, JHA, or growth regulator of plant origin, is the lack of materials supplied through hemolymph to the growing ovaries (Telfer *et al*. 1981) or due to the lack of materials made by the ovaries themselves (Indrasith *et al*. 1988). The lack of compounds like proteins, lipids, and carbohydrates may lead to abnormal oogenesis (Kunkel and Nordin 1985; Kanost *et al*. 1990). The decrease of two major biochemical compounds: lipid and protein, in the ovaries of the present insect may confirm this assumption. Shaya *et al*. (1993) reported that the growth of ovaries in *P. interpunctella* in early pupa is under the influence of high titer of ecdysone in hemolymph but vitellogenesis is under the influence of low titer of ecdysone. Hence, another reason for the low rate of growth in the ovaries of the present insect may be due to a discrepancy in ecdysone production in the presence of excess JHA after larval treatment. We found that incorporation of pyriproxyfen had a direct role on fecundity and fertility of treated insects, therefore it may be concluded that this compound had an effect on the growth of ovaries and oogenesis. Reduction in fecundity has been reported by Richardson and Lagos (2007) on *Aphis glycines* and Chanhang *et al*. (2008) on *Rhyzopertha dominica*. The results of Perween and Miyata (2000) showed that topical incorporation of Chlorfluazuron on ultimate instar larva of *S. litura* reduced the fecundity and fertility of adults. They speculated that reduced ovarian growth and oogenesis is responsible for reduced fecundity. The same reason might be the cause of the reduced fecundity and fertility in the studied insect. In the present investigation, it became clear that the lipids and proteins of ovaries in treated insects were reduced compared to the controls. These findings correspond to the results obtained by Perveen and Miyata (2000) where protein content in treated *S. litura* by Chlorfluazuron in ovaries was reduced. They speculated that the reduction of protein in the ovaries may be due to interruption of the compound with controlling mechanisms in yolk incorporation. Total protein and lipid in *P. interpunctella* in whole larva was similarly reduced by 20-hydroxyecdysone and azadirachtin (Rharrabe *et al*. 2008). They concluded that the

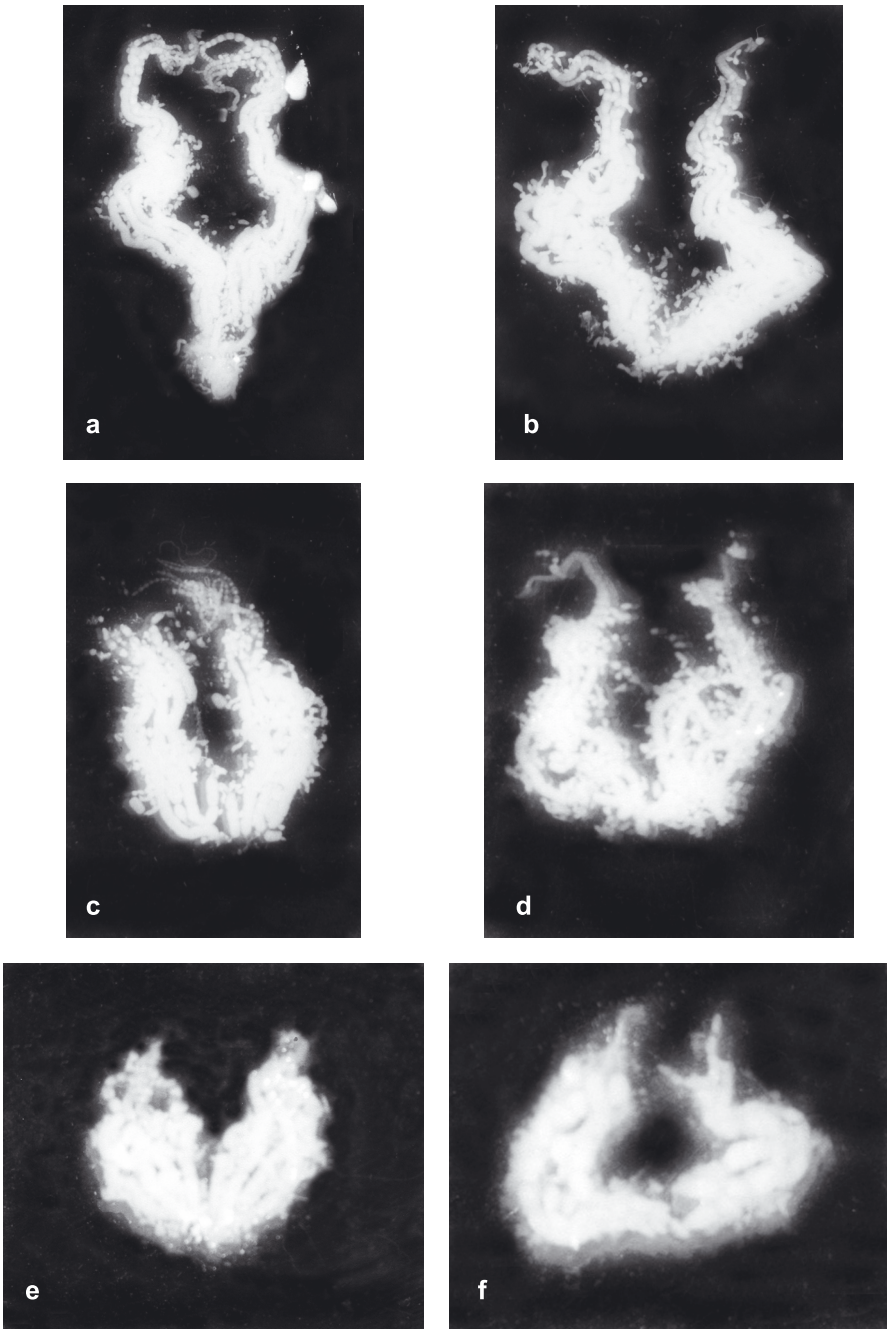

Fig. 3. Various developmental abnormalities in ovaries of treated (b–f) *vs.* the control (a), 0.02 ppm JHA (b), 0.04 ppm (c), 0.08 ppm (d), 016 ppm (e) and 0.3 ppm (f) (600 X)

depletion of these biochemical constituents could be due to major mobilization of these substances in response to the midgut as well as reduction of their synthesis.

In the future we can expect additional development and more advanced final adjustment of the insecticides and application techniques. We can also expect to obtain new knowledge about physical-chemical properties that determine the destiny of the insecticides in the environment and in biological systems (Zibaee *et al*. 2009). In this study, it is almost clear that pyriproxyfen mimics the action or natural JH and maintains the insect in an immature state. This action keeps the insects from molting successfully or reproducing normally. It is one of the insecticides recommended by WHO as an addition to drinking water for public health purposes (Sullivan and Goh 2008). Hence, we believe that its incorporation in food commodities for controlling stored product pests are appreciated.

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POLISH SUMMARY

WPŁYW PYRIPROXYFENU NA ZMIANY FIZJOLOGICZNE I BIOCHEMICZNE W MOLU MĄCZNYM *PLODIA INTERPUNCTELLA* (HÜBNER) (LEPIDOPTERA: PYRALIDAE)

Regulatory wzrostu owadów zwykle mają selektywne działanie na docelowe owady i praktycznie nie mają oczywistego działania ubocznego na organizmy niedocelowe, zwłaszcza kręgowce. Regulatory wzrostu mogą nadawać się odpowiednio do zwalczania szkodników w magazynowanych produktach. Dziesięciodniowe larwy indyjskiego mola mącznego *Plodia interpunctella* Hübner, były eksponowane na działanie analoga hormonu młodocianych stadiów pyriproxyfenu w celu uzyskania działania na wzrost, metamorfozę, reprodukcję, zawartość lipidów i białka w jajnikach. Larwy traktowano hormonem (IHA) obecnym w sztucznej diecie, a osobniki kontrolne otrzymywały w diecie sam aceton. Wyniki wskazywały na istotne różnice w trwaniu wzrostu, w średnim okresie trwania wylęgłych osobników dorosłych, w procesie wylęgłych normalnych osobników dorosłych, nienormalnych poczwarek, wylęgłych larw i w średnim tempie składanie jaj, w dodatku do lipidów i białka w jajnikach, w porównaniu do kontroli. Stężenie wywołujące inhibicję o wartości 50 (IF50) dla zapobieżenia wylęgu osobników dorosłych wynosiło 0,134 ppm. Pyriproxyfen powodował istotne defekty w nogach i skrzydłach niektórych osobników dorosłych, i duże zmiany morfologiczne w jajnikach wylęgłych dorosłych. Wyniki wykazały, że pyriproxyfen może być stosowany jako insektycyd w celu zmniejszenia szkód wywołanych przez indyjskiego mola mącznego w magazynowanych produktach. Pyriproxyfen może być używany, wywołując niskie efekty uboczne w stosunku do ludzi.