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FORAGE QUALITY IN *Pisum sativum*, TREATED BY BIOLOGICAL AND SYNTHETIC ACTIVE COMPOUNDS

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Abstract

The influence of single and combined biological active compounds of three insecticides NeemAzal, Pyrethrum (biological insecticides) and Nurelle D (synthetic pyretroid), two growth regulators – Polyversum (biological regulator) and Flordimex (synthetic regulator) and organic foliar fertilizer Biofa on the chemical composition and enzyme *in vitro* digestibility of dry matter of spring forage pea (*Pisum sativum* L.) are established (2011 – 2013) at the IFC, Pleven, Bulgaria. The results demonstrate that single and combined application of studied biological and synthetic active compounds have a positive impact on composition and digestibility of spring forage pea. A single treatment in budding stage increase CP, CF and hemicellulose content and decrease plant cell walls fiber components content - NDF, ADF, ADL and hemicallulose. Significant increase of CP is established at Polyversum and Flordimex (by 10.1 and 11.9%), while increase of CF is slightly expressed. Optimal combination of expressed decrease in plant cell walls fiber components content with significant increase of digestibility is established after applying of Biofa and combination Pyrethrum + Biofa. Digestibility reaches 71.0 and 70.4%, respectively with increase from 12.5 and 11.5%. Double application of biological active compounds in budding and flowering stages have significant influence on the composition and digestibility, but the influence is expressed in lower degree.

Key words: Pea *(Pisum sativum* L.)*,* chemical composition, enzyme *in vitro* digestibility, biological active compounds

Forage quality and feeding value are influenced by many factors as environmental conditions, phonological stages of harvesting, insect pests and pesticides treatment (Masoero et al., 2010; Niwińska et al., 2005). Insecticides applying for control of economically important pests and protection of forage crops in field conditions influence forage feeding value (Naydenova et al., 2010; 2011). Forage quality is the best defined by the forage feeding value in animal production of milk, meat and wool after consumption and intake by animals, but time and cost of large animal feeding trials limits their use in routine analyses of forage samples (Norris et al., 1976). These analyses determine feeding value on the bases of chemical composition (crude protein, crude fibers, detergent fiber) and *in vitro* digestibility. The nitrogen concentration in plant tissues is indicator of protein and protein feeding value estimated. The Neutral detergent fiber as the total fiber amount in plant cell walls is indicator of intake by ruminants (Naydenova et al., 2010; 2011). Digestibility of dry matter and cell walls fiber components present information for energy feeding value estimated (Johnstone et al., 1999). Description of the content of nutrients, and their rumen and intestinal digestibility are essential for prediction of their nutritive value for ruminants (Niwińska et al., 2005).

The aim of the study is establishment influence of biological and synthetic compounds, applied single or in combination in different vegetative stages from plant development on the chemical composition, plant cell walls fiber components content and enzyme *in vitro* digestibility of dry matter of spring forage pea.

MATERIAL AND METHODS

The effect of three insecticides – NeemAzal, Pyrethrum (biological insecticides) and Nurelle D (synthetic pyrethroid) applied alone and in combination with growth regulators - Polyversum

(biological growth regulator and fungicide) and Flordimex (synthetic growth regulator) and an organic foliar fertilizer, known as Biofa on forage quality and feeding value at spring forage pea (*Pisum sativum* L.) variety „Pleven 4" is studied. The trial was conducted during the period 2011 – 2013 in the experimental field of the Institute of Forage Crops, Bulgaria by the split plot method with sowing rate of 120 seeds m^{-2} in 4 replications and plot size of 6.5 m^2. The treatment was conducted once at budding and twice at budding and flowering stages. Variants of the trial: 1. control (treated with distilled water); 2. Biofa - 50 ml/da (dose); 3. Polyversum - 10 g/da; 4. Flordimex - 5 ml/da; 5. NeemAzal - 50 ml/da; 6. Pyrethrum - 5 ml/da; 7. Nurelle D - 40 ml/da; 8. NeemAzal - 50 ml/da + Biofa - 50 ml/da; 9. Pyrethrum - 5 ml/da + Biofa - 50 ml/da; 10. NeemAzal - 50 ml/da + Polyversum - 10 g/da; 11. Pyrethrum - 5 ml/da + Polyversum - 10 g/da; 12. Nurelle D - 40 ml/da + Flordimex - 5 ml/da.

The chemical composition of aboveground mass (milky ripeness of seeds in low pods) is determined by standard methods at Weende system (AOAC, 2001) and included crude protein (CP), by Keldahl (N x 6.25) and crude fiber (CF). The content of plant cell walls fiber components is analysed as neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) according to Goering and Van Soest (Goering et al., 1970), and the lignification degree is presented by coefficient as ADL/NDF x 100. Enzymatic *in vitro* digestibility of dry matter (IVDMD) is determined as two-stage pepsin-cellulase method by Aufrere (Todorov et al., 2010).

RESULTS AND DISCUSSION

The results from the chemical analyses of aboveground dry mass of spring forage pea, treated in the budding vegetative stage (Table 1) are connected with increase of CP content from 2 g kg^{-1} (NeemAzal) to 12 g kg^{-1} (Flordimex) in comparison to the control (138 g kg^{-1}). It is obvious that once treatment with growth regulators Polyversum and Flordimex, respectively 15.2 and 15.4 g kg^{-1} (exceeding by 10.1 и 11.9%) cause the highest CP values. It is followed by the combination Polyversum + Pyrethrum – 151 g kg^{-1} (by 9.5%). The CF content usually slightly exceeds that of untreated variant (261 g kg^{-1}) and is in the referent limits 262 (NeemAzal + Polyversum) – 281 g kg^{-1} (NeemAzal). Exception is registered in Polyversum, Nurelle D and its combination with Flordimex, which has values lower than the control. These biological active compounds reduce fiber content with 7, 16 and 12 g kg^{-1}.

Plant cell walls fiber components content of forage pea also is influenced in different degree by the type of biological or synthetic active compounds used. The significance of NDF, ADF, ADL and cellulose for forage quality evaluation is their low content which in the following experiment has values, lower than that of the control. The Neutral detergent fiber comprise total amount of plant cell walls fiber components (lignin, cellulose, hemicellulose) vary from 377 to 435 g kg^{-1}. It is necessary to mark that a more significant decrease which exceeds 10% compared to untreated variant (435 g kg^{-1}) it is established in Biofa, Polyversum and their combinations with Pyrethrum (by 51, 49, 58 и 57 g kg^{-1}).

The Acid detergent fiber and Acid detergent lignin determine forage digestibility and their content is lower than Neutral detergent fiber. The ADF content in spring forage pea is in the referent limits 275 – 353 g kg^{-1} dry matter of biomass and ADL – 45 – 65 g kg^{-1}. These fractions are influenced in lower degree than NDF as the differences concerning the control has low values. Exception represents the biological insecticide Pyrethrum, applied along which has the lowest values concerning the fiber fractions ADF – 275 and ADL – 45 g kg^{-1} of dry matter and decrease their content in highest degree by 22.0 and 27.1%, respectively. Strong influence and similar decrease is established even after treatment with Biofa – with 4.2 (ADL) and 10.0% (ADF) and NeemAzal + Biofa – with 9.1 (ADL) and 11.0% (ADF).

According to Niwińska et al. (2005) averagely about 80% of ADF is cellulose, which require longer retention time in the rumen and is degradable in lower degree than hemicellulose. In the following study the cellulose content in treatment with Pyrethrum is the lowest – 230.2 g kg^{-1} (decrease with 61 g kg^{-1}), which stimulates forage digestibility by ruminants. An objective of interest, present the variants Biofa and NeemAzal + Biofa with a more significant decrease of 33 g kg^{-1}. While the influence of biological active compounds on the cellulose content is similar to those of ADF and ADL, the results regarding to the content of completely digestible by ruminants, hemicellulose are controversial. A tendency of decrease in the values to the control (82 g kg^{-1}), prevails

which is most strongly expressed in the combinations of Pyrethrum with Biofa and Polyversum – with 43 and 48 g kg^{-1}. Independent applying of NeemAzal and Pyrethrum distinguishes with significant increase in hemicellulose content by 35 and 37 g kg^{-1}.

The degree of lignification is influenced by biological active compounds applied has ten-

Table 1. Composition and digestibility of spring forage pea, influenced by Biological active compounds at budding stage

№	Ash	CP	CF	NDF	ADF	ADL	Hemi	Cellu	Lignif	DMD	DMO
Treatment in Budding stage											
1	79.5	137.8	260.9	434.7	352.5	61.6	82.2	290.9	14.2	63.14	62.43
2	81.6	146.0	281.2	384.4	317.4	59.0	67.0	258.4	15.3	71.04	70.55
3	81.4	151.7	253.8	386.1	341.9	61.7	44.2	280.2	16.0	68.24	67.81
4	82.3	154.2	262.1	399.6	339.0	60.8	60.6	278.2	15.2	67.13	66.96
5	77.5	139.7	283.8	454.9	338.1	59.0	116.8	279.1	13.0	68.17	67.86
6	76.3	149.8	265.7	394.0	275.1	44.9	118.9	230.2	11.4	66.93	66.67
7	80.8	145.3	244.5	403.1	320.6	58.9	82.5	261.7	14.6	69.13	68.89
8	81.1	140.4	272.3	414.7	313.8	56.0	100.9	257.8	13.5	67.38	66.99
9	82.9	143.2	263.6	377.4	337.9	59.6	39.5	278.3	15.8	70.43	69.76
10	83.6	147.4	261.9	404.8	332.4	60.2	72.4	272.2	14.9	66.32	66.12
11	78.8	150.9	262.5	377.9	343.9	65.1	34.0	278.8	17.2	67.69	67.27
12	77.9	146.1	249.3	429.7	328.3	59.2	101.4	269.1	13.8	63.88	63.26
Min	76.3	137.8	244.5	377.4	275.1	44.9	34.0	230.2	11.4	63.14	62.43
Max	83.6	154.2	283.8	454.9	352.5	65.1	118.9	290.9	17.2	71.04	70.55
Mean	80.2	146	263.5	405.1	328.4	58.8	76.7	269.6	**14.58**	**67.46**	**67.05**
SD	*2.3*	*5.1*	*11.6*	*24.3*	*20.3*	*4.8*	*29*	*15.9*	*1.53*	*2.31*	*2.35*
CV	**2.9**	**3.5**	**4.4**	**6.0**	**6.2**	**8.3**	**37.8**	**5.9**	**10.5**	**3.4**	**3.5**

Table 2. Composition and digestibility of spring forage pea, influenced by Biological active compounds at budding and flowering stages

№	Ash	CP	CF	NDF	ADF	ADL	Hemi	Cellu	Lignif	DMD	DMO
Treatment in Budding stage and Flowering stage											
1	84.1	132.5	254.0	428.0	351.0	63.7	77.0	287.3	14.9	63.85	64.46
2	72.2	137.4	262.6	401.3	342.8	63.6	58.5	279.2	15.8	70.25	69.84
3	76.3	131.3	266.9	399.2	336.3	60.6	62.9	275.7	15.2	68.89	68.17
4	78.7	132.1	261.6	409.0	339.7	62.2	69.3	277.5	15.2	70.34	70.12
5	75.0	153.5	266.1	425.0	350.2	64.9	74.8	285.3	15.3	68.10	67.59
6	81.7	138.4	256.7	401.0	331.6	61.4	69.4	270.2	15.3	66.51	65.34
7	78.0	154.4	247.4	407.6	345.8	61.4	61.8	284.4	15.1	68.33	67.90
8	74.8	152.9	259.7	417.3	344.7	60.0	72.6	284.7	14.4	69.99	69.74
9	77.1	138.2	263.9	374.7	319.3	59.1	55.4	260.2	15.8	70.65	70.12
10	82.4	144.7	242.1	368.6	317.7	55.5	50.9	262.2	15.0	63.39	62.72
11	85.2	152.7	256.4	402.3	308.2	56.5	94.1	251.7	14.0	64.46	63.59
12	67.8	142.7	267.2	404.2	310.4	56.0	93.8	254.4	13.8	65.43	64.51
Min	67.8	131.3	242.1	368.6	308.2	55.5	50.9	251.7	13.8	63.39	62.72
Max	85.2	154.4	267.2	428	351	64.9	94.1	287.3	15.8	70.65	70.12
Mean	77.8	142.6	258.7	403.2	333.1	60.4	70.0	271.1	**15.0**	**67.52**	**66.92**
SD	*5.1*	*8.9*	*7.8*	*17.5*	*15.4*	*31.2*	*13.7*	*13.6*	*6.30*	*2.68*	*2.72*
CV	**6.5**	**6.3**	**3.0**	**4.3**	**4.6**	**5.2**	**1.9**	**5.0**	**4.2**	**4.0**	**4.1**

dency for increase predominantly. Significantly more favorable influence on this process has the treatment with Biofa, Polyversum and their combinations with Pyrethrum (increase from 7.7 to 21.1%). The independent application of NeemAzal and Pyrethrum, as well as combinations NeemAzal + Biofa and Nurelle D + Flordimex suppress the lignification process.

The pepsin-cellulase *in vitro* digestibility of dry matter (IVDMD/DMD) is measure for the relative amount digestible cell soluble matters, proteins, hemicelluloses) and non digestible (lignin, cellulose) components in the forage. Increased digestibility is the indicator for high forage quality. Digestibility of dry matter is increased influenced by the biological active products used and varies between 63.9 – 71.0%. According to Bamualim et al. (1980) the best index for preliminary evaluation digestibility of dry matter in forage legume crops is the lignin content as the authors establish a strong negative relation between them. Absorption is expected to be higher in the variations with lower lignin content. This tendency is clearly expressed after treatment with biological foliar fertilizer Biofa, where the plant cell walls fiber components content is lower and digestibility reaches 71.0%, exceeding the control by 12.5%. With high digestibility of 70.4% is distinguished Biofa + Pyrethrum exceeded non treated variant with 11.5%. The combination is characterized by most significant decrease in NDF content. After application of other products digestibility is decreased averagely with 5.8% and has a similar values. Analogical is the tendency concern digestibility of total amount of dry matter, which varies from 63.3 to 62.4%.

Chemical composition and enzyme digestibility of spring forage pea is influenced by the multiplicity of treatment as the influence of biological active compounds in double application in the budding and flowering stages is less significantly expressed (Table 2). The crude protein content in insecticides application and their combinations exceeds the control (13.3 g kg^{-1}) I higher degree from 5 to 21 g kg^{-1} compared to growth regulators and foliar fertilizer. The crude fiber content varies in short limits 242 – 267 g kg^{-1} and influence of the biological agents applied usually exceed the control from 2 (Pyrethrum + Polyversum) to 13 g kg^{-1} dry matter (Polyversum and Nurelle D + Flordimex).

Structural plant cell walls fiber components NDF, ADF, ADL and cellulose decrease their content but the differences to the control are smaller in comparison to single treatment. The NDF content varies from 369 to 425 (control) g kg^{-1}. A more significant decrease is observed in combinations Pyrethrum + Biofa and NeemAzal + Polyversum with 53 and 59 g kg^{-1} dry matter (with 12.5 and 13.9%). The influence of the other biological active compounds is associated with average decrease of 19 g kg^{-1} (4.3%). Fiber components ADF, ADL and cellulose have values that usually reach the respective controls as the increase slightly varies from 0.2 to 6.0%. Exception is established in four combinations with insecticides where the decrease of the fractions is significantly demonstrated. The application of Pytethrum in combination with Biofa and Polyversum, NeemAzal + Polyversum and Nurelle D + Flordimex decrease ADF in cell walls with 32, 43, 33 and 41 g kg^{-1} to the control (351 g kg^{-1}) or averagely by 10.6%. The decrease of ADL in combinations is respectively by 5, 8, 9 and 8 g kg^{-1} to the control (64 g kg^{-1}) or averagely 10.9%. The influence is similar on the cellulose content as to the non treated variant (287 g kg^{-1}) the decrease is with 27, 35, 25 and 33 g kg^{-1} dry matter (mean 10.5%).

The influence of applied biological active compounds on the digestible polyosid hemicellulose is connected to decrease of its content as only the combinations Pytethrum + Polyversum and Nurelle D + Flordimex exceed the control (77 g kg^{-1}) with 17 g kg^{-1}.

The process of lignification is influenced in significantly by double treatment as a more significant increase is accounted at Biofa and Polyversum + Biofa with 6.0%.

Enzyme degradability/digestibility of dry matter (IVDMD) of pea forage follow the tendency of increase and is in the limits 63.4 – 70.7%. Increased digestibility exceeding 70% is established after application of Biofa, Pyrethrum + Biofa and Flordimex. The biological active compounds mentioned increase the digestibility of the total dry matter in the highest degree.

CONCLUSIONS

The independent and combine application of biological and synthetic biological active compounds positively influence chemical composition and enzyme *in vitro* digestibility of dry mass of spring forage pea.

Single treatment in the budding stage increase crude protein, crude fiber and hemicel-

lulose content and decrease plant cell walls fiber components content – NDF, ADF, ADL and cellulose. Significant increase of crude protein is established in Polyversum and Flordimex (by 10.1 and 11.9%), while the crude fiber content increasing is slightly expressed. Optimal combination of expressed decrease in plant cell walls fiber components content with significant increase of forage enzyme in vitro digestibility is establish after applying of Biofa and combination Pyrethrum + Biofa. Digestibility reaches 71.0% and 70.4% respectively with increase from 12.5% and 11.5%.

Double application of biological active compounds in budding and flowering vegetative stages, have similar influence on the chemical composition and enzyme pepsin-cellulase *in vitro* digestibility, but the influence is expressed in lower degree.

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Качество на фураж при *Pisum sativum,* третиран с биологични и синтетични продукти

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Резюме

През периода 2011 – 2013 г. в ИФК, Плевен е проучено влиянието на самостоятелното и комбинирано действие на 3 инсектицида – Нимазал, Пиретрум (биологични инсектициди) и Нурелe Д (синтетичен пиретроид), 2 растежни регулатора – Поливерзум (биологичен регулатор) и Флордимекс (синтетичен регулатор) и биологичен листен тор Биофа върху химичния състав и смилаемост на суха маса при пролетен фуражен грах (*Pisum sativum*). Резултатите показват, че самостоятелното и комбинирано използване на проучваните биологични и синтетични продукти оказва положително влияние върху химичния състав и ензимната смилаемост на сухата маса при пролетен фуражен грах. Еднократното третиране във фаза бутонизация повишава съдържанието на суров протеин, сурови влакнини и хемицелулоза, а намалява структурните влакнинни компоненти (полиозиди) в клетъчните стени – NDF, ADF, ADL и целулоза. Значително повишаване на суровия протеин се установява при Поливерзум и Флордимекс (с 10,1 и 11,9%), докато повишението на суровите влакнини е слабо изразено. Оптимално съчетаване на изразено намаление в съдържанието на влакнинните компоненти с изявено повишаване на ензимната смилаемост на фуража се установява след прилагане на Биофа и комбинацията Пиретрум + Биофа. Смилаемостта достига 71,0% и 70,4% съответно с повишение от 12,5% и 11,5%. Двукратното прилагане на препаратите във фази бутонизация и цъфтеж оказват сходно влияние върху химичния състав и ензимната смилаемост, но въздействието е по-слабо изразено.