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Toxicity of Some Pyrethroids and Neonicotinoids used for Individual and Simultaneous Control of *Bruchus Pisi* L. and *Acyrthosiphon Pisi* Kalt

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***Abstract* – A study was conducted to evaluate the toxicity of few neonicotinoids, namely Mospilan 20 SP - 3000 g/ha (200 g a.i./kg acetamiprid); Calypso 480 SC-300 ml/ha (480g a.i./l thiacloprid); Proteus 110 OD - 700 ml/ha (100g a.i./l thiacloprid + 10g a.i./l deltamethrin) and pyrethroids: Duet 530 EC -500 ml/ha (50g a.i./l cypermethrin + 480g a.i./l chlorpyrifos-ethyl); Nurele Hlorsirin D 550 EC - 400 ml/ha (50g a.i./l cypermethrin + 500g a.i./l chlorpyrifosethyl) and Fury 10 EC - 100 ml/ha (100g a.i./l zeta-cypermethrin) used for individual and simultaneous chemical control of against *Bruchus pisi* L. and *Acyrthosiphon pisi* Kalt. The treatment with insecticides was committed after the findings of the first pea weevil eggs on pods located on bottom two nodes. The pest species numbers were reported on the first, third, fifth and seventh day after treatment. The method of sweeping with entomological net was used. It was found, that the best protection against *Bruchus pisi* on spring forage pea (*Pisum Sativum* L.) provided acetamiprid, followed by zeta-cypermethrin and thiacloprid. The control of *Acyrthosiphon pisi* was the most efficient by treatment with cypermethrin+chlorpyrifos-ethyl, followed by acetamiprid, thiacloprid and thiacloprid+deltamethrin, which showed the fast initiation effect and long-lasting activity. Simultaneous chemical control of pea weevil and pea aphid and increased grain yield by 24.22 and 24.14% may be implemented by using acetamiprid and thiacloprid, and rotation of the insecticides according to the necessary numbers of treatments, which is a recognized anti-resistance strategy.**

***Keywords* – Insects, Neonicotinoids, Pyrethroids, Toxicity.**

I. INTRODUCTION

Bruchus pisi L. is one of the economically most dangerous pea pest for the environmental conditions of Bulgaria. A number of authors ascertain enormous damage of the pea weevil, which can reduce grain yield by 40% or more [1], [4]-[6], [17]. It is necessary an annual control to this pest, to ensure high yield and quality. Dangerous pea pest is the pea aphid Acyrthosiphon pisi Kalt too. In order Hemiptera it occupies over 90% of the total number of piercing-sucking insects. The population density of the pea aphid is high especially in favorable combination of weather conditions, when the species reproduce massively and causes substantial damage to crops [18].

Still the main method to control the weevils and aphids is treatment with insecticides. Effect to insecticides on the pest depend on a development stage of insects, mechanisms of action of insecticide and on a way of intoxication. Application of preparations with active substance cypermethrin turns out to be effective against the imago of Bruchus pisi, causes up to 90% reduction of population density and reduces damage by the larvae on average by 11% [3], and [9]. The treatment against the all forms of pea aphid with dimethoate, benzofosfat and thiamethoxam also provides good protection of pea plants as the preparations on the basis of dimethoate are effective as well against *B. pisi* [3], [10], and [22].

Some of the insecticides, as a result of incorrect use, cause resistance of this pest. In accordance with current European standards and Directive 91/414 EU other plant protection products no longer apply that require testing of new active substances that are effective and also environmentally friendly.

The aim of this study was to evaluate the toxicity of some neonicotinoid and pyrethroid insecticides for individual and simultaneous chemical control of *Bruchus pisi* and *Acyrthosiphon pisi*.

II. MATERIAL AND METHODS

The trial was conducted with spring forage pea variety Pleven 4 in 2011-2012 in the experimental field of the Institute of Forage Crops, Bulgaria. The experiment was realized by the split plot method with sowing rate 120 seeds/m^2 in 4 replications and plot size of 6.5m^2.

The subject of the study were to evaluate the toxicity of few neonicotinoids: Mospilan 20 SP - 3000 g/ha (200 g a.i./kg acetamiprid; Producer: Nippon Soda, Japan); Calypso 480 SC-300 ml ha^{-1} (480g a.i./l thiacloprid; Producer: Bayer Crop Science -Germany); Proteus 110 OD - 700 ml/ha (100g a.i./l thiacloprid + 10g a.i./l deltamethrin; Producer: Bayer CropScience -Germany) and pyrethroids: Duet 530 EC -500 ml/ha (50g a.i./l cypermethrin + 480g a.i./l chlorpyrifos-ethyl; Producer: Agria corporation -Bulgaria); Nurele Hlorsirin D 550 EC - 400 ml/ha (50g a.i./l cypermethrin + 500g a.i./l chlorpyrifosethyl; Producer: Dow AgroSciences-Indiana, USA); Fury 10 EC - 100 ml/ha (100g a.i./l zeta-cypermethrin; Producer: FMS -USA)) used for individual and simultaneous chemical control of *Bruchus pisi* L. (*Coleoptera*: *Bruchidae*) and *Acyrthosiphon pisi* Kalt. (*Hemiptera*: *Aphidinea*).

The treatment with insecticides was committed after the findings of the first pea weevil eggs on pods located on bottom two nodes according to method of Horne and Bailey *[9]*.

The pest species numbers were reported on the first, third, fifth, and seventh day after treatment. The method of sweeping with entomological net was used. The efficacy of the insecticides was calculated according to the formula of Henderson and Tillton *[7]*.

The statistical processing of experimental data was conducted using the Statgraphics Plus software program and ANOVA for statistical analysis.

III. RESULTS

The efficacy of insecticides against Bruchus pisi on the first day after treatment in 2011 was high and varied in the range of 70.40-90.45% (Table I). The most expressed toxic effect was established in the plot, treated with Mospilan (90.45%), followed by Fury (88.40%) and Calypso (84.30%). The efficacy of these insecticides was statistically significant higher compared with the other insecticides. On the third day after treatment with the highest significant value stood out Mospilan (93.50%), whereas standard Nurele Hlorsirin D (56.60%) - with the lowest. The results of the 5th and 7th day after the treatment showed that the best protection against the attack of B. pisi provided Mospilan and Fury, followed by Calypso (the mortality ranged from 70.60 to 84.60%). Low efficacy of Nurele D Hlorsirin, Duet and Proteus (47.10, 50.00 and 55.80% respectively) was found of the 7th day after treatment.

A similar trend was observed in 2012 as the efficacy of insecticides had relatively lower values in compared to 2011. From the first to the seventh day after treatment Mospilan again showed the fast initiation effect and long-lasting activity, as well as the highest efficiency (from 42.90 to 63.77%). There was significant differences

Table I: Efficacy of some insecticides against Bruchus pisi

Insecticides	g a.i. kg^{-1}	Dose g/ml ha^{-1}	First day		Third Day		Fifth day		Seventh day	
			2011							
Mospilan 20 SP	200 g acetamiprid	300 g	90.45	b [a]	93.50	d	84.60	c	72.10	b
Calypso 480 SC	480 g thiacloprid	300 ml	84.30	b	83.80	c	70.40	c	64.50	b
Proteus 110 OD	100 g thiacloprid + 10 g deltamethrin	700 ml	76.10	a	70.70	b	58.70	b	55.80	a
Duet 530 EC	50 g cypermethrin + 480g chlorpyrifosethyl	500 ml	72.50	a	74.00	b	55.80	b	50.00	a
Nurele D Hl. 550 EC	50 g cypermethrin + 500g chlorpyrifosethyl	400 ml	70.40	a	56.60	a	47.60	a	47.10	a
Fury 10 EC	100 g zeta-cypermethrin	100 ml	88.40	b	82.10	c	78.70	c	70.60	b
LSD $_{5\%}$			6.248		6.838		6.617		9.187	
			2012							
Mospilan 20 SP	200 g acetamiprid	300 g	63.77	d	64.05	d	52.88	d	42.90	c
Calypso 480 SC	480 g thiacloprid	300 ml	58.59	cd	67.46	d	39.07	bc	36.44	b
Proteus 110 OD	100 g thiacloprid + 10 g deltamethrin	700 ml	47.56	b	40.65	b	34.93	b	34.88	b
Duet 530 EC	50 g cypermethrin + 480g chlorpyrifosethyl	500 ml	34.44	a	38.11	ab	23.72	a	28.75	a
Nurele D Hl. 550 EC	50 g cypermethrin + 500g chlorpyrifosethyl	400 ml	40.48	a	32.55	a	27.37	a	27.08	a
Fury 10 EC	100 g zeta-cypermethrin	100 ml	55.25	c	53.78	c	46.04	cd	40.60	bc
LSD $_{5\%}$			6.964		6.605		7.129		6.116	

[a] Means in a column followed by the same letter are not significantly different (α= 0.05, LSD)

between the high mortality caused by Mospilan and the low mortality caused by Proteus, Duet and Nurele D Hlorsirin in all reported days after treatment. High toxicity was established after the treatment with Fury and Calypso.

The efficacy of the insecticides in regard to *Acyrthosiphon pisi* differed substantially in compared to reported results about Bruchus pisi - Table II. During the first year of the study (2011), it varied within a wide range of 91.96-69.59% on the 1st day after the treatment to 74.12-17.05% of the 7th day after treatment. The highest mortality was caused by Duet on the 1st, 3rd, 5th and 7th day after treatment (respectively 91.96, 90.91, 85.25 and 74.12%). Successful protection against the pea aphid provided treatment with Proteus, Calypso and Mospilan which exhibited fast initial effect (respectively 86.18, 79.26 and 78.80%) and long-lasting activity (respectively 69.41, 57.95 o 63.00%).

The lowest statistically significant efficacy during the reporting period showed Nurele D Hlorsirin (69.59% on the first day and 41.24% on the seventh day), followed by Fury, which exhibited satisfactory initial effect (78.34 and 77.27% on the first and third day), but significant short effect (33.12 and 17.05% on the fifth and seventh day respectively).

Table II: Efficacy of some insecticides against Acyrthosiphon pisi

Insecticides	g a.i. kg^{-1}	Dose g/ml ha^{-1}	First day		Third Day		Fifth day		Seventh day	
			2011							
Mospilan 20 SP	200 g acetamiprid	300 g	78.80	b [a]	80.68	bc	78.82	cd	63.00	cd
Calypso 480 SC	480 g thiacloprid	300 ml	79.26	b	84.82	c	82.35	cd	57.95	c
Proteus 110 OD	100 g thiacloprid + 10 g deltamethrin	700 ml	86.18	c	80.36	bc	76.14	c	69.41	de
Duet 530 EC	50 g cypermethrin + 480g chlorpyrifosethyl	500 ml	91.96	c	90.91	d	85.25	d	74.12	e
Nurele D Hl. 550 EC	50 g cypermethrin + 500g chlorpyrifosethyl	400 ml	69.59	a	55.29	a	50.00	b	41.24	b
Fury 10 EC	100 g zeta-cypermethrin	100 ml	78.34	b	77.27	b	33.12	a	17.05	a
LSD $_{5\%}$			6.724		5.696		8.983		7.468	
			2012							
Mospilan 20 SP	200 g acetamiprid	300 g	57.92	bc	56.07	b	51.63	b	51.07	d
Calypso 480 SC	480 g thiacloprid	300 ml	55.88	bc	69.98	c	49.00	b	35.08	c
Proteus 110 OD	100 g thiacloprid + 10 g deltamethrin	700 ml	56.45	bc	52.64	b	48.35	b	45.46	d
Duet 530 EC	50 g cypermethrin + 480g chlorpyrifosethyl	500 ml	62.99	c	57.73	b	53.28	b	44.84	d
Nurele D Hl. 550 EC	50 g cypermethrin + 500g chlorpyrifosethyl	400 ml	40.01	a	31.79	a	28.75	a	23.71	b
Fury 10 EC	100 g zeta-cypermethrin	100 ml	48.96	ab	50.61	b	19.38	a	9.80	a
LSD $_{5\%}$			9.150		8.722		10.387		8.088	

[a]Means in a column followed by the same letter are not significantly different (α=0.05, LSD)

A similar trend was observed in 2012, despite of a lower efficacy of insecticides and less expressed difference between them. Relatively high protective effect showed Duet

from 1st to 7th day after treatment (the efficiency ranged from 44.84 to 62.99%). Similar results were observed after the treatment with Mospilan, Calypso and Proteus.

The lowest mortality of aphids was caused by Nurele Hlorsirin D from the first (40.01%) to the seventh (23.71%) day after treatment with significant differences to the other insecticides.

Fury had unsatisfactory effect on the fifth and seventh day after the treatment.

The results followed by different trends regarding the toxicity of insecticides against both pests than individual control (Table III). In 2011 all insecticides showed fast initial effect and significantly higher efficacy on the first and third day after treatment, which ranged from 82.24-86.72% to 76.83-88.69% respectively, compared with standard Nurele Hlorsirin D (respectively 70.99 and 56.45%). Similar results observed on the fifth day as with the highest efficiency was distinguished Mospilan (82.71%), followed by Calypso (78.38%). An exception was observed for Nurele D Hlorsirin and Fury, which differed with the lowest toxicity, which was maintained during the last reporting day. Mospilan and Calypso were the only insecticides that showed a high efficacy on the seventh day after treatment (71.35 and 70.93% respectively) and the differences were statistically significant compared with the other insecticides. Proteus and Duet took an intermediate position.

In 2012 results were more indicative independently of the lower efficacy of insecticides. The highest statistically significant toxicity and provided long term protection simultaneously against the pea weevil and pea aphid showed the neonicotinoid insecticides Mospilan (51.79-68.95%) and Calypso (52.46-70.02%) through the duration of the seven-day reporting period. Statistically the lowest mortality was caused by Nurele D Hlorsirin and the results were unsatisfactory for the combined control of both species. Proteus, Duet and Fury had a short term protection.

Table III: Efficacy of some insecticides against Bruchus pisi and Acyrthosiphon pisi

Insecticides	g a.i. kg^{-1}	Dose g/ml ha^{-1}	First day		Third Day		Fifth day		Seventh day	
			2011							
Mospilan 20 SP	200 g acetamiprid	300 g	86.72	b [a]	88.69	d	82.71	e	71.35	c
Calypso 480 SC	480 g thiacloprid	300 ml	83.58	b	85.61	cd	78.38	de	70.93	c
Proteus 110 OD	100 g thiacloprid +10 g deltamethrin	700 ml	82.24	b	76.83	b	68.72	c	61.33	b

Duet 530 EC	50 g cypermethrin + 480g chlorpyrifosethyl	500 ml	83.53	b	83.96	cd	71.83	cd	62.86	b
Nurele D Hl. 550 EC	50 g cypermethrin + 500g chlorpyrifosethyl	400 ml	70.99	a	56.45	a	49.40	a	44.37	a
Fury 10 EC	100 g zeta-cypermethrin	100 ml	84.67	b	80.39	bc	57.01	b	44.73	a
LSD $_{5\%}$			4.958		5.443		7.178		4.393	
			2012							
Mospilan 20 SP	200 g acetamiprid	300 g	68.95	c	61.66	c	60.26	d	51.79	c
Calypso 480 SC	480 g thiacloprid	300 ml	65.04	c	70.02	d	58.04	d	52.46	c
Proteus 110 OD	100 g thiacloprid + 10 g deltamethrin	700 ml	53.11	b	47.95	b	42.94	c	34.89	b
Duet 530 EC	50 g cypermethrin + 480g chlorpyrifosethyl	500 ml	50.02	b	49.42	b	39.80	bc	37.60	b
Nurele D Hl. 550 EC	50 g cypermethrin + 500g chlorpyrifosethyl	400 ml	41.25	a	31.67	a	28.66	a	25.60	a
Fury 10 EC	100 g zeta-cypermethrin	100 ml	53.41	b	52.90	b	33.81	ab	26.10	a
LSD $_{5\%}$			5.446		7.248		7.025		6.668	

[a] Means in a column followed by the same letter are not significantly different (α= 0.05, LSD)

The division of the tested insecticides into two groups: pyrethroids and neonicotinoids showed that the neonicotinoids had a higher efficacy and provided better protection against combined attack by *B. pisi* and *A. pisi*.

Tested insecticides had a positive impact on the productivity of spring peas. The greatest increase in grain yield compared with the untreated plants was found for Mospilan and Calypso – with 24.22 and 24.14% on average (Table IV). Duet was effective too and it increased the productivity with 16.39%. Statistically significant differences between these variants and the control were found over the years, as well as average yield for the period.

Proteus, Fury and Nurele D Hlorsirin increased grain yields from 8.76 to 11.87%. Depending on the applied insecticide was not observed a statistical difference relative to the control in one of the experimental years, unlike average yield.

Table IV: Grain yield (kg ha^{-1}) depending on the tested insecticides

Insecticides	**2011**		**%**	**2012**		**%**	**Mean**		%
Mospilan 20 SP	2734.46	cd [a]	20.9	2420.12	d	28.2	2577.29	d	24.22
Calypso 480 SC	2801.26	d	23.9	2349.77	cd	24.4	2575.52	d	24.14
Proteus 110 OD	2489.19	b	10.1	2024.01	ab	7.2	2256.60	b	8.76
Duet 530 EC	2712.80	cd	20.0	2116.62	b	12.1	2414.71	c	16.39
Nurele D Hl. 550 EC	2572.25	bc	13.8	2069.60	ab	9.6	2320.92	bc	11.87
Fury 10 EC	2397.69	ab	6.1	2171.43	bc	15.0	2284.56	b	10.11
Control	2260.96	a	-	1888.53	a	-	2074.75	a	-
LSD $_{5\%}$	181.674			196.722			102.903		

[a] Means in a column followed by the same letter are not significantly different (α= 0.05, LSD)

Table V: Analysis of variance for grain yield

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean square	
Total	55	50155.6	-	-	-
Varieties	13	42972.5	85.6	3305.6	*
Factor A Year	1	24922.6	49.7	24922.6	*
Factor B -Insecticide	6	16247.9	32.4	2708.0	*
AxB	6	1802.0	3.6	300.3	-
Pooled error	39	6524.6	-	167.3	

The analysis of variance in terms of grain yield showed that the years (factor A) had the strongest influence on this parameter - 49.7% from the total variation of the variants (Table V). We were conditioned by the unequal reaction of the variants to the change of the environmental conditions. The reason for that was the large differences in the meteorological conditions during the years of this study that affected efficacy of the tested insecticides. The strength of influence for the insecticides (factor B) was also high and statistically significant - 32.4% and it was determined by the mechanism of action and the active substance of pesticide. The interaction between the

conditions of years and the preparations (AxB) was not significant (3.6%).

IV. DISCUSSION

The efficacy of the studied pyrethroid and neonicotinoid insecticides against *Bruchus pisi* and *Acyrthosiphon pisi* was different and it was determined by the mechanism of action, the kind of the toxic substances and the meteorological conditions.

The sum of the rainfall during the vegetation period of spring pea in 2012 was 21.3% higher compared with 2011. In the second and third decade of June, when was treated with insecticides and reported their toxic effect, the amount of rainfall reaching 27.2mm compared with 13.2mm for 2011. In this case substantially the higher rainfall amount in the second year of the study with 106.6% was determinative for exhibited lower biological efficacy of the insecticides than in 2011.

Nurele D Hlorsirin is frequently used insecticide for chemical control of the economically important pea pest B. pisi and A. pisi in Bulgaria. In this study, the insecticide exhibited relatively low efficacy and provided little protection of plants compared with the other tested formulations. Probable this is associated with the emergence of resistance in populations of the both pest, as a result of its frequent use.

The best protection against pea weevil attack was provided by Mospilan (acetamiprid) compared with the other tested insecticides. It was due to the fast initial effect and long-lasting activity after the 7th day of the treatment, which ensured the realization of high productivity too.

We were found that Mospilan, which belongs to the group of neonicotinoids, showed not only toxic activity against the adults, but also exhibited ovicidal effect. It was concluded, that acetamiprid was very selective without having detrimental effects on non-targets and had also demonstrated ovicidal activity against many pest species [13]. Acetamiprid' s products for control of the weevils, which juvenile stages occur inside the seed or fruit recommended by other authors [19], [21].

The high efficacy among neonicotinoids was observed after application of Calypso (thiacloprid). Neonicotinoid insecticides: thiacloprid as Calypso 480 SC, and acetamiprid as Mospilan 20 SP gave good control of other weevil as strawberry root weevil [12]. The efficacy of Calypso and Mospilan was 88.2 and 80.7% respectively between tenth and twentieth day after the treatment and pest reduction was at the level 80-92.8%.

In the present study Fury (zeta-cypermethrin), which belongs to the group of synthetic pyrethroids showed knock-down effect and a high efficiency, comparable to that of Mospilan and Calypso, and it was one of the most suitable insecticides against pea weevil. Zeta-cypermethrin provided successful control of B. pisi [8], [9], and [24].

The protection against other major pest on spring forage pea - *Acyrthosiphon pisi* was most efficient by treatment with Duet (50 g/l cypermethrin +480 g/L chlorpyrifos-ethyl), which belongs to the group of synthetic pyrethroids, that block a sodium channel in nervous cell membranes [15]. Duet exhibited fast initiation effect and long-lasting activity after 7th day on the treatment, followed by Mospilan (acetamiprid), Calypso (thiacloprid) and Proteus (thiacloprid + deltamethrin) – to the group of neonicotinoid insecticides.

A number of authors found that acetamiprid, thiacloprid, cypermethrin + chlorpyrifos showed a high efficacy against different aphid, thrips, cicada and weevil species and contributed significantly to the reduction of pest populations below the economic threshold level. The authors recommend for their inclusion in integrated pest management systems [2], [11], [14], [16], [23], [25], [27]-[29].

The neonicotinoid insecticides works by disrupting the nervous system by acting as an inhibitor at nicotinic acetylcholine receptors [20]. The neonicotinoids, the newest major class of insecticides, such as acetamiprid, thiacloprid, clothianidin, imidacloprid, have outstanding potency and systemic action for crop protection against piercing-sucking pests [26].

Pea weevil and pea aphid are major economically important pests on spring forage pea, which occur usually every year and often reach high population density both. This requires a need for simultaneous control with suitable insecticides, when these pests occur in complex.

From a pest management perspective and realization of high grain yields, the current study showed potential for simultaneous management of pea weevil and pea aphid by applying of Mospilan and Calypso under field conditions. Similar results in terms of efficacy and grain yield were also observed after the use of Duet.

Depending on the number of treatments for pea pest control is necessary rotation of insecticides with different mechanisms of action, which is an effective long-term anti-resistance strategy.

V. CONCLUSIONS

The best protection against *Bruchus pisi* on spring forage pea was provided acetamiprid, followed by zeta-cypermethrin and thiacloprid.

The control of *Acyrthosiphon pisi* was the most efficient by treatment with cypermethrin + chlorpyrifos-ethyl, followed by acetamiprid, thiacloprid and thiacloprid + deltamethrin, which showed the fast initiation effect and long-lasting activity.

Simultaneous chemical control of pea weevil and pea aphid and increased grain yield by 24.22 and 24.14% may be implemented by using acetamiprid and thiacloprid, and rotation of the insecticides according to the necessary numbers of treatments, which is a recognized anti-resistance strategy.

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