Gesunde Pflanzen (2018) 70:21–29
https://doi.org/10.1007/s10343-017-0409-5

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ORIGINAL ARTICLE

Vegetative, Chemical Status and Productivity of Zucchini Squash (*Cucurbita pepo* L.) Plants in Responses to Foliar Application of Pentakeep and Strigolactones Under NPK Rates

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Received: 31 August 2017 / Accepted: 25 October 2017 / Published online: 13 November 2017

Abstract In order to determine the effect of two bio-stimulants under NPK fertilizer rates on growth and nutrient content and yield of zucchini (squash), an experiment was conducted at Mendel university during 2014. The treatments included four NPK rates namely, 0, 75, 100 and 150% of recommended rate in combination with foliar application of Pentakeep super and strigolactones (alone and in combination) were used. This study was arranged in a split plot design in three replications. The results showed NPK at 150% led to the maximum significant increase of growth characters i. e., leaf number, fresh and dry weight of leaves relative to unfertilized control. Chlorophyll reading increased significantly with increase in NPK supply up to NPK75%. The highest contents of N, K and nitrate were found in zucchini leaves treated with NPK at 150% of recommended rate. Yield and its component increased significantly with increase in NPK supply up to 100% of recommended rate. The combined application of Pentakeep super and strigolactones significantly maximized growth characters and fruit yield as compared to control treatment. The highest content of N generated in plants sprayed with Pentakeep super while application of Pentakeep and strigolactones did not affect the P and K contents. Whereas, control plants effectively significantly increased the chlorophyll and nitrate content of leaves.

Keywords NPK rates · Pentakeep super · Strigolactones · Zucchini

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Vegetativer chemischer Status und Produktivität von Gartenkürbispflanzen (*Cucurbita pepo* L.) in Reaktion auf die Blattanwendung von Pentakeep und Strigolactonen unter NPK-Raten

Zusammenfassung Um die Wirkung zweier Biostimulanzien unter NPK-Düngerwerten auf das Wachstum und den Nährstoffgehalt sowie den Ertrag von Gartenkürbissen zu bestimmen, wurde an der Mendel-Universität im tschechischen Brünn 2014 ein Experiment durchgeführt. Die Behandlung umfasste die vier NPK-Werte 0, 75, 100 und 150 % des empfohlenen Werts in Kombination mit der Blattanwendung von Pentakeep super und Strigolactonen (einzeln und in Kombination). Diese Studie war nach einem Split-Plot-Design mit drei Replikationen aufgebaut. Die Ergebnisse zeigten, dass NPK bei 150 % zum maximalen signifikanten Anstieg in den Wachstumsmerkmalen führt, d. h. Blattzahl, Frisch- und Trockengewicht der Blätter im Verhältnis zur ungedüngten Kontrolle. Die Chlorophyllanalyse stieg signifikant mit dem Anstieg in der NPK-Versorgung bis zu NPK 75 %. Die höchsten Konzentrationen an N, K und Nitrat wurden in Kürbispflanzenblättern gefunden, die mit NPK zu 150 % der empfohlenen Rate behandelt worden waren. Der Ertrag und dessen Bestandteile steigerten sich signifikant mit Erhöhung der NPK-Zufuhr bis 100 % der empfohlenen Rate. Die kombinierte Anwendung von Pentakeep super und Strigolactonen maximierte die Wachstumsmerkmale und den Fruchtertrag im Vergleich zur Kontrollbehandlung signifikant. Der höchste N-Gehalt wurde in Pflanzen erzielt, die mit Pentakeep super besprüht wurden, während die Anwendung von Pentakeep und Strigolactonen keine Auswirkung auf den P- und K-Gehalt hatte. Die Kontrollpflanzen erhöhten effektiv signifikant ihren Chlorophyll- und Nitratgehalt in den Blättern.

Schlüsselwörter NPK-Raten · Pentakeep super · Strigolactone · Gartenkürbis

Introduction

Squash (*Cucurbita pepo* L.) is an economically important vegetable crop. Zucchini squash is classified as a high fertilizer input vegetable, because of its ability to produce a lot of biomass and its nutrient requirements are generally considered to be high (Colla and Saccardo 2003). Nevertheless, its fertilization is often excessive, due to the poor knowledge on crop nutrient uptake.

It is well known that excessive N fertilization may increase plant vigor and extend the plant cycle, causing delayed harvest, nitrate accumulation in plant tissues and environmental contamination (Kant et al. 2011).

In modern agriculture and horticulture, the use of biostimulators for obtaining the maximum yields and qualities of the crops is recommended (Calvo et al. 2014). Any improvement in agricultural system that results in higher production should reduce the negative environmental impact of agriculture and enhance the sustainability of the system. Bio-stimulation is carried out using growth stimulators (bioactivators, bio-stimulators). One such approach is the use of bio-stimulants, which can enhance the effectiveness of conventional mineral fertilizers. Bio-stimulators can change a number of physiological and biochemical processes in plant cell metabolism (Yaronskaya et al. 2006; Tilly-Mándy et al. 2010). One of exogenous plant bio-stimulants is Pentakeep-super, a nitrogen liquid fertilizer that is blended with 5-aminolevulinic acid (or ALA), which promotes plant photosynthesis. ALA is an important amino acid in the bodies of plants and animals, a substance that is a precursor to chlorophyll in plants. Pentakeep-super has been confirmed that conventional fertilizer and ALA have synergy effects for plant growth. ALA is an essential biosynthetic precursor of all porphyrin compounds, including chlorophyll and heme (Kosáry 2008). ALA is also a natural organic acid presented in all living organisms (Tanaka et al. 2005). Hotta et al. (1997) suggest that ALA has plant growth regulating properties at low concentrations and may enhance agricultural productivity. It increases the photosynthetic efficiency, impact on the nitrogen metabolism—increases activity of nitrate reductase, decreases the content of nitrates. Foliar application of ALA resulted in a higher content of chloroplast pigments as well as increased photosynthetic and antioxidant activity in pakchoi (Memon et al. 2009). The improved of chlorophyll content and photosynthetical activity of Pentakeep was noted also by Kisvarga et al. (2015). The effect of ALA or fertilizers containing this compound (e. g. Pentakeep® fertilizers) on mineral nutrition is still vaguely defined and only few reports concern this question. Diverse effects of foliar application of Pentakeep® on the content of N, Cu and Zn in Phoenix dactylifera L. palm leaves were described (Awad 2008). Smoleñ and Sady (2010) found that using Pentakeep-V decreased calcium, sodium, and iron content on spinach, and increased potassium content. Suitable concentration of ALA had promotive effects on the growth rates and photosynthesis and also crop yields were enhanced by the application of ALA at the leaf-stage in the life cycle of rice, barley, potato and garlic (Tanaka et al. 1992) ALA is also becoming popular in the agriculture and horticulture sectors for its unique ability to promote the growth and quality of plants without harming living organisms or contaminating soil (Akram and Ashraf 2013). Hotta et al. (1997) proposed that, at low concentrations foliar application of ALA increased the growth and yield of barley, garlic, potato and kidney bean.

Recently, strigolactones (SLs) which are fit for the classical definition of a plant hormone can act as a new hormone to inhibit shoot branching (Umehara et al. 2008; Gomez-Roldan et al. 2008). Sls plant hormone group has roles both as an endogenous hormone and a rhizosphere signal, have been discussed as a potential regulator/integrator of nutrient acquisition (Umehara et al. 2015; Foo and Reid 2013). The strong regulation of strigolactone production by the macronutrients nitrogen and phosphorous (Yoneyama et al. 2010).

It is likely that SLs are mainly produced in roots and move upward to inhibit axillary bud outgrowth, since all natural SLs have been detected from root exudates so far (Xie et al. 2010). About 19 different forms of SLs have been detected so far (Koltai and Prandi 2014), but detection and quantification of this hormone is difficult, as SLs are only present in the plant system at extremely minute quantities (Boyer et al. 2012). Strigolactones have attributes that are related to some of the types of benefits have been found when seaweed extracts (Seasol™) are applied to specific plants.

From an agronomic perspective, the exogenous application of these plant stimulants (brassinosteroids and strigolactones) increased plant productivity (Hayat et al. 2012; Ha et al. 2014). Strigolactones are now known to modify many plant traits that are of great importance to agricultural and horticultural industries including adventitious rooting, wood formation, branching and crop yield and quality (Mason 2013).

The objective of this study was to investigate different rates of NPK fertilization with two bio-stimulants i. e., Pentakeep-uper and synthetic strigolactone-Fenyl 7-izomer application in order to evaluate the possibility to reduce the fertilizer application rates and improve the qualitative and quantitative yield characteristics of the zucchini plants.

Table 1 Principal characteristics of the soil analysis

	0–30	30–60
EC µS/cm	96	88
pH	6.37	5.95
P (mg/kg)	130	121
K (mg/kg)	276	255
Na (mg/kg)	1.6	1.8

Materials and Methods

Field Experiment

The present study was conducted in summer season of 2014 at the Faculty of Horticulture, Mendel University in Brno, Lednice, Czech Republic, (GPS location 48°47′36, 858″N 16°49.526″E) to study the interactive effect of two bio-stimulantus, i. e., Synthetic strigolacton (named as Fenyl 7-isomer, origin VUOS s. a., CZ) and Pentakeep® Super and their combination under different levels of NPK on growth, chemical constituents and yield of squash. Seeds of Zucchini squash (*Cucurbita pepo* L.) cv Nefertiti were used in this study. The seeds were sown on 6, June in the open field on drippers line with a distance of 30 cm between planted seeds with 3 m length and 1.0 m between each two dripper lines. Each experimental plot area consisted of one dripper line which equals 3 m^2 (10 plants/plot). The soil in the experimental pots was collected from a conventional farming field, and its principal characteristics are given in Table 1.

Treatments and Experimental Design

The experiment contained 16 treatments which were all combination of four NPK fertilization and four bio-stimulants and their combination. The treatments were arranged in a split plot design, with three replications. NPK fertilizer rates assigned as the maim plot while, bio-stimulants treatments were assigned as the subplot factor.

NPK Fertilizer Application

The NPK fertilizer levels were: 0%, 75%, 100% and 150% of the recommended fertilizer rate (designated as NPK_0, NPK_{75}, NPK_{100} and NPK_{150}). The NPK fertilizer was applied on a N-equivalent basis i. e., 120 kg/ha^{-1} as a recommended rate using mineral fertilizer of 18.13.8.

The quantity of NPK fertilizer was divided into four equal portions and added to each plot after three weeks from sowing and repeated with 2 weeks' intervals.

Bio-Stimulants Application

Four foliar application including two bio-stimulants treatments., viz Synthetic strigolacton (Fenyl 7-izomer) was prepared by synthesis of 3-methyl-2 (5H)-furanone and phenyl-butyrolactone. Fenyl 7 in a mixture with citric acid has been converted into a mixture of surfactant (MDGE PEGSHO + 1: 1). Fenyl 7-izomer has been used for spraying (dose 2 ml/L of water). Pentakeep® Super at the rate of 2 m/L according to the manufacturer's recommendation (Cosmo Seiwa Agriculture Co., LTD. Japan) and their combination as well as check treatment (water) (designated as F, P-s and F+P). Spraying treatments were started three weeks after sowing date and repeated 3 times 15 days' intervals throughout the growing season.

Evaluated Parameters

Vegetative Characteristics Measured

Growth parameters data were taken one week following the last foliar application for measurements. These include leaf number and fresh and dry weight of leaves from the three plants from each replicate.

Leaf Chlorophyll Content and Chemical Constitutes

Was determined using the fourth full mature upper leaf, one week following the last foliar application, a chlorophyll meter (SPAD-502, Minolta corporation, Ltd., Osaka, Japan) was used to take readings from the fully expanded functional leaves. Twentyone leaves were measured randomly per plot and averaged to a single SPAD value for each treatment.

The leaf samples were oven-dried at 70 °C. Samples of dry leaves were digested using concentrated sulfuric acid and H_2O_2 and analyzed for N-P-K nutrients as described in Cottenie et al. (1982). N-No_3 in zucchini fruit was determined by using Merck test strips as described by Jemison and Fox (1988).

Yield and its Components

Edible fruits from each subplot were harvested for eight times number, weight of fruits were recorded and fruit yield was obtained by summing up the successive harvesting intervals and expressed in Kg/m^2.

Statistical Analysis

All data were statistically analyzed by ANOVA using the SAS package (1996). Duncan's multiple range test was per-

Table 2 Mean comparison of the interaction effect of bio-stimulants and NPK rates on leaf number

Treatments	Leaves number				
	NPK rates				
	0	75%	100%	150%	Mean
Control (water)	27.38[a]	37.54[a]	40.88[a]	42.16[a]	*36.99[B]*
F	35.8[a]	39.52[a]	43.63[a]	44.43[a]	*40.85[AB]*
P-s	33.27[a]	38.85[a]	44.81[a]	42.61[a]	*39.89[AB]*
F+P	37.09[a]	41.70[a]	46.47[a]	47.71[a]	*43.24[A]*
Mean	*33.38[B]*	*39.41[A]*	*43.95[A]*	*44.23[A]*	–

Values with common letter in the same column are not significantly different at 5%level
F Fenyl 7-izomer, *P-s* Pentakeep super

formed at $P = 0.05$ on each of the significant variables measured.

Results and Discussion

Plant Growth

Results presented in the Table 2 showed the effect of four fertilization rates and application of two bio-stimulants either alone or it's combination as well as the interaction effect on vegetative growth characters of zucchini plants, expressed as leaf number and fresh and dry weight of leaves.

In term of fertilizer rates, there were several effects seen. There was a general trend of increase in growth characters with increase in fertilizer up to the highest level of NPK_{150}. Plants that received NPK_{100} and excessive NPK_{150} led to significant increase of growth characters with clear superiority to NPK_{150} relative to unfertilized control (except of NPK_{100} for leaves fresh weight which statistically lacking). However, the leaves dry weight of the plants received NPK_{150} was almost double of the unfertilized plants. The improvement of vegetative characteristics with increase in fertilizer rate could be attributed to high N supply and increased uptake of N and its associated role in chlorophyll synthesis and hence the process of photosynthesis and carbon dioxide assimilation (Jasso-Chaverria et al. 2005). Nitrogen has been reported to govern plant growth by virtue of being a major constituent of chlorophyll, protein, amino acids and which plays a crucial role in photosynthetic activity (Sumeet et al. 2009). Similarly, based on higher leaf biomass, results can be attributed to the aforementioned increase in the leaf number and may be due to the role of nitrogen in creating the plant fresh and dry matter as well as many energy-rich compounds which regulates photosynthesis and plant production (Wu et al. 1998). However, Gent et al. (2005) reported that further increases in nitrogen enhance growth and yield, which could be attributed to the fact that plants have the ability to take up N that is not necessarily transformed into dry matter and hence growth. Our findings coincide with those of earlier studies carried out on squash reported that the plants treated with 80, 120 and 160 kg N ha-1 were different from the control (0 *N*) by about 21.0% in influencing the number of leaves and biomass yield from 120 and 160 kg N ha-1 was about 99% higher than the control Ng'etich et al. (2013).

As for bio-stimulants, there were several effects seen, leaf number and fresh and dry weight of zucchini leaves were effectively increased with the two bio-stimulants application either solely or in combination relative to untreated treatment. These effects were significant and greater with application of Fenyl 7-izomer when coupled with Pentakeep Super.

The magnitude effect with the combination of F+P than single factor may be attributed to the integrated of each other and such this integration could be suggested to increase the effect of the individual application upon growth characters. Therefore, these results may be also attributed to the stimulatory effect upon photosynthetic pigments and minerals content (Table 3 and 4).

How strigolactones can affect different physiological processes within plants we are only just beginning to understand the relevance of strigolactone effect on growth characters.

However, in a few cases, the mechanisms behind the physiological and biochemical effects of bio-stimulants are unknown, since the heterogeneous nature of raw materials and substances that are used for bio-stimulant production do not allow to understand the mode of action. The interaction effect between NPK rates and bio-stimulants for leaf number and fresh and dry weight of leaves was insignificant, indicating that both factors act separately.

SPAD Reading

Results presented in the Table 4 showed the effect of four fertilization rates and application of two bio-stimulants either alone or it's combination as well as the interaction effect on chlorophyll content. Compared with respective control, chlorophyll reading increased significantly with increase in NPK supply up to NPK_{75} then tended to decrease slightly as nitrogen levels increased further up to recom-

Table 3 Mean comparison of the interaction effect of bio-stimulants and NPK rates on fresh and Dry weight of leaves

Treatments	Leaves fresh weight (gm/plant) NPK rates					Leaves dry weight (gm/plant) NPK rates				
	0	75%	100%	150%	Mean	0	75%	100%	150%	Mean
Control	739.92^{a}	1230.70^{a}	1416.49^{a}	1749.96^{a}	*1284.27^{C}*	56.43^{a}	80.37^{a}	108.56^{a}	131.05^{a}	*94.10^{B}*
F	1438.33^{a}	1641.78^{a}	1848.26^{a}	1917.29^{a}	*1711.41^{B}*	78.99^{a}	104.92^{a}	144.62^{a}	202.44^{a}	*132.74^{A}*
P-s	1269.04^{a}	1538.89^{a}	1349.15^{a}	1427.22^{a}	*1396.08^{C}*	76.74^{a}	100.15^{a}	129.67^{a}	145.01^{a}	*112.89^{AB}*
F+P	1673.62^{a}	1843.33^{a}	2107.44^{a}	2369.10^{a}	*1998.35^{A}*	104.25^{a}	126.63^{a}	145.00^{a}	162.65^{a}	*134.63^{A}*
Mean	*1280.23^{B}*	*1563.67^{AB}*	*1680.33^{AB}*	*1865.87^{A}*	–	*79.10^{C}*	*103.02^{BC}*	*131.96^{AB}*	*160.28^{A}*	–

Values with common letter in the same column are not significantly different at 5%level
F Fenyl 7-izomer, *P-s* Pentakeep super

Table 4 Mean comparison of the interaction effect of bio-stimulants and NPK rates On Spad reading and nitrate content

Treatments	Spad NPK rates					Nitrate content NPK rates				
	0	75%	100%	150%	Mean	0	75%	100%	150%	Mean
Control	424^{c}	478^{a}	480^{a}	451^{b}	*458^{A}*	148$^{g–i}$	164$^{e–g}$	267^{b}	305^{a}	*221^{A}*
F	416^{cd}	428^{c}	425^{c}	414^{cd}	*421^{B}*	141$^{h–j}$	125^{j}	170^{ef}	181^{e}	*154^{D}*
P-s	361^{f}	410^{cd}	380^{e}	337^{g}	*372^{C}*	133^{ij}	146$^{g–i}$	177^{ef}	211^{cd}	*167^{C}*
F+P	412^{cd}	424^{c}	419^{cd}	400^{d}	*413^{B}*	183^{e}	158$^{f–h}$	201^{d}	221^{c}	*191^{B}*
Mean	*403^{C}*	*435^{A}*	*426^{B}*	*400^{C}*	–	*151^{C}*	*148^{C}*	*204^{B}*	*230^{A}*	–

Values with common letter in the same column are not significantly different at 5%level
F Fenyl 7-izomer, *P-s* Pentakeep super

mended rate. While, an excessive level of NPK supply up to 150% of the recommended rate has a negative effect and kept the chlorophyll level at the same concentration of standard control. The significant response of chlorophyll content leading to more effective use when both are at a satisfactory level (NPK_{75} and NPK_{100} of recommended rate) may be an indication that nitrogen was taken up by the plant and subsequently utilized in cell multiplication, amino acid synthesis and energy formation that acts as structural compound of the chloroplast which carries out photosynthesis. Nitrogen has been reported to be a constituent of chlorophyll (Lawlor 2002). However, nitrogen insufficiencies have been reported to reduce the individual leaf area index, and total leaf area resulting to reduced surface light interception for photosynthesis (Cechin and Fumis 2004).

On the other hand, the chlorophyll reduction under high fertilization levels (NPK_{150}) may be attributed to the increase of N up to a certain threshold increases up to a maximum level, but the further increase of N supply beyond the threshold affects their content negatively (Kirnak et al. 2005). As for bio-stimulants effect, generally, the results showed that spraying zucchini plants with F or P-s either alone or in combination decreased chlorophyll content compared to control. The most effective and significant values were recorded by F+P. A tight relation was found between ALA content in cells and photosynthetic activity in plants (Yaronskaya et al. 2006; Memon et al. 2009). The improved chlorophyll content and photosynthetical activity of Pentakeep, noted also by Kisvarga et al. (2015).

The interaction effect between NPK fertilizer and bio-stimulants application on chlorophyll content was significant, indicating that both factors did not act separately. The best combination of treatments were NPK_{75} and NPK_{100} of the recommended rate associated with the mixture of F+P with no significant differences between them compared with all possible combinations tested.

Nitrate Content

As for NPK rates effect, the results showed that, increasing NPK rates to 75% of the recommended rate did not affect nitrate content as compared to NPK_0. Conversely, as expected nitrate content significantly increased rapidly up NPK_{100} and successive increases in NPK level up to 150% were associated with a corresponding and significant increases in nitrate content Table 4. The possible explanation may be argued due to NPK application amplified the nitrate content and significantly higher under high NPK level. These results in accordance with the results reported by Liu et al. (2006) indicated, the values of soluble solids and VC in cucumber fruits decreased at the highest level of N, whereas the nitrate content increased.

The nitrate content of zucchini fruits was significantly lowered by individual application of F and it rapidly significantly increased when sprayed plants with P-s either alone

Table 5 Mean comparison of the interaction effect of bio-stimulants and NPK rates on NPK content

Treatments	Nitrogen Content% NPK rates					Phosphorus Content% NPK rates					Potassium Content% NPK rates				
	0	75%	100%	150%	Mean	0	75%	100%	150%	Mean	0	75%	100%	150%	Mean
Control	2.22^{f}	3.09$^{c–e}$	2.82^{e}	3.25$^{c–e}$	*2.85*^{B}	0.39^{d}	0.63$^{a–c}$	0.66^{ab}	0.43 cd	*0.53*^{A}	3.79^{a}	4.06^{a}	4.98^{a}	4.72^{a}	*4.39*^{A}
F	2.82^{e}	2.76^{ef}	3.56$^{a–d}$	3.64$^{a–c}$	*3.17*^{A}	0.56$^{a–d}$	0.53$^{a–d}$	0.56$^{a–d}$	0.67^{ab}	*0.58*^{A}	3.77^{a}	4.15^{a}	4.68^{a}	5.25^{a}	*4.46*^{A}
P-s	2.68$^{e–f}$	2.99^{de}	4.01^{a}	4.11^{a}	*3.45*^{A}	0.45$^{b–d}$	0.64$^{a–c}$	0.71^{a}	0.60$^{a–c}$	*0.60*^{A}	3.40^{a}	3.93^{a}	4.99^{a}	4.76^{a}	*4.27*^{A}
F+P	3.45$^{a–d}$	3.13$^{c–e}$	3.09$^{c–e}$	3.85^{ab}	*3.38*^{A}	0.46$^{b–d}$	0.69^{a}	0.67^{ab}	0.61$^{a–c}$	*0.61*^{A}	3.76^{a}	3.89^{a}	4.25^{a}	4.76^{a}	*4.16*^{A}
Mean	*2.76*^{D}	*2.97*^{C}	*3.37*^{B}	*3.71*^{A}	–	*0.47*^{B}	*0.62*^{A}	*0.65*^{A}	*0.58*^{A}	–	*3.68*^{B}	*4.0*^{A}	*4.73*^{A}	*4.87*^{A}	–

Values with common letter in the same column are not significantly different at 5%level
F Fenyl 7-izomer, *P-s* Pentakeep super

Table 6 Mean comparison of the interaction effect of bio-stimulants and NPK rates on yield and its components

Treatments	Number of fruits/Plant NPK rates					Fruit weight NPK rates					Total yield kg/plot (10 plants) NPK rates				
	0	75%	100%	150%	Mean	0	75%	100%	150%	Mean	0	75%	100%	150%	Mean
Control	6.13^{d}	7.14$^{b–d}$	8.08$^{a–d}$	7.19$^{b–d}$	*7.13*^{D}	0.27^{f}	0.34^{de}	0.48^{b}	0.44^{bc}	*0.36*^{C}	16.49^{h}	23.52^{g}	35.03^{de}	34.63^{de}	*27.32*^{B}
F	6.51^{cd}	7.78$^{a–d}$	8.92^{ab}	7.95$^{a–d}$	*7.69*^{C}	0.33^{e}	0.56^{a}	0.48^{b}	0.55^{a}	*0.48*^{A}	21.10^{g}	40.29$^{b–d}$	42.88^{bc}	46.4^{ab}	*37.67*^{A}
P-s	7.87$^{a–d}$	8.64$^{a–c}$	8.98^{ab}	8.58$^{a–c}$	*8.52*^{A}	0.36^{de}	0.38^{de}	0.47^{b}	0.47^{b}	*0.41*^{B}	28.28^{f}	31.52^{ef}	40.97^{bcd}	41.26$^{b–d}$	*35.51*^{A}
F+P	7.64$^{a–d}$	7.97$^{a–d}$	9.30^{a}	8.48$^{a–c}$	*8.35*^{B}	0.40^{cd}	0.46^{b}	0.55^{a}	0.43^{bc}	*0.46*^{A}	29.246^{ef}	38.57^{cd}	49.59^{a}	38.68^{cd}	*39.03*^{A}
Mean	*7.04*^{D}	*7.87*^{C}	*8.82*^{A}	*8.05*^{B}	–	*0.34*^{D}	*0.44*^{C}	*0.49*^{A}	*0.47*^{B}	–	*23.78*^{C}	*33.47*^{B}	*42.12*^{A}	*40.25*^{B}	–

Values with common letter in the same column are not significantly different at 5%level
F Fenyl 7-izomer, *P-s* Pentakeeo super

or in combination with F and such effects were more distinct by F+P treatment. These results might be explained as the Pentakeep-super is classified as a liquid N fertilized and foliar treatment can induce more accumulation of N-NO_3 in plant.

As for the interaction effect, nitrate showed increased content in Zucchini fruits with the higher fertilizer rate (150%) associated with exogenous application of F+P.

Nutrients Contents

Irrespective to bio-stimulants treatments, the results showed that, by increasing NPK supply from 75 up to 150% from recommended rate the N content increased and the differences among of them were insignificant. This indicates that zucchini responded to fertilizer application by enhancing leaves biomass due to higher N content (Table 5).

As for bio-stimulants effect the results showed that, spraying zucchini plants with P-s or F either alone or in combination increased N content compared to corresponding untreated plants.

These results may be attributed to P-s considered independently of N fertilization and foliar treatment can induce more accumulation of N in plant tissue.

As for Interaction between NPK rates and bio-stimulants, results showed that sole application of P-S in combination with the NPK150 were more effective and gave the highest N content (Table 5).

A similar trend was noted respecting to P content that, all NPK rates significantly increased P content with no significant differences among of them. This result is logic based on the increased P supply level an increase in the P content could be expected.

Pentakeep-S or Fenyl 7-izomer either in single application or in combination did not significantly affect P concentration in plants in comparison to plants without foliar nutrition.

The interaction effect between NPK rates and bio-stimulants for P content was significant, indicating that both factors did not act separately. It was found that sole applications of P-s associated with NPK100 supply showed the higher content of P.

As for K content, compared to unfertilized treatment, a significant increase trend in K content with increasing NPK rate up NPK150 but still statistically similar to another NPK rates (75 and 100%) Treatments with the various biostimulants and their interaction with NPK rates did not have any significant influence on the K content of the leaves, as can be seen in Table 5.

Yield and Yield Components

The yield data of the treatments are presented in Table 6, there were eight harvests which started at 44 days after sowing and seven harvests were carried out. Zucchini yield and its components varied widely under different levels of NPK

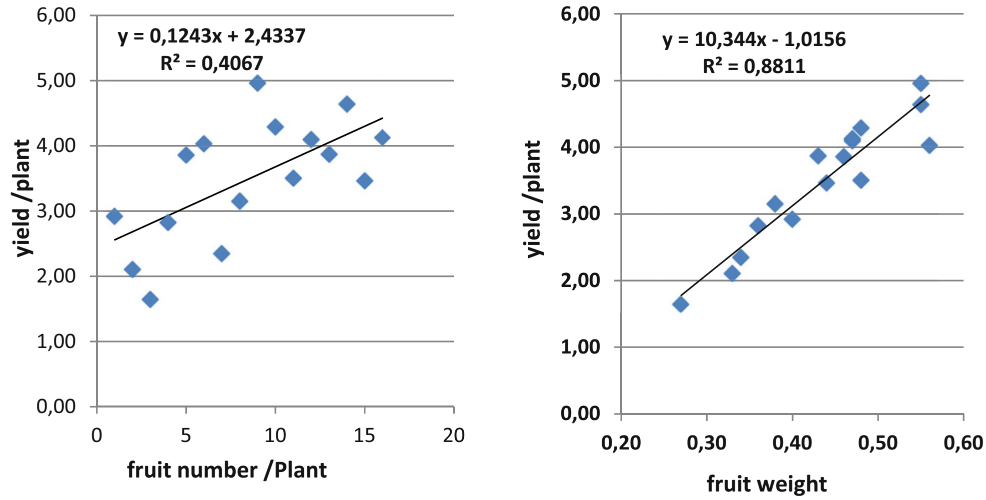

Fig. 1 Relationship between fruit yield and fruit number per plant (**a**), and average fruit weight (**b**), of zucchini grown under different NPK and bio-stimulants

and bio-stimulants application. The results showed that, fertilizer levels significantly ($P < 0.05$) influenced zucchini fruit number, weight and yield. Yield and its component increased significantly with increase in NPK supply up to 100% of recommended rate, then tended to decrease slightly as nitrogen levels increased further. Plants that received the 100% of recommended rate resulted in higher fruit yield being (42.25 kg m-2). However, there were significant reductions on yield and its components when applying less or more of 100%. compared to other fertilizer levels. This was 76.8, 25.8 and 4.6% higher than that of NPK0, NPK.75 and NPK150 of the recommended rate, respectively.

The results of this study revealed that the application of nitrogen fertilizer increases fruit yield of zucchini to a point where further increase in fertilizer results into a decline in productivity. The decline in yield at high NPK rates could be explained by the fact that a high concentration of soluble N increases the osmotic potential of the soil solution, causing reduction in water uptake by the plant roots (Onyango 2002). According to Wei et al. (2009) excess nitrogen application causes osmotic stress, which can cause oxidative damage injuring many important cellular components, such as lipids, protein, DNA and RNA leading to reduced growth and eventual yield of plants. Also it is known that the increase of N produces an increase in the yield to reach a maximum value, but if the supply continues increasing, the production is affected in a negative way (Purqueiro et al. 2003; Kirnak et al. 2005). Zotarelli et al. (2008) reported that when the N amount was excessive, an increase in all components of the N balance (uptake, soil content, leaching, etc.) was produced. N uptake is the only one of these factors that could affect productivity, but this excess N uptake resulted in a luxury consumption and higher growth, but not in higher yields. However, our results are line with Ng'etich et al. (2013), reported that, Maximum zucchini fruit yield achieved from plants subjected to 120 kg Nha1 and then decline with high N rate (160 Nha-1).

Irrespective of the fertilizer treatments, the productivity of zucchini squash was significantly influenced positively by the application of all bio-stimulants, which are the object of this study. However, the order of productivity was the same: F+P > F > P > control. The significant increases of total yield over the control induced by these treatments were 42.9, 37.9 and 30%.

The interaction effects between NPK rates and bio-stimulants application reflect significant response in fruit yield. The data shown that at NPK rate up 100% and all bio-stimulants either singly or in combination were associated with a corresponding and significant increases in fruit. The combined treatment, which included 100% of NPK plus foliation with the mixture of F+P can be considered the best treatment and it gave the highest fruit yield. The increase in the total yield of zucchini plants was about 200.7% compared the least yield in the combination of plants grown with NPK0 and sprayed with water. From a legal point of view, the bio-stimulants can contain traces of natural plant hormones, but their biological action should not be ascribed to them, otherwise they should be registered as plant growth regulators. However, the correlation analysis between fruit yield and fruit number was weak (R2 = 0.4076), indicating that fruit number was not the main reason for yield increment (Fig. 1a). On the other hand, the correlation analysis between fruit yield and average fruit weight showed a strong correlation (R2 = 0.8811), which indicates that the increase in fruit yield in different treatments was attributed mainly to the increase in fruit weight (Fig. 1b).

One of the important function of strigolactones is inhibiting of side shoots growth and thus the plant branching. Such a limitation was desirable for tomatoes, where the plant could rather invest its growth potential in fruits formation instead of branching (Umehara et al. 2008). Similar effect could occur in zucchini where the plant weight was increased after Fenyl or Fenyl+Pentakeep application regardless of the fertilisation level.

In order to increase crop, yield the combination of reasoned fertilization in combination with bio stimulants could be a possible way for proper integrated plant production.

Conclusions

Based on the present study, it could be concluded that NPK at the recommended rate (100%) gave the best fruit yield and its components. The combination of Fenyl 7-izomer plus Pentakeep-super treatment had the best effect on total yield. While, individual application of Pentakeep-super gave the highest fruit number.

Funding This work was supported by grant TA02020544 of The Technology Agency of the Czech Republic.

Conflict of interest R. Pokluda, S.M. Shehata and T. Kopta declare that they have no competing interests.

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