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Assessment of the Commercial Botanical Insecticides Against the desert Locust, *Schistocerca gregaria* (FORSK.) (Orthoptera: Acrididae)

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ABSTRACT

Desert locust, *Schistocerca gregaria* is a serious agricultural pest that causes considerable damage to food crops and pasture grasses**.** This study was carried out to evaluate the commercial botanical insecticides, Sabadilla, Limonene, Rotenone, and Azadirachtin on *Sch. gregaria*. Results indicated that the 4th nymphal instars were affected after feeding on clover leaves treated with the various concentrations of the tested compounds. Early 24hrs. moulted 4th nymphal instar was more sensitive to azadirachtin followed by rotenone, sabadill and finally limonene botanical. The recorded LC_{50} value was 3.4, 3.7, 3.9 and 4.2% by the effect of azadirachtin, rotenone, sabadilla and limonene respectively against 4th nymphal instars 72hrs. post-treatment. While LC90 was 15.2, 18.7, 26.3 and 28.1% in the same previous compound respectively. The higher prolongation of nymphal duration was 17.6 days with azadirachtin at a concentration of 15% and lower prolongation was 10.5 with limonene at a concentration of 5% compared to 10.2 days of control. Also, nymphs suffered from weight loss for all tested botanical insecticides particularly at higher concentrations then died. The morphogenetic effect was obtained at different concentrations of the tested botanical insecticides. Deformation was in the form of abnormal nymphs and nymphal-adult intermediate stages.

INTRODUCTION

The insect pests particularly the desert locusts, which belong to Family Acrididae, *Schistocerca gregaria* (Forsk.) are considered the most serious pests around the world. Locusts and other species of grasshoppers always cause considerable economic problems (Bullen 1970; Hamdy and Nassar 2013). *Schistocerca gregaria* predominates in desert and scrub regions of northern Africa, the Arabian Peninsula and the southwest of Asia (Steedman, 1988). In recent years such insecticides have come under increasing attack due to their hazard to the environment, insect resistance, and affected non-target organisms (Tingle, 1996) and humans (Pretty, 1996 and Nassar *et al.*, 2014**)**. Consequently, the search for new friendly environmentally sounds safe insecticides became an important task (Nassar

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et al., 2017, and 2018). Many workers reported that plants are considered one of the richest sources of natural bioinsecticides which can be used as pest control agents. They attended to use botanical toxicants, repellents synergists, growth regulators and antifeedants against many insect pests (Disha Varijakzhan *et al.*, 2020, El-Shazly *et al.*, 2019 and El-Sonbati *et al.*, 2014). The present study is an attempt through the laboratory to evaluate the efficacy of some commercial botanical insecticides such as Azadirachtin, Rotenone, sabadella, and Limonene against desert locust, *Schi*. *gregaria*.

These commercial botanical insecticides act quickly, degrade rapidly and have low mammalian toxicity (Lomer *et al.*, 2001).

On the other hand, the mode of action of any chemical refers to which biological process the pesticide interrupts (Khambay *et al.*, 2003; Lushchak *et al.*,2018). Commercial botanical insecticides can induce various modes of action including repellence, growth inhibition, and modifications in their physiology and structure. The botanical pesticides could be divided into two generations: The 1st generation included Nicotine, Rotenone, Sabadilla, Ryania, Pyrethrum, and Plant essential oils; while the 2nd generation included Synthetic Pyrethroids and Azadirachtin, as well as new botanicals of plant origin (Roger, 2005). Their highly versatile chemical structures arise from the enormous biosynthetic capabilities of plant origin (Wafukho *et al.*, 2013). Botanical insecticides represent promising alternatives in the present and future of integrated pest management programs (IPM).

MATERIALS AND METHODS

Insect Colony:

The susceptible strain of desert locust, *Schistocerca gregaria* obtained from the Ministry of Agriculture, Dokki, Giza being used throughout the present investigation. A colony was established in the laboratory according to Hoste et al. (2002) with some modifications. The rearing was performed in cages 50 cm × 50 cm × 70 cm provided with wire gauze sides. The bottom was covered with a sterilized sand layer of 10 cm thickness for egg laying. These cages have small doors on the front side to facilitate daily routine feeding and cleaning. They were fed on clover (*Trifolium alexandrinum*) from October till May and then on *Sesabania aegyptiaca* (Fabales: Fabaceae) introduced daily as feeding materials, along with dry wheat bran fortified with yeast powder as a source of vitamins. The cages were cleaned daily to avoid contamination and kept under conditions at a temperature of 30 ± 2 ◦C and 50–60% relative humidity (RH).

Botanical Insecticides:

The 4th nymphal instars 12hrs. old of *Schhisocerca gregaria* were feeding on the treated clover leaves (dipping technique) with different concentrations of the use of botanical insecticides. Considering the commercial botanical insecticides as 100%, a known volume of the botanical insecticides, Azadirachtin, Rotenone, Sabadilla, and Limonene was added to a similar volume of the acetone solvent to obtain a stock solution. Five concentrations were prepared for each botanical insecticide (5, 10, 15, 20 and 25%). Clean clover leaves were dipped in each concentration for 25 seconds and were left to evaporate the acetone solvent.

Bioassay and Statistical Analysis:

Ten 4th nymphal instars of *Schi. gregaria* were transferred into a 150 mL glass beaker covered with muslin in order to feed on treated clover leaves with different botanical concentrations (three replicates containing 10 insects of each concentration). For the control experiment, the 4th nymphal instars of *Sch. gregaria* were fed on clover leaves without treatment. Percent mortality of treated insects was recorded after 72 hrs. and corrected mortalities were determined according to Abbott's formula (Abbott, 1925). Nymphal duration, weight and morphogenesis effects were recorded.

RESULTS AND DISCUSSION

Toxicity Effect of the Commercial Botanical Insecticides on *Schistocerca gregaria*:

The toxicity effect of botanical insecticides on the 4th nymphal instars of *Sch. gregaria* was recorded in Figure (1). Results show all the tested commercial botanical insecticides have potential mortality against *Sch. gregaria* stages. The higher mortality was 100, 95, 84 and 78% after 72 hrs. post feeding 4th nymphal instars on treated clover leaves with the higher concentration (25%) of azadirachtin, rotenone, sabadilla and rotenone respectively. While the lower concentration, 5% caused 65, 63, 62 and 55 mortalities with the same previous botanical insecticides respectively (Fig. 1).

In order of toxicity, there is a significant effect observed at LC_{90} and no significant clear at LC_{50} (Table 1). Higher toxicity was 3.4 and 15.2 which occurred by the effect of azadirachtin on the 4th nymphal instars of *Schi. gregaria* at LC_{50} and LC_{90} respectively. While the lower toxicity of 4th nymphal instars post-treatment with limonene was 4.2 and 28.1% of LC_{50} and LC_{90} respectively (Table 1).

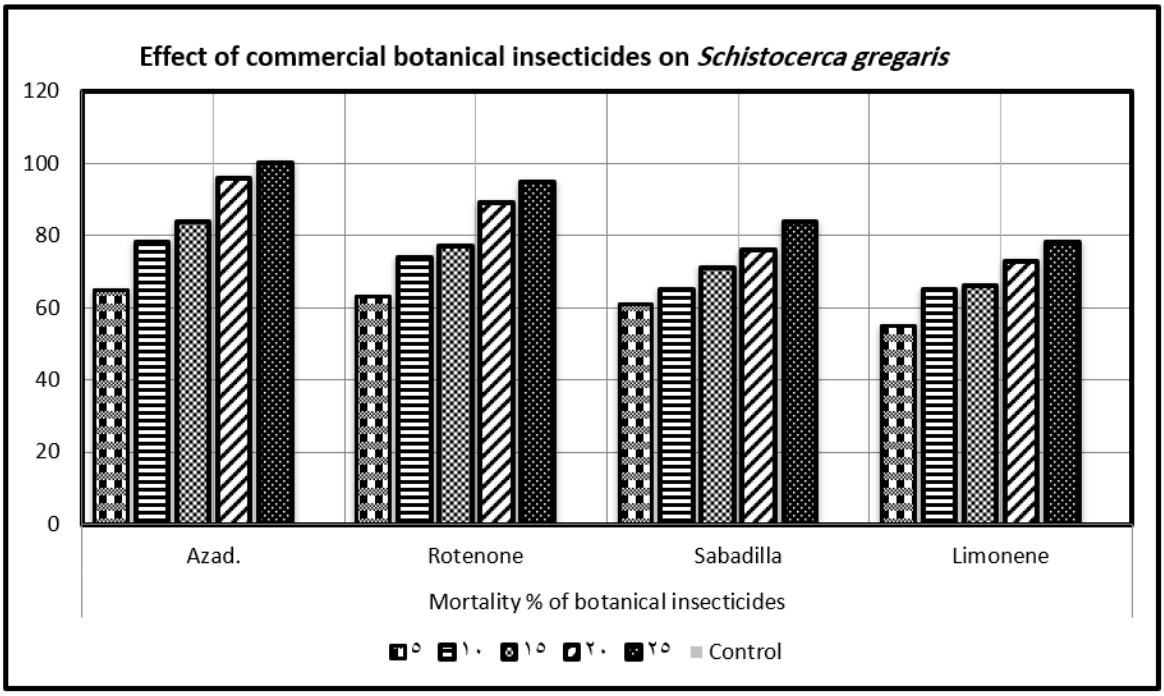

Fig. 1: Effect of commercial plant insecticides on 4th nymphal mortality% after 72 hrs post-treatment of 4th nymphal instars of *Sch. Gregaria.*

Table 1: Toxicity effect of botanical insecticides after 72 hrs on *Sh. gregaria* 4th nymphal instars.

Toxicity test	Toxicity effect of Botanical insecticides after 24hrs			
	Aadirachtin	*Rotenone*	*Sabadilla*	*Limonene*
LC_{50}	3.4	3.7	3.8	4.2
LC_{90}	15.2	18.7	26.3	28.1

The toxicity value of botanical insecticides may be obtained due to the chemical components of the active ingredient of the botanical insecticides. Many scientific academic institutions have been engaged in great efforts have achieved for using natural compounds of plant origin which may have toxic, repellent, antifeedant, or anti-hormonal characteristics (Bagari *et al.,* 2015). Mortality of *Sh. gregaria*, like many other insects, depends basically on the botanical extract type and its mode of action (Nassar 2000 and El-Shazly & Nassar 2005). Botanical extracts mostly have delayed effects on insects (Schmutterer, 1990). Obtained results revealed that the azadirachtin and rotenone were more potent than the other tested commercial botanical insecticides could be attributed to the active ingredients, as well as the mode of action. It is now well established that the activity of many plants including *Azadirachta indica* is attributed to the presence of saponin components that perhaps affects cell membranes as well as reduce digestion and absorption (Bogumil and Wieslaw, 2006 and De Geyter *et al.,* 2012). According to Stark and Rangus (1994), neem acts slowly. While Ghazawy *et al.*, (2007) reported that LC_{50} within 24 hrs on the 2nd nymphal instars of *Sch. gregaria* and 4th, 5th and 6th of *Heteracris littoralis* instars, was dose-dependent and they died on the time of ecdysis. Asiri (2015) showed that the methylene chloride extract of *A. indica* is the most potent extract against *Schistocerca gregaria*. More extent of injury by commercial botanical insecticides may occur depending on the susceptibility of tissues to particular poisons. Some poisons the lipid layers of membranes resulting in destroying the permeability of plasma membrane due to water loss and appearing of vacuoles (Sharaby *et al.,* 2012). Despite the plenty of literature about the bioactivity of commercial botanical insecticides and other plant extracts against several insect species, were caused toxicity by higher concentrations. The oils are pressed from the seeds of *Azadirachta. indica* achieved mortality rates of 65-100 % in *Sch. gregaria* (Schmutterer and Freres, 1990; Nicol and Schmutterer, 1991). Also, the high mortality of *Sch. gregaria,* red locust *Nomadacris septemfasciata* and variegated grasshopper *Zonocerus variegates* was caused by the neem oil (Schmutterer *et al.,* 1993).

Azadirachtin, Rotenone, Sabadilla and limonene seem to have a potent toxic and antifeedant effect on *Sch. gregaria*. In harmony with the present results was obtained on a large number of pest insects, including Orthopterans El-Shazly *et al.,* (2008) and Sharaby *et al.,* (2012) on *Heteracris littoralis.*

On the other hand, sabadilla reduced the feeding behavior of *Diaprepes abbreviates* weevils and deterrence due to sabadilla alkaloids. Sabadilla alkaloids appear to be similar to that pyrethrins in that they work on voltage-sensitive sodium channels (Stephen *et al.,* 2010). Sabadilla is approved for use in the USA as an organic insecticide, as well as for other uses, by the Organic Materials Review Institute (OMRI). Sabadilla caused a significant reduction in the feeding behavior of the grasshopper, *Sch. americana* which has similar properties to azadirachtin against different orders of insects, including Orthoptera (Aerts and Mordue 1997, Capinera and Froeba 2007). Toxicity by sabadilla triterpenoid could be the result of blockage of the input from neurons that detect phagostimulatory compounds such as carbohydrates (Winstanley and Blaney, 1978, against *Schistocerca gregaria*). In this study, rotenone was effective as a toxic insecticide on *Sch. gregaria*. Sabadilla, azadirachtin and ryanodine effectively deterred *Sch. americana* whereas rotenone, sabadilla and ryanodine reduced the feeding activity of *D. abbreviatus* under field conditions (Andres, 2009). There is evidence that some species of grasshoppers are able to detect flavonoids (Bernays and Chapman 2000). Chapman *et al.* (1991) detected that salicin, a phenolic glycoside that stimulates a deterrent neuron in *Sch. americana,* had a phagostimulatory effect on this grasshopper at low concentrations. Rotenone showed a strong antifeedant effect against adults and larvae of the wheat weevil, *Sitophilus granarius* L., adults of the confused flour beetle, *Tribolium confusum*, and larvae of the khapra beetle, *Trogoderma granarium* Everts (Nawrot *et al.,* 1989). Rotenone is one of several isoflavonoid compounds produced in the roots of the tropical legumes Derris, Lonchocarpus and Tephrosia (Leguminosae). Rotenone is a mitochondrial poison that blocks the electron transport chain and prevents energy production (Andres 2009, Isman 2006, Rosell *et al.*, 2008). Rotenone can act as a feeding deterrent against stored product insect pests (Nawrot *et al.*, 1989) and polyphagous noctuid species (Wheeler *et al.*, 2001).

The present study revealed that commercial limonene insecticides also caused the mortality of *Sch. gregaria*. Mortality may be associated with the failure of the nymph to moult and the nymphs died. Also, a significant reduction of the nymphal survival of *Sch. gregaria* was observed with the effect of limonene. In harmony with these results, D-limonene had higher mortality rates than control fleas and did not lay any eggs. D-limonene-treated fleas also produced fewer feces and probably fed less while on their hosts (Collart, and Hink. 1986). Limonene is a naturally occurring monoterpene found in citrus and other fruits. The toxicity and neurotoxic effects of limonene are discussed by Coats *et al.* 1991, and the suitability of limonene for the control of insect pests has been reviewed by Ibrahim *et al.* (2001). Several reports mention using limonene for the control of plant pests (Tiberi *et al.*, 1999 and Hollingsworth 2005). The limonene mode of action in insects is not fully understood, it may cause an increase in the spontaneous activity of sensory nerves, this heightened activity sends spurious information to motor nerves and results in twitching, and lake of coordination (Weinzierl and Henn 1994). And convulsions, the central nervous system may also be affected, resulting in additional stimulation of motor nerves, which may lead to rapid knockdown paralysis (Weinzierl and Henn 1994). Limonene is a monoterpene usually found in many plant species, against *Tribolium confusum* du Val (Antonino *et al.*, 2016). On the other hand, Limonene has broad application prospects in antibacterial and food preservation due to its broad-spectrum bactericidal activity, safety, and low mammalian toxicity (Young *et al.*, 2013). Finally, the commercial botanical insecticides, Rotenone, sabadilla, azadirachtin and Limonene have potency toxic effects against *Sch. gregaria* instars. Also, it is worth that, botanical insecticides make it a potential tool for the integrated management of grasshoppers.

Effect of the Commercial Botanical Insecticides on The Nymphal Weight of *Schistocerca gregaria*:

As listed data in Table (2) penultimate instar nymphs gained somatic weight less than that of control congeners. However, the most drastic suppressing effect on the weight gain was exhibited atthe higher concentrations level compared with that of the control nymphs. The higher loss of nymphal weight was significant by 245.3mg at a concentration level of 15% of azadiractin insecticides vs 354.7mg of control. While at concentrations of 20 and 25% all nymphs died after nymphal treatment with azadirachtin however at 25% only all nymphs died for all botanical insecticides. Nevertheless, the lower loss of nymphal weight was 268.6 after nymphal treatment with limonene at a concentration of 20% vs. the control treatment. Also, the 4th nymphal weight was in dose-dependent terms, decreased by increasing concentrations of the applied botanical insecticides prohibited to gain normal somatic growth (Table 2). These datacould be confirmed by Schmidt *et al.* (1997 and Schmidt 1999). Because body weight and hence weight gain is one of the valuable indicators for evaluating growth (Armbruster and Hutchinson, 2002). After a feed of the 4th nymphal instars of *Sch. gregaria* on treated clover leaves with azadirachtin, rotenone, sabadilla and limonene the somatic weight gain was less than of control nymphs. Similar to these results were obtained for the migratory locust *L. migratoria* after treatment with a compound from the neem tree *Az. indica* (Sieber and Rembold, 1983, and Rembold and Annadurai, 1993). In contrast, no significant effect of a neem extract on the body weight gain of *Pieris brassicae* larvae was observed (Osman, 2009). Concerning the growth inhibition in the present study by studying commercial botanical insecticides, treatment of *Sch. gregaria* 4th instar nymphs resulted in suppressed body weight gain.

Table 2: Effect of commercial plant insecticides on nymphal weight (mg ± SD) after treatment of 4th nymphal instars of *Sch. Gregaria.*

Concentration %	Nymphal weight (mg ± SD) of *Sch. gregaria* by the effect of commercial botanical insecticides			
	Azadirachtin	Rotenone	Sabadilla	Limonene
5	315.8 ± 52.4	326.4 ± 51.5	331.6 ± 44.7	338.7 ± 56
10	296.6 ± 44.6	317.6 ± 44.2	325.5 ± 43	314.4 ± 55
15	245.3 ± 35.0	266.4 ± 48	277.6 ± 61	308.7 ± 48
20	-	238.4 ± 55	251.6 ± 54	268.6 ± 53
25	-	-	-	-
Control	**354.7 ± 66.4**			

Effect Of Botanical Insecticides on Nymphal Duration:

Results obtained in Table (3) showed that commercial botanical insecticides exhibited an inhibitory effect on the duration of 4th instar nymphs of *Schi. gregaria*. Since nymphs in the penultimate instar were significantly prolonged, particularly at the concentration level: of 10, 15 and 20%. Data presented in Table (3) cleared that the nymphal duration was significantly ($p<0.05$) prolonged by the application of botanical insecticides and more prolongation was obtained after nymphal treatment with azadirachtin at a concentration of 10 and 15 (16.7 and 17.6 days respectively vs. 10.2 days of control). While the same previous concentrations caused nymphal duration by (15.4 and 17.6 days of rotenone), (14.3 and 12.6 days of sabadill), and (the lowest nymphal duration was 12.3 and 11.3 days in the case of limonene) respectively table (3). Botanical insecticides prolonged the nymph duration so that nymphs were unable to be adults, especially at higher concentrations. Successful control of desert locusts will require the development of an integrated pest management program using a variety of products that can be applied with a range of techniques that are appropriate in different habitats and circumstances (Magor *et al.,* 2008). This must be associated with reduced pesticide application, economic costs, environmental risks, and duration and extent of the locust threat (Showler 2002). The increased developmental duration was observed in offspring nymphs of *Schi. gregaria* treated with biopesticides, while a reduction in adult longevity was also detected (Waqas *et al.,* 2022). The obtained results in this study were not in harmony with the data reported for some plant species such as *M. volkensii* on *Culex pipiens* (Al-Sharook, *et al.,* 1991) and Jojoba oil on *M. domestica* (Amer *et al.,* 2004)

Table 3: Effect of commercial botanical insecticides on nymphal duration (day ± SD) after treatment of 4th nymphal instars of *Sch. gregaria* **.**

Concentration %	Nymphal duration of *Sch. gregaria* (day ± SD) by the effect of commercial botanical insecticides			
	Azadirachtin	Rotenone	Sabadilla	Limonene
5	12.3 ± 1.4	11.3 ± 2.3	11.2 ± 2.5	10.5 ± 2.4
10	16.7 ± 1.5	15.4 ± 2.4	14.3 ± 2.4	12.3 ± 18
15	17.6 ± 2.2	13.7 ± 2.3	12.6 ± 2.6	11.3 ± 2.3
20	-	16.7 ± 2.4	15.4 ± 3.1	13.5 ± 2.4
25	-	-	-	-
Control	**10.2 ± 2.8**			

Description of Nymphal Performance Affected by Botanical Insecticides:

All commercial botanical insecticides azadirachtin, rotenone, sabadilla and limonene induced morphogenesis effect on *Schistoceca gregaria* after treatment of 4th nymphal instars. Various degrees of different malformation which obtained in the form of nymphal-adult intermediate stages at all concentrations. The majority of such malformations appeared as severely or slightly curled bodies, with some cases antennal or leg deformities observed. Such deformation occurred due to nymphs not moult into the next nymphal instar or moult reaching malformed adult stages. In the present study, the nymphal morphogenesis program of *Sch. gregaria* was disrupted by the effect of commercial botanical insecticides, azadirachtin, rotenone, sabadilla, and limonene after treatment of the penultimate instar nymphs of *Sch. gegaria*. These deformations could be generally observed in the following forms, a-nymph failure to completely get rid of the last nymphal exuvia because the nymphal exuvia remained attached to its body, b- Form like an adult with curled legs and coiled incompletely developed short wings, c- the appearance of the nymphal-adult intermediate stage. Many Authors recorded an inhibitory action of botanical extracts on the immature adult transformation forms while others reported no effects or even contradictory effects, depending on the activity of theplant species and the susceptibility of the insect species (Hashem and Youssef, 1991; Jagannadh and Nair, 1992; Khalaf and Hussein, 1997; Hassan, 2002; Al-Dali *et al.*, 2003, and Nassar *et al.*, 2018). Also, some nymphal-adult intermediates were observed in the orthopteran *Sch. gregaria* by essential oil of *A. conyzoides* (Pari *et al.*, 2000) and by *C. rotendus* (El-Sokkary, 2003). Inaddition, botanical insecticide treatment leads to the inhibition of neurosecretion (prothoracicotropic causing the inhibition or delay of a number of physiological processes, suchas metamorphosis (Josephrajkumar *et al.*, 1999). Hence azadirachtin, rotenone, sabadilla and limonene affected the hormonal system in *Sch. gregaria* as well as juvenile andeclosion hormones. It is also May reasonable to suggest the existence of juvenilizing, and subsequently antigregarizing, substances in the commercial botanical insecticides used. The present results are in agreement with results reported by Schmutterer *et al.* (1993) who observed morphogenic defects in adults of *Sch. gregaria* after treatment of the last instar nymphs with neem oil. Adult deformities of *Sch. gregaria* were caused by essential oil from *Ageratum conyzoides* (Pari *et al.*, 2000). Topical application of the ethanolic extract from *Cyprus rotendus* onto the penultimate instar nymphs of *Sch. gregaria* resulted in the formation of defected adults (Bakr *et al.*, 2008 and 2009). Disturbance of Sch. performance may be due to the modification of the ecdysteroid titer, which in turn leads to changes in lysosomal enzyme activity causing overt morphological abnormalities (Josephrajkumar *et al.*, 1999). To a great extent, similar results had been obtained by azadirachtin in *Bombyx mori* (Koul *et al.*, 1987), *Spodoptera litura* (Mittal *et al.*, 1995; Naqvi, 1986), *M. domestica* (Wilps, 1989) and for some other botanicals in *M. stabulans* (El-Shazly *et al.*, 1996).

CONCLUSION:

The use of pesticides produced synthetically is still hazardous to environmental health, animals, and human beings. It is important to remember and convey the risks associated with using natural insecticides. Concerning their regenerative nature and contribution to human and environmental protection, botanicals must be reconsidered and their effectiveness in controlling crop pests. Large-scale agriculture could be practiced in marginal lands where food is not in abundance to escape the competition with source plant extracts. The chemical features of botanical pesticides, notably repellents, feeding dissuasive agents, toxicants, and growth retardants and their impact on insects in various forms. As a result, we advocated for the use of botanical insecticides, which has been encouraged, and research is underway to identify new botanical insecticide sources.

Future research needs to explore commercial botanical insecticides with other control agents including chemical insecticides as a means of integrated pest management of locusts in the field.

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