

The Influence of synthetic strigolactones and plant extracts on the morphological parameters of onion (*Allium cepa*)

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: In recent years there has been frequent reference to the significance of strigolactones as a new group of hormones which might have a significant effect on horticultural production. The aim of this work was to find an ideal combination of stable synthetic strigolactones and plant extracts with potential effects on onion plants. The synthetic strigolactone Fenyl 7 (dihydro-3-[[2,5-dihydro-4-methyl-5-oxo-2-furanyl]oxy]-methylene]-5-phenyl-2(3H)-furanone) was tested in a carrot macerate, with citric acid and with salicylic acid. From the results it was confirmed that increasing the pH of the preparation leads to improving the stability of Fenyl 7. Evaluation has repeatedly confirmed the effect of the preparation, combining synthetic strigolactone and a macerate of carrot in a mixture of surfactants with added citric acid. In all the experiments this combination showed a statistically demonstrable influence on leaf weight (increased by 12-31%) and length (increased by 6-13%) in comparison with the controls.

1. Introduction

In several countries fertiliser use on agricultural soil results in serious problems associated with the accumulation of residues in the food chain and groundwater pollution (Hegazi *et al.*, 2010). The increased costs of chemical fertilisers and the massive input of non-renewable resources have led to promote the research in the field of alternative preparations (Board, 2004). Several technological innovations have been proposed in order to enhance the sustainability of production systems through a significant reduction in the use of chemicals. An effective tool would be the use of biostimulants (Hamza and Suggars, 2001) a group of compounds that act neither as fertilisers nor as pesticides, but have a positive impact on plant performance when applied in small quantities (Calvo *et al.*, 2014).

Carotenoids are essential photosynthetic pigments also identified as

precursors of different signalling molecules and hormones such as strigolactones. Strigolactones, recently recognised as a new class of plant hormones, play a key role in different developmental processes such as plant architecture, the number of shoot branches and nutrient availability (Al-Babili and Bouwmeester, 2015). Several studies have reported the ability of these molecules to stimulate root hair elongation (Kapulnik *et al.*, 2011) and root growth (Arite *et al.*, 2012), but also to inhibit adventitious root formation (Rasmussen *et al.*, 2013; Urquhart *et al.*, 2015). Furthermore, strigolactones increase stem thickness by stimulating secondary growth in interaction with auxin (Agusti *et al.*, 2012), positively regulate internode length (De Saint Germain *et al.*, 2013) and accelerate leaf senescence (Yamada *et al.*, 2014).

Strigolactones are also signalling molecules, which participate in communication with mycorrhizal fungi, initiating the arbuscular mycorrhiza formation (García-Garrido *et al.*, 2009). Through mycorrhiza strigolactones are also involved in plant nutrition increasing the reduction of the phosphates in soils (García-Garrido *et al.*, 2009).

Moreover, studies have shown the role of this plant hormone on seed germination (Toh *et al.*, 2012) and early seedling development (Tsuchiya *et al.*, 2010). Strigolactones stimulate the germination of parasitic plants, for example witchweed or broomrape, which frequently survive for years in a dormant state. These seeds can be stimulated by preparations containing strigolactones obtained from plant roots showing effects already in extremely low concentrations: for example, a concentration less than 10^{-10} mol l⁻¹ is sufficient to stimulate the germination of parasitic plants (Humphrey *et al.*, 2006).

Plant extracts are mostly rather complex mixtures, and therefore individual components can function antagonistically but also synergistically. The assumption that plant extracts stimulate the growth with similar effects to strigolactones was tested in experiments with the germination of the *Striga hermonthica* plants. This test is a known method for ascertaining the presence of strigolactones and is standardised for the effects of the synthetic strigolactone GR24 (Sato *et al.*, 2005; Yoneyama *et al.*, 2008).

Strigolactones are now known to modify positively many plant traits important for crop yield and quality (Mason, 2013) and many commercial opportunities could arise (wood production, shoot architecture, root development, nutrient acquisition and more). Already it is possible to identify certain strigolactones that affect one process and not another.

However, one challenge is to identify stable strigolactones or methods of delivery that enhance their stability (Beveridge, 2014).

The aim of this work was to find a suitable combination of stable synthetic strigolactone and plant extracts with a positive effect on onion growth parameters. In the experiment the stability of the synthetic strigolactone Fenyl 7 was evaluated and the application of several types of bio-additive in onion seedlings and the influence on morphological parameters was followed.

2. Materials and Methods

Fenyl 7 (dihydro-3-[[2,5-dihydro-4-methyl-5-oxo-2-furanyl]oxy]-methylene]-5-phenyl-2(3H)-furanone) is a synthetic strigolactone protected as utility model No. 29064 by Industrial Property Office and produced in VUOS (Research Institute for Organic Synthesis, Rybitvi) by synthesis from 3-methyl-2(5H)-furanone and phenylbutyrolactone. The obtained compound, which occurs in two stereoisomers, was purified with the aid of column chromatography. The compound has a similar structure to other synthetic strigolactones (e.g. GR5, GR7 and GR24), but is simpler and cheaper to produce. Fractions of individual isomers were obtained and also a fraction with a mix of isomers in a concentration of 10^{-2} mol l⁻¹ was used for additive preparation.

Given the instability of Fenyl 7 in water a method was sought to increase the stability of this substance in solution. Aside from using surfactants a possible means of increasing stability is to change the pH of the application solution. Natural strigolactones arise from the biosynthesis of carotenoids, and so for this purpose carrot macerate (pH = 5.2) was used, which presents its positive effects (López-Ráez *et al.*, 2010). To further reduce pH and also to conserve the macerate, citric or salicylic acid were added (pH = 3.6). Fenyl 7 was dissolved in the mixture of surfactants or in the carrot macerate and converted with the aid of a vacuum evaporator to a 3 g l⁻¹ mixture of surfactant. The resulting concentration for application on plants was 10^{-5} M. The carrot macerate was prepared by the maceration of carrot roots for 3-5 days in ethyl acetate. For the experiment a 1:1 mixture of MDGE (diethylene glycol monomethyl ether) and PEGSHO (poly(ethylene glycol)sorbitol hexaoleate) was used.

First the stability of Fenyl 7 in buffers (MDGE and PEGSHO) of pH 4, 7 and 9, according to OECD (2004),

was followed. These solutions were left at a temperature of 4°C and the decline in Fenyl 7 was steadily monitored over a period of 30 days or until the drop was greater than 90%. Liquid chromatography diode-array detection (HPLC-DAD) was used to determine the Fenyl 7. The determination was carried out on a LiChrospher RP-18 column of length 125 mm and internal diameter of 4 mm. The average particle size in the column packing was 5 µm. The column temperature was set at 30°C. A wavelength of 239 nm was used for the detection of measurements. A mixture of 40% MeOH (component A) and 100% MeOH (component B) was used as a mobile phase. The flow of the mobile phase was set at 0.9 ml min⁻¹. Analysis of one sample with a volume of 10 µl last 15 min. The gradient of the mobile phase was set at: 0 min 0% component B, 10 - 18 min 50% component B and 21 min 0% component B.

The influence of preparations selected according to previous laboratory research (Table 1) on the morphological parameters of onion seedlings (*Allium cepa*) was then followed. The experiment was carried out in three periods during two years. The experiments labelled 1, 2 and 3 were evaluated on the dates 12/5/2014, 17/7/2014 and 13/5/2015. The variety Lusy (SEMO, CZ) was sown in the substrate Klasman TS3 Fine 416, the pH was 6.0 and the electrical conductivity of the substrate was 30 mS m⁻¹. The nutrient content of the substrate was as follows: 100 mg l⁻¹ N, 70-150 mg l⁻¹ P₂O₅, 140-300 mg l⁻¹ K₂O and 60-100 mg l⁻¹ Mg (PASIC, CZ). The experiment took place in a greenhouse in type 96 trays (cell volume 27 ml). 100 ml per tray (dilution of 2 ml l⁻¹ water), which corresponds to a dose of 2 ml per plant, was applied as a foliar spray. Only water was used to spray the control treatment in the same period. For treatments, the variants given in Table 1 were

Table 1 - Composition of bio-additives used and labelling of experiments

Label	Composition of mixture used
BIOA-1	Fenyl 7 (isomer 1) in mixture of surfactants
BIOA-3	Fenyl 7 (mix of isomers) in mixture of surfactants
BIOA-7	Fenyl 7 (mix of isomers) in mixture of surfactants with added salicylic acid (20 g l ⁻¹)
BIOA-10	Fenyl 7 (isomer 1) and carrot macerate in mixture of surfactants
BIOA-11	Fenyl 7 (isomer 1) and carrot macerate in mixture of surfactants with added citric acid (20 g l ⁻¹)

selected in two applications (the first in the phase of the first true leaves, and the second after 14 days). The determination of fresh leaf weight (g), leaf length (m) and their number (pcs) was carried out 8 weeks from sowing on 25 plants.

Data were analysed by analysis of variance and evaluated by using the LSD test at a probability of $p = 0.05$ (Statistica 12.0, StafSoft).

3. Results and Discussion

Given the instability of Fenyl 7 in water, a means was sought for increasing the stability of this compound in water. Accordingly, aside from the use of surfactants the pH of the environment was modified. The assumption that making the pH more acidic would lead to greater stability of Fenyl 7, as stated by (Babiker *et al.*, 1988) in the case of GR7 in soil, was confirmed in this experiment. In fact, as clearly reported in figure 1, in the case of a solution with pH 9 there was a degradation of Fenyl 7 by more than 90% in three days. With reduced pH there was a reduction in the degradation of Fenyl 7. The lowest level of degradation was determined in the variant with a pH 4, where the reduction in the concentration was only by 8.5%. The ascertained state corresponds to the summary from Zwanenburg and Pospíšil (2013), that a lower pH does not lead to the hydrolysis of the tested synthetic strigolactones (GR7, GR24).

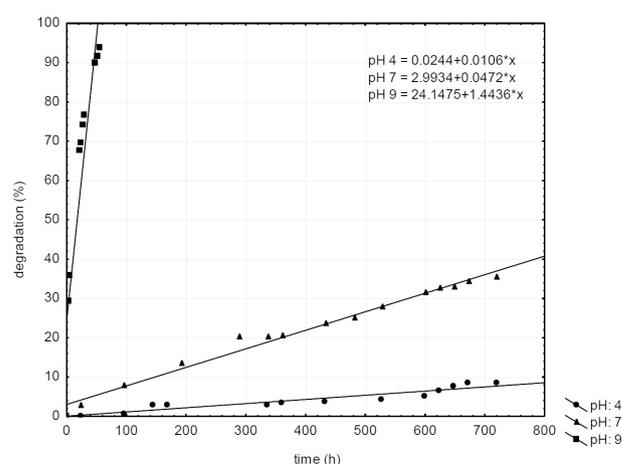


Fig. 1 - Degradation of Fenyl 7 in dependence on pH.

In the tested bio-additives (Table 1) it is possible to conclude the presence of an influence on the evaluation of morphological parameters as it is shown in

Table 2. All the treatments had a positive effect which however was not manifested in repeated experiments with all variants.

The number of leaves in onion was positively influenced only in experiment No. 2 (summer 2014) in variants BIOA-1, 3, 7 and 10 while a reduction in number of leaves was found in experiment No. 1 (spring 2014) only in variant BIOA-3. On the contrary, in the experiment No. 3 (spring 2015) the number of leaves was not influenced in comparison to control conditions. From the results obtained it is not possible to conclude that there was a positive influence of the bio-additive used on the number of leaves in onion plants.

tion it follows that the total sunlight hours for March to May was 7% higher in 2014 than in 2015. Similarly, the average temperature for the same period was 11% higher in 2014 than in 2015.

The positive effect of the external application of strigolactones on leaf length might be related to the report of (Pasare *et al.*, 2013) who stated that plants with significantly reduced strigolactone content showed, among others, decreased in plant height.

Although the pea plants supplied with 3 µM of synthetic strigolactone (GR24) via hydroponics for 16 days did not significantly increase the internode length (De Saint Germain *et al.*, 2013) the effect on onion leaf weight and length has been proven with

Table 2 - Average values of the monitored morphological parameters

Experiment number	Evaluated factor	Treatment					
		Control	BIOA-1	BIOA-3	BIOA-7	BIOA-10	BIOA-11
1 (spring 2014)	leaf mass (g)	2.61 a	2.77 a	2.68 a	3.61 b	2.68 a	3.43 b
	leaf length (cm)	32.92 a	34.27 ab	33.20 ab	36.80 c	32.86 a	34.73 b
	Number of leaves (pcs)	3.80 b	3.73 b	3.25 a	3.52 ab	3.55 ab	3.55 ab
2 (summer 2014)	leaf mass (g)	1.08 ab	1.56 d	1.02 a	1.24 bc	1.29 c	1.35 cd
	leaf length (cm)	23.88 a	28.12 d	25.70 bc	25.20 ab	26.40 bc	27.06 cd
	Number of leaves (pcs)	2.47 a	3.23 d	2.77 bc	3.00 cd	2.85 bc	2.60 ab
3 (spring 2015)	leaf mass (g)	1.27 ab	1.19 a	1.39 bc	1.27 ab	N/A	1.43 c
	leaf length (cm)	28.87 a	29.73 ab	30.33 bc	30.60 bc	N/A	31.27 c
	Number of leaves (pcs)	3.10 a	3.10 a	3.20 a	3.00 a	N/A	3.10 a

The letter after the value indicates the demonstrable difference according to the LSD test at p=0.05 (different letters in rows indicate significant differences between treatment variants).
N/A= not evaluated.

From the evaluation over more replicates however it is possible to confirm the positive effect of preparation BIOA-11. In all the experiments this combination showed a statistically demonstrable influence on the leaf weight and length, which attained higher values compared to the controls, as is apparent from Table 2. The recorded increase in the leaf weight was of 12-31% and in the leaf length was of 6-13% within all the evaluations. From the overall evaluation of the all tested bio-additives there is an evident tendency for the more frequent manifestation of a positive influence in the later (summer) sowings compared to the spring sowing. The recorded increase in all the evaluated parameters in the spring period of 2014 in comparison with the same period in 2015 can be assigned to the higher sunlight and higher average temperature recorded, which could have had the effect in accelerating the plant growth. According to the meteorological data from the sta-

10 µM of Fenyl 7.

From the experiments carried out we obtained the following findings. Essentially the use of one single isomer or a mixture (BIOA-1 vs. BIOA-3) does not have an influence on the evaluation of the morphological parameters. From the results it is not possible to conclude that there is an unequivocal difference between these variants. Also in itself the use of the carrot macerate does not have a fundamental influence on some of the morphological parameters (BIOA-1 vs. BIOA-10). Against this, clearly better results are achieved when the pH was modified with the aid of one of the acids (BIOA-7 and BIOA-11). While BIOA-7 was positively evaluated in two out of the three experiments, BIOA-11 was positively evaluated in all the experiments carried out. It can thus be concluded that acidification with citric acid and the presence of carrot macerate ensures the repeated effectiveness of Fenyl 7 on the morphological para-

meters in onion plants.

4. Conclusions

From the experiments it was confirmed that changing the pH of the solution into acidic territory leads to higher stability of Fenyl 7. In the case of a solution with a pH 9 the degradation of Fenyl 7 was more than 90%. With a drop down in pH there was a reduction in the degradation of Fenyl 7. The least degradation was recorded in the variant with a pH 4, where there was a reduction in concentration by 8.5%. It is also possible to summarise the positive effect of preparation BIOA-11 (Fenyl 7 and carrot macerate in a mixture of surfactant with added citric acid) on the growth parameters of onion seedlings. In repeated tests an increase in leaf weight (by 12-31%) and leaf length (by 5-8%) was verified in comparison with the control variant. Accelerated production through an appropriate bio-additive, leading to a reduction in the pre-cultivation period could have, especially in the spring period, marked economic benefits for growers. It would be of benefit in future experiments to verify to what extent the effects on seedlings will be manifested in the ensuing vegetation.

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