Efficiency of Agroecosystem Compounds against the desert locust Schistocerca gregaria (Forskal) and the African migratory locust Locusta migratoria migratorioides (Reiche and Fairmaire)

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ABSTRACT

The efficacy of Agroecosystem Compounds, fipronil, spinosad and chloraantraniliprole as alternatives to the conventional insecticides on the mortality rates and haemolymph protein, lipid and carbohydrate contents were assessed in Schistocerca gregaria and Locusta migratoria migratorioides. One day old 5th nymphal instars of each of the mentioned species were treated by various concentrations of tested compounds by the feeding technique. The results indicated that Locusta migratoria migratorioides is more sensitive to all tested insecticides than Schistocerca gregaria. In both species, fipronil was the most effective followed by spinosad, and chloraantraniliprole was the least effective. LC30 concentrations of the three compounds were used to treat the 5th instar hoppers of both species to examine the effects of sub lethal concentrations of the mentioned insecticides on the haemolymph total proteins, total lipids and total carbohydrates. It was found that haemolymph proteins, lipids and carbohydrates contents were dramatically reduced for the treated hoppers when compared to the untreated ones of both species. Malformed hopper and wrinkled winged adult were seen in insects treated by sub lethal concentrations of fipronil and spinosad.

KEYWORDS: Insects, Schistocerca gregaria, Locusta migratoria migratorioides, proteins, lipids, carbohydrates.

1. INTRODUCTION

The Desert Locust, Schistocerca gregaria (Forskal) and the African Migratory Locust, Locusta migratoria migratorioides (Reiche & Fairmaire) are two of the most economically important pest species in Egypt as well as other African countries. S. gregaria can invade Egypt during the winter and spring breeding seasons from outside the country and breeds if the conditions are favorable, and the resulted breeding can invade the farmed crops in the cities of the Nile valley. L. migratoria migratorioides has become a major pest in the western desert of Egypt, mainly in the large land reclamation projects of Sharq El-Owinat and Toshka. Both of these two key pests cause great damages to crops when they increase in numbers and form hopper bands or swarms.

Currently; chemical control of locusts using the organophosphorous pesticides and synthetic pyrethroids is still the main tool to control locusts, while organochlorines were banned due to their persistence and severe effects on the environment (Lecoq 2001). The widespread use of the synthetic pesticides has considerable negatives such as handling hazards, the development of insect resistance to insecticides, increased costs, concerns about insecticide residues, and great threats to both human and environmental health (Garriga and Caballero 2011), which occurs due to pollution by the chemical pesticides such as toxicity to non-target organisms (Tingle 1996) and to humans too (Pretty, 1996). All this resulted in searching and development of new alternatives to control locusts.

This study aims to find humanly and environmentally safer and fast alternatives to the conventional insecticides. The study suggests new alternative compounds from various groups of pesticides to control the two key agricultural pests in Egypt including fipronil from phenylpyrazoles, spinosad as a biological agent and chloraantraniliprole from the antranilic diamides group.

2. MATERIALS AND METHODS

2.1. Test insects

Two insect species were used in the present study, S. gregaria and L. migratoria migratorioides were reared under laboratory conditions. S. gregaria individuals were obtained from the stock culture maintained for several generations at the Locust Research Section, Plant Protection Research Institute (PPRI), ARC, Dokki, Giza Egypt. The insects were reared according to (Robert et al., 2002) in wooden framed cages measuring (55 x 55 x 60cm). The L. migratoria migratorioides insects
were collected from infested field farms in Sharq El-Owinat area (southwest Egypt) in May 2016 before the beginning of control campaigns, and were reared in the same way as *S. gregaria* insects. The first generation of *L. migratoria migratorioides* was left to breed and give a second generation from which the hoppers were used for the bioassay. The insects of *S. gregaria* were fed on branches of clovers, *Trifolium alexandrinum*, while the insects of *L. migratoria migratorioides* were fed on maize leaves, *Zea mays*. The locusts' cages were kept at 30 ± 5 °C and 20-25 % R.H.).

### 2.2. Bioassay methods (Toxicity trials)

Leaf dipping technique was used to examine the different concentrations of chlorantraniliprole as 20% Suspension Concentrate (Coragen®), Spinosad as 24% SC (Tracer®), and Fipronil as 20% SC (Coach®) on the mortality rates of one day old 5th instar nymphs of *S. gregaria* and *L. migratoria migratorioides* under laboratory conditions. Clovers leaves were submitted to *S. gregaria* and maize (corn) leaves submitted to *L. migratoria migratorioides*. The hoppers in both species were subdivided, in each treatment, into five replicates with ten nymphs for each replicate. The hoppers were starved for 24 hours and were feed on the leaves dipped for 10 seconds in: 30 ppm, 60 ppm, 120 ppm, 180 ppm of spinosad, 0.1 ppm, 0.5 ppm, 1 ppm and 2 ppm of fipronil, and 60 ppm, 240 ppm, 480 ppm and 600 ppm of chlorantraniliprole. The leaves were air dried under room temperature for one hour before providing to the nymphs, and water dipped leaves were provided to the hoppers of the control treatment. After 24 hours of treatment; the treated leaves were replaced with non-treated leaves. Mortality was recorded after 1, 2 and 3 days post treatment, and observations on the insect feeding and general behavior were noted. LC50, LC90 and LC99 were calculated using (Ldp line) software according to the method of Finney (1971).

### 2.3. Biochemical changes in haemolymph

The 5th instar hoppers, haemolymph total proteins, total lipids and total carbohydrates were estimated in both species after treatment with sub-lethal concentrations (LC30) of chlorantraniliprole, spinosad and fipronil. The experiments were carried out by treatment of 1-day old 5th instar nymphs under laboratory conditions which were starved for additional 24 hours. A group of each species containing one hundred and fifty treated nymphs were divided into five replicates. The hoppers were fed for 24 hours on treated lettuce leaves in case of *S. gregaria* hoppers and were fed on treated maize leaves in the case of *L. migratoria migratorioides*. The control insects were fed on non-treated leaves and placed under the same conditions in 14:10 hours (light: dark) (Robert et al., 2002).

Haemolymph samples of control and treated hoppers were taken at different intervals of 2, 4, and 6 days post treatment. The haemolymph was collected through a fine puncture in the hind leg membrane and from beneath the dorsal pronotal shield membrane and transferred into dry centrifuge tubes (Metawe et al., 2001). The haemolymph was taken from each treatment using treated hoppers from all replicates and put into the Eppendorf tubes which were kept in the freezer. The remaining hoppers in each treatment were left to moult into adults and complete the life cycle, while observations on their feeding and general behavior activity were noted.

### 2.4. Determination of total proteins

Total proteins were estimated using the method explained by Bradford (1976) method. Sample solution (50 µl) or for preparation of standard curve 50 µl of serial concentrations containing 10 to 100 µg bovine serum albumin were pipetted into test tubes. The absorbance at 595 nm was measured after 2 min and before 1 hour against blank prepared from 1 ml of phosphate buffer and 5 ml protein reagent.

\[
\text{mg protein} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{absorbance of standard}
\]

### 2.5. Determination of total lipids

Total lipids were estimated by the method of Knight et al. (1972), by preparation of phosphovanillin reagent and standard solution, and the developed color was measured at 525 nm against reagent blank after 45 min. 250 µl was treated in the manner as the sample solution, the amount of mg lipids = \(\frac{\text{absorbance of test sample}}{\text{absorbance of standard}}\) x absorbance of standard.

### 2.6. Determination of total Carbohydrates

Total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of Dubios et al. (1956). Blanks were prepared by substituting distilled water for the sugar solution. The absorbance of characteristic yellow – orange colour is measured at 490 nm against blank. Total carbohydrate is expressed as: µg glucose / gm fresh weight. Carbohydrate concentration was expressed as mg glucose / 100 ml haemolymph.

\[
\text{mg Carbohydrates} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times \text{absorbance of standard}
\]
3. Results and discussions

3.1. The mortality rates of *S. gregaria* and *L. migratoria migratorioides* hoppers

The efficacy of chlorantraniliprole, spinosad and fipronil was calculated by using Abbott’s formula (1925) as follows:

Corrected % mortality = \((1 - \frac{n}{n_{in\ T\ after\ treatment}}) \times 100\), where \(n\) is insect population, \(T\) is treated and \(Co\) is control.

Data in table (1) indicates the corrected mortality rates of one-day old 5th nymphal instar after 3 days of treatment with 60, 240, 480 and 600 ppm of chlorantraniliprole were (20 % and 33.33%), (43.33% and 60%), (53.33% and 70%) and (66.67% and 80%) for *S. gregaria* and *L. migratoria migratorioides*, respectively. Chlorantraniliprole (Prevathon®) was found to be effective under laboratory conditions against 3rd instar hoppers of the oriental migratory locust, *Locusta migratoria* L., in a trial by Cao et al., (2017). The results agrees also with those found under field conditions by Bradshaw et al., (2013) who found that chlorantraniliprole (Coragen® and Prevathon®) as anthranilic diamide compounds could reduce the populations of rangeland grasshoppers at least as well as other standard products.

Additionally, the data in table (1) illustrates that the corrected mortality rates 3 days post treatment with 30, 60, 120 and 180 ppm of Spinosad were (34.48% and 51.72%), (55.17% and 89.66%), (86.21% and 96.55%) and (100% and 100 %) for the 5th nymphal instar of *S. gregaria* and *L. migratoria migratorioides* respectively. The results agrees with the finding by Kamel (2018) who found that spinosad (Tracer®) caused 100% mortality of the 4th instar hoppers of *S. gregaria* after 48 hours of treatment using leaf dipping technique. Similar results also were found by Said (2014) who found that Spinosad caused similar levels of mortality in the laboratory experiments when 5th instar nymphs of *S. gregaria* were exposed to spinosad dipped clover leaves.

Furthermore; table 1 shows that the corrected mortality rates, 3 days post treatment with 0.1, 0.5, 1 and 2 ppm of fipronil were (51.85% and 73.33%), (85.19% and 95.93%), (96.30% and 100%) and (100 and 100%) for the 5th nymphal instar of *S. gregaria* and *L. migratoria migratorioides* respectively. Ibrahim (2018) found that fipronil (Coach®) at concentration of 0.5 ppm achieved 100 % mortality after 24 hours to the 4th nymphal instar of *S. gregaria* under laboratory conditions. The results are also in agreement with those found by Abdel-Fattah et al. (2012), when applying fipronil (Regent®) in the field against the nymphal instars of *S. gregaria* and *Euprepocnemis plorans plorans*. The finding also agrees with that by Abdel-Fattah and Ammar (2005) in the field against the nymphal instars of *S. gregaria*.

Table 1. Corrected mortality percentages of 5th nymphal instars of *S. gregaria* and *L. migratoria migratorioides* using different concentrations of chlorantraniliprole, spinosad and fipronil

<table>
<thead>
<tr>
<th>Treats.</th>
<th>Days</th>
<th>Conc. (ppm)</th>
<th>1 day</th>
<th>2 days</th>
<th>3 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorantraniliprole</td>
<td>60</td>
<td>10</td>
<td>23.3</td>
<td>16.7</td>
<td>10.0</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>23.3</td>
<td>46.7</td>
<td>33.3</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>480</td>
<td>40</td>
<td>56.7</td>
<td>43.3</td>
<td>63.3</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>46.7</td>
<td>66.7</td>
<td>53.3</td>
<td>73.3</td>
</tr>
<tr>
<td>Spinosad</td>
<td>30</td>
<td>13.3</td>
<td>22</td>
<td>23.2</td>
<td>33.4</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>23.3</td>
<td>36.7</td>
<td>45.2</td>
<td>66.3</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>43.3</td>
<td>56.7</td>
<td>68.9</td>
<td>78.9</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>67.7</td>
<td>69.7</td>
<td>83.6</td>
<td>89.7</td>
</tr>
<tr>
<td>Fipronil</td>
<td>0.1</td>
<td>20</td>
<td>46.7</td>
<td>29.6</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>53.67</td>
<td>76.67</td>
<td>77.78</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>66.67</td>
<td>96.67</td>
<td>88.89</td>
<td>99.63</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>93.33</td>
<td>100</td>
<td>96.30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2 shows the values of LC30, LC50 and LC90 for *S. gregaria* and *L. migratoria* after 3 days of treatment with above mentioned concentrations for each of the selected pesticides.

Table 2. LC30, LC50 and LC90 values calculated after 3 days of treatments

<table>
<thead>
<tr>
<th>Period</th>
<th>chlorantraniliprole</th>
<th>spinosad</th>
<th>fipronil</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. gregaria</td>
<td>LC30</td>
<td>LC50</td>
<td>LC90</td>
</tr>
<tr>
<td>L. migratoria</td>
<td>116.26</td>
<td>325</td>
<td>4008.38</td>
</tr>
<tr>
<td></td>
<td>86.10</td>
<td>210</td>
<td>1855.46</td>
</tr>
</tbody>
</table>

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3.2. Observations on feeding and general behaviour

It was observed in both species that hoppers ceased feeding shortly after they began consuming the food treated with chlorantraniliprole and faintness occurred as hoppers were not able to move normally, but they cured gradually starting from 5th day post treatment and started to feed normally starting from the 6th day. Whereas; insects fed on spinosad treated leaves were feeding normally till the symptoms of poisoning appeared within 12 hours and ceased feeding till they died. Also; hoppers treated by fipronil did not feed well except at low concentrates (0.5 ppm). Additionally; Treatment of hoppers at sub lethal concentrations of fipronil and spinosad caused malformation, failure to moult (figures 1) and wrinkled winged adults (figure 2) which will not fly well and this will affect their mobilization.

The above mentioned results indicate that 5th instar hoppers of L. migratoria migratorioides were more susceptible to all tested insecticides than those of S. gregaria, and this may be due to the fact that L. migratoria migratorioides insects are softer than those of the other species. The results show that fipronil was the most effective compound against both species, followed by spinosad, while chlorantraniliprole showed weaker effect to both species than the two other compounds, but it showed better effect on L. migratoria migratorioides than in the case of S. gregaria.

Fig 1. Hopper malformation caused by spinosad

Sub lethal concentrates of spinosad caused malformed hoppers and some hoppers could not fledge into adults. “S. gregaria on the left hand side and L. migratoria migratorioides on the right hand side”

Fig 2. wrinkled winged adults caused by fipronil

Wrinkled wing adults resulting from sub lethal concentration of fipronil treated hoppers in both species. “S. gregaria on the left hand side and L. migratoria migratorioides on the right hand side”
3.3. Effects on haemolymph protein, carbohydrate and lipid contents

Proteins, lipids and carbohydrates are the main nutrients which provide insects with energy needed for movements and flights; also they are essential for building important insect tissues. In this study, the effects of sub-lethal concentrates (LC50, as shown in table 2) of the tested compounds on the levels of haemolymph total proteins, carbohydrates and lipids in the 5th instar nymphs were evaluated 2, 4 and 6 days post treatments.

3.3.1. Effect on haemolymph total protein

Data in table 3 shows that total protein levels were significantly low in S. gregaria nymphs 2 days post treatment with chlorantraniliprole, spinosad and fipronil, recording 74.93, 45, 67.63 mg/ml respectively compared to 80.33 mg/ml for the untreated nymphs. Also, the total protein levels decreased significantly in the L. migratoria migratorioides treated hoppers with chlorantraniliprole and fipronil, recording 66.10, 78.47 mg/ml respectively compared to 93 mg/ml for the untreated nymphs which was slightly higher than spinosad effect (91.47 mg/ml). After 4 days of treatment, the haemolymph total protein was significantly deceased in the S. gregaria treated nymphs treated with chlorantraniliprole, spinosad and fipronil, recording 91.33, 75.33, 76.33 mg/ml respectively compared to 116 mg/ml for the untreated nymphs. Whereas; in the case of L. migratoria migratorioides, the protein levels decreased significantly in the chlorantraniliprole and spinosad treated hoppers, recording 49.4, 98.90 mg/ml respectively compared to 112.33 mg/ml for untreated nymphs that was slightly higher than fipronil effect, which recorded 110.83 mg/ml. Additionally; the levels of haemolymph total protein were significantly deceased in the treated nymphs 6 days post treatment with chlorantraniliprole and fipronil, recording 80.33, 48.27 mg/ml respectively compared to 117.67 mg/ml for the untreated nymphs, but the total protein increased in hoppers treated with Spinosad (127 mg/ml). While; in the case of L. migratoria migratorioides treated hoppers, the total protein levels decreased significantly in the chlorantraniliprole, spinosad and fipronil, recording 104.8, 86.07, 71.50 mg/ml respectively compared to 112 mg/ml for untreated nymphs. Similar results were found by Said (2014) as spinosad caused a reduction of haemolymph total protein in the 5th nymphal instar of S. gregaria 2, 4 and 6 days post treatment.

Table 3. Means of haemolymph total proteins in 5th instar nymphs of S. gregaria and L. migratoria migratorioides

<table>
<thead>
<tr>
<th>Days post</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S. gregaria</td>
<td>L. migratoria</td>
<td>S. gregaria</td>
</tr>
<tr>
<td>Control</td>
<td>80.33a</td>
<td>93.00a</td>
<td>116a</td>
</tr>
<tr>
<td>Coragen</td>
<td>74.93a</td>
<td>66.10c</td>
<td>91.33b</td>
</tr>
<tr>
<td>Spinosad</td>
<td>45.00c</td>
<td>91.47a</td>
<td>75.33d</td>
</tr>
<tr>
<td>Fipronil</td>
<td>67.63b</td>
<td>78.47b</td>
<td>76.33c</td>
</tr>
<tr>
<td>F (calculated)</td>
<td>164.71*</td>
<td>140.65*</td>
<td>105.72*</td>
</tr>
<tr>
<td>LSD 5%</td>
<td>10.46</td>
<td>3.47</td>
<td>6.50</td>
</tr>
</tbody>
</table>

Same letters are not significantly different

3.3.2. Effect on haemolymph total lipids

Data in table 4 indicates that haemolymph total lipid in S. gregaria 5th instar nymphs were not significantly decreased in the treated hoppers 2 days post treatment with chlorantraniliprole (7.93) mg/ml compared to 7.67 mg/ml for the untreated nymphs. However; both levels were significantly higher than that in spinosad and fipronil treated hoppers which recorded 5.42 and 5.57 mg/ml respectively. On the contrary, the total lipid levels increased significantly in the L. migratoria migratorioides hoppers after 2 days of treatment with chlorantraniliprole, spinosad and fipronil, recording 18.77, 13.03 and 17.63 mg/ml respectively compared to 10.03 mg/ml for the control nymphs.

Additionally, the levels of haemolymph total lipids were significantly deceased in the treated S. gregaria 5th instar nymphs 4 days post treatment with chlorantraniliprole, spinosad and fipronil, recording 6.05, 5.33 and 5.77 mg/ml respectively compared to 8 mg/ml for the untreated nymphs. While 4 days post treatment; the lipid levels were significantly higher in the 5th instar nymphs of L. migratoria migratorioides treated hoppers with chlorantraniliprole, spinosad and fipronil, recording 14.27, 17.20 and 18.97 mg/ml respectively compared to 12.63 mg/ml for untreated nymphs.

Additionally; the levels of haemolymph total lipids were significantly deceased in the treated nymphs 6 days post treatment with chlorantraniliprole and fipronil, recording 5.74, 6.50 mg/ml respectively compared to 7.75 mg/ml for the untreated nymphs, but the level of total lipids was significantly increased in hoppers treated with Spinosad which recorded 9.74 mg/ml. While the
The levels of haemolymph total lipids of S. gregaria and L. migratoria migratorioides treated hoppers increased significantly after 6 days of treatment with chlorantraniliprole, spinosad and fipronil, recording 12.47, 12.7 and 18.43 mg/ml respectively compared to 11.13 mg/ml for untreated nymphs. The results are also in agreement with those found by Said (2014) who found that spinosad caused significant decrease in total lipids of 5th nymphal instar of S. gregaria after treatment. Also, Rashwan (2013) found that rynaxypyr (Coragen) caused significant decrease on total lipids of 5th larval instar of Spodoptera littoralis after 24 hours of treatment. In another study by (Upadhyay et al., 2010), fipronil caused a significant decrease in lipid levels after 8 and 4 hours of treatment with 40% and 80% of LD90, in the Indian white termite Odontotermes obesus.

### 3.3.3. Effect on haemolymph total carbohydrates

Data in table 5 indicates that haemolymph total carbohydrate levels in S. gregaria 5th instar nymphs were not significantly different 2 days post treatment in the hoppers treated with chlorantraniliprole (46.63) mg/ml and the untreated nymphs (52.37 mg/ml). However, carbohydrate levels were significantly lower than that in spinosad treated hoppers which recorded 68.33 mg/ml, while fipronil caused significant decrease in total carbohydrate levels recording 22.53 mg/ml. On the other side, the levels of total carbohydrate in haemolymph in the 5th instar nymphs of L. migratoria migratorioides were significantly decreased in after 2 days of treatment with chlorantraniliprole and fipronil, recording 25.73 and 39.07 mg/ml respectively compared to 50.87 mg/ml for the untreated nymphs. On the other side, the levels of total carbohydrate in haemolymph in the 5th instar nymphs of L. migratoria migratorioides were significantly deceased after 4 days of treatment with chlorantraniliprole and fipronil, recording 27 and 51.97 mg/ml respectively compared to 55.67 mg/ml for the untreated nymphs, which was slightly lower than the level in hoppers treated with spinosad which recorded 58 mg/ml.

Additionally; there were no significant differences in the levels of haemolymph total carbohydrates between the untreated hoppers (44.37 mg/ml) and the treated nymphs after 6 days of treatment with chlorantraniliprole and spinosad which recorded 45.56 and 47.80 mg/ml respectively, but fipronil treatment caused significant decrease in total carbohydrate levels (20.20 mg/ml). While the levels of total carbohydrates in the 5th instar nymphs of L. migratoria migratorioides increased significantly after 6 days of treatment with chlorantraniliprole and spinosad recording 61.47 and 45.43 mg/ml respectively compared to 45.73 mg/ml for untreated nymphs, but fipronil caused significant decrease (38.40 mg/ml) in total carbohydrates.

The results are also in agreement with those found by Said (2014) who found that a reduction in haemolymph total carbohydrates occurred in the 5th nymphal instar of S. gregaria after treatment with spinosad. Also, rynaxypyr (chlorantraniliprole) caused significant decrease in the haemolymph total carbohydrates of 5th larval instar of Spodoptera littoralis after 24 hours of treatment (Rashwan 2013).

The present study assessed the efficiency of chlorantraniliprole, spinosad and fipronil as three of the new alternative compounds to the currently used conventional insecticides against two key pests of family acrididae in Egypt. It can be inferred from the above mentioned results that the tested compounds caused good mortality rates to both 5th instar hopper species at the tested concentration, and fipronil was the strongest followed by spinosad, and chlorantraniliprole achieved accepted mortality rates against L. migratoria migratorioides but weaker.
performance was noticed against S. gregaria.

It is suggested that further tests should be executed before recommending chlorantraniliprole against S. gregaria.

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