EFFICACY EVALUATION OF INSECTICIDES ON LARVAE OF THE TOMATO BORER *TUTA ABSOLUTA*, MEYRICK (LEPIDOPTERA: GELECHIIDAE) UNDER LABORATORY CONDITIONS

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Abstract

The tomato borer Tuta absoluta (Meyrikc) is a serious pest of tomato. In Bulgaria it was recorded in 2009 and currently is found in all tomato-producing regions. The control is difficult because of the resistance to many of the known insecticides. With the aim to evaluate the efficacy of some prospective insecticides for control of the pest, experiments were conducted in 2012 - 2013 under laboratory conditions (25 ± 2 ºC, RH 60–70%,16:8 h L:D) at the Agricultural university-Plovdiv. The efficacy of 11 plant protection products with different mode of action was tested on larvae 2nd and 3th instar with two variants: treatment of tomato leaves with larvae in the mines (variant A), and treatment of leaves and release of larvae on the treated leaves (variant B), each with 15 replicates and control. In both variants 100% biological efficacy were recorded for azadirachtin, emamectin benzoate, spinosad, metaflumizone and chlorantraniliprole.

Key words: *tomato borer, control, insecticides, laboratory conditions*

1. INTRODUCTION

The tomato borer *Tuta absoluta* is originating from South America, where it is considered one of the most serious pests on tomato (Barrientos et al. 1998, Estay 2000, EPPO 2006). It attacks plants during the entire growing season. Larvae develop in leaves, stems, apical buds, flowers and fruits causing significant loss of yield (López 1991, Apablaza 1992). In Europe, the species has been found at the end of 2006 in the province of Castellón de la Plana (Eastern Spain) (Urbaneja et al. 2007). Subsequently, its presence was reported from most countries in Europe, North Africa and the Middle East (Russell IPM 2009, Desneux et al. 2010, Garzia et al. 2011, Baniameri & Cheraghian 2012). In the summer of 2009 *T. absoluta* was recorded in Bulgaria (Harizanova et al. 2009).

Historically, the most common practice for control of the tomato borer was the use of chemical insecticides (Lietti et al. 2005, Bielza 2010). In South America initially organophosphates were used which later were supplemented with pyrethroids. At the early 80s the cartap hydrochloride alternating with pyrethroids and thiocyclam proved very effective in the control of the pest. In the 90s some new insecticides such as abamectin, spinosad, chlorfenapyr, tebufenozide and acylurea IGR (inhibitors of chitin biosynthesis) were included in the schemes for plant protection. Various extracts of plants from the Meliaceae family, which possess insecticidal properties, have been tested as potential control products: *Melia azedarach* (Brunherotto & Vendramim 2001), *Neem* (Azadirachta indica) (Gonçalves Gonçalves-Gervásio & Vendramim 2007) and *Trichilia pallens* (da Cunha et al. 2005). The experiments showed that despite of the reported biological activity, when applied alone these products do not lead to sustainable management of the tomato borer in South America.

Among the different entomopathogens *Bacillus thuringiensis* var. *kurstaki* (Btk) causes larval mortality at all instars and all commercial formulations can be successfully applied in the schemes for control of the pest (Giustolin et al. 2001, Theoduloz et al. 2003, Niedmann & Meza-Basso 2006, González-Cabrera et al. 2011).

Since the early 80s, as a result of repeated and continuous applications, the efficacy of organophosphates as the primary tool for management of *T. absoluta* gradually decreased in countries such as Bolivia, Brazil and Chile (Salazar & Araya 1997, Siqueira et al. 2000, 2001). In Chile in addition to pest resistance to organophosphates, resistance also to pyrethroids was established (Salazar et al. 1997) and in Brazil – to abamectin, cartap hydrochloride, methamidophos and permethrin (Siqueira et al. 2000, 2001). Resistance of the pest to deltamethrin and abamectin was reported from Argentina (Lietti et al. 2005).

After detailed studies on the resistance of *T. absoluta* to different pesticides, in the southern, southeastern and prairie parts of Brazil the use of abamectin, cartap hydrochloride, chlorfenapyr, phenthoate, methamidophos, spinosad and indoxacarb was recommended, while in the Northeast of the country only chlorfenapyr, phenthoate and spinosad were recommended to use (IRAC 2007). In Argentina, the recommendation was to use triflumuron and *Bacillus thuringiensis* (Bt) against the larvae as part of the integrated pest management system, which includes also parasitoids (Riquelme et al. 2006).

In Spain indoxacarb, spinosad, imidacloprid, deltamethrin and Bacillus thuringiensis var. kurstaki (Btk) (FERA 2 009, Russell IPM 2009) were applied and in Italy - chlorpyrifos and pyrethrins (Garzia et al. 2009). Since existing methods for control of T. absoluta in some parts of Spain had proved ineffective the use of four additional active substances – chlorantraniliprole, flubendiamide, emamectin benzoate and metaflumizone (MARM 2010) was temporarily allowed. For outbreaks in Malta abamectin, indoxacarb, spinosad, imidacloprid, thiacloprid, lufenuron and Bacillus thuringiensis (Bt) (Mallia 2009) were recommended and in France - indoxacarb and Bt (FREDON-Corse 2009). In Bulgaria against tomato borer officially was permitted to use imidacloprid, acetamiprid, deltamethrin, metaflumizone, pyrethrins and azadirachtin. In 2012, the list of permitted plant protection products in Bulgaria was complemented with the active ingredients abamectin and chlorantraniliprole (BFSA 2012).

Currently in Europe hard work is dedicated to developing new insecticidal formulations which could successfully control the populations of *T. absoluta*, being at the same time selective to beneficial insects. This is imperative, considering the advantage of the integrated pest management as the primary method for the production of clean food and protection of the environment against pollution caused by chemicals.

The rapid spread of the tomato borer in all vegetable-growing regions of Bulgaria and the not effective control require urgent study on the biological efficacy of different groups of insecticides, depending on the different production areas - organic, integrated or conventional.

2. MATERAIL AND METHODS

The studies were carried out during the summer months of 2012 and 2013 in the laboratory of the Dept. of Entomology, Agricultural university-Plovdiv, Bulgaria. The biological efficacy of 11 active substances of insecticides with different mode of action, recommended for control of pests from Lepidoptera, including *T. absoluta* was tested: azadirachtin (BFSA 2012), *B. thuringiensis* var. *kurstaki* (Ave 2010), emamectin benzoate (Liguori et al. 2010), spinosad (FERA 2009), diflubenzuron (Chiot et al. 2011), metaflumizone (BFSA 2012), etofenprox (Giulianotti 2010), flubendiamide (Ebbinghaus et al. 2007), chlorantraniliprole (BFSA 2012), imidacloprid (BFSA 2012) and thiacloprid (Mallia, 2009). The mode of action, the trade name and the doses of application are presented in Table1.

The experiment was designed with two variants – directly sprayed tomato leaves with mines and live larvae inside (variant A) and released larvae on treated leaves (variant B). The plant protection products were tested on larvae, second instar, reared at laboratory conditions (25±2ºC, RH 60–70% and 16:8 h L:D).

In variant A the mined leaves with live larvae were treated with a solution of the products, dried on a grid and placed in plastic containers (⃠ - 90 mm). In variant B, the method for testing the biological efficacy of insecticides by IRAC (2009) was applied. Each tomato leaf was dipped for 5 seconds in the solution of the respective plant protection product at the selected dose, after which were dried on a grid and placed in a plastic container. In the each container a larva was released using a fine brush.

Table 1. Insecticides, mode of action, trade name and doses of application used in the study

Active Ingredients	Mode of Action		Trade Name	Dose ml (g) / hl water
	Group	Description		
azadirachtin	un	Compounds of unknown or uncertain MoA	NeemAzal®–T/S	300 ml/hl
Bacillus thuringiensis var.	11 A	Microbial disruptors of insect midgut membranes	DiPel® WP	150 g/hl

kurstaki				
emamectin benzoate	6	Chloride channel activators	Affirm®	250 g/hl
spinosad	5	Nicotinic acetylcholine receptor (nAChR) allosteric activators	Laser® 240 SC	25 ml/hl
diflubenzuron	15	Inhibitors of chitin biosynthesis, type 0	Dimilin®	40 g/hl
metaflumizon	22 B	Voltage-dependent sodium channel blockers	Alverde® 240 SC	100 ml/hl
etofenproks	3 A	Sodium channel modulators	Trebon® 30 EC	65 ml/hl
flubendiamide	28	Ryanodine receptor modulators	Belt® 24 WG	25 g/hl
chlorantraniliprole	28	Ryanodine receptor modulators	Coragen® 20 SC	16 ml/hl
imidacloprid + deltamethrin	4 A + 3 A	Nicotinic acetylcholine receptor (nAChR) agonists + Sodium channel modulators	Confidor Energy® OD	80 ml/hl
thiacloprid + deltamethrin	4 A + 3 A	Nicotinic acetylcholine receptor (nAChR) agonists + Sodium channel modulators	Proteus® 110 OD	60 ml/hl

The plastic containers were closed tightly with a lid and stored at 25±2ºC, RH 60-70% and 16:8 h L:D without direct sunlight. The experiments were set in 15 replications and 2 controls. Larval mortality was recorded at 3-rd, 6-th, 9-th day after treatment.

The biological efficacy (Eff.%) was calculated by Abbott's formula (1925): Eff.% = ((x - y) / x). 100, wherein:

Eff. – biological efficacy,%;

x – number of live specimens in the control after treatment;

y – number of live specimens in the variant after treatment.

3. RESULTS AND DISCUSSION

Most of the tested insecticides demonstrated high biological activity against the larvae of the tomato borer applied on the mined leaves (variant A) and when placing the larvae on treated leaves (variant B). Azadirachtin, emamectin benzoate, spinosad and metaflumizone in both variant A and variant B showed 100% biological efficacy by the 9-th day (Table 2 and 3). In variant B the same biological efficacy of flubendiamide was established (Table 3). A progressive increase in larval mortality was observed for most of the used active ingredients.

In variant A (Table 2) the rapid initial effect of the active ingredient spinosad which penetrates translaminarly in plant tissues was best demonstrated reaching larval mortality of 73.33% on the 3-rd day after treatment. Comparatively quick result was observed after treatment with emamectin benzoate, chlorantraniliprole, *Bacillus thuringiensis* var. *kurstaki* (*Btk*) and metaflumizone reaching 100%, 93.33%, 80% and 53.33% respectively on the 6-th day after treatment. On the 9-th day 100% biological efficacy was recorded for azadirachtin, emamectin benzoate, spinosad, metaflumizone and chlorantraniliprole. Azadirachtin, which is a biological product and penetration in the plant tissues was slower in comparison with the synthetic insecticides, had retarded effect.

Table 2. Efficacy of insecticides in variant A (treatment on mined leaves with larvae of the tomato borer)

Insecticides	Day after treatment								
	3-rd			6-th			9-th		
	Live	Dead	Eff., %*	Live	Dead	Eff., %*	Live	Dead	Eff., %*
azadirachtin	15	0	0.00	6	9	60.00	0	15	100.00
B. thuringiensis var. *kurstaki*	12	3	20.00	5	10	66.67	1	14	93.33
emamectin benzoate	0	15	100.00	0	15	100.00	0	15	100.00
spinosad	1	14	93.33	1	14	93.33	0	15	100.00
diflubenzuron	15	0	0.00	15	0	0.00	14	1	6.67
metaflumizon	4	11	73.33	2	13	86.67	0	15	100.00
etofenproks	7	8	53.33	6	9	60.00	4	11	73.33
flubendiamide	4	11	73.33	0	15	100.00	0	15	100.00
chlorantraniliprole	7	8	53.33	0	15	100.00	0	15	100.00
imidacloprid + deltamethrin	15	0	0.00	15	0	0.00	12	2	20.00
thiacloprid + deltamethrin	15	0	0.00	14	1	6.67	14	1	6.67
control	2	0	0.00	2	0	0.00	2	0	0.00

* Abbott's efficacy.

Table 3. Efficacy of insecticides in variant B (release of larvae of the tomato borer on treated leaves)

Insecticides	Day after treatment								
	3-rd			6-th			9-th		
	Live	Dead	Eff., %*	Live	Dead	Eff., %*	Live	Dead	Eff., %*
azadirachtin	15	0	0.00	6	9	60.00	0	15	100.00
B. thuringiensis var. *kurstaki*	12	3	20.00	5	10	66.67	1	14	93.33
emamectin benzoate	0	15	100.00	0	15	100.00	0	15	100.00
spinosad	1	14	93.33	1	14	93.33	0	15	100.00
diflubenzuron	15	0	0.00	15	0	0.00	14	1	6.67
metaflumizon	4	11	73.33	2	13	86.67	0	15	100.00
etofenproks	7	8	53.33	6	9	60.00	4	11	73.33
flubendiamide	4	11	73.33	0	15	100.00	0	15	100.00
chlorantraniliprole	7	8	53.33	0	15	100.00	0	15	100.00
imidacloprid + deltamethrin	15	0	0.00	15	0	0.00	12	2	20.00
thiacloprid + deltamethrin	15	0	0.00	14	1	6.67	14	1	6.67

control	2	0	0.00	2	0	0.00	2	0	0.00

* Abbott's efficacy.

In variant B (Table 3) the biological efficacy of almost all ingredients is higher compared to that recorded in variant A as the larval mortality was due to the contact action of the insecticides and not on their ability to penetrate and move in the plant. The fastest effect was observed after treatment with emamectin benzoate and spinosad. The larvae placed on treated leaves died during penetration in plant tissues without being able to form mines and therefore the reported death on the third day after treatment was 100% and 93.33% respectively. On the 6-th day larval mortality increased significantly and reached 100% for emamectin benzoate, flubendiamide and chlorantraniliprole; 93.33% for spinosad, 86.67% for metaflumizone; 66.67% for Btk; 60% for azadirachtin and etofenprox. At the end of the period (9-th day after treatment) the efficacy of the tested insecticides was high (100%) for azadirachtin, emamectin benzoate, spinosad, metaflumizone, flubendiamide and chlorantraniliprole and slightly lower for *Btk* (93.33%) and etofenprox (73.33%).

In both variants of the study (Table 2 and 3) very low biological activity for diflubenzuron (6.67%) was observed which is an insecticide from the group of IGR and its application is most appropriate in the phase "black head" of eggs. The biological efficacy of the combined insecticides Confidor Energy® OD and Proteus® 110 OD was low. The active ingredients imidacloprid and thiacloprid is moving in the plant tissues from base to apex (acropetal) and should be applied through irrigation systems and deltamethrin is in very low concentration, which is not sufficient to cause death of the larvae of *T. absoluta.*

4. CONCLUSIONS

At laboratory conditions the insecticides causing 90-100% larval mortality in both variants were azadirachtin (NeemAzal®-T/S), *Bacillus thuringiensis* var. *kurstaki* (DiPel® WP), emamectin benzoate (Affirm®), spinosad (Laser® 240 SC), metaflumizone (Alverde® 240 SC) and chlorantraniliprole (Coragen® 20 SC). Each of them could be included in a system of IPM in tomato because they are selective to beneficial insects.

The active ingredient flubendiamide (Belt® 24 WG) also has a good biological activity against the larvae of the tomato borer (variant A - 80% and variant B - 100% on the 9-th day after treatment), but due to its lower ability to penetrate the plant tissue it is desirable to be applied at the beginning of the vegetation period at the establishment of the pest on the crop.

Etofenprox (Trebon® 30 EC) showed lower biological effectiveness compared to the rest of the tested insecticides (variant A - 40% and variant B - 73.33% on the 9-th day after treatment), but it is very selective for beneficial insects and could be used as a preventive measure in the systems of integrated pest management.

The bioinsecticides NeemAzal®-T/S (azadirachtin) and DiPel® WP (*Bacillus thuringiensis* var. *kurstaki*) could be used in organic production of tomato, eggplant, potato and other crops, attacked by *T. absoluta*.

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