Toxicity and Bioefficacy of Cyromazine on Growth and Development of the Cotton Leafworm Spodoptera littoralis (Lepidoptera: Noctuidae)

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Abstract: The present work was conducted to evaluate the effects of Cyromazine on survival, growth, development and metamorphosis of Spodoptera littoralis. A series of concentrations (200.0, 100.0, 50.0, 10.0, 1.0, 0.1, 0.01 & 0.001 ppm) was applied on the newly moulted larvae of 5th (penultimate) and 6th (last) instars through the fresh food plant. After treatment of penultimate instar larvae, Cyromazine caused larval and pupal mortalities only at the higher concentration levels. No adult mortality was observed. LC$_{50}$ was 74.44 ppm. After treatment of last instar larvae, Cyromazine failed to exhibit a pupicidal activity, regardless the concentration level and caused adult mortality only at 1.0 and 0.1 ppm. LC$_{50}$ was 82.91 ppm. The larval growth was drastically suppressed, regardless the time of treatment and the concentration level. The developmental duration had been slightly prolonged indicating regressed developmental rate, regardless the time of treatment. Treatment of penultimate instar larvae with Cyromazine concentration levels, other than the lower two ones, caused prohibition of pupation in a dose-dependent course. Also, the pupation program was impaired since some larval-pupal intermediates had been produced. Cyromazine failed to affect the adult emergence, at the majority of the concentration levels except 100 ppm. Except the lower three concentration levels, treatment of last instar larvae with other concentration levels resulted in prohibited pupation rate and impaired pupation program. At only 100 and 50 ppm of Cyromazine, the adult emergence was partially blocked.

Keywords: adult, emergence, growth, intermediates, larva, metamorphosis, mortality, pupa

1. INTRODUCTION

The cotton leafworm Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) is an extremely dangerous pest, the larvae of which can defoliate many economically important crops cutting across over 40 families [1] or 112 plants belonging to 44 families [2-4] in a broad geographical area including Southern Spain, the Middle East, and both Northern and Central Africa [5,6]. S. littoralis inflicts excessive damage when it occurs in masses during certain years, commonly referred-to as "cotton worm monsoons" [7]. In Egypt, it is destructive phytophagous lepidopterous pest causing various ravages not only for cotton plants [8,9] but also for other field crops, vegetables [10], ornamentals and orchard trees [11,12] all over the year in Egypt [13]. The infested plants include 73 species recorded from Egypt [14].

To control the attacks of this pest several types of insecticides have been used, including synthetic pyrethroids, organophosphates, and non-steroidal compounds [15]. The extensive use of these insecticides has caused resistant insect strains to emerge [16,17] and serious toxicological problems to humans and the environment [18,19]. In Egypt, S. littoralis developed resistance to organophosphorus, synthetic pyrethroids, carbamates and other insecticides have been used, with appearance of resistance and cross resistance in many cases [20-23]. An outcry is exhibited against the use of pesticides due to their hazardous effects on human as well as environment [24].

On the bases of the mode of action, insect growth regulators (IGRs) had been grouped in three categories: (i) chitin synthesis inhibitors (CSIs) or moul inhibitors; (ii) ecdysone agonists and (iii) juvenile hormone analogues (JHAs) [25-29]. Recently, [30] classified IGRs into: CSIs and substances that interfere with the action of insect hormone (i.e. JHAs, and ecdysteroids). In the late decades, some new BPU analogues (considered in the CSIs group), such as: Novaluron, Bistrifluron, Fluzuron, and Noviflumuron were discovered [31-33]. Two other IGRs, Buprofezin and Cyromazine had been synthesized. These analogues have chemistries different from BPUs but they also interfere with moulting and chitin biosynthesis [32, 34].
Cyromazine (Trigard, Neoprex, Vetrazin) is widely used as an agricultural control agent inhibiting the moulting processes. It was assessed against several pests such as *Leptinotarsa decemlineata* [35,36], *Lucilia cuprina*, *Manduca sexta*, and *Lymantria dispar* [37-39], *Drosophila melanogaster* [40], *Liriomyza cicerina* [41], *Tribolium castaneum* and *T. confusum* [41], *Callosobruchus maculates* [42], *Stomoxys calcitrans* [43], *Culex pipiens* [44], etc. It was found as a promise control agent. The early investigation showed that Cyromazine is harmless to mammalian and poultry [45]. The present work was carried out aiming to evaluate the effects of Cyromazine on survival, growth, development and metamorphosis of the dangerous pest *S. littoralis*.

2. MATERIALS AND METHODS

2.1. Experimental Insect

A sample of *S. littoralis* pupae was kindly obtained from the culture of susceptible strain maintained for several generations in Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt. In laboratory of Entomology, Faculty of Science, Al-Azhar University, Cairo, a culture was established under laboratory controlled conditions (27±2°C, 65±5% R.H., photoperiod 14 h L and 10 h D). Rearing procedure was carried out according to Ghoneim [47] and improved by Bakr et al. [48]. Larvae were provided daily with fresh castor bean leaves *Ricinus communis*. The emerged adults were provided with 10% honey solution on a cotton wick as a food source. Moths were allowed to lay eggs on branches of *Nerium oleander*, then the egg patches were collected daily, and transferred into Petri dishes for another generation.

2.2. Bioassay of Cyromazine

**Cyromazine** [N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine] was supplied by Sigma-Aldrich Chemicals (https://www.sigmaaldrich.com). A series of concentration levels: 200.0, 100.0, 50.0, 10.0, 1.0, 0.1, 0.01 & 0.001 ppm was prepared using distilled water. Bioassay tests were carried out using the newly moulted larvae of 5th (penultimate) and 6th (last) instars. Fresh castor bean leaf discs were dipped in each concentration for 5 minutes and air dried before introduction to larvae for feeding. Control congeners were provided with water-treated leaf discs. Ten replicates of treated and control larvae (one larva/replicate) were kept separately in glass vials. The larvae were left to feed for 24 hrs and then all biological parameters were recorded daily.

2.3. Criteria of Study

2.3.1. Toxicity Test

All mortalities of treated and control (larvae, pupae and adults) were recorded every day and corrected according to Abbott’s formula [49] as follows:

\[
\text{% of corrected mortality} = \frac{\text{% of test mortality} - \text{% of control mortality}}{100 - \text{% of control mortality}} \times 100
\]

The LC\(_{50}\) value was calculated for general mortality by Microsoft office Excel, 2007, according to Finny [50].

2.3.2. Growth, Development and Metamorphosis

- **Growth:** Each individual larva (treated and control) was carefully weighed every day using a digital balance for calculating the growth as follows:

  Initial weight (before the beginning of experiment) - final weight (at the end of experiment).

- **Developmental rate:** Dempster’s equation [51] was applied for calculating the developmental duration, and Richard’s equation [52] was used for calculating the developmental rate. The pupation rate was expressed in % of the successfully developed pupae.

- **Deranged metamorphosis:** Deranged metamorphosis program of the cotton leaf worm was observed and calculated in larval-pupal or pupal-adult intermediates (%). Also, pupal deformation was calculated in %. Features of impaired development were recorded in photos.

- **Adult emergence:** number of successfully metamorphosed adults was expressed in % according to Jimenez-Peydro et al. [53] as follows:

  \[
  \left[ \frac{\text{No. of completely emerged adults}}{\text{No. of pupae}} \right] \times 100
  \]
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### 2.4. Statistical Analysis of Data

Data obtained were analyzed by the Student's *t*-distribution, and refined by Bessel correction [54] for the test significance of difference between means.

### 3. RESULTS

#### 3.1. Lethal Effects of Cyromazine on *S. littoralis*

After treatment of penultimate instar larvae of *S. littoralis* with eight concentration levels (200.0-0.001 ppm) of Cyromazine, data of lethal effects on all developmental stages were presented in Table (1). Depending on these data, Cyromazine, at its lower two concentration levels, failed to cause mortality, regardless the developmental stage. It exhibited a larvicidal activity on the treated larvae only at 200.0 and 0.10 ppm (20.0%). The successfully moulted last instar larvae suffered the lethal potency of Cyromazine only at the higher five concentration levels (62.5, 41.40, 33.30, 10.0 and 20.0%, respectively). In respect of the pupal mortality, data of the same table shows that Cyromazine failed to exhibit a pupicidal activity except at the higher two concentration levels (17.50 and 33.33%, respectively). Moreover, no adulticidal activity of Cyromazine was recorded for the successfully emerged adults. However, the corrected total mortality was 100.0% at the highest concentration level and then varied between 22.22 and 77.78% at concentration levels other than the lower two ones. LC$_{50}$ was calculated in 74.44 ppm.

According to data assorted in Table (2), treatment of last instar larvae of *S. littoralis* with different concentration levels of Cyromazine resulted in various larval and adult mortalities. No mortal potency of Cyromazine could be exhibited on the insect at the lower two concentration levels, regardless the developmental stage. With regard to the treated larvae, Cyromazine, at the highest concentration level, caused complete (100.0%) mortality but failed to exhibit a lethal effect at its lower three concentration levels. However, at other concentration levels, Cyromazine caused 10.0-40.0% larval mortalities. It, generally, failed to exhibit a pupicidal activity, regardless the concentration level. The successfully emerged adults suffered a lethal action of this compound only at 1.0 and 0.1 ppm (16.73 and 10.0% mortality, respectively). Although no certain trend could be detected for larval or adult mortality, the corrected total mortalities had been recorded in a dose-dependent course, regardless the lower two concentration levels. LC$_{50}$ was calculated in 82.91 ppm.

#### 3.2. Growth and Developmental Effects of Cyromazine on *S. littoralis*

The most important growth and developmental criteria of *S. littoralis*, after treatment of penultimate instar larvae with eight concentration levels (200.0-0.001 ppm) of Cyromazine, are assorted in Table (3). According to these data, growth of the treated larvae was drastically suppressed because their weight gain was seriously reduced, regardless the concentration level of Cyromazine. The strongest reducing effect on weight gain was exhibited at 100.0 ppm (8.92±1.61 vs. 95.56±0.52 mg of control larvae) while the least reducing effect was recorded at 10.0 ppm (27.24±2.12 vs. 95.56±0.52 mg of control larvae). Beside the remarkably affected growth of larvae, their developmental duration had been insignificantly prolonged at the lower two concentration levels while the prolongation was statistically significant at other concentration levels of Cyromazine. The maximally prolonged larval duration was measured in 2.80±0.42 days (vs. 2.22±0.44 days of control congeners) at the highest concentration level. The prolongation of duration was reflected in regressed developmental rate. However, the fastest developmental rate was recorded at the highest concentration level (40.00 vs. 45.05 of controls) but the slowest rate was estimated at both 100.0 and 1.00 ppm (35.71 vs. 45.05 of controls).

In the light of data of the same table, growth and development of the successfully moulted last instar larvae was slightly disturbed by Cyromazine. The growth of larvae was unexpectedly promoted at its lowest concentration level since their weight gain slightly increased (164.23±5.13 vs. 153.74±5.80 mg of control larvae). Nevertheless, treatments with other concentration levels prominently resulted in significantly prohibited growth as expressed in reduced somatic weight gain. The most powerful inhibitory effect of Cyromazine was exhibited at its higher two concentration levels (87.01±3.79 and 95.50±4.54 mg, at 200.0 and 100.0 ppm, respectively, compared to 153.74±5.80 mg of control larvae). In respect of the affected development of successfully moulted last instar larvae, their duration was statistically prolonged at the higher three concentration levels (8.33±0.58, 8.67±0.58 and 8.60±0.55 mg of control larvae)....
days, at 200.0, 100.0 and 50.0 ppm, respectively, vs. 6.89±0.78 days of control larvae). On the other hand, lengthened larval duration was recorded at other concentration levels of Cyromazine. Such duration lengthening had been expressed in slow developmental rate of treated larvae. The slower developmental rates were calculated for larvae at the higher three concentration levels (12.00, 11.53 and 11.63 at 200, 100 and 50 ppm, respectively, vs. 14.51 of control congeners).

Table (4) contains data of affected growth and development of *S. littoralis* after treatment of last instar larvae with different concentration levels of Cyromazine. As clearly shown in this table, Cyromazine exhibited a predominant inhibitory effect on growth, regardless the concentration level since the somatic weight gain was remarkably reduced in a dose-dependent course (ranged between 58.18±4.12 and 130.10±3.05 mg, compared to 153.74±5.80 mg of control congeners). Another prevalent inhibitory effect of Cyromazine was exhibited on the development because the larval duration was insignificantly prolonged at the lower five concentration levels but considerably lengthened at the higher three concentration levels (8.38±0.92, 8.67±0.58 and 8.20±0.45 days, at 200, 100 and 50 ppm, respectively, vs. 6.89±0.78 days of control larvae). Such finding was supported by data of the developmental rate which was severely regressed at these higher three concentration levels (11.93, 11.53 and 12.20, respectively, vs. 14.51 of control larvae).

### 3.3. Effect of Cyromazine on Metamorphosis of *S. littoralis*

After treatment of penultimate instar larvae of *S. littoralis* with different concentration levels of Cyromazine, some of the major parameters of metamorphosis program are presented in Table (3). Three parameters of the disturbed program had been recorded: pupation rate, production of larval-pupal intermediates and adult emergence%. Treatment of larvae with the highest concentration level of Cyromazine resulted in completely prevented pupation but no effect was determined after treatment with the lower two concentration levels. At other concentration levels, Cyromazine prohibited the pupation in a dose-dependent course. Cyromazine, at its lower two concentration levels, failed to affect pupation program since treated larvae metamorphosed into morphologically perfect pupae. On the contrary, treatment with other concentration levels led to impaired program since larval-pupal intermediates had been produced. The impairment of pupation program run in no certain trend but Cyromazine exhibited its strongest impairing effect at the highest concentration level (37.50% intermediates). Various forms of larval-pupal intermediates had been demonstrated in Plate (1). Because no larvae could pupate after treatment with the highest concentration level, no adults could be observed. However, the adult emergence was hindered only after treatment of larvae with 100.0 ppm (66.67 vs. 100% emergence of control adults). Thus, Cyromazine failed to affect the adult eclosion at the majority of its concentration levels.

Considering the metamorphosis program after treatment of last instar larvae of *S. littoralis* with different concentration levels of Cyromazine, some of the major parameters are presented in Table (4). These data exiguously reveal various degrees of impairing effect of this compound because treatment with its highest concentration level resulted in completely prevented pupation. At the lower three concentration levels, Cyromazine failed to affect the pupation while it prohibited the pupation rate at 100, 50, 10 and 1 ppm in a dose-dependent manner (30, 50, 60 and 80%, respectively, vs. 100% pupation of controls).

The pupation program was influenced by Cyromazine only at the higher four concentration levels since larval-pupal intermediates had been produced in 40, 30, 20 and 30%, respectively (see Plate 1 for various impaired pupation). Because the pupation was completely prevented after treatment with the highest concentration level, no adults could be developed. Cyromazine failed to block the adult emergence at the majority of its concentration levels. The adult emergence was partially blocked only after treatment of larvae with 100 and 50 ppm (66.7 and 80.0% of emerged adults vs. 100% emergence of control adults).
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Conc.: concentration levels, ---: Develop.: Developmental, Inter.: Intermediate. Mean ± SD followed with the same letter (a): insignificantly different (P >0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

**Table 2.** Lethal effect (%) of Cyromazine on *S. littoralis* treated as newly moulted last (6$^{th}$) instar larvae.

**Table 3.** Growth and development of *S. littoralis* after treatment of the newly moulted penultimate instar larvae with Cyromazine.

**Table 4.** Growth and development of *S. littoralis* after treatment of the newly moulted last instar larvae with Cyromazine.

Conc.: concentration levels, ---: Develop.: Developmental, Inter.: Intermediate. Mean ± SD followed with the same letter (a): insignificantly different (P >0.05), (b): significantly different (P<0.05), (c): highly significantly different (P<0.01), (d): very highly significantly different (P<0.001).

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Plate (1). Larval-pupal intermediates of S. littoralis as features of disturbed program of metamorphosis by Cyromazine larval treatments regardless the treated instar or concentration level. (A) Normal last instar larva. (B) Normal pupa. (C, D, E & F) Various larval-pupal intermediates.

4. DISCUSSION

4.1. Affected Survival Potential of S. littoralis by Cyromazine

Cyromazine is wildly used as an agricultural control agent. The early investigation showed that this compound is harmless to mammalian and poultry [46]. It exhibited insecticidal activity against some insects, such as Ciratitis capitata [55-57], D. melanogaster [58], Anopheles gambiae, Culex quinquefasciatus and Aedes aegypti [59], Liriomyza sativae [60]. The available literature contains many reported results of toxic effects of several IGRs on various insect species. In respect of S.
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littoralis, different larval and pupal mortalities had been recorded after treatment of larvae of certain instars with some IGRs, such as diflubenzuron [61-63], triflumuron [48, 62, 64, 65], chlorfluazuron [64,66-68], flufenoxuron [48,65,68-70], lufenuron [48,71-75], buprofezin [66,76,77], ecdysone agonist tebufenozide [78,79], ecdysone agonist methoxyfenozide [79], Novaluron [80], etc.

In the present study on the same insect, Cyromazine failed to cause larval mortality at the lower two concentration levels but at the higher ones, after treatment of penultimate instar larvae. At only the higher two concentrations, Cyromazine exhibited a pupicidal activity. No adult mortality was observed. LC50 was 74.44 ppm. After treatment of last instar larvae, Cyromazine exhibited similar mortal potency on larvae but failed to exhibit a pupicidal activity, regardless the concentrations and caused adult mortality only at 1.0 and 0.1 ppm. LC50 was 82.91 ppm. The current results of Cyromazine toxicity, at higher concentrations, are in agreement with the reported toxic effects on other insect species by various IGRs, such as Choristoneura fumiferana by tebufenozide and methoxyfenozide [81]; Musca domestica by Diofenolan [82]; Eurygaster integriceps by pyriproxyfen [83]; Dysdercus koenigii by flufenoxuron [84]; Papilio demoleus by Diofenolan [85]; Halyomorpha halys by diflubenzuron [86]; Spodoptera litura by chlorfluazuron [87]; Locusta migratoria var. manilensis by flufenoxuron, RH-5849 and pyriproxyfen [88]; C. piriens by kinoprene [89]; Agrois ipsilon by flufenoxuron and methoprene [90].

IGRs exhibit some toxic effects on the insects but with mode of action other than that of the conventional synthetic insecticides. Three sites have been proposed for describing the mode of action of CSIs namely: inhibition of chitin synthetase (or its biosynthesis), inhibition of proteases (or its biosynthesis) and inhibition of UDP-N-acetylglucosamine transport through the membrane [91]. Further, it was suggested that the CSI interferes with the transport system of UDP-N-acetyl amine across the membrane [92]. Although the disturbance of hormonal regulation by IGRs was reported [93, 94]. Larval and pupal mortalities of S. littoralis after treatment with the higher concentrations of Cyromazine, in the present study, may be related to some factors or causes, such as suffocation, bleeding and desiccation due to imperfect exuvation, failure of vital homeostatic mechanisms, etc. [95]. Moreover, the larval deaths of S. littoralis, in the current work, may be attributed to the inability of moulting larvae to swallow volumes of air to split the old cuticle and expand the new one during ecdisis [96]. Also, the actual cause of death by Cyromazine may be due to an inhibition of feeding and continuous starvation [97, 98]. On the other hand, adult mortality after treatment of last instar larvae of S. littoralis with only 1.0 and 0.1 ppm of Cyromazine, in the present study, can be explained by the retention and distribution of it in the insect body as a result of rapid transport from the gut of treated larvae into other tissues, the direct and rapid transport of the haemolymph to other tissues, and/or to lower detoxification capacity against the tested CSI [99].

4.2. Influenced Growth and Development of S. littoralis by Cyromazine

As reported in the literature, many IGRs exhibited some inhibitory effects on growth and development of S. littoralis. The growth of S. littoralis was inhibited by the ecdysone agonist tebufenozide [100], flufenoxuron [48, 70], lufenuron [74], triflumuron [70] and Novaluron [80]. On the contrary, buprofezin failed to affect the growth of this insect [77]. Also, development of the same insect was retarded by various IGRs, such as diflubenzuron [61, 63], chlorfluazuron [101], methoprene and Fenoxycarb [102], lufenuron [75]. In accordance with the majority of these reported results, the present work revealed various degrees of inhibited growth and retarded development of S. littoralis by Cyromazine because treatment of the penultimate instar larvae resulted in drastically suppressed because the weight gain was seriously reduced, regardless the concentration level. The developmental duration had been slightly prolonged indicating reduced developmental rate. After treatment of last instar larvae with Cyromazine, a predominant inhibitory effect was exhibited on growth, regardless the concentration level. Also, Cyromazine exhibited a prohibition effect on the development because the larval duration was slightly or considerably prolonged.

To a great extent, these results agree with many reported prohibited growth and development of other insect species by different IGRs, such as C. capitata by Cyromazine [56], P. demoleus by Diofenolan [85], S. litura by chlorfluazuron [87], A. aegypti [103] and C. piriens [104,105] by Novaluron, C. piriens by kinoprene [89] and A. ipsilon by methoprene and flufenoxuron [90]. In contrast, shortened developmental duration, and subsequently enhanced developmental rate, was reported for some insect species by various IGRs, such as Rhynchophorus ferrugineus by lufenuron and Diofenolan [106], A.
ipsilon by flufenoxuron [107] and Schistocerca gregaria by lufenuron [108]. Likewise, some IGRs failed to affect the growth of various insects, such as M. domestica [109,110], Periplaneta americana and Oncopeltus fasciatus [111], Spodoptera exempta, Spodoptera exigua, and L. decemlineata [95].

Retarded or enhanced development, as expressed in prolonged or shortened durations, of insects may be attributed to diverse effects of IGRs on the release of ecdysteroids indirectly by interference with neuroendocrine organs responsible for the synthesis and release of tropic hormones, like prothoracicotropic hormone [112]. The inhibited growth of S. littoralis by Cyromazine, in the current study, may be a result of the blocked release of morphogenic peptides, causing alteration in the ecdysteroid and juvenoid titers [113]. Also, Cyromazine may affect the tissues and cells undergoing mitosis [114]. The retarded development of S. littoralis, in the current study, may be explicated by a delaying effect of Cyromazine on ecydisis and transformation [96,115].

4.3. Disturbed Metamorphosis of S. littoralis by Cyromazine

Different symptoms of the impaired metamorphosis of S. littoralis, after treatment with various IGRs, had been reported in the literature. The major symptoms and features can be described as reduction of pupation and adult emergence, production of larval-pupal and/or pupal-adult intermediates, deformed larvae and/or pupae and production of supernumerary larval instars. However, all or some of these features were observed in this insect after treatment with several IGRs, such as Diflubenzuron [61, 62, 116], chlorfluazuron [64, 71, 101, 117, 118], triflumuron [62, 64, 70], lufenuron [72, 74, 118], flufenoxuron [48, 69, 70], methoprene and Fenoxycarb [102].

In the present study, treatment of penultimate instar larvae of S. littoralis with Cyromazine concentration levels, other than the lower two ones, resulted in prohibited pupation in a dose-dependent course. Also, the pupation program was impaired since some larval-pupal intermediates had been produced. Cyromazine failed to affect the adult emergence, at the majority of the concentration levels. Except the lower three concentration levels, treatment of last instar larvae with other concentration levels resulted in prohibited pupation rate and impaired pupation program. At only 100 and 50 ppm of Cyromazine, the adult emergence was partially blocked. Neither malformed larvae nor malformed pupae were observed, regardless the time of treatment and concentration level. To some extent, these results are consistent with many reported results of impaired metamorphosis of several insect species by different IGRs, such as M. domestica [119], C. capitata [55, 56]; T. castaneum and T. confusum [42], Liriomyza trifoli [120] and C. maculates [121] by Cyromazine; H. armigera [122], Phlebotomus papatasi [123], A. aegypti [124, 125], M. domestica, Haematobia irritans and S. calcitrans [126] by Novaluron; Blattella germanica [127], Ch. fuligenerana [128] by Fenoxycarb; Lipaphis erysimi by pyriproxifen [129]; Rh. fargnigueus [106] and P. demoleus [85] by Difenolan; Lobesia botrina by lufenuron [130]; C. pipiens by kinoprene [89]; etc.

As reported by [131], the effects caused by IGRs on the metamorphosis of insects may be important from a practical standpoint because they could result in various morphogenic defects as well as mortality. Lepidoptera belong to the most sensitive groups of insects regarding the growth regulating effects of these compounds. Disturbed metamorphosis of S. littoralis by Cyromazine, in the present study, can be interpreted by its interference with the hormonal regulation of programs of pupation, and to some extent the adult eclosion, since the disturbance of such vital process by IGRs was reported [93, 94]. In other words, Cyromazine may affect these programs leading to an inhibition of metamorphosis via an ecdysteroid reduction, interference with the release of eclosion hormone or/and inhibition of the neurosecretion [132]. In addition, production of larval-pupal intermediates in S. littoralis can be explained by an inhibitory effect of Cyromazine on the chitin biosynthesis, chitin synthase [133-135] and DNA synthesis [136]. Whatever the mode of action, Cyromazine suppressed the chitin synthesis and prevented the normal deposition of new cuticle during apolysis leading to the production of moulting abnormalities [137].

5. Conclusion

Cyromazine exhibited a lethal effect at LC$_{90}$ values of 74.44 and 82.91 ppm after treatment of penultimate and last instar larvae, respectively. It pronouncedly prohibited the larval growth and development, especially at the higher concentrations. Various degrees of the pupation prohibition had been recorded. Degrees of the adult eclosion blockage depended on the time of larval treatment and the concentration level. Therefore, the present CSI can be included in the integrated pest management program for S. littoralis.
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REFERENCES


[37] Abdel-Monem A.H., Cameron E.A. and Mumma R.O., Toxicological studies on the molt inhibiting insecticide (EL- 494) against the gypsy moth and effect on chitin biosynthesis. J. Econ. Entomol. 73, 22-25(1980).


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[78] Smaghe G. and Degheelee D., Comparative toxicity and tolerance for the ecdysteroid mimic tebufenozide in a laboratory and field strain of cotton leafworm (Lepidoptera: Noctuidae). J. Econ. Ent. 90(2), 278-282(1997).


Toxicity and Bioefficacy of Cyromazine on Growth and Development of the Cotton Leafworm Spodoptera littoralis (Lepidoptera: Noctuidae)


[100] Gobbi A., Budia F., Schneider M., Estal P. del, Pineda S. and Viñuela E., Tebufenozide effects on Spodoptera littoralis (Boisduval), Mythimna unipuncta (Haworth) and Spodoptera exigua (Hübner). Boletín de Sanidad Vegetal, Plagas 26(1), 119-127(2000).


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