Impact of natural products on *Acyrthosiphon pisum* density on *Pisum sativum* L. and forage quality

Ivelina Nikolova

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

Corresponding author: imnikolova@abv.bg

Received: 31 March 2021

Accepted: 27 April 2021

SUMMARY

A field trial was conducted at the Institute of Forage Crops (Pleven, Bulgaria) from 2015 to 2017. It studied the effects of natural products on *Acyrthosiphon pisum* density, as well as changes in the chemical composition, content of plant fibre components and enzyme degradability in forage pea. Treatments with the natural insecticides Madex and Agricolle, applied alone or in combination with the organic fertilizers Lithovit and Nagro were performed twice - at the beginning of the flowering stage and one week later. The fertilizers used in the trial are environmentally safe and approved for use in organic production. The synthetic products Kristalon, a foliar fertilizer, and Proteus 110 OD, an insecticide, were used for comparison. The application of natural products, either alone and in combination, resulted in a reduction in pea aphid density. Applying Agricolle with Nagro, followed by Lithovit with Agricolle, led to the highest aphid number decrease (70.0 and 51.1%, respectively). An optimal combination of decrease in the content of plant cell wall fibre components, cellulose and lignification degree with a significant increase in forage *in vitro* enzyme digestibility was established after applying Agricolle with Lithovit and Agricolle with Nagro. Digestibility reached 71.8 and 69.8%, respectively, an increase of 8.2-5.2%, while ADF, cellulose and lignification degree decreased from 7.1 and 7.7%, 8.0 and 23.4%, and 10.5 and 6.8% after applying Agricolle with Lithovit and Agricolle with Nagro, respectively. In comparison, the synthetic products Kristalon and Kristalon with Proteus increased forage quality, but to a relatively lesser extent. A stronger linear relationship was found between aphid density and dry matter digestibility, compared to the content of neutral detergent fibres. Pea forage with low content of plant cell wall fibre components, cellulose and lignification degree, high protein content, and digestibility after treatment with the natural product Agricolle, and its combinations with Lithovit and Nagro, make it a very good complement to other forages in dairy cow rations.

Keywords: pea aphid, natural products, pest control, forage pea, chemical composition, forage quality
INTRODUCTION

Legumes usually result in higher intake and animal production than grass silages of comparable digestibility. This is true both for silage (Dewhurst et al., 2003) and pasture herbaceous plants (Fraser et al., 2004). An additional benefit of legumes is that the rate of decrease in digestibility is lower than in grasses as maturity is progressing (Dewhurst et al., 2009).

Peas are very tasty and digested completely, which leads to greater intake and profit. In any case, pea provides equal or improved animal productivity. The advantage of using it for feeding is that it can be purchased for less than the comparative value of other forages based on nutrient content. Virtually all producers who have used pea in animal diets appreciate the positive results and nutrition, safety and palatability of this legume (Anderson et al., 2006). An increase in pea growing area under organic production would help to increase the pea supply on the feed market.

Organic production is known as a specific way to safeguard the production environment, in particular biodiversity, and provide healthy and quality nutrition (Grigorova & Arabska, 2013). Pea plants provide a valuable source of nitrogen in organic farming (Gerdgikova et al., 2012). Organic cultivation of *Pisum sativum* and the use of organic products in Bulgaria is at an early stage and needs further research. It is necessary not only to evaluate the productive potential of this crop in organic agriculture but analyse additionally its chemical composition, nutritive value, energy yield and feed units.

Pea aphids have become a serious pest of feed and grain peas in recent years in Bulgaria. Research has focused mainly on understanding the potential for aphid damage and how to manage them in relation to pea productivity. Feed infestation with these species has been extremely severe, especially in organic farming, but there are no available data on actual damage to yield and feed quality from these infestations.

The aim of the current study was to determine the effect of natural products on *Acyrthosiphon pisum* density, as well as changes in the chemical composition, content of plant fibre components and enzyme degradability of forage spring pea.

| Variants | Application rates, per ha | Active ingredients | Producer |
|----------|----------------------------|-------------------|----------|
| 1. Control | treated with distilled water |          |          |
| 2. Lithovit natural leaf nano-fertilizer | 2000 g ha<sup>-1</sup> | contains calcium carbonate from natural reserves with micronutrients: 79.19 % CaCO<sub>3</sub>; 4.62 % MgCO<sub>3</sub>; 1.31 % Fe | Ctheo Vita Ltd., Germany |
| 3. Nagro bio-organic nano-fertilizer | 500 ml ha<sup>-1</sup> | contains micro- and macro-elements (molybdenum, magnesium, cobalt, manganese, zinc, iron, copper, boron, nitrogen and phosphorus), meso elements, microhumates, vitamins, fulvo acid, amino acids, phytohormones, organic solvents, silicon compounds, organic calcium, antioxidants, adaptogens, metabolites, nitrogen fixators | Scientific Production Association “Bioplant”, Russian Federation |
| 4. Madex | 100 ml ha<sup>-1</sup> | Cydia pomonella Granulovirus - CpGV-V1S 3 x 10<sup>13</sup> granules/liter | Switzerland |
| 5. Agricolle | 300 ml / 100 l water | natural polysaccharides for sticking small insects | Cal-Agri products LLC, USA |
| 6. Lithovit+ Madex | 2000 g ha<sup>-1</sup> + 100 ml ha<sup>-1</sup> |          |          |
| 7. Lithovit+ Agricolle | 2000 ml ha<sup>-1</sup> + 300 100 l water |          |          |
| 8. Nagro+ Madex | 500 ml ha<sup>-1</sup> + 100 ml ha<sup>-1</sup> |          |          |
| 9. Nagro+Agricolle | 500 ml ha<sup>-1</sup> + 300 100 l water |          |          |
| 10. Kristalon | 2000 g ha<sup>-1</sup> | nitrogen 17.0%, nitrate nitrogen 8.0%, ammonium nitrogen 9.0%, phosphorus (P<sub>2</sub>O<sub>5</sub>) 6.0%, potassium 18.0%, magnesium 2.0% | Nu 3 BV, Netherlands |
| 11. Proteus 110 OD | 600 ml ha<sup>-1</sup> | Thiacloprid 100 g/l; Deltamethrin 10 g/l | Bayer CropScience |
| 12. Kristalon+Proteus | 2000 g ha<sup>-1</sup> + 600 ml ha<sup>-1</sup> |          |          |
MATERIAL AND METHOD

A study was conducted from 2015 to 2017 to examine the effects of natural products, applied either alone or in combination, on pea aphid control (Acyrthosiphon pisum, Hemiptera, Sternorrhyncha Aphididae) in spring forage pea (Pisum sativum L.), variety Pleven 4, in the experimental field of the Institute of Forage Crops, Pleven, Bulgaria. Synthetic products, a foliar fertilizer and an insecticide, were used for comparison. Trial variants and product characteristics are shown in Table 1. The bio-organic universal nano fertilizer Nagro is an environmentally safe fertilizer, allowed for use in organic production according to producers (Chekmarev et al., 2018). Lithovit is a suitable fertilizer for use in organic farming under Council Regulation (EEC) No. 2092/91 – European Community. Treatments were performed twice: at the beginning of the flowering stage and one week later. Aphid density was calculated by counting them per individual plant every two to three days over a period beginning one day before treatment until the harvest of green fodder mass.

Products with different levels of efficacy against pea aphids were used either alone or in combination, as well as the control, to create different degrees of infection with aphids. The experiments were set out using the long plot method with three replications of each variant, and the plot size was 6.50 m². Each product used and the untreated check were applied to the randomized replicated plots.

The chemical composition of aboveground biomass harvested at the flowering stage was determined by standard methods and included: crude protein (CP) by Keldahl (N x 6.25) and crude fibre (CF) by Weende system (AOAC, 2007). The content of plant cell wall fiber components was analysed as neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid-detergent lignin (ADL), according to Goering and Van Soest (1970), and degree of lignification coefficient=ADL/NDFx100. In vitro enzyme digestibility of dry (IVDMD) and organic (IVOMD) matter was determined using a two-stage pepsin-cellulase method proposed by Aufrere (1982, cited by Todorov et al., 2010).

The data were summarized and presented as average for 2015-2017. The data were exposed to one-way ANOVA, the mean being compared by Tukey’s test at 5% probability (p ≤ 0.05). Simple correlation and regression analyses were used to establish the relationship between pea density and some forage quality components (NDF, ADF and IVDMD).

RESULTS AND DISCUSSION

Forage pea plots were checked weekly during May for initial infestation of A. pisum. At the beginning of May, pea aphids were found in the test plots, and one week later treatments were performed.

Impact of natural products on Acyrthosiphon pisum density

Aphid density in treated plots and untreated check were similar during the three-year study period, and the differences were statistically insignificant when plants were treated with the foliar fertilizer Lithovit (F11,59=13.593; p=0.021) (Table 2). In contrast, aphid numbers after treatment with the natural fertilizer Nagro decreased by significant 52.2% and approached the density noted after using the bioinsecticide Agricolle. Nagro is a highly concentrated complex liquid bio-organic fertilizer that has the properties of a bioinsecticide with antiferromonic action. In addition to increasing resistance to unfavourable climatic factors (drought, low temperatures) and increasing leaf area, and subsequently the intensity of plant photosynthesis (according to the manufacturer), Nagro also had a repellent effect against aphids, which resulted in a reduction in their numbers.

| Products                        | 2015-2017 | Decrease % |
|---------------------------------|-----------|------------|
| 1. Control                      | 70.8 d    | –          |
| 2. Lithovit                     | 60.3 cd   | –14.8      |
| 3. Nagro                        | 33.8 ab   | –52.2      |
| 4. Madex                        | 57.2 c    | –19.2      |
| 5. Agricolle                    | 31.8 b    | –55.0      |
| 6. Lithovit + Madex             | 55.4 c    | –21.8      |
| 7. Lithovit + Agricolle         | 34.6 ab   | –51.1      |
| 8. Nagro + Madex                | 37.6 b    | –46.8      |
| 9. Nagro + Agricolle            | 21.3       | –70.0      |
| 10. Kristalon                   | 72.2 d    | +1.9       |
| 11. Proteus 110 OD              | 28.6 ab   | –59.6      |
| 12. Proteus + Kristalon         | 23.6 a    | –66.6      |

*Means in each column marked by the same letter are not significantly different (p<0.05)
The biological insecticides Madex and Agricolle decreased aphid numbers to different degrees, based on their modes of action. Madex applied alone reduced their numbers by 19.2%, and the difference was significant compared to the untreated check. A significantly lower density and considerable reduction of 55.0% was found after Agricolle treatment. The product has a natural impact, which is based on the properties of some natural polysaccharides for sticking small insects (such as aphids, whiteflies, fleas, etc.) on plants, soil or other surfaces.

The interaction of bioinsecticides with the natural fertilizers Lithovit and Nagro had different effects on *A. pisum* density. The use of Lithovit with Madex decreased aphid density by 21.8%, while the difference between Madex and Lithovit+Madex combination was insignificant. The application of Lithovit with Agricolle was associated with a considerably stronger reduction - an average of 51.1% due to the bioinsecticide action. Compared to Agricolle used alone, the difference was insignificant.

Applying Agricolle with Nagro led to the best interaction of active substances, compared to the other combinations, and the largest reduction in pea aphid density. The reduction in numbers by an average of 70.0% was the highest decrease among all other products and combinations, and a statistically significant decrease was observed compared to all treatments with Madex. The most successful combination was Nagro with Agricolle, followed by Lithovit with Agricolle.

The combination of the synthetic product Proteus 110 OD with Kristalon provided a marked reduction in the population density of *A. pisum* by an average of 66.6%, and differences in treatment with Agricolle and Nagro, and the combined use of Agricolle were insignificant.

Product applications revealed statistical differences between treatments and the untreated check. Those differences in aphid density created different degrees of damage over time until forage plots were harvested, and affected the forage quality components.

**Impact of natural products on forage quality**

Forage quality reflects the ability of forage to meet nutritional needs of consuming animals. Plants differ regarding quantitative contents of different nutrients, depending on aphid damage. Nutrients vary in the amounts of fats, proteins, carbohydrates, fibre and other micro-nutrients that are present in plant tissues.

Dry matter contains the essential nutrients in a feed. Nutrient contents of feeds are determined on the basis of ash (moisture included) or dry matter (no moisture) contents. Nutrient content will always be higher in DM, compared to ash in each feed. The determination of DM can be used to assess whether the moisture content in a feed is within expected limits. Moisture content should not exceed 15% of dry forage, as this amount of moisture is necessary for promoting mould growth.

In the present study, moisture did not exceed the defined limit, and dry matter after treatment with natural products ranged from 93.24 - 94.6% (Table 3).

| V | DM | Ash | CP | CF | NDF | ADF | HEMI | CELLU | LIGNIF | IVMD | IVOMD |
|---|----|-----|----|----|-----|-----|------|-------|--------|------|-------|
| 1 | 93.81 abc | 6.08 ab | 171.2 a | 242.3 c | 395.1 c | 329.1 c | 66.0 bcd | 263.1 c | 13.3 abc | 66.33 cd | 66.75 cd |
| 2 | 94.31 bc | 7.11 c | 184.0 d | 240.9 e | 431.2 ef | 363.6 e | 67.6 cde | 296.0 e | 15.6 cd | 58.97 def | 59.73 def |
| 3 | 94.04 abc | 6.78 bc | 190.4 e | 223.5 c | 376.7 ab | 319.7 b | 57.0 abc | 262.7 b | 13.5 ab | 66.91 cde | 67.28 de |
| 4 | 94.60 c | 6.82 bc | 190.4 e | 223.5 c | 376.7 ab | 319.7 b | 57.0 abc | 262.7 b | 13.5 ab | 66.91 cde | 67.28 de |
| 5 | 93.41 bc | 7.07 c | 197.4 f | 241.4 e | 408.2 d | 340.4 d | 68.8 cde | 271.6 c | 15.2 cd | 61.46 b | 61.96 b |
| 6 | 93.39 bc | 7.07 c | 197.4 f | 241.4 e | 408.2 d | 340.4 d | 68.8 cde | 271.6 c | 15.2 cd | 61.46 b | 61.96 b |
| 7 | 93.71 ab | 6.58 bc | 170.5 a | 223.8 c | 369.3 a | 305.7 a | 63.6 bc | 242.1 a | 11.9 ab | 71.79 g | 72.28 h |
| 8 | 93.90 ab | 6.12 ab | 178.5 c | 262.7 f | 409.2 d | 340.4 d | 68.8 cde | 271.6 c | 15.2 cd | 61.46 b | 61.96 b |
| 9 | 93.24 ab | 6.11 ab | 176.8 c | 224.8 c | 405.6 d | 303.6 a | 102.0 f | 201.6 a | 12.4 ab | 69.76 f | 69.86 g |
| 10 | 92.94 a | 6.10 ab | 173.8 b | 218.8 b | 424.6 e | 305.5 a | 121.1 j | 182.4 a | 11.1 a | 68.42 ef | 69.32 fg |
| 11 | 93.46 bc | 5.69 a | 170.4 a | 237.5 d | 389.8 c | 317.5 b | 72.3 cde | 245.2 a | 15.1 bcd | 65.59 c | 65.70 c |
| 12 | 93.67 c | 6.39 abc | 177.4 c | 237.7 d | 369.2 a | 325.9 c | 43.3 a | 282.6 b | 15.4 cd | 68.55 ef | 68.74 efg |

V – Variant; DM - dry matter, %; CP - Crude protein, g kg⁻¹; CF - Crude fibre; NDF - Neutral-detergent fibre; ADF - Acid-detergent fibre; HEMI - Hemicellulose; CELLU - Cellulose; LIGNIF - Degree of lignification; IVDMD - In vitro dry matter digestibility, %; IVOMD - In vitro total matter digestability, %; 1 - Control; 2 - Lithovit; 3 - Nagro; 4 - Madex; 5 - Agricolle; 6 - Lithovit+Madex; 7 - Lithovit+Agricolle; 8 - Nagro+Madex; 9- Nagro+ Agricolle; 10- Kristalon; 11- Proteus; 12- Kristalon+ Proteus; *Means in each column marked by the same letter are not significantly different (p<0.05)
Although different hypotheses have been proposed about the use of crude protein as a feed quality measure, it continues to be a frequently used parameter. The crude protein content is very different in feeds, but higher protein concentration is usually associated with higher quality in various feeds. As feed plants mature, their crude protein is diluted by increasing fibre content.

The results demonstrate that the applied natural products provided significantly higher contents of crude protein (F\textsubscript{11,2}=2.506; p=0.001) (from 1.5 to 15.3%) and lower crude fibre contents (F\textsubscript{11,2}=2.446; p=0.017) (0.6 – 12.4%), compared to the untreated control. Exceptions were observed for Agricolle and Lithovit + Agricolle, where crude protein and fibre had lower contents, and insignificant differences compared to the control. The optimal combination for aphid control, associated with the most pronounced increase in crude protein and decrease in crude fibre was found after the use of Nagro, Madex and Nagro + Agricolle.

The application of synthetic products impacted slightly the values of crude protein and crude fibre, compared to the natural products. Only Proteus led to a greater decrease in crude fibre (by 9.7%).

Sulc et al. (2015) reported comparable results. According to them, crude protein content in untreated alfalfa was lower (p < 0.05) than it was in plants subjected to an early insecticide treatment against Empoasca fabae Harris. According to other studies, the use of biologically active substances had positive effects on protein content and increased crude protein productivity (Štakhova et al., 2000; Zhelyazkova, 2007).

The detergent feed analysis system is used to characterize fibre or total cell wall content of forage or feed. That portion of forage is termed neutral detergent fibre (NDF), which contains primary components of plant cell walls, namely hemicellulose, cellulose, and lignin. Another parameter of fibre is acid detergent fibre (ADF), a subset of NDF. Acid detergent fibre contains poorly digestible cell wall components, namely cellulose, lignin, and other very resistant substances. NDF and ADF are good indicators of feed quality, and their lower values in feed suggest higher-quality feed and in vitro dry matter digestibility. According to Fahey and Hussein (1999), structural polysides in forage plants account for 300 to 800 g kg\textsuperscript{-1} (30-80 %) of forage dry matter and they are the main source of nutritional energy for ruminants, although less than 50% of them are digestible and utilized.

In the present study, differences in pea aphid density had a different impact on forage quality and the content of structural fibre fraction (polysides) in plant cell walls. The impact of the products was diverse, depending on their origin.

Treatments with Lithovit, Madex and its combinations with natural fertilizers increased the NDF and ADF components due to weak protective effects against \textit{A. pism} and pronouncedly stronger aphid infection compared to other treatments (Table 2). A similar trend was observed regarding cellulose (Table 3).

The use of Nagro, as well as the natural insecticide Agricolle, alone or in combination with natural fertilizers, had entirely positive effects on feed quality, associated with statistically significant reductions in NDF (F\textsubscript{11,2}=8.193; p=0.001), ADF (F\textsubscript{11,2}=5.994; p=0.034) and cellulose (F\textsubscript{11,2}=6.918; p=0.018) values. That was due to the protective effect of Agricolle and Nagro, which led to a strong reduction in \textit{A. pism} density. The most favourable effect, expressed as a significant reduction in fibre content, was found after treatment with Lithovit and Agricolle (decrease by 6.5, 7.1 and 8.0% for NDF, ADF and cellulose, respectively), Agricolle (by 3.6, 7.5 and 13.3% for NDF, ADF and cellulose) and Nagro (by 4.7, 2.9 and 0.2% for NDF, ADF and cellulose). Compared to them, the synthetic products reduced fibre components and cellulose to a lesser extent, and NDV and cellulose values for Kristalon and its combination with Proteus significantly exceeded the control value, respectively.

The ADF values were found to be highly correlated (p < 0.05) with CP (r=0.700), and ADF with cellulose (r=0.845). Similar results were reported by Jančík et al. (2008).

Total digestible animal polyside hemicellulose in pea dry mass was several times lower - from 43.3 to 121.1 g kg\textsuperscript{-1}, compared to that of non-digestible cellulose - from 182.4 to 296.0 g kg\textsuperscript{-1}, a trend confirmed in this study as well. There was an increasing trend in hemicellulose contents, while a significant decrease was noted for Kristalon + Proteus 110 OD only, and insignificant for Nagro, and the combinations Lithovit + Madex and Lithovit + Agricolle (F\textsubscript{11,2}=10.060; p=0.033).

Lignification extent was not impacted by the activity of products, and not found to be significantly different in comparison to the control, except for Madex (F\textsubscript{11,2}=3.274; p=0.001).

Decreasing the content of fibre components, determined as NDF, ADF and cellulose, and increasing \textit{in vitro} digestibility of dry matter, are considered as important effects in forage quality evaluation.

The variation range of \textit{in vitro} dry matter digestibility (IVDMD) was from 66.33 to 71.79%. The treatment process for lower aphid density did not lead to increased \textit{in vitro} digestibility in all variants. The digestibility of forage pea investigated was higher after application of the bioinsecticide Agricolle and its combinations, as well as Nagro.
Forage quality in terms of IVDMD was the highest when the combination Lithovit with Agricolle was used (an increase of 8.2%), followed by Agricolle with Nagro (an increase of 5.2%), and the difference in comparison to control data was significant ($F_{11,2}=3.581; p=0.011$). Increase in IVDMD values in other treatments mentioned above was insignificant and varied from 0.9 to 2.4%.

According to Vilela et al. (2020), higher crude protein content and digestibility resulted in their experiment in a higher predicted intake and high-quality feed because of lower contents of cellulose, hemicellulose and lignin. That trend was confirmed in the present study.

Identical trends were noted regarding in vitro total matter digestibility, where the combined use of Lithovit with Agricolle, and Agricolle with Nagro led to significant increase of 8.3 and 4.7%, respectively ($F_{11,2}=3.677; p=0.027$). Compared to organic products, treatments with Kristalon, and Kristalon + Proteus increased the IVDMD and IVOMD values, but to a lesser extent.

Figure 1 shows the relationship between pea aphid density and some forage quality components. *Acyrthosiphon pisum* infestation had a negative effect on forage quality associated with an increase in the content of neutral detergent fibres and decrease in dry matter digestibility. A stronger linear relationship ($R^2 = 0.59$) was found between aphid density and dry matter digestibility. Linear regression indicated there is a 0.18% loss in IVDMD for each number increase in density. The relationship between pea aphid density and other feed quality components was weak.

Therefore, the presented data indicate that the level of aphid infestation caused during the flowering development stages impacted the quality of forage pea at harvest. The use of the bioinsecticide Agricolle, alone or in combination with biofertilizers, protected plants and improved forage quality.

Various authors have reported that crude protein, lignin, ADF, and IVDMD were affected by insecticide treatments, as well as by damage from sucking insect pests in untreated control. All parameters were negatively affected by insect damage in the untreated variant. For example, Singh et al. (2007) assessed qualitative losses in sorghum following *Pyrrhula perpusilla* Walker infestation and revealed a significant decrease in in vitro dry matter digestibility of the whole plant. The increasing fibre components (NDF and ADF) were acting as an additional factor in reducing dry matter digestibility.

Banyal et al. (2015) reported data on cowpea quality parameters which clearly revealed that the incidence of insects-pests was directly correlated with forage quality. In the present study, there was a significant increase in dry matter, crude protein, ash and in vitro dry matter digestibility in the protected crop, as compared to unprotected crop. The NDF, ADF, hemicellulose and phenolics were lower in the protected crop than in unprotected crop.

In a later study, Bynum and Bell (2019) confirmed a positive impact of insecticides on forage quality by controlling aphid density - a trend also found in the present study after treatment with natural insecticide products. The authors reported that sugarcane aphid damage impacted negatively sorghum silage quality. The studied components in terms of forage quality, such as ADF, lignin, IVTDMD, milk per ton and relative feed quality, had statistically significant levels of difference in the untreated variants when compared to insecticide treatments. According to these authors, heavy aphid pressure had a negative effect, resulting in increase in ADF and lignin values, and decrease in IVTDMD, milk per ton and relative feed quality. Additionally, they found that ADF and lignin were positively correlated to sugarcane damage, indicating that plants became lignified under increased aphid pressure, decreasing forage quality and its digestibility.

High-quality forage pea is palatable and often maximizes intake and productivity by dairy cows. Low
fibre, high protein content and digestibility of that forage after treatment with the natural product Agricolle, and its combinations with Lithovit and Nagro, made it a very good complement to grains and other forages in dairy rations.

**CONCLUSIONS**

- The application of natural products, either alone or in combinations, resulted in a reduction in pea aphid density. Applying Agricolle with Nagro, followed by Lithovit with Agricolle, led to the highest aphid number decrease (70.0 and 51.1%, respectively).
- An optimal combination of decrease in the content of plant cell wall fibre components, cellulose and lignification degree with a significant increase in forage enzyme *in vitro* digestibility was established after applying Agricolle with Lithovit, and Agricolle with Nagro. Digestibility reached 71.8 and 69.8%, respectively, increasing 8.2 and 5.2%, while ADF, cellulose and lignification degree decreased 7.1 and 7.7%, 8.0 and 23.4%, and 10.5 and 6.8% after applying Agricolle with Lithovit and Agricolle with Nagro, respectively.
- A stronger linear relationship was found between aphid density and dry matter digestibility compared to the content of neutral detergent fibres.

**ACKNOWLEDGMENT**

This study received a grant from the Agricultural Academy, Bulgaria, under the project POZM-219.

**REFERENCES**

Anderson, V.L., White, L., & Ilse, B. (2006). The feeding value of field peas. *Carrington Research Extension Center Feedlot Research Report*, 29, 42-45.

AOAC (2007). *Official methods of analysis* (18-th ed.) Association of Analytical Chemists, Gaithersburg, MD, USA. http://files.b00kpedia.com/download/download+official+methods+of+of+analysis+of+aoac+international+18th+edition.pdf

Banyal, D., Chaudhary, J., & Katech, R. (2015). Effect of diseases and insect-pest on forage quality of cowpea. In M.M. Roy, D.R. Malaviya, V.K. Yadav, Tejveer Singh, R.P. Sah, D. Vijay, & A. Radhakrishna (Eds.), *The 23rd International Grassland Congress “Sustainable Use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection*, Theme 2: Grassland production and utilization, (pp 1-3). New Delhi, India: Range Management Society of India.

Bynum, E. & Bell, J. (2019). Sugarcane aphid damage to forage sorghum silage yield and quality induced by different infestation levels for the Texas High Plains. Texas Grain Sorghum Board Final 2019 Report. Texas A&M AgriLife Extension, 1-32. Retrieved from https://www.sorghumcheckoff.com/assets/media/2017-2019%20TGSB%20Final%20Report%20SCA%20Forage%20Sorghum%20Damage_distribution.pdf

Chekmarev, P.A., Glinushkin, A.P., & Startsev, V.I. (2018). Production of organic products - competitive position of the Russian Federation APRK. *Achievements of Science and Techniques APK*, 32(3), 5-6.

Dewhurst, R.J., Delaby, L., Moloney, A., Boland, T., & Lewis, E. (2009). Nutritive value of forage legumes used for grazing and silage. *Irish Journal of Agricultural and Food Research*, 48(2), 167-187.

Dewhurst, R.J., Fisher, W.J., Tweed, J.K.S., & Wilkins, R.J. (2003). Comparison of grass and legume silages for milk production. 1. Production responses with different levels of concentrate. *Journal of Dairy Science*, 86, 2598-2611. doi: https://doi.org/10.3168/jds.S0022-0302(03)73855-7

Fahey, G.C., & Hussein, H.S. (1999). Forty years of forage quality research: Accomplishments and impact from an animal nutrition perspective. *Crop Science*, 39, 4-12. doi: https://doi.org/10.2135/cropsci1999.0011183X03900010002x

Fraser, M.D., Speijers, M.H.M., Theobald, V.J., Fychan, R., & Jones, R. (2004). Production performance and meat quality of grazing lambs finished on red clover, lucerne or perennial reygrass swards. *Grass and Forage Science*, 59, 345-356. doi: https://doi.org/10.1111/j.1365-2494.2004.00436.x

Gerdgikova, M., Videva, M., Pavlov, D., & Dobrev, A. (2012). Chemical composition, nutritive value, energy yield and feed units of the winter pea grain grown after different predecessors using conventional and organic production. *Agricultural Science and Technology*, 4(3), 271-276.

Goering, H.K., & Van Soest, P.J. (1970). Forage fiber analyses (apparatus, reagents, procedures, and some applications). *Agriculture Handbook No. 379*. Washington, WA: US Department of Agriculture.

Grigorova, Z., & Arabska, E. (2013). Opportunities of organic farming for biodiversity preservation and sustainable development. *New Knowledge Journal of Science*, 2(1), 136-145.

Jančík, F., Homolka, P., Čermák, B., & Lád, F. (2008). Determination of indigestible neutral detergent fibre contents of grasses and its prediction from chemical composition. *Czech Journal of Animal Science*, 53(3), 128-135.
Singh, S., Luthra, Y. & Joshi, U. (2007). Biochemical differences in some forage sorghum varieties in relation to Pyrilla perpusilla Walker infestation. *Acta Phytopathologica et Entomologica Hungarica*, 42(1), 17-23. doi: https://doi.org/10.1556/aphyt.42.2007.1.3

Stakhova, L.N., Stakhov, L.F., & Ladygin, V.G. (2000). Effect of exogenic folic acid on the yield and amino acid composition of the seeds of *Pisum sativum* L. and *Hordeum vulgare* L. *Applied Biochemistry and Microbiology*, 36(1), 85-89. doi: https://doi.org/10.1007/BF02738142

Steg, A., van Straalen, W.M., Hindle, V.A., Wensink, W.A., Dooper, F.M., & Schils, R.L.M. (1994). Rumen degradation and intestinal digestion of grass and clover at two maturity levels during the season in dairy cows. *Grass and Forage Science*, 49, 378-390. doi: https://doi.org/10.1111/j.1365-2494.1994.tb02014.x

Sulc, R.M., McCormick, J.S., Hammond, R.B., & Miller, D.J. (2015). Forage yield and nutritive value responses to insecticide and host resistance in alfalfa. *Crop Science*, 55, 1346-1355. doi: 10.2135/cropsci2014.09.0658

Todorov, N., Atanassov, A., Ilchev, A., Gantchev, G., Mihailova, G., Gigrinov, D.,... Tchobanova, S. (2010). *Practices in animal nutrition*. Sofia, Bulgaria: East-West Publishing House.

Vilela, A., González-Paleo, L., Ravetta, D., Murrell, E. & Van Tassel, D. (2020). Balancing forage production, seed yield, and pest management in the perennial sunflower *Silphium integrifolium* (Asteraceae). *Agronomy*, 10(1471), 1-14. doi: 10.3390/agronomy10101471

Zhelyazkova, C. (2007). Study the influence of some growth regulators on productivity, the chemical composition and nutritional value of spring peas (*Pisum sativum* L.) and spring vetch (*Vicia sativa* L.). Ph.D. thesis. Faculty of Plant Technology, Agrarian University, Plovdiv, Bulgaria.

---

**Uticaj prirodnih preparata na brojnost *Acyrthosiphon pisum* na *Pisum sativum* L. i kvalitet stočne hrane**

**REZIME**

U Institutu za krmno bilje (Pleven, Bugarska) u periodu 2015-2017 izveden je poljski ogled kako bi se ispitao uticaj prirodnih proizvoda na brojnost *Acyrthosiphon pisum*, kao i promene u hemiskom sastavu, sadržaju komponenti biljnih vlakana i degradaciju enzima u krmnom grašku. Tretmani prirodnim insekticidima Madex i Agricolle primenjeni su samostalno i u kombinaciji sa organskim dubrivima Lithovit i Nagro dva puta, na početku cvetanja i sedam dana kasnije. Primjenjena dubriva su ekološki bezbedna i odobrena za primenu u organskoj proizvodnji. Sintetički folijarno dubrivo Kristalon i insekticid Proteus 110 OD, bili su primenjeni uporedno. Primena prirodnih preparata samostalno ili u kombinaciji dovelo je do redukcije brojnosti zelene graškove vaši. Primena Agricolle sa Nagro, a zatim Lithovit sa Agricolle, dovela je do najvećeg smanjenja brojnosti vaši (70,0 i 51,1%, respektivno). Optimalna kombinacija smanjenja sadržaja biljnih vlakana čelijskog zida, celuloze i stepena lignifikacije sa značajnim povećanjem enzimske svarljivosti *in vitro* dobijena je nakon primene preparata Agricolle sa Lithovit, kao i Agricolle sa Nagro. Svarljivost je dostigla 71,8 i 69,8%, respektivno, što je porast od 8,2-5,2%; dok su ADF, celuloza i stepen lignifikacije opali 7,1 i 7,7%, 8,0 i 23,4%, 10,5 i 6,8%, respektivno. U poređenju sa tim, sintetički proizvod Kristalon, kao i Kristalon + Proteus, povećali su kvalitet stočnog graška, mada u relativno manjem stepenu. Dobijena je veća linearna zavisnost između brojnosti vaši i svarljivosti suve mase nego sadržaja neutralnih vlakana deterdženta. Stočni grašak sa niskim sadržajem komponenti vlakana čelijskog zida, celuloze i stepenom lignifikacije, kao i visokim sadržajem proteina i svarljivošću nakon tretmana prirodnim proizvodom Agricolle, kao i njegovim kombinacijama sa preparatima Lithovit i Nagro, čine ga dobrim dodatkom drugim krmivima za ishranu krava muzara.

**Ključne reči:** zelena graškova vaš, prirodni proizvodi, suzbijanje štetočina, krmni grašak, hemijski sastav, kvalitet stočne hrane