INTRODUCTION

Helicoverpa armigera (Hübner) is a widespread insect pest, especially warm in area, which it exists in different regions of Africa, Asia, Australia and Europe (Mathews and Turnstile, 2001). This insect has been recorded in Egypt since 1905 and has turned into a lesion in 1972 and is considered as one of the most economic pests in Egypt (Ibrahim *et al.*, 1974).

H. armigera is an important pest of cotton, particularly in Australia and China (King, 1994). All parts of the cotton plant are vulnerable to attack. Cotton yields were reduced by 50-60% by *H. armigera* each year from 1980-1990 in China (Xiao *et al*., 2002). In Queensland Australia, *H. armigera* damage accounted for 7% yield loss in cotton in spite of pest control costs a 800 U$/ha in 1998 (Sequeira, 2001). In Andhra Pradesh region of India, *H. armigera* reduced yields of seed cotton from 436 kg/ha in 1986-87 to 168 kg/ha in 1987-88 (Sekhar *et al*., 1996& Loganathan *et al.*, 1999). Significant tomato crop loss also occurred in Burkina Faso, India and New Zealand, particularly in unsprayed or late season varieties (Tewari and Prasado Rao, 1987; Bouchard *et al.*, 1992; Cameron *et al.*, 2001). In New Zealand, *H. armigera* attacked Monterey pine and consumed more than 50% of the foliage off about 60% the trees (CABI/EPPO 1997). Pigeon pea and chickpea are severely damaged in India, where losses up to 90-100% in the 1992/93 and 1997/98 growing seasons have been reported. Worldwide, annual losses from this pest on chickpea are approximately 10%, equaling $300 million dollars

(Shanower *et al*., 1997, Mulimani &Sudheendra 2002 and Sidde Gowda *et al*., 2002). Protection Organization as an A2 quarantine pest and is considered a quarantine pest by the Caribbean Plant Protection Commission (CPPC), Organismo Internacional Regional de Sanidad Agropecuaria (OIRSA), and the country of Brazil (EPPO, 2000).

This insect was presented in traditional crops and reclaimed area and infest a large number (more than 200 hosts) of economically important crops such as cotton , corn , tomatoes , peppers , beans , sunflower and sorghum (Fitt ,1989 ; Matthews, 1999 and Xiulian *et al*., 2004) and cause injury , decline in the quantity and quality of the crop damage as it occurs in the flowers , buds and fruits , so as to convey the larvae from another part of the plant and also from one plant to another plant in the field.

This pest is a highly polyphagous of many economic crops because it affects alarge number of crops. It has many common names: scarce bordered straw worm, corn earworm, African cotton bollworm, American bollworm, and tomato worm (Zhang ,1994 & Begemann and Schoeman, 1999). This range occupied by the species includes tropical, dry, and temperate climates (CAB, 2000).

The global distribution of *H. armigera* suggests that the pest may be most closely associated with deserts and xeric shrublands; Mediterranean scrub; temperate broadleaf and mixed forests; tropical and subtropical grasslands, savannas, and shrublands; and tropical and subtropical moist broadleaf forest based on the distribution of climate zones.

The fight against this cotton pest by using conventional

pesticides for long periods led to build up resistant strains of *H. armigera* to these pesticides beside occuring imbalance in the biological system, which led to reducing the role of natural enemies. The occurrence of resistance and cross-resistance in addition to persistence of insecticides in the environment resulted in expiration of the biotic potential of many insecticides.

Therefore, during the past three decades, effort have been made to find biorational insecticides with novel modes of action which have non cross-resistance with the old insecticides. Most of these insecticides are preferable to the conventional insecticides because of their specificity to target pests, their effectiveness at low rates and their nonpersistent characteristics in the environment. These new classes of insecticides include emamectin benzoate, chlorantraniliprole, indoxacarb, spinosad, pyridalyl and insect growth regulators (IGR, s).

Accordingly, this study aims to evaluate the toxicological and biological effects of some nonconventional insecticides compared with conventional insecticide (chlorpyrifos) on *H.armigera* (Hűbner),compared with *Pectinophora gossypilla* (Saund) and *Earias insulana* (Boisd).

The following studies were carried out to achieve these goals:

1. Toxicological effect of methoxyfenozide, emamectin benzoate, spinosad, chlorpyrifos, chlorantraniliprole, pyridalyl and indoxacarb in laboratory on *H.armigera* and *P. gossypila*.
2. The effect of the four tested insecticides (emamectin benzoate, chlorpyrifos, spinosad and methoxyfenozide) on some biological aspects such as larval mortality, pre-pupal and pupal mortality, total

mortality, larval and pupal durations ,adult emergence percentages, moth life span, fecundity, hatchability and sterility percentages.

3- The latent effect LC_{50} of the tested insecticides on certain biological aspects of the American bollworm.

4- Moreover, field trials were conducted to evaluate the efficacy of the tested insecticides, emamectin benzoate, indoxacarb, chlorpyrifos, pyridalyl, chlorantraniliprole and spinosad against bollworms on two successive seasons.

REVIEW OF LITERATURE

1. Toxicological studieson the effect of different groups of insecticides on American bollworm and cotton bollworms.

a. Insect growth regulators Methoxyfenozide (Runner 24%SC).

Methoxyfenozide is an insect growth regulator which mimic the action of hormones on the growth and development of insect pests (Beckage, 2000). Insect growth regulators are in general much slower in providing full efficacy against pest insects compared to insecticides interfering with neuronal target sites. This class of compounds was introduced to the insecticide market in 1993 and represents the only new major contribution to the group of insect growth regulators since the introduction of the chitin biosynthesis inhibitors (CSIs) in the mid-seventies. Ecdysone receptor agonists or moulting accelerating compounds (MACs) are non-steroidal ecdysone analogs and mimic the natural function of the endogenous moulting hormone 20-hydroxy-ecdysone. This is released at physiologically strictly defined time-points before larval/larval or larval/pupal moults and initiates a cascade of biochemical reactions that leads to the formation of a new cuticle and shedding of the old cuticle at the end of the moulting process. MACs bind to ecdysone receptor-ultra spiracle protein heterodimer complexes. This ligand heterodimer complex then trans activates a cascade of genes by binding to a DNA ecdysone response element initiating molting (Nauen and Bretschneider, 2002). This leads first to

the cessation of feeding and weight gain and then, at the end of the intoxication process, to premature head capsule slippage and death.

Smagghe and Degheele (1994a) tested the effect of non-steroidal ecdysteroid agonist tebufenozide (RH-5992) on larval stages of a number of lepidopteran species by topical application and by feeding on treated leaves. LC50 values in the range 0.03-0.10 mg litre $^{-1}$were obtained for third to sixth instars of *Spodoptera exempta*(Walker) when insects were fed on leaves dipped in aqueous emulsions of the compound; while first to fifth instars of *Spodoptera exigua*(Hüb.) were less susceptible, their LC_{50} values were in the range 2.5-10.5 mg litre^{-1}. They found that when insects were topically treated, susceptibility of tested last instar larvae of order Lepidoptera were decreased *S.exempta, Mamestra brassica L., Spodoptera littoralis (Boisd.), S.exigua and Galleria mellonella*. tebufenozide induced a premature and lethal larval moult in larval Lepidoptera within 24hrs. of treatment. Most larvae died in their old larval cuticle.

Smagghe and Degheele (1994b) assessed the biological activity of RH 5849 and RH 5992 against last-instar larvae of *S. exigua* (Hüb.) (Lepidoptera: Noctuidae) . Results indicated that both compounds affected growth and development of treated larvae in a dose-dependent manner. Within the first 24hrs. after treatment by continuously offering leaves dipped in a water solution of >50 mg/l RH 5849 and >0.5 mg/l RH 5992, symptoms of a prematurely induced larval moult and head capsule apolysis were visible. They showed that, intoxicated larvae died shortly afterwards, showing signs unsuccessful ecdysis. LC5o values of RH 5849 and RH 5992 for fifth-

instars *S. exigua* larvae were 110 and 2.5 mg/l, respectively. Pyriproxyfen alone affected the larval stage and disturbed normal metamorphosis. One supernumerary larval instar occurred occasionally. LC50 value for pyriproxyfen was 1.7 mg/l. Larvae simultaneously treated with RH 5849 or RH 5992 and pyriproxyfen, continued to grow until they attained a size and weight about 2-3 times that of the controls. This growth was accompanied by at least one and sometimes two supernumerary moults.

Khan *et al*. (2005) determined the toxicity of the two conventional insecticides chlorpyrifos (Lorsban 40%EC) and profenofos (Curacron 50%EC) and the three new chemical insecticides lufenuron (Match 5%EC), emamectin benzoate (proclaim 1.9%EC) and methoxyfenozide (Runner 25%EC) against the adult of the parasitoid *Bracon hebetor* at selected the recommended dose applied for *S. litura*, beside two other doses 10% above and below the recommended one . One day old freshly emerged adults of *B. hebetor* used for bioassay in the vial method. Data indicated that, chlorpyrifos proved to be toxic, yielding 100% mortality at 24 hours on 10% above the recommended dose. Profenofos exhibited 100% mortality after 36 hours at higher dose rate. Emamectin benzoate showed least mortality ;*i.e*., 21.75, 25.25 and 28.25% at all the selected doses after 48 hours of exposure.

Anwar and Abdel-Mageed (2005) conducted a work to study the susceptibility of both laboratory and field strain of 2^{nd} and 4^{th} instars larvae of the cotton leaf worm *S. littoralis*(Boisd.) to six insect growth regulators (diflubenzuron, tebufenozide, hexaflumuron, flufenoxuron,

chlorofluazuron and lufenuron). The obtained data revealed that, resistance varied considerably according to the chemical structure of the studied IGR's and the treated instar of larvae.

Abahussain (2006) assessed the non-steroidal ecdysone agonist, RH-5849, as IGR against the false stable fly *Muscina stabulans* (Fallen). A dose range of 0.025, 0.100, 0.400, 1.000 and 4.000 µg/insect was topically applied on 1 day old 3rd larval instar and the newly moulted pupae. The larval mortality was increased by increasing RH-5849 doses; while the pupal and adult mortalities which resulted from treated larvae were increased by decreasing doses. On the other hand, the higher of pupal deformation (40.3%) was obtained due to the effect of dose 1.0 µg/larvae. Although some various deformities of the adult stage, there is no certain trend could be encountered for the adult deformities. No perfect adult could be emerging as affected by RH-5849 at the dose levels of 4.0 and 1.0 µg/larvae. In case of treated pupae with RH-5849, the percentage of pupal mortalities were increased by increasing the dose levels.

Pineda *et al*. (2007) studied the susceptibility of *S. littoralis* (Boisduval) larvae to mothoxyfenozide through exposure of neonate and fourth instars to dipped and sprayed leaves of pepper , *Cpsicum annum*. Mothoxyfenozide and spinosad were tested against adults of this pest by oral, residual, and topical application. Results illustrated that larval weight of fourth instars fed for 48 hrs. on pepper. leaves containing methoxyfenozide was significantly suppressed. Spinosad and methoxyfenozide reduced , in a dose-dependent manner the fecundity and fertility of *S. littoralis* adults when treated orally and

residually. Likewise, when mothoxyfenozide was demonstrated orally in three different adult crosses, the fecundity was strongly affected, independently of the treated sex. They concluded that, the combination of lethal and sub-lethal effects of mothoxyfenozide and spinosad might exhibited significant effects on the population dynamics of *S.littoralis*.

Faheem *et al*. (2013) reported that methoxyfenozide recorded low resistance (03-14 folds) to *H. armigera* ; emamectin benzoate and spinosad recorded (01-42 folds) and (01-07 folds), respectively, when use leaf dip technique in treatment.

Sabera *et al*. (2013) reported that methoxyfenozide showed higher potential for killing the American bollworm *H. armigera* when it fed on insecticide-treated artificial diet.

b. Emamectin benzoate compounds (Radical 0.5%EC).

Abamectin is a natural product produced by the soil microorganism *Streptomyces avermitilis*. It is chemically unrelated to any other currently registered insecticides. It has a unique mode of action and was effective against insect's resistant to organophosphates, carbamates, pyrethroids and other types of insecticides. It is non-phytotoxic at the recommended dose rate on virtually all crops on which it has been tested. It is reserved within the tested plant. This contributes to the products long lasting activity. It is not considered disruptive to natural predators and parasitoids or beneficial insects. Abamectin binds tightly to soil and is degraded rapidly by soil microorganisms and does not bioaccumulate in the environment (Dybas, 1983; Campbell, 1981 and 1989).It acts by stimulating the pre-

synaptic release of the inhibitory neurotransmitter, gamma-amino butyric acid (GABA), binding to the post-synaptic receptor. In arthropods, Abamectin inhibits signal transmission at the neuromuscular junctions via the same mechanism of the amplifying GABA action. The susceptible insects become irreversibly paralyzed and are thereby killed.

Abamectin does not affect the cholinergic system, as do most insecticides so that cross-resistance to other pesticides is highly unlikely. Turner *et al.* (1989) reviewed the mode of action of Avermectins (Abamectin and other analogues) in invertebrates (electro-physiology, binding sites, chloride uptake, acetylcholine release, interaction with retinal binding proteins and inhibition of chitin synthesis) and vertebrates (neurotransmitter release, binding sites, effect on [3H] GABA binding, effect on benzodiazepine binding sites, chloride uptake). Deng and Casida (1992) reported that avermectins and their analogues (channel openers) act in the GABA- gated chloride channel of the housefly head at a site closely coupled to that for EBOB [ethnylbicycloorthobenzoate(a channel blocker)]. Emamectin derived from abamectin via a five-step synthesis, was discovered after screening several hundred avermectin derivatives in an invivo screen using tobacco budworm, *H virescens*, and southern army worm, *Spodoptera eridania* (Cramer). This compound was subsequently selected for further development in crop protection. Later studies found that benzoate salt of emamectin had improved thermal stability and greater water solubility compared with the original hydrochloride salt.

As such emamectin has a broader spectrum of insecticidal activity than abamectin.

Bariola (1984) reported that abamectin was highly toxic to male and female pink bollworm, *Pectinophora gossypiella* moths under laboratory conditions when applied topically on either the dorsal thorax or the ventral surface of the last abdominal segment of the moths at LC50 of 0.032 to 0.041 mg/insect.

Also, Wright *et al*. (1985) showed that in bioassay against *Heliothis spp*, the first visible symptom of exposure of the first instar larvae to abamectin was paralysis of the hind legs within 24 hours, followed by larval mortality over a 48 to 72- hours period.

Anderson *et al.* (1986) reported a 400-fold difference in the toxicity of abamectin to *H. viresens* larvae compared to *S. eridania* at the LC50 level in a foliage bioassay test. Also they showed that abamectin was toxic to lepidopteran larvae via ingestion than by contact with product residues. Abamectin was one fourth as toxic as the pyrethroid fenvalerate to *S. eridania* larvae in the foliage ingestion bioassay, whereas by topical application, abamectin was nearly 1000-fold less toxic to *S. eridania* than fenvalerate.

Christie and Wright (1990) found that marked changes in the relative toxicity of topically-applied abamectin were found between larval instars of *S. littoralis*. Toxicity decreased up to the 5th instar but increasing over 500-fold (at the LD50 level) in the 6th instar. In contrast, the toxicity of abamectin remained constant from the 5th to the 6th instar in *H. armigera*. There was also an increase in the toxicity of 2 chemically unrelated insecticides, malathion (4-fold) and lambda-

cyhalthrin (2.5-fold), from the 5th to 6th -instar in *S. littoralis*. The toxicity of injected abamectin to 5th -instar larvae of *S. littoralis* was greater (20-fold) than with topical application; but injected abamectin was less toxic (2-fold) to 6th -instar larvae of *S. littoralis* and had no significant effect on 5th -instar larvae of *H.armigera*. It is suggested that differential toxicity of abamectin is due in part to greater metabolism and reduced penetration of 5th -instar larvae than in 6th -instar larvae of *S. littoralis* or 5th -instar larvae of *H. armigera.*

Rui *et al.* (1995) tested the toxic effects of abamectin on the cotton bollworm, *H.armigera*; the red spider mite *Tertanychus cinnabarinus*; the green peach aphid, *Myzus persicae* , the cabbage aphid, *Brevicoryne brassicae* and the armyworm *Leucania separata*. The LC50 were 7.6966, 1.3526, 1.0848, 0.2140 and 0. 1626 ppm for *H. armigera, B. brassicae. L separata, M. persicae* and *T. cinnabarinus*, respectively.

Wang *et al.* (1998) evaluated the toxicity of Avermectins against neonate, 1st, 3rd and 4th instar larvae of *H.armigra*, using a diet incorporation bioassay. LC50 for neonate, 1st, 3rd and 4th instar larvae were 0.002, 0.049, 0.58, 1.14 µg/g (48 hrs. exposure) and 0.05, 0.021, 0.64, 1.13 µg/g (96 h.), respectively.

Biddinger and Hull (1999) reported that azinphosmethyl, diflubenzuron, tebufenozide, fenoxycarb and abamectin were highly toxic to neonate larvae of the laboratory susceptible strain of tufted apple bud moth, *Platynota idaeusalis* (Walker) through ingestion of diet treated on the surface.

Abd El-Rahman *et al*. (2002) conducted a study to evaluate the toxicological effects of es-fenvalerate and abamectin on *Earias insulana* (Boisd.). Results indicated that, the natural product abamectin and synthetic pyrethroides-fenvalerate provide high insecticidal activities on *E. insulana*. Abamectin was more effective insecticide against young larvae, eggs and adults; while es-fenvalerate showed more potency against pupae. Data also indicated that the young larvae were the most susceptible stage followed by egg, adult and pupal stages.

Ishaaya *et al.* (2002) reported that emamectin benzoate is considered an important component in pest-management programmes for controlling field crop pests. It is a powerful compound for controlling the cotton bollworm *H.armigera* (Hüb.). A spray concentration of 25mg a.i. / litre in a cotton field resulted in over 90% suppression of *H. armigera* larvae up to day 28 after treatment, while similar mortality of the Egyptian cotton leaf worm *S.littoralis* (Boisd), under the same conditions, was maintained for 3 days only.

Korrat *et al.* (2012) compared the effects of conventional (profenofos) and nonconventional (emamectin benzoate, spinosad and chlorfluazuron) insecticides at their LC_{10}, LC_{25} and LC_{50} were evaluated against 2^{nd} instar larvae of cotton leaf worm, *S. littoralis* (Boisd.) under laboratory conditions. After 3 days of the treatment, emamectin benzoate was the most effective insecticide (LC_{50} = 0.017 ppm) followed by chlorfluazuron (LC_{50} = 0.42 ppm) and profenofos (LC_{50} = 10.9 ppm) and finally spinosad which showed the lowest toxic effect (LC_{50} = 19.9 ppm).

C . Spinosad (Tracer 24%SC).

Spinosad (a mixture of spinosyns A and D) belongs to a new class of polyketide-macrolide insecticides derived by fermentation from the metabolites of a new species of actinomycetes. It has a novel mode of action, acting primarily at the nicotinic acetylcholine receptor in the nerve synapse (Salgado *et al.,* 1998).

Radwan (2002) studied the toxic effect of diple2x, agrine (*Bacillus thuringiensis*) and spionsad (Actinomycete product) compared with a novel insecticide chlorofenapyr (Pyrazole analogue) on larvae of the spiny bollworm. The neonates of *E. insulana* were highly susceptible to all tested insecticides than the last instar larvae. Spionsad was more effective insecticide against neonates and last instar larvae than Diple2x, Agrine and chlorofenapyr.

Ahmad *et al.* (2003) determined the toxicity of new chemistries viz. fipronil, chlorfenapyr, indoxacarb, spinosad, abamectin and emamectin-benzoate,which having novel modes of action and tested them by using an insecticide resistance action committee (IRAC) leaf-dipping method against Pakistani field populations of *H.armigera*, which were highly resistant to conventional chemistries. Whereas the majority of populations exhibited susceptibility close to the baselines, there were, nevertheless, signs of resistance development to the new chemistries as demonstrated by a low level of tolerance in many populations. This may be due to the absent of a cross-resistance from their resistance mechanisms, particularly metabolic, already selected against older chemistries. If used judiciously and in rotation, the new

chemistries can restore the profitability of crop production by counteracting and preventing insecticide resistance in *H. armigera* and other pests in the future.

Ahmed *et al.* (2004) studied the chemical control of *H. armigera* on chickpea in the laboratory. Arrivo (cypermethrin), Lannate (methomyl) 40%SP, Tracer (spinosad) 24%SC and Steward (indoxacarb) 15%SC.were tested through treated plants with different concentrations of the insecticides. Data showed that the Tracer (1 ppm) was toxic to 2nd instar larvae of the pest and Arrivo (216 ppm) was least effective.

Nirmal and Mahal (2005) tested five insecticides for their relative toxicity against third instar larvae (30–50 mg) of *H. armigera* (Hűbner) on cotton using leaf disc dipping bioassay technique. The LC50 values for spinosad, chlorpyriphos, endosulfan, acephate and cypermethrin were 0.4, 6.6, 80.4, 629.0, and 2087.0 µg ml - ^{1}, respectively, indicating that spinosad was highly effective followed by chlorpyriphos. cypermethrin and acephate were ineffective in controlling *H. armigera*, whereas, endosulfan was intermediate in this respect.

Aydin and Gurkan (2006) measured the susceptibility of the cotton leaf worm, *S. littoralis* (Boisduval) larvae to spinosad, which were collected from commercial cotton production fields. The susceptibility of the field strain was also compared to the susceptible strain (S) of *S. littoralis*. Lethal dose bioassays were performed with third instar larvae using the leaf dipping method. The LC50 values for