

EFFECT OF BIOSTIMULATORS ON SEED QUALITY, YIELD AND OIL CONTENT IN SUNFLOWER

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ABSTRACT

The effect of five biostimulators on seed quality, yield and oil content in sunflower was tested in this study. Seed was treated with biostimulators Amalgerol, Slavol, Иммуноцитифит, ТАБ, Raykat Start and НИКФАН, ж, as well as with fungicide Apron XL 350 ES and insecticide Cruiser 350 FS with added polymer Sepiret. Seed treatment with particular biostimulators had significant effect on the germination energy and germination. This effect was especially visible in the second trial year, when seeds lost their germination due to a long storage period. Treatments with pure Slavol and НИКФАН, ж showed the most significant positive effect. Positive effect was completely reduced when fungicide and insecticide were used with biostimulators. The largest average seed yield was achieved in treatment with НИКФАН, ж+insecticide and fungicide (4467 kg/ha), while the highest average oil content was achieved in treatment with pure НИКФАН, ж (53.34%). However, the effect of all treatments on seed yield and oil content was weak.

Key words: Sunflower, Biostimulators, Germination Energy, Germination, Seed Yield, Oil Content

INTRODUCTION

The most important target of sunflower production is high seed yield and high oil yield. In order to achieve this, it is necessary to grow high-yielding hybrids using optimal cultivation practices. Apart from these standard measures, various biostimulators are more often used via seed treatment or foliar treatment, with various degrees of success. Biostimulators are substances that can enhance the immunity of cultivated crops, benefit their metabolism (Kolomaznik et al., 2012), and decrease the effects of stress. The type of the biostimulator, its application, genotype and environmental conditions all affect its performance.

Using different foliar biostimulators based on 2-(1-Naphthyl) acetic acid, naphthalene derivatives, etc., Tahsin and Kolev (2005) found significant increase of sunflower seed yield with treatment in the flowering phase, but not with treatment in the budding phase; additionally there was no significant effect on the oil content. Beltrano et al. (1994) used gibberellic acid and benzyladenine and recorded yield increase of 25% through the increase of 1000-seed weight and pollination in the middle part of the sunflower head. Using various biostimulators on different oil crops, Ghosh et al. (1991) reached yield increase of 10-40%, but it was inconsistent throughout the trial years. Foliar application of amino acids had positive effect on the head diameter and pollination in sunflower in drought conditions (Kheybari et al., 2013). With foliar application of Fertileader Gold (patented extract of sea algae with addition of nitrogen, boron and molybdenum), Glijin et al. (2013) found significant increase in plant height and head diameter. By treating the seed with BION (active matter BTH), Buschmann and Sauerborn (2002) achieved induced resistance of sunflower to broomrape infection.

Jakienė and Liakas (2013) treated the soil with Azofit and Amalgerol and recorded significant increase in sugar beet root yield (7.26-9.67%), sugar content and sugar yield. Boteva (2014) found that fertilization with bioproducts Biofa and Amalgerol on background Biosol resulted in increased number of fruits in pepper – on average 3.1 fruits per plant. The increase of pepper yield was recorded from 6.2% (background Lumbrikal) to 16.9% (background Biosol+Amalgerol). The foliar products Amalgerol+Cereal mix, Foliar extra and KTS were the most effective for wheat grain yield, and the increase of yield compared to untreated control was 39.3%, 38.1% and 36.2%, respectively (Kostadinova et al., 2015). Šimunić et al. (2011) reported that foliar application of Amalgerol caused increased sunflower oil yield per hectare by 7.26% and soybean grain yield by 2.56%. On the other hand, under the conditions of extremely high temperatures during the growing season and severe soil drought in the region of Dobrudzha, Milev and Todorova (2014) found that foliar application of Almagerol on soybean did not have a significant positive effect neither on seed yield nor on 1000-seed weight. Treatment of growth stimulator Amalgerol premium with herbicides Goal, Raft, Wing, Pledge and Modown as tank mixtures increased the selectivity of these herbicides (Delchev, 2013).

The seed of Nadine F1 lettuce, treated with Slavol before sowing, sprung up two days earlier than the seed that was not treated at all (Kaliderović and Mirecki, 2013). Treating sunflower seed with Slavol (indole-3-acetic acid) and Bioplant Flora (mixture of humic and fulvic acids, amino acids, macro and micro elements) Miladinov et al. (2014a) recorded increased length of sprout root (but not sprout shoot) in individual sunflower genotypes, but also found negative effects in certain treatments. Miladinov et al. (2014b) applied the same products while testing germination energy and germination, and found positive effect in certain treatments, higher on filter paper than on sterile sand.

Чухланцев (2010) reported that sunflower seed treatment with Vermikulen ŽK (3 l/t) + Иммуноцитифит, ТАБ (0.5 g/t) VDB (0.2 l/t) provided biological efficiency in the management of root white rot and fusariosis. Treatment of sunflower seed with a mixture of Иммуноцитифит and several other formulations increased yield, 1000-seed weight and number of seeds per head in sunflower (Высоцкая, 2013), as well as assimilating leaf area by 8.9-9.1% and seed yield by 320-360 kg/ha (Фирсов et al., 2010). Иммуноцитифит stimulated the mass germination of the sunflower seeds and increased their germination ability. The yield obtained from such plants (treated seeds + threefold treatment during the vegetation: in bud formation phase + two fold treatments every 15 days with addition of 0.5% Kristalon 18, 18, 18) was on average higher by 23.54% than control (Masheva et al., 2012).

Maize seed was treated with НИКФАН and germination increased by 20-40%, fresh weight yield increased by 22-32%, quality of fresh weight also increased (Маркелова et al., 2011). Петров and Шершнеv (2007) found that maize seed treated with Agat – 25K and НИКФАН had better plant development and shorter growing season (by 7-8 days) and significant increase in seed yield.

Савенкова (2011) found that treatment of *Galega officinalis* seed with Raykat Start enhanced germination and root growth. Агафонов and Шабалдас (2013) reported increased yield of soybean seed treated with Raykat Start and several other products. However, this was not in agreement with Гракова (2011).

The aim of this study was to assess the effects of treating sunflower seed with biostimulators Amalgerol, Slavol, Иммуноцитифит, ТАБ, Raykat Start and НИКФАН, on seed yield, oil content and seed quality parameters.

MATERIAL AND METHODS

The trial was set up as split-plot design at the experimental field Rimski šančevi of the Institute of Field and Vegetable Crops in Novi Sad, Serbia in 2012 and 2013. The seed of sunflower hybrid Baća produced in 2011 was used in the trial and regular cultivation practices were performed.

The seed was treated with the systemic insecticide Cruiser 350 FS (1 l per 100 kg seed) and fungicide Apron XL 350-EC (300 ml per 100 kg seed), with addition of polymer and colorant Sepiret (300 ml per 100 kg seed), according to the regular sunflower seed processing procedure at the Institute of Field and Vegetable Crops. Additionally, the seed was treated with biostimulators in doses recommended by the manufacturers: Amalgerol at a concentration of 2%, Slavol at a concentration of 25%, Иммуноцитифит, ТАБ - one tablet in 10-15 ml of water per 5 g seed, Raykat Start - 0.5 l per 1000 kg seed, НИКФАН, ж- 0.6 l in 10 l of water per 1000 kg seed.

Amalgerol is an organic stimulator and soil enhancer. It contains essential oils, plant extracts and plant oils, marine algae extracts and mineral oil distillates. Slavol is a liquid microbiological fertilizer and growth stimulator certified for organic and traditional agricultural crop production. This product contains no chemical additives and has beneficial effect on the crops, soil and the environment. Иммуноцитифит, ТАБ is a plant growth regulator with active matter arachidonic acid ethyl ester. Raykat Start is a special fertilizer for the initial plant growth, used as seed / tuber dressing (free amino acids 4%, polysaccharides 15%, cytokine 0.05%, nitrogen (N) a single 4%, phosphorus pentoxide (P₂O₅) water-soluble 8%, potassium (K₂O) soluble in water 3%, iron (Fe) chelate EDDHA 0.1%, zinc (Zn) chelate of EDTA - 0.02%, boron (B) water-soluble 0.03%). НИКФАН, ж is an environmentally-friendly fertilizer, a product of microbiological synthesis mushroom-producing properties with strong stimulator for plant growth and development.

Oil content in clean seed was determined by nuclear magnetic resonance (NMR) method, according to Granlund and Zimmerman (1975). Sunflower seed yield was calculated to t/ha and corrected to 11% moisture. Laboratory analyses were performed in 2012 and 2013 at the Laboratory for Seed Testing of the Institute of Field and Vegetable Crops according to randomized block design in four repetitions, and the tested parameters were determined by standard laboratory methods. The data were processed in GENSTAT, and two-factorial analysis of variance was used for assessing results.

RESULTS AND DISCUSSION

The treatment of sunflower seed with the tested biostimulators did not show significant effect on the oil content in seed (Table 1). The highest oil content on average for both trial years was found in seed treated with pure НИКФАН, ж (53.34%), and the lowest in seed treated with the combination of Raykat Start with Apron and Cruiser (51.78%). Between these two treatments the differences were significant, but not highly significant, and in individual years there were no significant differences among the treatments (Table 2). No significant differences were found between the control and the treatments. In 2013 there was a higher average oil content than in 2012, but the differences were not significant.

Sunflower seed treatment with the tested biostimulators did not significantly affect the seed yield (Table 3). The highest seed yield was found in seed treated with НИКФАН, ж + Apron and Cruiser (4467 kg/ha), and the lowest in seed treated with Slavol + Apron and Cruiser (3846 kg/ha). The differences between these two treatments were significant, but not highly significant (Table 4). There were no significant differences in relation to the control, and there were no significant differences among all treatments in individual trial years. The

highest average seed yield was achieved in 2013 (4431 kg/ha), and the lowest in 2012 (3853 kg/ha), there were no significant differences between the trial years.

Table 1. ANOVA for oil content in hybrid Baća seed

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	74.64	74.64	6.71	0.122 ^{ns}
Error Y	2	22.25	11.13	10.14	-
Treatment (T)	15	18.56	1.24	1.13	0.353 ^{ns}
Y x T	15	7.60	0.51	0.46	0.950 ^{ns}
Error T	60	65.84	1.10	-	-
Total	93	188.89	-	-	-

**significant at 1% level; * significant at 5% level; ^{ns}not significant

Table 2. Effect of year and biostimulators on oil content (%) in hybrid Baća seed

Treatments (T)	Trial year (Y)		Mean (T)
	2012	2013	
Control	53.64	51.64	52.64
Amalgerol	53.18	50.98	52.08
Amalgerol+Apron XL 350 ES	54.35	52.08	53.21
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	54.50	52.11	53.30
Slavol	53.09	51.80	52.45
Slavol+Apron XL 350 ES	54.00	51.28	52.64
Slavol+Apron XL 350 ES+Cruiser 350 FS	53.61	51.63	52.62
Иммуноцитифит,ТАБ	53.70	51.72	52.71
Иммуноцитифит,ТАБ+Apron XL 350 ES	54.09	52.04	53.06
Иммуноцитифит,ТАБ+Apron XL 350 ES+Cruiser 350 FS	53.55	51.38	52.46
Raykat Start	53.27	52.17	52.72
Raykat Start+Apron XL 350 ES	53.90	52.68	53.29
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	52.30	51.26	51.78
НИКФАН,ж	53.66	53.02	53.34
НИКФАН,ж+Apron XL 350 ES	53.62	51.83	52.72
НИКФАН,ж+Apron XL 350 ES+Cruiser 350 FS	52.92	51.57	52.25
Mean (Y)	53.59	51.82	

	Y	T	Y x T
LSD _{0.05}	2.93	1.21	2.35
LSD _{0.01}	6.76	1.61	3.30

Both seed treatment and trial year showed highly significant effect on the germination energy of sunflower seed (Table 5). The highest average of germination energy was found in seed treated with pure Slavol (90.62%), and the lowest in seed treated with the combination Amalgerol + Apron and Cruiser (80.12%). The differences between these two treatments were highly significant and the treatment with Slavol gave significantly higher germination energy than the control (Table 6). In 2012 there was highly significantly higher average of germination energy (91.89%) than in 2013 (78.48%). In 2012 no treatment showed significantly higher germination energy than the control, but there were several combinations with highly significant differences. Namely, highly significantly higher germination energy

was found in seed treated with НИКФАН, ж + Apron than in Raykat Start + Apron and Cruiser. In 2013 the differences among treatments were much higher – treatments with only Slavol and НИКФАН, ж were highly significantly higher or significantly higher than the control, but there were also significant reductions in some treatments, mostly in Amalgerol + Apron and Cruiser. These results imply that seed treatment with biostimulators showed more effect on the seed with lower average of germination energy, as was the case in 2013. It was discovered that in treatments with certain biostimulators which showed positive effect, the positive effect was lacking in combinations of biostimulator with fungicide and insecticide. Since seed treatment with fungicides (and insecticides as well) is a mandatory measure in seed processing, the practical possibility of biostimulator application is questionable.

Table 3. ANOVA for seed yield of hybrid Baća

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	8015126	8015126	5.21	0.150 ^{ns}
Error Y	2	3076814	1538407	7.92	-
Treatment (T)	15	3328390	221893	1.14	0.341 ^{ns}
Y x T	15	1022172	68145	0.35	0.986 ^{ns}
Error T	60	11649289	194155	-	-
Total	93	39227240	-	-	-

** significant at 1% level; * significant at 5% level; ^{ns} not significant

Table 4. Effect of year and biotimulators on seed yield (kg/ha) of hybrid Baća

Treatments (T)	Trial year (Y)		Mean (T)
	2012	2013	
Control	3817	4317	4067
Amalgerol	3817	4692	4255
Amalgerol+Apron XL 350 ES	3994	4725	4360
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	4053	4279	4166
Slavol	3661	4237	3949
Slavol+Apron XL 350 ES	3918	4139	4028
Slavol+Apron XL 350 ES+Cruiser 350 FS	3718	3975	3846
Иммуноцитифит,ТАБ	4013	4585	4299
Иммуноцитифит,ТАБ+Apron XL 350 ES	3764	4630	4197
Иммуноцитифит,ТАБ+Apron XL 350 ES+Cruiser 350 FS	3530	4221	3875
Raykat Start	3868	4554	4211
Raykat Start+Apron XL 350 ES	3901	4740	4320
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	4081	4518	4299
НИКФАН,ж	3826	4319	4072
НИКФАН,ж+Apron XL 350 ES	3531	4186	3858
НИКФАН,ж+Apron XL 350 ES+Cruiser 350 FS	4156	4778	4467
Mean (Y)	3853	4431	

	Y	T	Y x T
LSD _{0.05}	1089	509	918
LSD _{0.01}	2513	677	1270

Seed treatment and trial year showed highly significant effect on the seed germination (Table 7). The highest mean seed germination was achieved in seed treated with pure Slavol

(91.12%), and the lowest in seed treated with Amalgerol + Apron and Cruiser (81.12%). The difference was highly significant and the treatment with Slavol showed significantly higher germination than the control (Table 8). In 2012 mean seed germination (93.03%) was highly significantly higher than in 2013 (79.80%). In individual years, the trends were similar to the germination energy, i.e. the treatment was more effective in a year with lower mean germination. In 2012 no treatment showed significant difference in relation to the control, but there were highly significant differences among individual treatments. In 2013 the treatments with Slavol and НИКФАН, ж showed highly significant increase in seed germination than the control. In 2013 the treatment with pure biostimulators showed better results than the treatments with added fungicides and insecticides, while in 2012 this was not the case.

Table 5. ANOVA for the germination energy of hybrid Baća seed

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	5751.28	5751.28	446.68	<.001**
Error Y	15	1016.50	67.77	5.26	<.001**
Treatment (T)	15	675.72	45.05	3.50	<.001**
Y x T	93	1197.44	12.88	-	-
Total	124	8855.50	-	-	-

** significant at 1% level; * significant at 5% level; ^{ns}not significant

Table 6. Effect of year and biostimulators on the germination energy (%) of hybrid Baća seed

Treatments (T)	Trial year (Y)		Mean (T)
	2012	2013	
Control	93.75	80.00	86.88
Amalgerol	90.75	79.25	85.00
Amalgerol+Apron XL 350 ES	94.25	79.25	86.75
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	90.75	69.50	80.12
Slavol	92.75	88.50	90.62
Slavol+Apron XL 350 ES	90.25	73.25	81.75
Slavol+Apron XL 350 ES+Cruiser 350 FS	90.00	74.25	82.12
Иммуноцитифит, ТАБ	91.75	85.00	88.38
Иммуноцитифит, ТАБ+Apron XL 350 ES	91.50	78.50	85.00
Иммуноцитифит, ТАБ+Apron XL 350 ES+Cruiser 350 FS	94.00	76.50	85.25
Raykat Start	91.75	78.50	85.12
Raykat Start+Apron XL 350 ES	92.25	77.25	84.75
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	88.50	76.75	82.62
НИКФАН, ж	91.50	86.75	89.12
НИКФАН, ж+Apron XL 350 ES	95.50	79.00	87.25
НИКФАН, ж+Apron XL 350 ES+Cruiser 350 FS	91.00	73.50	82.25
Mean (Y)	91.89	78.48	

	Y	T	Y x T
LSD _{0.05}	1.26	3.56	5.04
LSD _{0.01}	1.67	4.72	6.67

It is evident that the sunflower seed treatment with the tested biostimulators did not generally result in a significant increase of oil content in seed and seed yield, which is contrary to the results on sunflower reported by Šimunić et al. (2011), Высоцкая (2013),

Фирсов et al. (2010), on maize by Маркелова et al. (2011), and on soybean by Агафонов and Шабалда (2013). Lack of biostimulator effect on soybean yield was reported by Milev and Todorova (2014). There was a certain effect in individual treatments, but it was difficult to deduce any regularity which could justify commercially viable recommendations for general use.

Table 7. ANOVA for seed germination of hybrid Baća

Source of variation	df	SS	MS	F	P
Year of study (Y)	1	5604.76	5604.76	504.45	<.001**
Error Y	15	784.18	52.28	4.71	<.001**
Treatment (T)	15	702.37	46.82	4.21	<.001**
Y x T	93	1033.29	11.11	-	-
Total	124	8269.05	-	-	-

** significant at 1% level; * significant at 5% level; ^{ns} not significant

None the less, the seed quality parameters showed different results. The effect of the treatment was much higher, especially in years with low mean values of germination and germination energy. The best effect was achieved with Slavol and НИКФАН, ж. The positive effects of individual biostimulators on the sunflower seed quality parameters were previously reported by Miladinov et al. (2014b), Masheva et al. (2012), and on other crops by Маркелова et al. (2011) and Савенкова (2011). The problem is that the combination of biostimulators with fungicides or fungicides and insecticides did not show any positive effects as pure biostimulators did, which greatly impedes the practical use of biostimulators.

Table 8. Effect of year and biostimulators on seed germination (%) of hybrid Baća

Treatments (T)	Trial year (Y)		Mean (T)
	2012	2013	
Control	94.00	81.00	87.50
Amalgerol	92.00	79.50	85.75
Amalgerol+Apron XL 350 ES	95.25	79.50	87.38
Amalgerol+Apron XL 350 ES+Cruiser 350 FS	91.50	70.75	81.12
Slavol	93.75	88.50	91.12
Slavol+Apron XL 350 ES	90.75	74.25	82.50
Slavol+Apron XL 350 ES+Cruiser 350 FS	91.50	76.75	84.12
Иммуноцитифит, ТАБ	92.75	85.25	89.00
Иммуноцитифит, ТАБ+Apron XL 350 ES	93.50	79.00	86.25
Иммуноцитифит, ТАБ+Apron XL 350 ES+Cruiser 350 FS	97.50	78.00	87.75
Raykat Start	93.00	79.25	86.12
Raykat Start+Apron XL 350 ES	93.75	78.50	86.12
Raykat Start+Apron XL 350 ES+Cruiser 350 FS	89.25	82.50	85.88
НИКФАН, ж	92.00	87.50	89.75
НИКФАН, ж+Apron XL 350 ES	96.00	79.25	87.62
НИКФАН, ж+Apron XL 350 ES+Cruiser 350 FS	92.00	77.25	84.62
Mean (Y)	93.03	79.80	

	Y	T	Y x T
LSD _{0.05}	1.17	3.31	4.68
LSD _{0.01}	1.55	4.38	6.20

In the current situation of slow increase of genetic yield and quality potential in new cultivars of many crops and the level of cultivation practices that cannot easily be revolutionized nor quickly improved, various biostimulators are more often being used. The results show that the positive effects were not as spectacular as marketed or reported in different studies. Individual biostimulators certainly hold their place in the improvement of individual crops cultivation, so investments into biostimulators application must be economically viable, which is only possible through detailed and objective studies in different agricultural environments using different genotypes.

CONCLUSIONS

Sunflower seed treatment with the tested biostimulators did not show significant effect on oil content in seed nor the seed yield. The highest oil content on average for both trial years was found in seed treated with pure НИКФАН, ж (53.34%), and the lowest in seed treated with Raykat Start in combination with Apron and Cruiser (51.78%). Between these two treatments the differences were significant, but not highly significant, and in individual years there were no differences between the treatments. The highest seed yield was found in seed treated with НИКФАН, ж + Apron and Cruiser (4467 kg/ha), and the lowest in seed treated with Slavol + Apron and Cruiser (3846 kg/ha). The differences between these two treatments were significant, but not highly significant.

Sunflower seed treatment with the tested biostimulators showed highly significant effect on the seed quality parameters. The highest mean first count was found in seed treated with pure Slavol (90.62%), and the lowest in seed treated with Amalgerol + Apron and Cruiser (80.12%). The differences between these two treatments were highly significant, and the treatment with Slavol showed significantly higher germination energy than the control. The highest mean seed germination was found in the seed treated only with Slavol (91.12%), and the lowest in seed treated with Amalgerol + Apron and Cruiser (81.12%). The difference was highly significant and treatment with Slavol showed significantly higher germination than the control.

The conclusion is that the tested biostimulators could be more applicable in seed production than in commercial (mercantile) production. Practical application of individual biostimulators for enhancement of seed quality parameters is restricted by the fact that the positive effects drastically drop when biostimulators are combined with fungicides and insecticides, which should further be studied. This indicates that biostimulators can be used more successfully in organic production.

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