Journal on New Biological Reports
JNBR 9(1) 68 – 78 (2020)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

Effect and selectivity of insecticides in forage pea and feed quality

Ivelina Mitkova Nikolova*, Natalia Anastasova Georgieva

Institute of Forage Crops, Pleven, "General Vladimir Vazov" str. 89, Pleven 5800, Bulgaria

Corresponding author: imnikolova@abv.bg

| **Received**: 3 February 2020 | **Accepted**: 15 March 2020 |

How to cite: Nikolova IM, Georgieva NA. 2020. Effect and selectivity of insecticides in forage pea and feed quality. J New Biol Rep 9(1): 68-78.

ABSTRACT

Chemical control remains a very important part of plant protection and still widely used to safeguard profitable yields. In this regard the toxicity of two insecticides (Biscaya and Proteus), used alone and in combination with fertilizers (Biomax and Kristalon) to control of pea aphid, *Acyrthosiphon pisum* and their selectivity on *Coccinella septempunctata* larvae in forage pea was studied. In addition, feed quality was determined. It was found that the application of Biscaya, alone and in combined with Biomax, leed to an overall efficacy of 74.5 and 83.7% respectively against A. pisum on cumulative insect-days based. The mixture had an additive interaction. Proteus treatments were significantly less pronounced - overall efficacy of 66.2 and 66.1%. According to the International Organization of Biological Control categorization, Biscaya (alone and in combination) was moderately harmful in 12.5% of cases, slightly harmful in 75% and harmless in 12.5% of cases on *Coccinella septempunctata* larvae over the years. Proteus treatments were classified as slightly harmful against seven-spot ladybird in 68.8% of cases and harmless in 31.2% of cases. The complex evaluation of the biochemical composition of the forage, with an expressed decrease in the plant cell fiber content, improved mineral composition and a considerable increase of digestibility, determined the treatment with Biomax and the combination of Biscaya and Biomax as the highest qualitatively. The use of Biscaya and Biomax combination is recommended for the practice and revealed the application possibility in IPM programs considering the high efficacy against pea aphid, the pronounced selectivity for ladybird larvae and improved forage quality.

Key words: Insecticides, fertilizers, toxicity, pea aphid, selectivity, forage quality.

INTRODUCTION

Chemical control remains a very important part of plant protection and still widely used to safeguard profitable yields. Pesticides, such as insecticides, fungicides, herbicides, and acaricides are important tools for crop management and play a significant role in agricultural production worldwide (Bueno and Bueno 2012). When used wisely, appropriately, they can provide excellent control with minimal risks of environmental and forage contamination with residual pesticide levels. The thrifty use of pesticides should be promoted within an integrated program in order to ensure a stable yield (Moonga 2015) and to reduce the possibility of developing resistance among insect pest populations.

Withdrawal and prohibition of certain pesticides from the country's licensed list necessitate continuous updating of the pesticide list and the addition of new efficient products, with a short persistence, selective for beneficial insects, and environmentally friendly.

An important prerequisite for the high efficiency of the chemical process, especially when using insecticides with a short toxicity period, is the rational choice of the chemical product and the time of its application (Saini 2014; Reitz and Funderburk 2015). A modern set of chemical products for plant protection, with a wide range of action, makes it possible to integrate their applications with the activities of natural enemies in pest management. Another feature of chemical control is that pest control can only be applied if the economic threshold of harmfulness is exceeded (Gianessi and Williams 2011; Papadopoulou 2014). Assessment of the insecticide efficacy is also crucial with regard to pesticide choice and resistance management (Reitz and Funderburk 2015).

Usually, insecticide toxicity is based on the Abbott equation for percent mortality. That is a good indicator of the product effect at a given time, but it is only indirectly measured by percent mortality without reporting the insect number that survived and caused plant damage. A point of extreme importance to crop protection is the duration of insect attack. That focuses attention on both the numbers of each species and the duration of the attack after the insecticidal product use.

The mixed-use of insecticides with growth regulators, stimulants and foliar fertilizers, as an element of the modern technologies in conventional production, increases the effectiveness of the preparations, allows reducing their doses and has a high economic effect saving time, energy and costs (Petroff 2008; Georgieva et al. 2014). Using the mixtures of pesticides is one of the effective ways to postpone the development of insecticide resistance or to struggle current resistance in a pest species especially when they are aggressive. The combination of insecticides with other products with various modes of action could either be synergistic, additive or antagonistic against an insect species. If the mixtures would be synergistic, the costs of excessive use of insecticides might effectively be reduced (Wolfenbarger and Cantu 1975).

Along with pest control, a key issue in forage production was its quality, which was determined mainly by the chemical composition. Evaluating the forage quality in conventional or integrated production will help to establish feeds with increased nutritional value and digestibility. Studies evaluating the nutritional value and quality of fodder crops treated with pesticides have been insufficient (Nikolova et al. 2015; Sulc et al. 2015). It is essential therefore to continue and expand research in that direction.

The aim of this study was to determine the toxicity effect on pea aphid in forage pea and selectivity of insecticides, used alone and in combination with foliar fertilizers, and to assess feed quality after treatments.

MATERIALS AND METHODS

The trial was performed in the experimental field of the Institute of Forage crops, Pleven, Bulgaria during 2018-2019. The effect of two insecticides (Biscaya and Proteus 110 OD), used alone and in combination with fertilizers (Biomax and Kristalon) to control of pea aphid, *Acyrthosiphon pisum* Harris (Hemiptera, Sternorrhyncha: Aphididae) and their selectivity on *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae) larvae in forage pea was studied. In addition, feed quality was determined. The experiments were laid out in a Randomized Complete Block (RCB) Design with three replications. The size of each plot was 3 x 6.50 m^2. Treatments were carried out at the beginning of the flowering stage. Trial variants and product characteristics are shown in Table 1. On the basis of the sweeping with the entomological net average number of aphids and seven-spot ladybirds were calculated on the first, third, seventh and ninth days after treatment.

Calculation of insect-days

The use of insect-days and cumulative insect-days as the index for the insecticide efficacy provides the ability to identify both the intensity and duration of the insect pest infestation.

The analyses of the individual insect-days served as indices of the efficacy of the insecticides during the period between samples whereas the cumulative insect-days served as indices of the overall efficacy of the treatments.

The insect-days was calculated following the methodology of Ruppel (1983) by the equation:

Insect-days = $(X_{i+1} + 1 - X_i) [(Y_i + Y_{i+1}) / 2]$, where

X_i and X_{i+1} are adjacent points of time,

Y_i and Y_{i+1} are the corresponding points of insect number.

The cumulative insect-days were calculated by sequentially summing the individual insect-days.

Calculation of product interactions

The expected mortality (ME) for the combination of the insecticide with fertilizer was accounted through formula according Tak and Isman (2015).

ME=MB+MA (1-MB),

MB signifying observed mortality caused by fertilizer; MA signifying observed mortality caused by insecticides. Results from a chi-square test, $\chi 2=(MAB- ME)^2/ME$, where MAB is the observed mortality for the combination of the insecticide with fertilizer, were compared to the chi-square table value and χ 2 with d.f. = 1 and $\alpha = 0.05$ is 3.84.

The interactions can be considered as synergistic when the χ 2 values > 3.84 of the mixture and having greater mortality than the expected, and as antagonistic with smaller observed mortality than the expected, or as an additive when χ 2 values < 3.84.

Table 1. Product characteristics

Products	**Characteristics**	**Dose of application**	**Producer**
Biscaya	Oil dispersion formulation containing 240 g/L (22.97% w/w) thiacloprid	600 ml ha^{-1}	Bayer CropScience
Biomax	Amino acids of enzymatic hydrolysis, Free amino acids: 9,0 % w/w. Total Nitrogen (N): 9,0 % w/w	3000 ml ha^{-1}	Agri Nova, Spain
Proteus 110 OD	oil dispersion containing Deltamethrirn 10 g a.i./l + Thiacloprid100 g a.i	600 ml ha^{-1}	Bayer Crop Science
Kristalon	Nitrogen /N/-18%, Ammonium nitrogen-3.3%, Nitrate Nitrogen-4.9%, Amide nitrogen-9.8%, Phosphorus /P_2O_5/-18%, Potassium /K_2O/-18%, Magnesium /MgO/-3%, Sulfur /SO_3/-5%, microelements /Boron, Iron, Manganese, Molybdenum, Zinc, Copper/	2000 g ha^{-1}	Nu 3 BV, Netherlands
Biscaya+ Biomax	–	600 ml ha^{-1} + 3000 ml ha^{-1}	
Proteus+Kristalon	–	600 ha^{-1}+2000 g ha^{-1}	
Control	Treatment with water		

Calculation of insecticide selectivity

In order to classify a chemical as selective or harmful to larvae of seven-spot ladybird *Coccinella septempunctata* were used protocols of the International Organisation for Biological Control (IOBC), where the toxic effect of pesticides on the beneficial organisms was calculated in four Classes: 1 = harmless (E<30%), 2 = slightly harmful (30<E <79%), 3 = moderately harmful (80<E< 99%), 4 = harmful (E>99%) (Hassan et al. 1985).

Evaluation of forage quality after treatments

The chemical composition is determined by standard methods of the Weende system (AOAC 2001) and includes crude protein (CP) by Kjeldahl method (crude protein is calculated on the formulae CP = total N x 6.25), crude fibers (CF), phosphorus – calorimetrically by hydroquinone, calcium – complexometrically (Sandev 1989). The enzyme degradability/digestibility in vitro of dry matter (IVDMD) was determined as percent by a two-stage pepsin-cellulase method of Aufrere, 1982 (Todorov et al. 2010).

The statistical processing of the data was done using an Anova for a one-factor case, the mean being compared by a Tukey test in 5% significance ($P \leq 0.05$). Statgraphics Plus software was used.

RESULTS AND DISCUSSION

Pea aphid, *Acyrthosiphon pisum*, is a main pest present in spring forage pea. Aphid population growth was largely dependent on weather conditions. The warm and wet weather resulted in outbreaks of aphids (Grigorov 1980).

The population dynamic of the pea aphid during the vegetation period had a characteristic course, but the environmental factors (temperature, rainfall) largely affected the species density. Due to the cool and wet weather in 2019 (lower temperatures and more rainfall of 4.7^0C and 96.7 mm compared to 2018, as well as 0.3^0C lower temperatures and more rainfall of 61.9 mm, compared to 10 year period (2007-2017), plant growth slowed down and aphids appeared late, at the end of the month – Figure 1. Rainfall of 116.8 mm during the spring month had a strong negative impact on the emergence and development of *A. pisum*, and often they were washed off the plant surface. April of 2018 was characterized by an optimal combination of a higher average daily temperature and an equal distribution of the amount of rainfall. These conditions determined earlier occurrence and a higher number of the aphids. In May of 2019, when plants were in the sensitive stage of flowering, cool and rainy weather (lower temperatures and more rainfall of 2.6^0C and 35.1 mm compared to 2018) suppressed aphid population growth and generation development and as a result, the density occupied lower values.

The results of Figure 2 showed the aphid number in the control for a one-month period in the area formed under the curve created by the interaction between insect-days (blocks) and cumulative insect-days (line). The heights of the insect-days are given as the mean number of insects between adjacent samples ($Y_i + Y_{i+1}$) / 2. The curve of a cumulative area on time formed the corresponding sigmoid curve. It is evident that the pea aphid density increased proportionally with plant development after the formation of the first flowers (17 May) in 2018. It reached a maximum value at the full pod formation (15 June). The population density of *A. pisum* during that period of 2018 was on average 75.6 individuals / 2m^2.

Due to weather conditions in 2019, the aphid number in the one-month period averaged 34.7 individuals / 2m2, or 54.1% lower density than the previous year. The presented sigmoid curve covered a smaller area and the cumulative insect days had considerably lower values (Fig. 3).

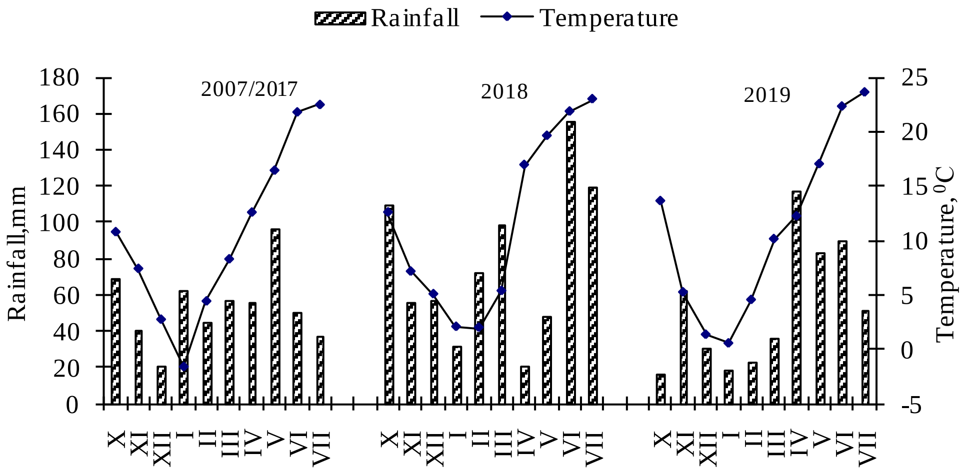

Fig.1. Meteorological characteristics for the Pleven region

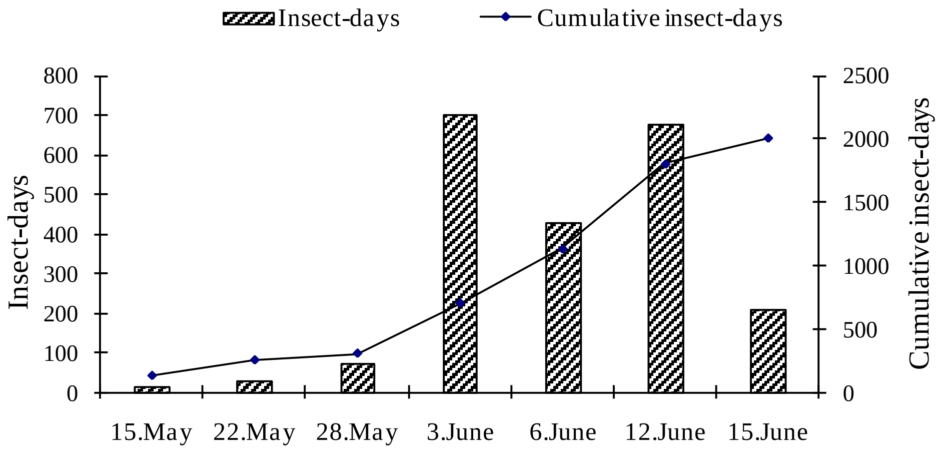

Fig. 2. Insect-days and cumulative insect-days of *Acyrthosiphon pisum* in the control, 2018 (aphid number per 2 m^2)

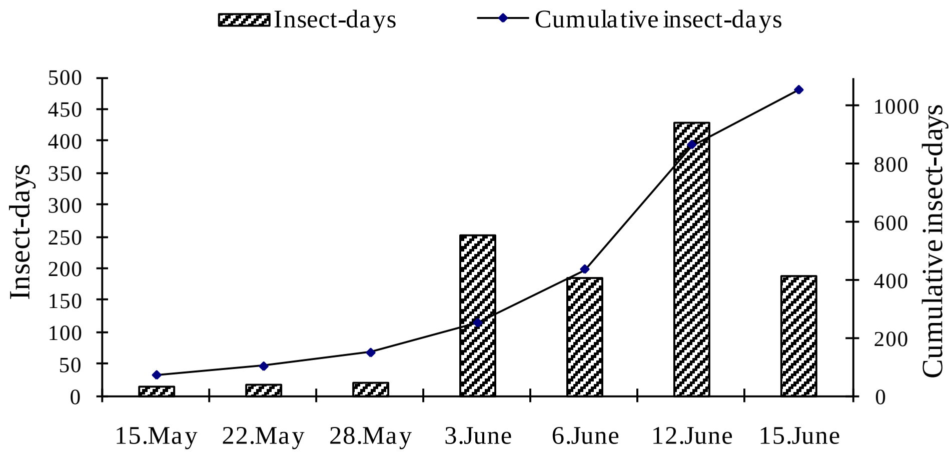

Fig. 3. Insect-days and cumulative insect-days of *Acyrthosiphon pisum* in the control, 2019 (aphid number per 2 m^2)

Meteorological conditions affected insecticidal action too and lower temperatures and more rainfall in 2019 resulted in lower product toxicity and a lower reduction in the aphid number.
Cumulative insect days decreased on the first day after treatment in 2018 and differences to the control were statistically significant (F6.5 = 38.430; *p* <0.001) (Table 2). The combined use of Biomax and Biscaya had the highest reduction rate of 95.0% followed by Biscaya (89.0%) with significant differences. The aphid decrease in Proteus treatments were below 70%. There were insignificant differences between alone and combination treatment with Proteus, as well as between Kristalon and Biomax applied alone. The outlined trend in the next reporting day was maintained, showing a significant decrease in A. pisum numbers in all treatments compared to control ($F_{6,5}$=52.137; *p*<0.0142).

Cumulative insect-days of the seventh and ninth day after product applications demonstrated significantly decreased relative to control (F6,5=46.281; p<0.0104; F6,5=58.713; p<0.001), while values in Kristalon treatments significant exceeded the control by 3.4 and 9.4% respectively. In the last two reporting days, the differences between Proteus applications were negligible, while the combination of Biscaya stood out significantly with the highest reduction in insect days.
The decrease in the aphid number in ascending order as follows: Kristalon, Biomax, Proteus, Proteus + Kristalon, Biscaya, and Biscaya + Biomax.

Table 2. Cumulative insect days (2m^2) in spring forage pea after treatment with products with different biological effect.

Products/Dates	**3 June**	**6 June**	**12 June**	**15 June**	**Average**
			2018		
Control	699.6±32.3 e* –	1128.6 ± 75.8f –	1803.6 ± 62.0 e –	2010.6 ± 138.5e –	1410.6 ± 84.7 –
Biscaya	110.0 ± 22.1 b -89.0	161.0 ± 18.5 b -85.7	281.0 ± 29.4 b -84.4	325.3 ± 35.2 b -83.8	219.3 ± 28.6 -85.7
Biomax	600.6 ± 42.5 d -14.2	963.6 ± 62.7 d -18.9	1587.6± 85.9 d -20.0	1838.1 ± 91.3d -15.7	1247.5 ± 123.1 -23.6
Proteus	234.2 ± 22.7 c -65.5	280.3 ± 24.3 c -75.2	422.4 ± 31.4 c -76.6	478.4 ± 29.1 c -76.2	353.8 ± 34.7 -73.6
Kristalon	611.1 ± 44.3 d -12.7	1018.7 ± 52.7 e -9.7	1864.2 ± 61.6 f +3.4	2200.5±107.7 f +9.4	1423.6 ± 79.8 -2.4
Biscaya+ Biomax	50.0 ± 13.5 a -95.0	51.5 ± 17.0 a -95.4	63.5 ± 11.4 a -96.5	77.0 ± 16.6 a -96.2	60.5 ± 20.8 -95.7
Proteus+ Kristalon	225.1 ± 24.1 c -67.8	263.6 ± 19.5 c -76.6	403.4 ± 38.5 c -77.6	463.5± 33.3 c -76.9	338.9± 29.9 -74.8
			2019		
Products	**10 June**	**13 June**	**19 June**	**21 June**	**Average**
Control	251.1 ± 20.6 d –	435.6 ± 28.5 d –	864.6 ± 43.1 d –	1053.6 ± 39.7f –	651.2 ± 31.3 –
Biscaya	95.1 ± 8.1 a -62.1	147.6 ± 18.5 b -66.1	321.6 ± 39.4 b -62.8	402.6 ± 35.2 b -61.8	241.7 ± 28.6 -62.9
Biomax	230.1 ± 18.4 c -8.4	396.6 ± 33.2 c -9.0	792.6 ± 62.0 c -8.3	966.6 ± 44.5 e -8.2	596.5 ± 23.7 c -8.4
Proteus	112.6 ± 17.2 b -55.2	164.7 ± 19.8 b -62.2	350.9± 29.6 b -59.4	443.0 ± 36.5 c -58.0	267.8 ± 20.7 -58.9
Kristalon	223.7 ± 20.4 c -10.9	390.8 ± 35.6 c -10.3	811.3 ± 40.5 c -6.2	1003.4 ± 47.7 e -4.8	607.3 ± 38.9 -8.0
Biscaya+ Biomax	86.1 ± 17.1 a -65.7	116.1 ± 27.0 a -73.3	224.1 ± 27.3 a -74.1	276.6 ± 20.6 a -73.7	175.7 ± 22.5 -73.0
Proteus+ Kristalon	106.2 ± 11.5 b -57.7	161.1 ± 23.6 b -63.0	379.5± 34.2 b -56.1	493.1± 39.8 d -53.2	285.0± 19.7 -56.2

*Means in each column followed by the same letters are not significantly different (*P* < 0.05)

The averaged cumulative insect days used as the indices of the overall efficacy of the product treatment over a nine-day period indicated that the highest reducing effect was achieved after the combined treatment of Biscaya and Biomax (95.7%) and Biscaya (85.7%). Biomax use did not lead to a satisfactory reduction of cumulative insect days (23.6%), but in combined treatment with the insecticide product, it contributed to better adhesion of the mixture on the leaf surface and the more rapid absorption by the plant organism.

In 2019, the trend of toxic effects of products and their impact on the reduction of cumulative insect-days remained, but it was less pronounced. On the first day after treatment, no significant differences were observed in Biscaya and Proteus applied alone and in combination ($F_{6,5}$ = 18.751; p<0.0237), but Biscaya treatments resulted in a significantly higher reduction than the Proteus treatments. On the third reporting day, the combined use of Biscaya and Biomax significantly reduced the cumulative insect days to the greatest degree, while the differences between the Proteus treatments and Biscaya were negligible ($F_{6,5}$ = 25,652; p<0.005). No significant differences were found after Biomax and Kristalon use.

The trend one week after treatment was maintained ($F_{6,5}$ = 33,533; p<0.001). In the last reporting day, with the lowest number of cumulative insect-days was distinguished the combination Biscaya and Biomax followed by the insecticide application with 73.7% and 61.8% reduction and significant differences ($F_{6.5}$ = 39.734; p<0.0141). A higher reduction in cumulative insect-days was observed after the use of Proteus compared to its combination with Kristalon and the difference between them was significant.

The average values for 2019 confirmed the results of the previous year, as the most pronounced toxic effect on pea aphid was found in the combination of Biscaya and Biomax and Biscaya used alone with a reduction of cumulative insect-days of 73.0 and 62.9% respectively.

Similar results for the high efficacy of thiacloprid against different pests reported by a number of authors. Hashem (2010), Kolařík and Rotrekl (2013), Hou and He (2017), Jagginavar et al. (2018) found a high bioefficacy of thiacloprid to thrips, weevil and moth control, and in addition, Jagginavar et al. (2018) reported a phytotoxicity absence of thiacloprid on *Coccinella* spp.

The application of Biscaya, alone and in combined with Biomax, leed to an overall efficacy of 74.5 and 83.7% respectively against *A. pisum* over a nine-day period based on cumulative insect-days on average for 2018-2019 while Proteus treatments were significantly less pronounced - overall efficacy of 66.2 and 66.1%.

Cumulative insect days reflected the overall plant protection effect provided by the use of different products during a certain period of time. The pattern of percent reductions in cumulative insect-days from those of the control was almost horizontal over time (Fig. 4), compared to change from the declines noted by using the Abbott equation (Fig. 5).

The insect-day system provides a single measure of the intensity of the insect attack and it focuses attention on the insects that survive treatment to damage the crop. The use of insect-days is, therefore use as an index of the efficacy of insecticides in crop protection.

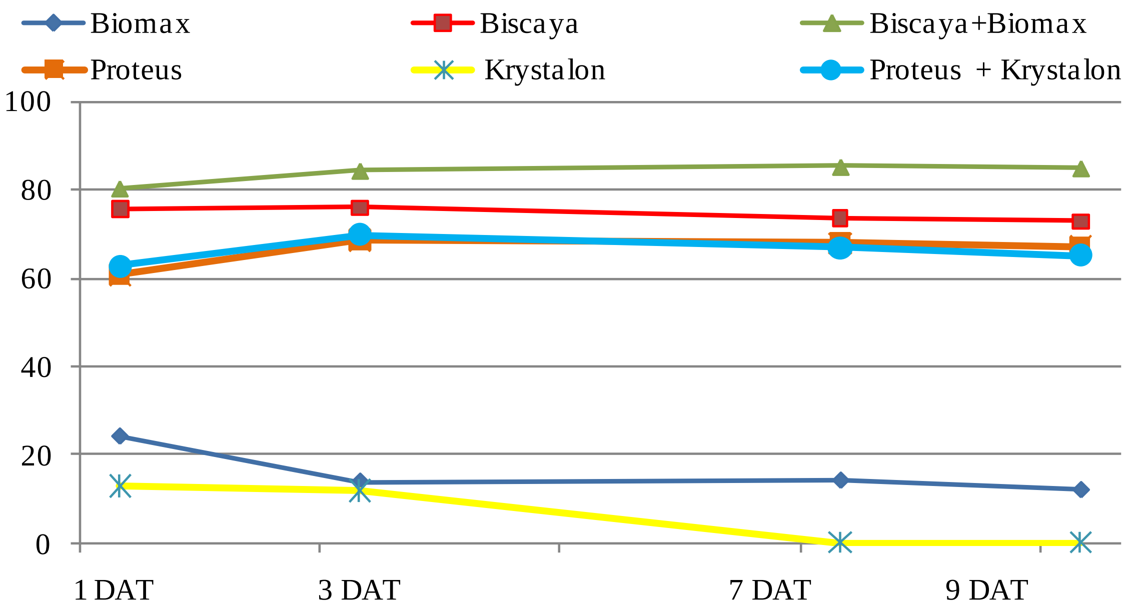

Fig. 4. Reduction of cumulative insect-days in *Acyrthosiphon pisum* after treatment with products with different biological effect, 2018-2019

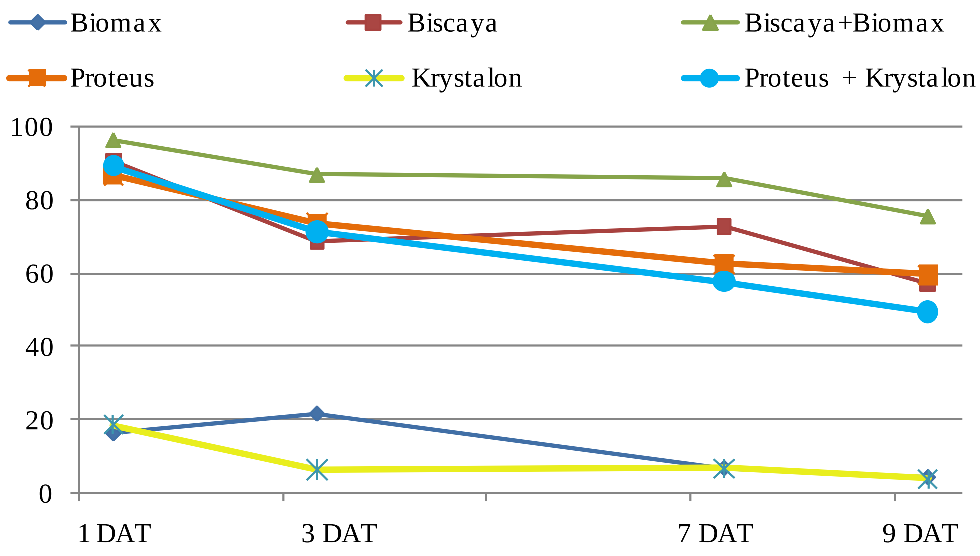

Fig. 5. Toxicity (%) of products with different biological effect against *Acyrthosiphon pisum* according to the Abbott formula, 2018-2019

The insect-day system is used in other author studies to find efficient products for pest control considering the insect attack intensity (Shafie et al. 2009; Moonga 2015). Modeling can be used to characterize the different components like effects of environmental and biotic factors on the phenology of insect pests (Milosavljević et al. 2018), to determine economic injury levels and economic thresholds for these insects (Santana et al. 2018).

Biomax is a fertilizer that improves the crop even in stressful situations (plagues, frosts, drought, etc.). Moreover, it forms a permeable film that protects the cutaneous surface of the plant, acting as a support to insecticides, especially in combine treatment. As a result, the productive performance of the crops and insect protection increases.

The enhanced insecticidal activity of Biscaya when the cuticular barrier was bypassed, combined with its increased penetration when admixed with Biomax can explain the additive interaction between the two compounds. This is clear when comparing the toxicities of mixtures and individual applications ((Table 3). These comparisons lead us to conclude that the positive effect between thiacloprid and amino acids largely results from the increased penetration of the compounds, especially that of thiacloprid in this particular combination. The interaction of Deltamethrirn + Thiacloprid and Krystalon can be considered as neutral.

Table 3. Toxicity of product combination on pea aphids, the average for 2018-2019

Combination	Observed mortality, % (MAB)	Expected mortality of combination, % (ME)	χ2	P	Interactions
Biscaya + Biomax	83.7	75.1	0.99	0.05	additive
Proteus + Krystalon	66.1	66.4	0.00	0.05	−

Legend: **χ2−** interactions of the compounds. The interactions can be considered as synergistic when the χ2 values > 3.84 of the mixture and as an additive when χ2 values < 3.84

Toxicological studies of conventional and broad-spectrum insecticides under field conditions have revealed that the use of such materials typically leads to reduced population densities and activity (Bueno et al. 2017). The decrease in the number of natural enemies caused by the use of non-selective insecticides may have serious consequences for pest population dynamics, such as resurgence and outbreaks of secondary pests (Fernandes et al. 2010). It is, therefore, necessary to assess the toxicity of the used products to the beneficial species.

In order to classify a chemical as selective or harmful to beneficial arthropods, it is of great importance to use a well-established methodology (Hassan et al. 2000; Bueno and Bueno 2012). In this context, the International Organization of Biological Control (IOBC) study pesticide selectivity to beneficial organisms.

Coccinella septempunctata L. were present in high numbers in the stands, which allowed for the monitoring of their reaction to products under field conditions.

The results of this study in 2018 indicated that Biscaya treatments were moderately harmful with respect to larvae of the predatory ladybird on the first reported day (80 <E <99%) (Figure 6). In the next reporting days, the effects on the predator population proportionally reduced, not exceeding 79%, but exceeding 30% and considered to be slightly harmful.

The synthetic insecticide Proteus showed a similar impact as Biscaya with a quick knockdown effect but was distinguished by a lower toxic effect on predatory larvae. Compared with the Biscaya, the toxicity of Proteus treatments from first to ninth day defined as slightly harmful.

The use of synthetic fertilizers alone was harmless and had no effect on *C. septempunctata* density.

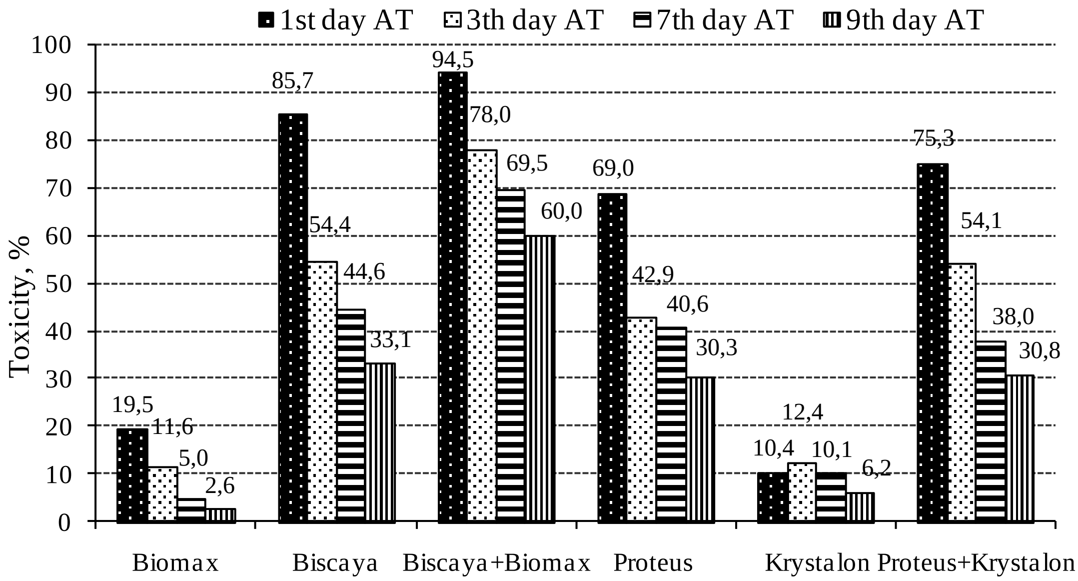

Fig.6. Toxicity of synthetic products on larvae of *Coccinella septempunctata* Linnaeus, 2018

A similar trend was observed in 2019 as the effect of synthetic products was less pronounced (Fig. 7). It was found some differences between the use of insecticides alone and in combination. While the toxic effect of the combination of Biscaya with Biomax was slightly harmful throughout the reporting period, the application of insecticide alone was slightly harmful on the first and third days after treatment and harmless on the fifth and ninth days. Proteus used alone was defined as harmless after the first reporting day (E <30%) and its combination with Krystalon - after the third reporting day.

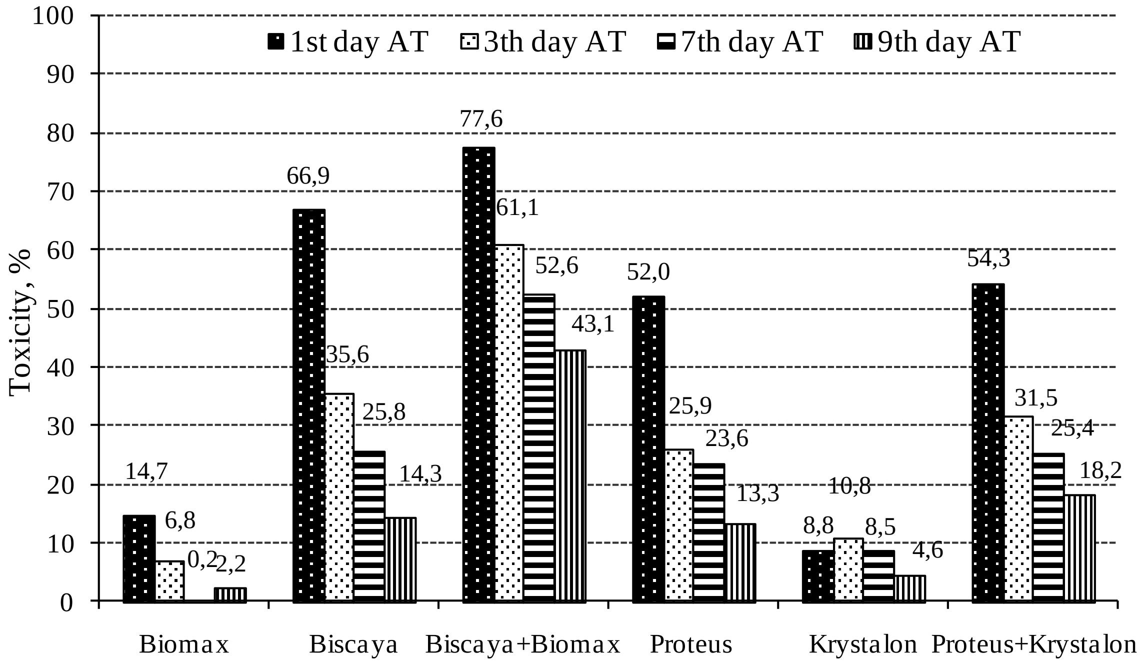

Fig.7. Toxicity of synthetic products on larvae of *Coccinella septempunctata* Linnaeus, 2019.

Abdel-Latif and Abdu-Allah (2012) recommend that neonicotinoids should avoid direct contact with ladybirds because of more contact toxicities than ingestion or these insecticides metabolized to non-toxic compounds in mouth and in the insect gut. That can explain the moderately harmful effect of insecticide treatments on the first day and slightly harmful or harmless effect in the next reporting days.

According to IOBC categorization, Biscaya (alone and in combination) was moderately harmful in 12.5% of cases, slightly harmful in 75% and harmless in 12.5% of cases on *C. septempunctata* larvae over the years. Because of its rapid initiation action and higher toxicity against pea aphid, the product caused higher predatory mortality in the first reporting days. Proteus treatments were classified as slightly harmful against seven-spot ladybird in 68.8% of cases and harmless in 31.2% of cases.

Although, many studies have been performed to assess the toxicity of pesticides on ladybirds in a laboratory and a majority of the insecticides tested, mainly pyrethroids, neonicotinoids, and organophosphorus compounds were highly toxic (Hautier et al. 2006; Jansen et al. 2008; Katsarou et al. 2009) some of them had slightly or no toxic effect in the semi-field or field investigation. For example, Jansen (2012) reported that in conditions closer to the field the thiacloprid toxicity on the larvae of *Adalia bipunctata* did not lead to significant mortalities for an average 21 days of development. In addition, no effect on adult development and fecundity was observed.

Similar to our results reported Nag et al. (2018) which concluded that among four conventional insecticides (Dimethoate 30EC, Imidacloprid 17.8 SL, Thiamethoxam 25 WG, and Thiacloprid 21.7SC) Imidacloprid followed by Thiacloprid showed least detrimental effect on *C. septumpunctata* adults and had high efficacy against potato aphid and whitefly. In addition, Sánchez-Baio et al. (2013) found that foliar applications of neonicotinoids appear to have lesser impacts on non-target communities than pyrethroids.

The presented results clarify some of the side effects of synthetic products on predatory larvae of *C. septempunctata* and in most the cases they were defined as slightly harmful or harmless. That reveals the possibility of their application as selective insecticides in integrated pest management (IPM) programs.

Forage quality is a pooled indicator defined by different, interrelated indicators including the main chemical composition of forage – a content of crude protein and crude fibers, microelements, enzyme in vitro degradability/digestibility of dry matter and others. Forage quality was affected positively at a different level after product treatments. The crude protein content significantly increased from 27.2 to 39.3% ($F_{6,2}$=3.125; p=0.058) and the fiber content significantly reduced in the range 21.0-33.0% $F_{6,2}$=2.313; p=0.082) (Table 4).
At the individual application of Biscaya and Proteus was formed forage with an increased protein content of 39.3 and 31.0% respectively while at the combine used, the increase was 28.8 and 29.9%. Biomax was distinguished with a significantly higher value (34.4% increase) comparing Krystalon (31.0%).

It is known that existed a negative correlation between crude protein content and those of crude fiber content (Pavlov 1996), which was confirmed by the investigation (r= −0.809).
The crude fiber content decreased in the highest degree at Biomax and Biscaya+Biomax (by 33.0 and 32.2 respectively), and the lowest – at Biscaya with a 21.0% decrease.

The result of the product applications was improved mineral composition, and the calcium concentration increased significantly in the range of 28.2 – 35.1% ($F_{6,2}$=0.018; p<0.001), and of phosphorus - 23.6 – 36.9% ($F_{6,2}$=0.013; p<0.001). A higher values as regards calcium were established after the application of Biscaya, Proteus and Biscaya+Biomax (35.1, 33.9 and 33.2% respectively), and in reference to phosphorus - after Biomax and Krystalon use (36.9 and 34.9% increase).

Increased digestibility is an indicator of high-quality forage. The digestibility of forage dry matter was in limits 70.36 – 76.47% and was an indicator influenced well by the effects of the applied products. The highest digestibility was found at Biomax treatment (76.47%), followed by combined application – Biscaya+Biomax (74.72%) with a significant difference between them ($F_{6,2}$=1.014; p=0.098). The increase was 8.7 and 6.2% respectively.

The complex evaluation of the biochemical composition of the forage, with an expressed decrease in the plant cell fiber content, improved mineral composition and a considerable increase of digestibility, determined the treatment with Biomax and the combination of Biscaya and Biomax as the highest qualitatively.

Table 4. Chemical composition (g/kg DM) of *Pisum sativum* forages after treatment with producs with different biological effect, average 2018-2019.

Products	**Crude protein**	**Crude fiber**	**Ca**	**P**	**IVDMD****
Control	110.7 a*	279.6 f	0.687 a	0.195 a	70.36 a
Biomax	148.8 d	187.3 a	0.881 c	0.267 e	76.47 e

Biscaya	154.2 e	221.0 e	0.928 d	0.255 cde	72.23 b
Biscaya+Biomax	142.6 bc	189.6 ab	0.915 d	0.252 bcd	74.72 d
Proteus	145.0 c	194.7 c	0.920 d	0.241 b	72.50 bc
Krystalon	140.8 b	190.3 b	0.833 b	0.263 de	72.36 bc
Proteus+Krystalon	143.8 bc	203.5 d	0.886 c	0.248 bc	73.31 c

Legend: *[*]Means in each column followed by the same letters are not significantly different (P < 0.05); **In vitro dry matter digestibility; %

CONCLUSION

The application of Biscaya, alone and in combined with Biomax, leed to an overall efficacy of 74.5 and 83.7% respectively against *Acyrthosiphon pisum* over a nine-day period based on cumulative insect-days. Proteus treatments were significantly less pronounced - overall efficacy of 66.2 and 66.1%. It was found an additive interaction between Biscaya and Biomax.

According to the International Organization of Biological Control (IOBC) categorization, Biscaya (alone and in combination) was moderately harmful in 12.5% of cases, slightly harmful in 75% and harmless in 12.5% of cases on *Coccinella septempunctata* larvae over the years. Proteus treatments were classified as slightly harmful against seven-spot ladybird in 68.8% of cases and harmless in 31.2% of cases. That reveals the possibility of product applications as selective insecticides in integrated pest management programs.

The complex evaluation of the biochemical composition of the forage, with an expressed decrease in the plant cell fiber content, improved mineral composition and a considerable increase of digestibility, determined the treatment with Biomax and the combination of Biscaya and Biomax as the highest qualitatively.

ACKNOWLEDGEMENTS

This work would not have been possible without the kind support of the management of the Institute of Forage Crops, Pleven. We are especially indebted to the technical staff to the who have been supportive during the field experiments.

REFERENCES

Abdel-Latif G, Abdu-Allah M. 2012. Toxicity of three new selective insecticides on a nine-spot ladybird beetle, *Coccinella novemnotata* (Herbst) in Sebha, Libya. Journal of Sebha University-(Pure and Applied Sciences) 10 (1): 40-48.

AOAC. 2001. Official methods of analysis, 18-th ed. Association of Analytical Chemists, Gaithersburg, Maryland, 2001, USA.

Bueno AF, Carvalho GA, Santos AC, Sosa-Gómez DR, Silva DM. 2017. Pesticide selectivity to natural enemies: challenges and constraints for research and field recommendation. Ciência Rural, Santa Maria, 47 (6): e 20160829.

Bueno AF, Bueno RCOF. 2012. Integrated pest management as a tool to mitigate the pesticide negative impact into the agroecosystem: the soybean example. In: JOKANOVIC, M. The impact of pesticides. Cheyenne: Academy Publish, 165: 190.

Fernandes FL. Bcci L, Fernandes MS. 2010. Impact and selectivity of insecticides to predators and parasitoids. EntomoBrasilis 3: 1-10.

Georgieva N, Nikolova I, Pavlov D, Zhelyazkova Ts, Naydenova Y. 2014. Energy assessment of forage pea production under influence of organic and synthetic products. Banat's Journal of Biotechnology, V (9): 15-22.

Gianessi L, Williams A. 2011. Insecticides Protect in Regions Where Parasites are Ineffective. CropLife Foundation, Pesticide Benefits Case Study 24, pp. 18-24.

Grigorov S. 1980. Aphids and their control. Zemizdat, Sofia, Bulgaria.

Hashem K. 2010. Insecticide efficacy Thiacloprid (Biskaya OD240) on codling moth control in the North Khorasan province. Khorasan Shomali Agricultural and Natural Resources.

Hassan SA, Bigler F, Blaisinger P, Bogenschütz H, Brun J, Chiverton P, Dickler E, Easterbrook MA, Edwards PJ, Englert WD, Firth SI, Huang P, Inglesfield C, Klingauf F, Kühner C, Ledieu MS, Naton E, Oomen PA, Overmeer WPJ, Plevoets P, Reboulet JN, Rieckmann W, Samsose-Petersen L, Shires SW, Stäubli A, Stevenson J, Tuset JJ, Vanwetswinkel G, Van Zon AQ. 1985. Standard methods to test the side-effects of pesticides on natural enemies of insects and mites developed by the IOBC/WPRS working group ‘Pesticides and Beneficial Organisms’. EPPO Bulletin 15: 214–255.

Hassan SA, Halsall N, Gray AP, Kuehner C, Moll M, Bakker FM, Roembke J, Yousef A, Nasr F, Abdelgader H. 2000. A laboratory method to evaluate the side effects of plant protection products on Trichogramma cacoeciae Marchal (Hym., Trichogrammatidae). In: CANDOLFI, M.P. et al. (Eds.). Guidelines to evaluate side-effects of plant protection products to non-target arthropods. Reinheim: IOBC/WPRS 107-119.

Hautier L, Jansen J.-P, Mabon N, Schiffers B. 2006. Building a selectivity list of plant protection products on beneficial arthropods in open field: a clear example with potato crop. IOBC-WPRS Bull. 29(10): 21-32.

Hou J, He FP. 2017. Analyzing damage and control measures of Eucryptorrhynchus chinensis and E. brandti. Chin Agric Technol 37: 67-74.

Jagginavar SB, Krishna Naik L, Karabantanal SS. 2018. Bioefficacy and phytotoxicity of Alanto 240 SC (Thiacloprid 240 SC) against thrips and natural enemies in Pomegranate. Int J Curr Microbiol App Sci 7(8): 1598-1602.

Jansen JP. 2012. Toxicity of two neonicotinoid insecticides via the food chain for larvae of the two spot ladybird Adalia bipunctata. Pesticides and Beneficial Organisms IOBC-WPRS Bulletin 82: 19-26.

Jansen JP, Hautier L, Mabon N, Schiffers B. 2008. Pesticide selectivity list to beneficial arthropods in four field vegetable crops. IOBC-WPRS Bull. 35: 66-77.

Katsarou I, Martinou A, Papachristos DP, Zoaki D. 2009. Toxic effects of insecticide residues on three aphidophagous coccinellid species. HPPJ 2: 101-106.

Kolařík P, Rotrekl J. 2013. Regulation of the abundance of clover seed weevils, Apion spp. (Coleoptera: Curculionidae) in a seed stand of red clover (Trifolium pratense L.). J Entomol Acarolog Res 45(3): e19. https://doi.org/10.4081/jear.2013.e19

Milosavljević I, Amrich R, Strode V, Hoddle MS. 2018. Modeling the phenology of asian citrus psyllid (Hemiptera: Liviidae) in Urban Southern California: Effects of environment, habitat, and natural enemies. Environ Entomol 47(2): 233-243.

Moonga MN. 2015. Integrated multiple-tactic management of the redbanded stink bug on soybeans in Louisiana. PhD tesses, University of Zambia, Louisiana.

Nag D, Gupta R, Bhargav P, Kumar Y, Bisen MS. 2018. Relative efficacy of insecticides against potato aphid (Myzus persicae Sulzer), white fly (Bemisia tabaci Genn.) and lady bird beetle. Int J Chem 6(1): 1182-1186.

Nikolova I, Georgieva N, Naydenova Y. 2015. Forage quality in Pisum sativum, treated by biological and synthetic active compounds. VI annual scientific conference with international participation “Innovations in agricultural science for efficient farming” September 24-25, 2015. Plant science LII (5): 94-98.

Papadopoulou S. 2014. Determination of insecticide application time in forage crops against Sitona humeralis Stephens based on its biology and ethology. Biotechnol Biotechn Equip 27 (2): 3665-3668.

Pavlov D. 1996. Productivity, nutritional value, quality characteristics in different groups of forage crops and possibilities for their prediction, Ph.D. thesis, Sofia (Bg).

Petroff R 2008. Pesticide interactions and compatibility. Montana State University.

Reitz SR, Funderburk J. 2015. Management strategies for western flower thrips and the role of insecticides. In: Perveen F (Ed.) Insecticides – Pest Engineering: 355-384.

Ruppel RF. 1983. Cumulative Insect-Days as an Index of Crop Protection. J Econ Entomol 76: 375-377.

Saini RK. 2014. Compendium of lectures delivered during the Advanced Training Course on “Novel Approaches in Pest and Pesticide Management in Agro-Ecosystem”, CCSHAU Press, Hisar.

Sánchez-Bayo F, Tennekes HA, Goka K. 2013. Insecticides - Development of Safer and More Effective Technologies. eBook (PDF) ISBN: 978-953-51-5348-1DOI: 10.5772/52831

Sandev S. 1979. Chemical methods for analysis of forages. Zemizdat, Sofia. (Bg)

Santana MV, Macedo RS. dos Santos TTM, Barrigossi JAF. 2018. Economic injury levels and economic thresholds for Tibraca limbativentris (Hemiptera: Pentatomidae) on paddy rice based on insect-days. J Econ Entomol 111(5): 2242-2249.

Shafie EL, Mudathir HAF, Khartoum M, Basedow TH. 2009. The possibilities of using different Neem preparations for pest control in vegetables in the Sudan. Proceedings of the 14TH workshop; Wetzlar, Germany, November 15TH - 16TH 2004: Biological Control of Plant, Medical and Veterinary Pests. R. Strang & H. Kleeberg (eds.): 61-72. Published by: Trifolio-m GMBH, 35633 Lahnau, DR.-Hans-Wilhelmi-We.

Sulc RM, McCormick JS, Hammond RB, Miller DJ. 2015. Forage yield and nutritive value responses to insecticide and host resistance in alfalfa. Crop Sci 55: 1346-1355.

Tak JH, Isman MB. 2015. Enhanced cuticular penetration as the mechanism for synergy of insecticidal constituents of rosemary essential oil in Trichoplusia ni. Cientific Reports 5: 12690. DOI: 10.1038/srep12690

Todorov N, Aleksiev A, Ilchev A, Ganchev G, Mikhailova G, Girginov D, Penkov D, Shindarskaya Z, Naydenova Y, Nedelkov K, Chobanov S. 2010. A workshop on animal feeding. East-West Publishing House, Sofia.

Wolfenbarger DA, Cantu E. 1975. Enhanced toxicity of carbaryl when combined with synergists against larvae of the bollworm, Heliothis zea and the tobacco budworm, *Heliothis virescens*. Flor Entomol 58:103-104.