An Experimental Study of Certain Neonicotinoid Insecticides on the Incidence of Early Infestation of the Spiny and the American Bollworms, Growth and Lint Yield Characteristics of Cotton Plants

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ABSTRACT

The cotton bollworms are major pests that reduce yield and quality of the growing cotton crop all over the world. The Spiny bollworm (SBW) *Earias insulana* (Boisd.) and American bollworm (ABW) *Helicoverpa armigera* (Hüb) are two common injurious bollworms of cotton in Egypt. A field experiment was conducted at Abo-Homos district, El-Beihara, Governorate during 2015 & 2016 cotton season to determine the efficacy of two neonicotinoid insecticides (imidacloprid and thiamethoxam) as seed dressing and seedling bed at the 3rd true leaf emergence of cotton plants (Giza 86). Reduction in the infestation and larval content/100 green bolls and/or plants of both pests was estimated through 30 - 90 days of germination. Furthermore, the total chlorophyll content, cotton yield and its fiber quality was determined. The results showed that imidacloprid and thiamethoxam caused a significant larval reduction (79.9, 75.5% & 66.9, 72.7%) of the SBW in both experimental seasons, respectively, at early infestation after 90 days of germination. In case of the ABW, thiamethoxam caused a gradual reduction (79.5, 70.8%) in both seasons, respectively. While, in imidacloprid treatment reduction was fluctuated (54.0 - 70.8%) after 90 days of germination in 2016 season. Most interestingly, the present results indicated that the application of imidacloprid and thiamethoxam as seed treatments significantly increased the total chlorophyll content in cotton leaves that enhance plant growth and improve cotton properties. In general, the selection of a suitable insecticide for controlling the cotton pests not only depends on its efficacy against the targeted insects but also on their profitable effects on cotton production and fiber quality.

**Keywords:** *Earias insulana* (Boisd.), *Helicoverpa armigera*, neonicotinoid, chlorophyll, fiber quality, yield.

INTRODUCTION

Cotton is the most important commercial fiber crops in the world and considers a prominent place in the national economy. The cotton plant seems to be highly attractive to injurious insects i.e. the cotton leafworm, pink bollworm, spiny bollworm, the American bollworm, cotton aphid, cotton flea hoppers, leafworm, pink bollworm, spiny bollworm, the highly attractive to injurious insects i.e. the cotton blooms may open prematurely and stay fruitless. Moreover, when the bolls are damaged, some of which will fall off and others will fail to produce lint or produce lint of an inferior quality. The annual worldwide costs for controlling this pest along with yield losses are estimated at US $ 5 billion (Lammers & Macleod 2013). Thus, great efforts have been made to improve the efficiency and specificity in insect control. Insecticides remain the most important element of integrated approaches in pest control. But unfortunately, insecticides used extensively tend to lose their effectiveness because of an increasing cycle of resistance. Lepidoptera, most notably cotton bollworms, had developed resistance to the major classes of insecticides, such as pyrethroids (Scott-Dupree et al. 2008; Achalke and Brevault, 2010, Nada et al. 2011); carbamates (Kranthi et al. 2001; Torres-Vila et al. 2002); and organophosphates (Kranthi et al. 2001; Martin et al. 2003, Ren et al. 2002, Torres-Vila et al. 2002). Moreover, cotton bollworms did not only demonstrated resistance to the conventional pesticides but also showed resistance to *Bacillus thuringiensis* (Bt) transgenic cotton (Akhurst et al. 2003; An et al. 2015; Ibargutxi et al. 2006; Nair et al. 2010; Tabashnik et al. 2012). So far, no resistance to the neonicotinoids has been documented in this insect pest.

Imidacloprid and thiamethoxam are chloronicotinyl class of insecticides that are nicotinic analogue; a neurotoxin which binds to the nicotinic acetylcholine receptor (nAChR) of insects (Matsuda et al. 2001), causing hyper-excitation that eventually leads to the insect’s death (Matsuda et al. 2005). Because of their systematic characteristic, they are applied against soil-living pests and seed as well as foliar treatments (Magalhaes et al. 2009; Lanka et al. 2013). In recent years, the use of systemic insecticide as seed treatments is considered one of the most effective...
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The infestation time of both SBW and ABW indicate that comparison to control in the 1st season 2015. In addition, the first infestation of SBW was observed after 70 days for imidacloprid and thiamethoxam in comparison to control in the 1st season 2015. In the 2nd season, 2016 (Tables 1 and 2).

MATERIALS AND METHODS

Experimental design

Two neonicotinoid insecticides were experimented against the most common bollworm-infested cotton in Egypt, SBW and ABW. A field experiment was conducted at Abo-homos district, El-Behaira, Government, Egypt, during the subsequent growing cotton season of 2015 and 2016. An area of about one feddan (feddan= 4200 square meter) was cultivated with cotton, *Gossypium barbadense* (Boisd.) and the American bollworm (ABW) *Helicoverpa armigera* (Hüb) infesting cotton( var.Giza 86) in Egypt. The infestation rate was determined after a period of 90 days post germination which is considered as a critical growth period of cotton plant development and boll formation. The growth, yield and the fiber quality of cotton plant were also determined.

Insecticides and seed treatment

1. **Imidacloprid:** (E) - 1 - (6-chloro -3- pyridylmethyl) - N-nitromidazolidin -2- ylidenameine. The formulation of this insecticide (Gaucho 7% WS) was obtained from Bayer Co. It was applied as seed dressing treatment at 7 g/kg seeds.

2. **Thiamethoxam:** (EZ) – 3 - (2-chloro-1, 3-thiazol-5-ylmethyl) - 5-methyl - 1, 3, 5-oxadiazinan-4-ylidene (nitro) amine. The formulation of this insecticide (Actara 25% WP) was obtained from Syngenta AG Co. It was applied as irrigation in seedling bed at 20mg/100-liter water after the emergence of the 3rd true leaf.

**Total chlorophyll content**

The mean of three registered readings by means of a portable chlorophyll meter SPAD-502 (Konica-Minolta, Japan) was calculated for each leaf disc of sampled individual leaves (10 leaves per plant) and pooled to obtain one SPAD measurement per disc. The leaf disc used to obtain a SPAD value provided sufficient tissue for total chlorophyll. Total chlorophyll content (nmol mg-1) of leaves was determined according to (Kartiya et al. 1982).

**Determination of cotton yield and fiber properties**

The cotton yield was measured as the weight of lint (kg/plot); transformed to Kentar/feddan, (Kentar equal 50 kilograms of lint cotton). The USTER® HVI1000 system was used to measure the most important cotton fiber properties for cotton classing purposes in particular, moisture content, micronaire, maturity index, length, strength, color and trash.

**Statistical analysis**

The obtained data were analyzed by one-way analysis of variance using SPSS program (version 11.0; SPSS Inc. Chicago, IL, USA, 2011). The means were compared using the LSD test at P < 0.05.

**RESULTS AND DISCUSSION**

a) Efficiency of applied imidacloprid and thiamethoxam seed treatments against the early bollworms infestation of cotton plants:

The insecticidal activity of each of experimented imidacloprid and/or thiamethoxam as seed treatments against the two bollworms; SBW and ABW was evaluated under field conditions during the following seasons of 2015 and 2016. Data presented in Tables (1 and 2) summarize the extracted means number of cotton bollworm larvae/100 plant throughout 90 days after seeds germination. It is obvious that imidacloprid and thiamethoxam induced highly efficient delayed toxic effect against bollworms. The results related to the infestation time of both SBW and ABW indicate that high critical time of infestation occurrence usually begins after 50 days of germination for ABW and 60 days for SBW while the first infestation of SBW was observed after 70 days for imidacloprid and thiamethoxam in comparison to control in the 1st season 2015. In the 2nd season, 2016 (Tables 1 and 2)
On the other hand, the first infestation incidence of ABW was recorded after 60 days for imidacloprid and 70 days for thiamethoxam in the 1st season 2015 while in the 2nd one of 2016 the first infestation of ABW was observed after 60 and 70 days for both of tested imidacloprid and thiamethoxam respectively, compared to the recorded first infestation of control which occurred after 50 days of seed germination in the both seasons. It means that each of imidacloprid and thiamethoxam caused significant delay of probable incidence of the cotton bollworms infestations that ranged from 10 to 20 days. Moreover, the comparatively fewer calculated mean number of SBW and/or ABW for each treatment was significantly lower when compared to the respective control. Also, the obtained data showed that both the separately tested insecticides, exhibited a significant reduction in the means numbers of both inspected cotton bollworms. For SBW, imidacloprid caused a 100% initial reduction after 50 days of germination and impairment of non-significantly decreased to reach 79.9 and 75.5 % after 90 days post germination in both season respectively (Tables 1 & 2). Regarding ABW, the exhibited data in Tables 1&2, also show that imidacloprid and thiamethoxam induced a significant reduction in the inspected number of infested plants by this insect. Reduction % infestation comprised 100.0 – 79.5 % and 100.0 – 83.1 % after 60 - 90 days of germination in 2015 & 2016 cotton season, respectively. In the other hand, imidacloprid induced gradual reduction ranged between 100.0 to 80.9% after 60 - 90 days of germination in 2015. In contrary, during 2016 season reduction% tumble to 54.0% after 60 days of germination then increased gradually to achieve 70.8 % after 90 days; anyhow the reduction value ranged between 100.0 to 70.8 % after 50 - 90 days of germination (Table 2).

Our obtained results clearly show that imidacloprid and thiamethoxam used separately as seed treatments gave highly effective protection against cotton bollworm larvae of early infestation. From these above cited results, it could be also noticed that imidacloprid had a slightly better toxic efficiency against both of inspected cotton bollworms than thiamethoxam, due to its highly systemic activity, especially through the root system. The obtained results were also in parallel with the pervious literary findings which showed that imidacloprid has a better efficiency against sap-sucking pests than thiamethoxam (El-sayed, 2017).

### Table 1. Insecticidal activity of imidacloprid and thiamethoxam applied as seed treatments against the early infestation of SBW & ABW on cotton plants (Giza 86) during the 2015 season.

<table>
<thead>
<tr>
<th>Inspection(s) Time</th>
<th>Earias insulana</th>
<th>Helicoverpa armigera</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 days</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>40 days</td>
<td>0.0±0.0</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>50 days</td>
<td>2.7±0.33</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>60 days</td>
<td>6.3±0.88</td>
<td>1.3±0.33</td>
</tr>
<tr>
<td>70 days</td>
<td>7.7±0.33</td>
<td>1.3±0.33</td>
</tr>
<tr>
<td>80 days</td>
<td>10.3±0.88</td>
<td>2.0±0.0b</td>
</tr>
<tr>
<td>90 days</td>
<td>13.0±1.15a</td>
<td>3.0±0.58b</td>
</tr>
</tbody>
</table>

Mean number (M.N.) %R (Percent of Reduction) = [(M.N. in the control – M.N. in the treatment)/ M.N. in the control] x 100

The mean inside each row followed by the same letter do not differ from each other significantly from each other by the LSD at the 0.05 level

### Table 2. Insecticidal activity of imidacloprid and thiamethoxam applied as seed treatments against the early infestation of SBW & ABW on cotton plants (Giza 86) during the 2016 season.

<table>
<thead>
<tr>
<th>Inspection(s) Time</th>
<th>Earias insulana</th>
<th>Helicoverpa armigera</th>
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<tr>
<td>30 days</td>
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<td>40 days</td>
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<td>0.0±0.0</td>
</tr>
<tr>
<td>50 days</td>
<td>3.0±0.58a</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>60 days</td>
<td>6.0±0.58a</td>
<td>0.0±0.0</td>
</tr>
<tr>
<td>70 days</td>
<td>7.7±0.88a</td>
<td>1.0±0.0b</td>
</tr>
<tr>
<td>80 days</td>
<td>10.0±0.88a</td>
<td>1.3±0.33b</td>
</tr>
<tr>
<td>90 days</td>
<td>11.3±0.67a</td>
<td>3.0±0.58b</td>
</tr>
</tbody>
</table>

Mean number (M.N.) %R (Percent of Reduction) = [(M.N. in the control – M.N. in the treatment)/ M.N. in the control] x 100

The mean inside each column followed by the same letter do not differ from each other significantly from each other by the LSD at the 0.05 level
Moreover, our exhibited results are in conformity with the previous carried out studies investigating the efficacy of imidacloprid as well as thiamethoxam on the larvae of European Corn Borer and Indian meal moth (Lepidoptera: Pyralidae). Yue, et al. (2003) found that all the fifth instar larvae of European corn borers died after 2 or 4 days of exposure to corn treated with 250 and 500 ppm, thiamethoxam. On the other hand, all second and third instars of Indian meal moth died after 5 days of exposure period to corn grain treated with imidacloprid or thiamethoxam at 50 ppm. This confirmed by Zhang et al. (2011) who provided that the cotton seeds treated with imidacloprid and thiamethoxam were effective against B. tabaci for up to 45 days under laboratory and greenhouse conditions, and up to 2 months under field conditions.

**b) Effect of imidacloprid and thiamethoxam on chlorophyll content, growth and lint yield of cotton plant**

The revealed effect of imidacloprid and thiamethoxam on the chlorophyll content of cotton plant is summarized in Table (3). The results showed a significant increasing trend in the chlorophyll content due to both performed treatments compared to the respective control. The higher calculated values of chlorophyll content comprised amounted to 44.5±1.21, 46.6±0.90 and 42.7±0.68, 42.2±0.52 nmol mg⁻¹ for imidacloprid and thiamethoxam in both the following seasons of 2015 and 2016 respectively, whereas the estimated value in control, was lower and equaled (36.6 and 37.7) nmol mg⁻¹. Thus, both treatments of imidacloprid and thiamethoxam more increased the photosynthesis levels and physiological of activity of cotton plant, which finally reflected on the chlorophyll content. The same performance was observed by many authors Gonias et al. (2006) in cotton Preetha and Stanley (2012) in cotton Baozhen et al. (2013) in maize and Huang et al. (2015) in oilseed rape. Preetha and Stanley (2012) provided that the neonicotinoid insecticides increased the soluble protein content of cotton and okra. This is reported to improve the ability of plants to fix carbon dioxide (CO2) effectively and thus increase photosynthesis. Baozhen et al. (2013) showed that the increase of chlorophyll content in maize differently affected by imidacloprid at different application times, its content mostly increased by morning application, followed by noon, and least by afternoon application. Gonias et al. (2006) found that imidacloprid enhanced the metabolism of cotton plant, which was recorded as improved photosynthesis. Also, Huang et al. (2015) revealed that thiamethoxam treatment induced significant increase in the chlorophyll content in the leaf of oilseed rape when compared to control.

The best remarkable knowledge about our research article is the first throwing light on the influence of imidacloprid and thiamethoxam on square appearance in the cultivated cotton-variety (Giza 86), *Gossypium barbadense*. The data presented in Table (3) illustrate that both imidacloprid and thiamethoxam reduced the elapsed period (per day) from planting to appearance of early square. The early detection of the square was observed after 50 days for both insecticides in both the following seasons, while in control treatment, the first appearance of square was recorded after 60.0 & 58.3 days for both consequent seasons. Herein, it could be concluded that the application of each of imidacloprid and/or thiamethoxam as seed treatment promoted the early appearance of cotton squares.

The present study also showed that cotton seed treatments of imidacloprid and thiamethoxam had a significant positive effect on green and dry boll weights as well as the lint yield (Table 3). The green and dry boll weights were significantly increased by (17.6, 17.9g and 4.6, 4.6g) post imidacloprid treatment and by (18.1, 18.5g and 5.8, 5.3g) for thiamethoxam, compared to (13.9, 13.5g and 3.1, 3.2g) in the untreated control in both seasons, respectively. The same trend of results had been detected for both insecticides on the measured lint yield in both seasons, where, the lint yield was merely equally increased by (8.5 - 8.7) kentar/feddan for both insecticides in the two subsequent seasons, compared to the untreated control plants that gave lint yield of (5.6 – 5.8) kentar/feddan only. The statistical analysis of data revealed the insignificant differences between imidacloprid and thiamethoxam. The present results are in agreement with that findings reported by Gonias et al. (2006) who studied the effect of imidacloprid on cotton growth and yield enhancement after foliar application in the absence of insects. They reported that imidacloprid increased lint yield, dry matter production as well as crop growth.

**c) The impact of imidacloprid and thiamethoxam on cotton fiber quality**

Data presented in Table (4) show the effect of imidacloprid and thiamethoxam on cotton fiber quality in the both seasons of 2015 and 2016. The results revealed that both applied insecticides significantly increased the examined cotton fiber properties compared to the untreated control. No significant differences were observed in fiber fineness (micronaire reading) as affected by both compounds in season 2016. Whereas, the highest value of micronaire reading (4.98, 4.92 micronaire unit) resulted post thiamethoxam followed that of imidacloprid which comprised (4.36, 4.98 micronaire unit) in both the subsequent seasons, respectively, while the lowest value of (3.14 micronaire unit) was found in the untreated control. It is clearly obvious that imidacloprid and thiamethoxam applications could improve the micronaire factor. Our results are coincide with those mentioned by Mohamed (2013) who observed that higher micronaire value was obtained in case of treatment with imidacloprid and acetamiprid for controlling cotton bollworms and Jassid insect. Also, maturity index was affected by both the tested insecticides, which gave value of maturity index comprising (0.84 - 0.89 index) compared to control (0.60 index). In regards to the measurements of fiber length, the results indicated that there were no significant differences in all performed measurements except the detected values of uniformity index which showed an interaction significant difference between thiamethoxam (85.2%), imidacloprid (84.8%) and control (83.6%) in the 2nd
Insecticides SPDA Chlorophyll content (nmol mg-1) Nitrogen % First square (day) Green boll weight (g) Dray boll weight (g) Lint yield/Feddan (kentar)

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>SPDA Chlorophyll content</th>
<th>Nitrogen %</th>
<th>First square (day)</th>
<th>Green boll weight (g)</th>
<th>Dray boll weight (g)</th>
<th>Lint yield/Feddan (kentar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>44.5±1.21 a</td>
<td>4.6±0.40 b</td>
<td>50.0±0.00</td>
<td>17.6±0.61 a</td>
<td>4.6±0.35 b</td>
<td>8.5±0.14 a</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>42.7±0.68 a</td>
<td>4.7±0.21 b</td>
<td>50.0±0.00</td>
<td>18.1±0.62 a</td>
<td>5.8±0.06 a</td>
<td>8.6±0.09 a</td>
</tr>
<tr>
<td>Control</td>
<td>36.6±0.41 b</td>
<td>5.7±0.37 a</td>
<td>60.0±0.00</td>
<td>13.9±0.35 b</td>
<td>3.1±0.21 c</td>
<td>5.6±0.32 b</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>2.89</td>
<td>0.68</td>
<td>1.87</td>
<td>0.82</td>
<td>0.72</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.** Effect of imidacloprid and thiamethoxam on the chlorophyll content, growth and lint yield during 2015 and 2016 cotton seasons.

<table>
<thead>
<tr>
<th>Insecticides</th>
<th>Micronaire</th>
<th>Maturity index</th>
<th>Uniformity index %</th>
<th>Short fiber index %</th>
<th>Fiber strength (g/tex)</th>
<th>Elongation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>4.36±0.07 b</td>
<td>0.84±0.00 a</td>
<td>84.7±1.07</td>
<td>7.3±0.44 b</td>
<td>36.1±1.91</td>
<td>6.6±0.18 a</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>4.98±0.09 a</td>
<td>0.89±0.06 a</td>
<td>84.8±0.58</td>
<td>7.2±0.26 b</td>
<td>40.0±0.23</td>
<td>5.4±0.23 c</td>
</tr>
<tr>
<td>Control</td>
<td>3.14±0.06 b</td>
<td>0.60±0.00 b</td>
<td>83.6±0.35</td>
<td>7.9±0.21 a</td>
<td>38.6±1.93</td>
<td>6.0±0.15 b</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.53</td>
<td>0.24</td>
<td>2.54</td>
<td>0.09</td>
<td>5.43</td>
<td>0.54</td>
</tr>
</tbody>
</table>

**Table 4.** Effect of imidacloprid and thiamethoxam applications on fiber quality properties during 2015 and 2016 cotton seasons.

Finally, it could be concluded that both of the tested imidacloprid and thiamethoxam as a seed treatment, are promising candidate compounds that could be used successfully to protect and reduce the cotton bollworm infestation in cotton, improve growth and increase lint yield. Moreover, further studies are needed to understand the impact of these insecticides on the behavioral and physiological changes of cotton bollworm larvae.

**ACKNOWLEDGMENT**

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