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Control of Cotton Spiny Bollworm, *Earias insulana* Boisduval, Using Three Bio-Insecticides, Bt, Spinosad and Neem-Azal

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Abstract: This study was conducted to evaluate, the more efficient insecticide, between three bio-insecticides used, in a chemical control of spiny bollworm and to compare a purely chemical control, with an integrated control of cotton boll worms, which was already studied by the same authors. Cotton variety vermin planted in a Complete Randomized Block Design (CRBD), with 6 treatments and 5 replications. The treatments were as follows: three concentrations of spinosad, 200, 4000 and 10000 ppm, Neem-Azal, 50 ppm, Bt., 3000 ppm and control. Insecticides were sprayed, with an interval of 15 days. For sampling, 10 plants were chosen randomly and weight and number of attacked, or healthy blossomed or blind bolls measured. Results of analysis of variance by MSTAT-C showed that, there is a significantly difference, between use of 200 ppm and use of each of 10000 and 4000 ppm of Spinosad. Between the treatments, those sprayed with 4000 and 10000 ppm of Spinosad had the lowest number of damaged bolls, the plots sprayed with 200 ppm Spinosad had the minimum number of blind damaged bolls, followed by plots, in which Neem-Azal and Bt., were sprayed.

Key words: Bt, Spinosad, Neem-Azal, *Earias insulana*

INTRODUCTION

Cotton, the world's major fiber crop is perhaps unique in the broad nature of the insect attack to which it is subjected and the control of cotton insect pests remains an unabated challenge (Johnstone, 2006). Cotton spiny bollworm is the larva of *Earias insulana*, belonging to the Noctuidae family. It is one of the most important pests of cotton, in all over the world and in Iran, the pest, is recorded to be spread in cotton farming areas (Esmaili *et al.*, 1995; Mirmoayedi, 2009). For control of the pest, we used three insecticides, Spinosad, Neem-azal and Bt. Spinosad proved to be harmless to predators and parasites in cotton field (Tillman and Mulrooney, 2000) and with minimum impact to bees (Cleveland, 2001) and in laboratory conditions, it was harmless to *C. carnea* eggs and pupae irrespective of concentrations or method of treatments (Mandour, 2009). In China, when Spinosad was used for control of pests, in vegetables such as eggplant, Chinese cabbages and Cotton the quantities found in sprayed plants didn't surpassed, the norm of that country (Gao *et al.*, 2007). Some researchers, observed resistance of pests toward this insecticide, for example, *Heliothis virescens* (F), which was collected from tobacco fields in North Carolina

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and reared in laboratory condition showed, a resistance toward Spinosad, which was 1068-fold greater than the parental generation (Young *et al.*, 2003). The microbial insecticide *Bacillus thuringiensis* (Bt) has become the mainstay of non-chemical control of Lepidopteron pests, either as sprays or through the incorporation of Bt. toxins into transgenic crops. Recent years, resistance to *B. thuringiensis* was observed in populations of many lepidopteron pests, between them, *Trichoplusia ni* (Janmaat and Myers, 2003) *Plutella xylostella* (Sayyed and Wright, 2001) *Helicoverpa armigera* (Akhurst *et al.*, 2003) *Helicoverpa zea*, *Spodoptera frugiperda*, *Spodoptera exigua* (Stewart *et al.*, 2001). The aim of this study was to compare the effectiveness of three bio-pesticides, Spinosad, Neem-azal and Bt., in control of cotton's spiny bollworm.

MATERIALS AND METHODS

This research was done, during spring and summer, in 2008, following a Complete Randomized Block Design (CRBD), with 6 treatments and 5 replications. The 6 treatments were as follows:

- Spinosad, with a concentration of 200 ppm
- Spinosad, with a concentration of 4000 ppm
- Spinosad, with a concentration of 10000 ppm
- Neem-Azal, with a concentration of 50 ppm
- *Bacillus thuringiensis*, Bt-H, with a concentration of 3000 ppm
- Control, without spraying

Cotton, variety vermin was planted individually by hand in a hole, 15 cm apart, in each of 2.5×4 m plots, each plot had 5 longitudinal rows, 50 cm apart. Seeds were disinfected, before planting, by a 1000 ppm concentration of Carboxin thiram (Vitavax®). In time of seed planting, 125 g of granulate urea, plus 1 kg of potassium phosphate was poured, in inter rows, of each plot. The plants were irrigated, weekly, during 24 weeks.

RESULTS

Analysis of variance made after logarithmic transformation of the original data, indicated that, there was no significantly difference, between means of the number of healthy and or attacked blind bolls, in different treatments (Table 1). This conclusion was also true for the healthy or attacked blossomed bolls. There was not also any significantly difference, comparing weight of fibers in different treatments. Use of DMRT to compare mean number of healthy blind bolls, in different treatments, showed no significantly difference, between them, although, there is a significantly difference between these plots and control treatment. Comparison of mean number of attacked blind bolls, indicates that, there is a significantly difference between means of them, at 5 and 1% levels of significance. There is a significantly difference between mean numbers of damaged blind bolls, in plots sprayed with 200 ppm, compared to those in plots sprayed with 4000 or 10000 ppm Spinosad (Table 2), but there is not a significantly difference between mean numbers of damaged bolls, in plots sprayed with 400 and or 10000 ppm Spinosad. Between blossomed healthy bolls, the maximum number of such bolls belonged to plots sprayed with 10000 ppm Spinosad. In addition, there is a significantly difference, when mean number of blossomed damaged bolls were compared, attacked bolls picked from plots which were sprayed with 10000 ppm Spinosad, their mean

Table 1: Analysis of variance of characters

MS of analyzed characters										
SV	DF	w1	w2	w3	w4	n1	n2	n3	n4	fw
Block	4	0.336ns	0.336ns	0.136ns	0.245ns	0.245ns	0.114ns	349.96ns	43.28ns	0.078*
T	5	0.202ns	0.202ns	0.088ns	0.32ns	0.32ns	0.06ns	505.07ns	85.52*	0.032ns
Error	20	0.192	0.192	0.108	0.16	0.16	0.091	1133.8	25.103	0.023

*: significantly difference, at level of 5% ns: Non significantly difference, SV: Source of variation DF: Degree of freedom, w1: Weight of blind healthy bolls, w2: Weight of blind damaged bolls, w3: Weight of blossomed healthy bolls, w4: Weight of blossomed damaged bolls, fw: Weight of fibers, n1: Number of blind healthy bolls, n2: Number of blind damaged bolls, n3: Number of blossomed healthy bolls, n4: Number of blossomed damaged bolls

Table 2: Comparison between means of different factors

T	w1	w2	w3	w4	n1	n2	n3	n4	fw
1	A1.75	A1.75	A 2.23	A 1.44	A 1.44	A15.60	A 13.80	A 93.80	A 2.13
2	A1.66	A 1.66	A 2.19	A 1.22	A1.22	B13.00	AB12.60	A 92.60	AB1.95
3	A 1.60	A 1.60	A 2.18	A1.10	C 1.10	C11.80	AB 12.40	A 83.80	B1.88
4	A1.54	A1.54	A 2.15	A 0.97	A 0.97	D10.20	BC 6.00	A 78.00	B1.88
5	A 1.14	A1.14	A1.86	A 0.95	A 0.95	E 8.00	BC5.60	A72.00	B 1.86
6	B 0.17	B 0.17	B 0.13	B 0.16	B 0.16	E 8.00	C 4.80	A70.40	C 0.06

Those items with identical alphabetic letters have no significantly difference, between them, at level of significance 5%

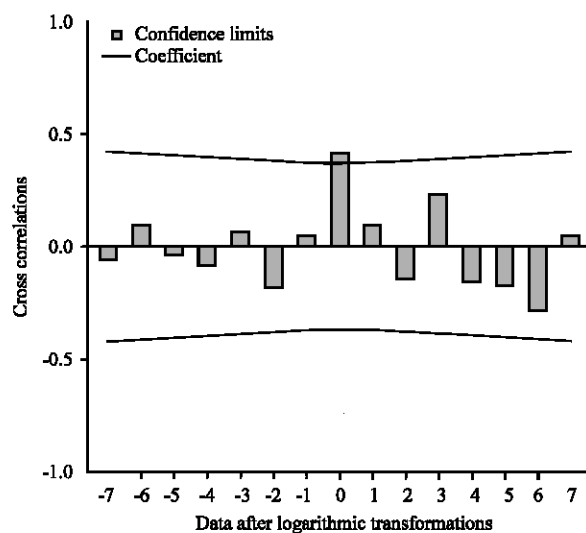


Fig. 1: Comparison between weight of cotton bolls, w3 = Weight of blossomed healthy bolls, w4 = Weight of blossomed damaged bolls. Graph was plotted, by the use of SPSS16 (Norušis, 2008) and data in Table 2

number, had a significantly difference from those sprayed either with Neem-Azal, or Bt and also, with those in control, without spray of any insecticide, at level of 5%. Weight of blossomed healthy bolls, was compared with weight of blossomed damaged bolls, in Fig. 1.

DISCUSSION

It seems that little by little, the industrialized countries adapt new procedures and strategies for control of agricultural pests. The global warming, besides the environmental pollution, forced these countries to ban the use of old synthetic pesticides and to choose other methods of control, which cause less harm to the nature and to encourage the

production of environment friendly, pesticides. Between them, we could mention pesticides, which are derivatives of bacteria, fungi, extract of plants, etc., these pesticides decompose rapidly after use, with little or no harmful impact for the environment. Insecticidal toxins from *Bacillus thuringiensis* (Bt) can now be deployed either as sprays or as transgenic plants. Some entomologists and environmentalists have argued that the sprays are preferable to plants because they are less likely to cause resistance. However, Bt. sprays are not generally competitive with chemical insecticides and seem unlikely to displace them (Roush, 1994). Transgenic plants are excellent replacement for chemical pesticides, today transgenic crops, such as transgenic rice, soya, corn and cotton are cultivated in more than 80 million hectares, of lands throughout the world. And although, in the first year of their use in 1998, anxieties existed between the users, concerning the persistence of genes transferred into these plants, such as Bt. crystal genes, but in 2005, after 8 years of continuous cultivation, resistance was not observed in target insect pests (Bates *et al.*, 2005) and also, citing another example, in Arizona in a 10 years study successful control of major cotton pest pink bollworm (*Pectinophora gossypiella*) was obtained by using such plants (Carriere *et al.*, 2003) and recently in 13 US Southern locations, with cotton plantations, Siebert *et al.* (2008) compared the damage caused by of *Helicoverpa zea* (Boddie) in Phytogen 440W, a transgenic cotton, with non-treated, non-Bt cotton (PSC355) and with management strategies in which supplemental insecticides targeting heliothines were applied, they found that, Phytogen 440W containing Cry1Ac and Cry1F provided consistent and much more better control of heliothines, than other varieties of non-transgenic cotton.

Many other researchers observed resistance of target pests, between them, Stewart *et al.* (2001) expressed use of transgenic cotton with two types of crystal proteins of Bt. should be more effective against pests of cotton versus varieties, containing only one type of such proteins and Akhurst *et al.* (2003) stated, the Resistance Ratio (RR) peaked approximately 300-fold at generation 21 of *Helicoverpa armigera* (Hübner) when the pests were fed on Bt. cotton in laboratory conditions. Another non-chemical alternative to control of spiny boll worm, was the use of late, or early sowing, using certain local varieties of cotton seeds as a control method, one of such practices was to counter attacks by *Earias insulana* in Harran plain (Unlu and Yildiz, 2003) although, this practice was accompanied with some success, but as a practice, is not applicable to be used worldwide, because every country generally use it's own local varieties of cotton seeds and many countries now, use transgenic cotton seeds, as a more successful replacement for local varieties. In Mexico, Tamez-Guerra *et al.* (2000) used Bt. (Dipel) as powder mixed with corn bran and lignin mixed with corn bran, or as encapsulated in gelatin, for control of *Ostrinia nubilalis* in corn and found that, Dipel, as encapsulated form is more resistant to UV and has a longer protection period and cause a mortality of 75% of larvae, but it's application as Dipel powder sprayed mixed with corn bran, caused only a mortality of 51%. (Tamez-Guerra *et al.*, 2000). In Iran, as cultivation of transgenic plants, are banned by the government, so, we used instead, formulation of wettable powder of Bt. to control cotton spiny boll worm. We observed different percentages of damage, caused by attack of spiny boll worm, in different plots of cotton, in which different concentrations of Spinosad was used, these damages, were significantly less than, those treatments, in which, Bt. was sprayed. So, we conclude, that, the use of Spinosad compared to the use of Bt. in cotton, has a better protective effect. Although, Diazinon was the insecticide of choice already used by us for control of *Earias insulana*. In a integrated control together with release of green lacewings (Mirmoayedi and Maniee, 2009) but as little by little, synthetic insecticides, are replaced by bio-insecticides, so, we propose Spinosad, to be used, instead of Diazinon, in any integrated

control of spiny bollworm, together with the use of green lacewings, or any other predators or parasites. But when we compared this study, to our previous study, we understood that, we have had a better control of the pest in our last study (Mirmoayedi and Maniee, 2009), than this one, because in that research work, we have used an integrated control of spiny bollworm, in which we have used two different control measures, to protect, cotton bolls, against spiny boll worms, primarily we controlled the bolls against the worm, by spray of Diazinon and the second time, one month later, the second control, was done by the release of the eggs and larvae of green lacewing *Chrysoperla lucasina*. So, as Diazinon, was a primary protective agent, to protect the bolls of cotton against the pest for a period of one month and then, with an interval of one month, the release of lacewings, helped us to protect them for the rest of their growth time, so the total control time could cover the total growth time of cotton, but in this new experiment, although Spinosad proved to be a more efficient bio-pesticide, between, three chemical used, but as it has a shorter protective period, than, Diazinon and it was sprayed only once and as a single application, so, we conclude, that in a purely, chemical control of cotton boll worm, a second application of this bio-pesticide, should be considered, to provide a better protection of cotton, toward the attacks of spiny boll worms.

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