DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 64, 2018 (2): 49−56

Original paper DOI: 10.2478/agri-2018-0005

VERMICOMPOST AND *EISENIA FOETIDA* AS FACTORS INFLUENCING THE FORMATION OF RADISH PHYTOMASS

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KOVÁČIK, P. – ŠALAMÚN, P. – WIERZBOWSKA, J.: Vermikompost and *Eisenia foetida* as factors influencing the formation of radish phytomass. Agriculture (Poľnohospodárstvo), vol. *64*, 2018, no. 2, pp. 49–56.

Many of the world's findings indicate the positive effect of earthworms on soil parameters, and consequently, on the formation of phytomass of cultivated plants. In our experiment we studied: A) the influence of soil itself, soil mixed with vermicompost in a ratio of 9:1; B) the influence of earthworms number (genus *Eisenia foetida*, 10 and 20 individuals per pot) supplied to soil mixed with vermicompost in the ratio of 9:1 on the dynamics of changes in the weight of radish roots, the total chlorophyll content in leaves and the selected qualitative parameters of the roots. The results obtained showed that one tenth proportion of vermicompost from the total weight of soil substrate caused the statistically significant increase in the total chlorophyll content in leaves, the increase in yield of radish roots, the reduction of the vitamin C content and the increase of nitrate content in the roots. The impact of earthworms on the chlorophyll content in leaves and on the root weight was negative. The addition of 10 individuals of earthworms into 20 kg of substrate (soil + vermicompost) resulted in the increased content of vitamin C and the decreased content of nitrates in the radish roots. Twenty earthworms added to vermicompost, compared to vermicompost alone, did not affect the vitamin C content and reduced the nitrate content.

Key words: earthworms, *Eisenia foetida*, radish, vermicompost, vitamin C

Despite the sharp increase in knowledge from the area of plant nutrition optimization and soil protection observed in the recent years, many data have only limited territorial applicability. For this reason, more attention should be paid to obtaining such information, which could be used in the largest possible agricultural area and effectiveness of it would be long-term.

From this aspect plant nutrition through vermicomposts enables biotechnologically and energetically undemanding utilization of by-products or waste products from a wide range of industrial and agricultural activities, which is one of the ways of increasing soil fertility, the use of which has assumptions of applicability on each agricultural land.

The product of composting with help of earthworms is a vermicompost. The vermicomposting is the utilization of the waste products by the technology of earthworm breeding, most frequently from the genus *Eisenia foetida*. The earthworms mix the predigested organic matters with soil in the alimentary tract. They create relatively waterproof aggregates excreted in the form of cylinders that have the positive impact on the soil parameters (Albanell *et al.*

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1988; Jouquet *et al*. 2010). The usage of vermicompost affects the firmness of cells of cultivated plants that are then more resistant to pests (Sinha & Valani 2011). The use of vermicomposts increases the content of total antioxidants, carotenes, lycopene, carbohydrates, vitamin C, proteins, dry matter, iron and zinc in cultivated plants (Gutiérrez-Micely *et al*. 2007; Shankar *et al*. 2009; Sinha *et al*. 2011; Ghosh *et al*. 2013).

The vermicompost production has been recognized for several decades (Chan & Griffiths 1988; Datar *et al*. 1997; Jeyabal & Kuppuswamy 2001). In spite of that, its production will have a significant impact also in the future decades because the contemporary society produces more and more new types of wastes, which have to be involved ecologically and energetically into the matter circulation. Therefore, the research of the effective production of vermicomposts and their further usage is still continuing.

Earthworms in soil are bio-indicators of the soil environment and quality of soils (Smith *et al.* 2008; Boyer & Wratten 2010; Fazekašová & Bobuľská 2012). The activity of earthworms increases the content of available nitrogen, phosphorus and potassium (Tripathi & Bhardwaj 2004; Garg *et al*. 2006; Doan *et al*. 2015), it also improves the soil structure (Adhikary 2012), but their impact on plants has not been examined sufficiently. Zero, positive and negative plant responses to the presence of earthworms were recorded (Eisenhauer *et al*. 2009; Doan *et al*. 2013; Nurhidayati *et al*. 2016). For this reason, the future research needs to be focused on testing the impact of individual species of earthworms on individual plant species.

The aim of our contribution is to give an answer to the question of how the application of the vermicompost without earthworms and with earthworms of the genus *Eisenia foetida* effect the quantity and quality of the radish yield.

MATERIAL AND METHODS

The pot experiment was carried out in the vegetation cage located in the area of the Slovak University of Agriculture in Nitra. The size of the cage was 20 m × 20 m × 5 m. On its sides and ceiling there was the metal mesh with the size of a mesh 15 mm × 15 mm, which protected the experiment against birds.

The experiment was established on March 13, 2017. Soil (20 kg – treatment 1) and soil with vermicompost (20 kg) mixed in the rate 9:1, i.e. 18 kg soil and 2 kg vermicompost (treatment 2, 3, 4) was put into the cylindrical pots of the height 35 cm and diameter 35 cm. Ten individuals of adult earthworms (*Eisenia foetida*) were placed to the pots of the treatment number 3, and twenty individuals of earthworms were introduced to the pot of the treatment 4. The used soil (Haplic Luvisol) was taken from the field located in Párovské Háje, (cadaster Nitra), in particular, from the upper horizon of soil 0.0–0.3 m. The weighed out pots were placed into the dishes, which were able to keep 1,000 ml of the leaked soil solution during the period of precipitation. The leaked through solution was returned back to the pots. The agrochemical parameters of the used soil and the soil mixed with vermicompost (VC) are indicated in the Table 1. We used the following analytical methods for the indication of the given parameters: N-NH_4^+ by Nessler's colorimetric method; N-NO_3^- by colorimetric method with phenol – 2,4 disulphonic acid, where the extract from

Table 1

Parameters of the soil substrates used in the experiment

Substrate	N_{min}	P	K	Ca	Mg	S	N_t	C_{ox}	C:N	EC [mS/cm]	pH_{KCl}
	[mg/kg]						[%]				
Soil	13.20	21.90	156	4,250	444	1.3	0.077	0.915	11.88	0.14	6.97
Soil + VC	91.65	87.78	3,925	4,270	966	938	0.367	4.908	13.37	1.23	6.99

VC – vermicompost

soil was achieved by using the water solution 1% K_2SO_4. N_{min}= N–NH_4^+ + N–NO_3^-. The contents of available P, K, Ca, Mg were determined by Mehlich 3 extraction procedure (Mehlich 1984). The content of P was determined by colorimetric method, K by flame photometry, Ca and Mg by atomic absorption spectrophotometry, S spectrophotometrically (in the leachate of ammonium acetate), N_t by distillation after the mineralization of strong H_2SO_4 (Kjeldahl - Bremner 1960), C_{ox} spectrophotometrically after the oxidation (Tyurin 1966), EC by the method of specific electrical conductivity and pH/KCl (in solution of 1.0 mol/dm^3 KCl) potentiometrically (Kováčik 1997).

The experiment was established according to the method of random arrangement of pots with the quadruple repetition. The model crop was radish (*Raphanus sativus* L.) cultivar Granát. The sowing was carried out on March 16. Subsequently, the experiment was irrigated to the level of 75% FWC. In the following three weeks all pots were irrigated by the same dose of water containing the minimal quantity of nutrients. During the last 14 days the treatments 2, 3 and 4 were irrigated by a higher dose of water, because in these treatments the plants vaporized more water as a result of the significantly larger leaf area. During the growth season (April 24, May 3, and May 9, i.e. in 27/39, 36/48 and 42/54 days after germination/sowing) three samplings of plant material were accomplished. (The plants of radish germinated after 12 days from sowing.) 10 average individuals were taken from each treatment and repetition, which served for the evaluation of the root weight.

For the analysis of the pigment content, the last fully developed leaves were used. The photosynthetic pigments were determined in the acetonic extract by spectrophotometric method using the equations of Lichtenthaler (1987). The diameter of the root thickness was measured by the slide calliper. The nitrate content and vitamin C content in the roots of the second and third sampling was detected. The quantity of vitamin C was determined by titration with 2,6-dichlorophenolindophenol. In order to determine nitrates we used ion-selective electrode of the type 07-35 and reference electrode of the type RCE 101 (Monokrystaly Turnov). The second and third samplings were carried out in six days interval because radish is usually harvested in two or more terms.

The acquired results were processed by mathematical and statistical method, by analysis of variance (ANOVA) and linear regression analysis using Statgraphics PC program, version 5.0. The differences between the treatments were evaluated subsequently by *LSD* test at the significance level α = 0.05.

RESULTS AND DISCUSSION

Growing of radish sown in the soil with 10% content of vermicompost (Trt. 2) in comparison with the soil without vermicompost (Trt. 1) increased considerably root weight. The positive impact of VC was measurable from April 24, i.e. 27 days from the beginning of germination (Table 2). On the 36th day of the growth season (May 3) there was the most

T a b l e 2

Impact of vermicompost on the dynamics of the changes in the weight of the radish roots

Treatment		24. IV.		3. V.		9. V.	
number	mark	[g/10ind.]	[%]	[g/10ind.]	[%]	[g/10ind.]	[%]
1	control	1.95	100.00	20.03^a	100.00	27.83^a	100.00
2	VC	2.83	145.13	148.70^c	742.39	185.80^c	667.62
3	VC + EW_{10}	1.87	95.90	106.13^b	529.86	141.95^b	510.06
4	VC + EW_{20}	1.53	78.46	95.15^b	475.04	127.88^b	459.50
$LSD_{0.05}$				12.686		20.527	

[g/10ind.] – g/10 individuals; VC – vermicompost; EW_{10} – ten earthworms; $LSD_{0.05}$ – least significant difference at the level α = 0.05

considerable difference. In this period of the growth season the root yield was 7.42 times higher in the treatment with 10% content of VC than in the control treatment. On the 42nd day of the growth season (May 9) the highest root yields were achieved in all treatments, however, the differences of the yields between the treatments were decreased.

The diameter of roots in the unfertilised treatment was so low that those roots were unmarketable, similarly on 36th as well as on 42nd day of the growth season. The unmarketability was related to the achieved root diameter. According to the Slovak Technical Standards 46 3120 the radish roots grown in the field conditions must have the diameter bigger than 2.0 cm. The average root size in the control treatment was smaller than 2.0 cm and it achieved from 1.44 to 1.68 cm (Table 3).

During the whole radish growth season the presence of earthworms in soil had the negative impact on the root weight (Trt. 2 versus Trt. 3 and Trt. 3 versus Trt. 4 – Table 2). The negative impact of earthworms was evident especially at the beginning of the growth season (April 24), when the root weight in the treatments 3 and 4 was lower compared to the treatment 1. The smallest root mass was in the treatment containing the most worms (Trt. 4). This fact is probably the consequence of the attack of the earthworms at root hair, which appears more considerably in younger plants.

In the period of technological ripeness (second and third sampling) the radish roots were 4.6 to 5.1 times heavier in the treatments with vermicompost and earthworms (Trt. 3 and 4) than in the control treatment (Trt. 1), however, the roots were smaller than in the treatment with vermicompost without earthworms (Trt. 2). The differences in root mass between treatments which contained earthworms and treatment without earthworms (Trt. 3 and 4 versus Trt. 2) were significant. The differences between the treatments with the earthworms (Trt. 3 versus Trt. 4) were not significant. Nevertheless, it can be stated that with the growth of the number of earthworms in the soil, the weight of the roots decreased. The recorded differences in the weight of the roots between the treatments with the number of 10 and 20 individuals of earthworms (Trt. 3 versus Trt. 4) were in the second sampling of 10.3% and in the third sampling at 9.9%. The discovered facts are not consistent with the major opinion of public and scientists on the positive impact of earthworm on the growth of plants (Friberg *et al*. 2005; Groenigen *et al*. 2014) but they are consistent with the opinion of Ayuke *et al*. (2017) who are stating that the mechanism of action of earthworms is not still well known. Our findings are consistent with the findings of Doan *et al*. (2013) who along with the positive effects of earthworms noted also the negative effects of earthworms on plants. Ayuke *et al*. (2017) did not notice the influence of earthworms on the formation of rapeseed phytomass (*Brassica napus*).

Depending on the agrotechnical conditions of plant cultivation and weather conditions during the growth season, the pigment contents usually increase, but the declines are recorded too (Vician *et al*. 2012). In this experiment on the 48th day after sowing the radish had a higher content of chloro-

T a b l e 3

Impact of vermicompost on the change in the diameter of the radish roots and on qualitative parameters of radish roots

Treatment		Roots diameter		Viatmin C		$NaNO_3$	
		3. V.	9. V.	3. V.	9. V.	3. V.	9. V.
number	mark	[mg/kg]				[cm]	
1	control	1.44^{a}	1.68^{a}	114.88^{c}	161.84^{c}	738^{a}	570^{a}
2	VC	3.13^{c}	3.29^{c}	91.92^{a}	115.00^{ab}	$1,649^{d}$	$1,480^{c}$
3	VC + EW_{10}	2.77^{bc}	2.80^{bc}	101.92^{b}	127.52^{b}	$1,032^{b}$	815^{b}
4	VC + EW_{20}	2.77^{b}	2.56^{b}	91.92^{a}	110.00^{a}	$1,131^{c}$	816^{b}
$LSD_{0.05}$		0.411	0.530	7.667	14.549	87.636	55.831

VC – vermicompost; EW – earthworms; $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%

phyll than the plants on the 54th day from sowing (Table 4).These discovered findings confirmed that after the period of growth, in the senescence period, the leaves of plants were losing gradually chlorophyll. The decrease in chlorophylls content was from 4.02% to 10.7% but the decrease in carotenoids content was more pronounced. It reached the values from 10.3% to 19.4%. It has been found out that the carotenoid contents in comparison with the total chlorophyll contents react more considerably to the ageing process of the radish leaves.

The vermicompost application increased significantly the content of total chlorophylls and did not affect the carotenoid contents in the radish leaves. However, the opposite conclusions were recorded by Ali (2007), who determined the decrease in chlorophyll content with an increasing proportion of vermicompost in the substrate. The significant decrease occurred only when the cultivation substrate consisted solely of vermicompost. Adding earthworms to vermicomposts reduced the total chlorophylls content. The size of the drop depended on the num-

T a b l e 4

Impact of vermicompost on pigments formation in radish leaves

Treatment		3. V. (48 days a.s)	9. V. (54 days a.s)	3. V.	9. V.
		Total chlorophyll		Carotenoids	
number	mark	[mg/m²]			
1	control	281.3[a]	270.0[a]	64.3[c]	51.8[a]
2	VC	335.0[c]	299.3[c]	59.7[b]	51.8[a]
3	VC + EW_{10}	325.0[bc]	292.0[bc]	59.2[ab]	53.2[a]
4	VC + EW_{20}	315.0[b]	286.0[b]	58.7[a]	52.5[a]
$LSD_{0.05}$		16.426	12.991	0.717	1.735

VC – vermicompost; EW – earthworms; $LSD_{0.05}$ – least significant difference at the level $\alpha = 0.05$; different letter behind a numerical value respond to the statistically significant difference at the level 95.0%; days a.s – days after sowing

T a b l e 5

Correlation coefficient r expressing the relationship between the radish quantitative and qualitative yield parameters and the total chlorophyll content occurring in the last fully developed leaves in the particular terms

Parameter		Correlation coefficient (r)	n
dependent	independent		
Weight of roots	total chlorophyll (3. V.)	0.935[++]	16
	total chlorophyll (9. V.)	0.832[++]	16
	total chlorophyll (3.V. and 9. V. together)	0.482[++]	32
Vitamin C	total chlorophyll (3. V.)	–0.666[++]	16
	total chlorophyll (9. V.)	–0.632[++]	16
	total chlorophyll (3.V. and 9. V. together)	–0.719[++]	32
$NaNO_3$	total chlorophyll (3. V.)	0.814[++]	16
	total chlorophyll (9. V.)	0.712[++]	16
	total chlorophyll (3.V. and 9. V. together)	0.753[++]	32
Roots diameter	total chlorophyll (3. V.)	0.875[++]	16
	total chlorophyll (9. V.)	0.796[++]	16
	total chlorophyll (3.V. and 9. V. together)	0.609[++]	32

[+]statistically significant ($P < 0.05$); [++]statistically high significant ($P < 0.01$); n – number of measurements ber of added earthworms. Ten earthworms per 20 kg of cultivation substrate had an insignificant impact. Twenty earthworms had a significantly negative impact. It can be assumed that the earthworms by consuming amino acids, organic nitrogenous substances found in the growing substrate, at various degrees of degradation, limited the amount of inorganic nitrogen but also other nutrients entering the plant. The relationship between the N content but also Mg and other nutrients and the amount of chlorophyll in the plant is generally known (Evans 1983; Bojovič & Markovič 2009; Liu *et al*. 2012; Saberioon *et al.* 2014; Gholizadeh *et al.* 2017). Earthworms did not affect the amount of carotenoids in the leaves.

The diameter of radish roots grown in the treatments with earthworms complied with the requirements of traders. It was more than 2.0 cm (Table 3). The small diameters and weights of radish roots detected in the treatment 1 emphasize the fact that radish grown in the soil with the relatively sufficient supply of N_{min}, K, Ca and Mg (Table 1), without the application of nutrients (inorganic or mineral fertilisers) did not create the prerequisites for the growing marketable yield (commercially realizable yield).

The influence of earthworms on the diameter of the radish roots was identical with the effect on chlorophyll content in leaves and on the weight of the roots. It is evident that the amount of chlorophyll influenced significantly the diameter of the radish roots, and consequently, the weight of the radish roots. This finding is supported by the values of the correlation coefficient *r* between the amount of total chlorophyll and the diameter of the root between the amount of total chlorophyll and the weight of the roots (Table 5). Similarly, Kim *et al*. (2015) have noticed a strong interaction between the morphology of tea tree root and earthworms.

The analysis of roots related to two qualitative parameters – content of vitamin C and content of nitrates - confirmed that there is the negative correlation between these two parameters (Kováčik 2014; Wang *et al.* 2017). The presence of vermicompost in the substrate (Trt. 2) increased the content of nitrates and decreased the content of vitamin C (Table 3). The effect of the earthworms added to the vermicompost depended on the number of earthworms supplied. Ten earthworms significantly increased vitamin C content and reduced nitrate content. Twenty earthworms had a negative effect on vitamin C content (Trt. 4 versus Trt. 3). It reduced the content. The effect on nitrate content was negative on the 48th day from sowing (increased the content) and on the 54th day it was insignificant. Similar findings were obtained by Nurhidayati *et al*. (2016), who by testing of three species of vermicomposts discovered that in the most cases with the growing number of earthworms in the cultivaiting substrate, the cabbage plants decreased both the vitamin C content and the sugar content.

The content of vitamin C in the treatment with 20 earthworms (Trt. 4) was the same as in the treatment with vermicompost without earthworms (Trt. 2) and the nitrate content in treatment 4 was lower than in treatment 2. In both treatments with earthworms (Trt. 3 and 4), there were significantly less nitrates in the radish roots than in the comparable treatment 2 without earthworms. The data from the Table 3 also show that on 54th day after sowing (which is the last suitable date for harvest) there were determined less nitrates and more vitamin C than 6 days ago, i.e. on 48th day after sowing. Based on this finding, we can say that the older plants contained less nitrates and more vitamin C than younger ones.

CONCLUSIONS

The impact of earthworms on the chlorophyll content in radish leaves and on the root weight and its diameter was negative. The addition of 10 individuals of earthworms (*Eisenia foetida*) into 20 kg of substrate (soil + vermicompost) resulted in the increased content of vitamin C and the decreased content of nitrates in the radish roots. Twenty earthworms added to vermicompost, compared to vermicompost free of earthworms, did not affect the vitamin C content and reduced the nitrate content in roots. The tenth ratio of vermicompost out of the total weight of soil substrate caused a statistically significant increase in the total chlorophyll content in leaves, the increase in yield of the radish roots, reducing the vitamin C content and increasing the nitrate content in the roots.

Acknowledgments. This article was created on the basis of the solution of project VEGA No. 1/0704/16.

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Received: November 2, 2017

DE GRUYTER OPEN
Agriculture (Poľnohospodárstvo), 64, 2018 (2): 57−64
Original paper DOI: 10.2478/agri-2018-0006

RESPONSE OF TWO POTATO VARIETIES TO IRRIGATION METHODS IN THE DRY MEDITERRANEAN AREA

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MUBARAK, I. – JANAT, M. – MAKHLOUF, M.: Response of two potato varieties to irrigation methods in the dry Mediterranean area. Agriculture (Poľnohospodárstvo), vol. *64*, 2018, no. 2, pp. 57–64.

Due to water scarcity and dry Mediterranean conditions, improving water use efficiency is a major challenge for sustainable crop production and environment protection. Field experiments were conducted for two consecutive years (2010 and 2011) to assess the effects of variety and irrigation method on potato crop, following a 2 × 4 factorial experiment type arranged in a split plot design with two spring potato varieties (Spunta and Marfona), and four irrigation methods (drip irrigation with two modes of dripper spacing/dripper flow: 30 cm at 4 l/h and 60 cm at 8 l/h, sprinkle irrigation, and furrow irrigation), with three replicates. Potato was irrigated when soil moisture in the active root depth was within the range of 75–80% of field capacity as determined by the neutron probe technique. Results did not show any differences between both varieties. Moreover, no differences in marketable yield, total dry matter, and harvest index were found between irrigation methods. However, results showed that sprinkle irrigation significantly enhanced nitrogen use efficiency. Furthermore, both water productivity and irrigation water use efficiency were significantly increased under drip irrigation compared with the other irrigation methods. They were about twice those under furrow irrigation, indicating that the employment of drip irrigation method can effectively address water shortage and sustainable potato production, in the dry Mediterranean region.

Key words: drip irrigation, sprinkle irrigation, water productivity, irrigation water use efficiency, ^{15}N, nitrogen use efficiency

Potato (*Solanum tuberosum* L.) is one of the most economical crops grown worldwide, with a total production of about 365 million ton (FAOSTAT 2014). It is a shallow-rooted crop and more sensitive to soil water stress than other deeper-roots crops. Its water requirements vary with locations, agricultural practices, and soil types. In regions where water resources are scarce, as in the dry Mediterranean region, efficient water use is an urgent need to meet a sustainable crop production for substantial food demands. Moreover, higher benefits may be acquired by adapting suitable irrigation techniques (Ati *et al.* 2012; Badr *et al.* 2012; Eskandari *et al.* 2013; El-Mokh *et al.* 2015; Matovic *et al.* 2016).

Drip irrigation method proved useful in improving water and fertiliser use efficiencies, enhancing yield, reducing the environmental pollution risk, and its amenability to conform to irregularly shaped fields, compared with sprinkle and surface irrigation (Tiercelin 2007; Matovic *et al.* 2016). Similar studies have reported that drip irrigation is well suited for row crops such as potato production (Ati *et al.* 2012; Eskandari *et al.* 2013; Cantore *et al.* 2014; Matovic *et al.* 2016). Moreover, several studies have shown that although no significant differences in tuber yield were observed between furrow and drip irrigation methods, the drip irrigated potato consumed less water relative to the other irrigation

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methods, resulting in higher water use efficiency. The huge amount of water applied when using sprinkle and furrow irrigation methods combined with the need to apply nitrogen fertiliser at high rates may result in the lose of nitrate and other nutritive components to deep percolation (Ati *et al.* 2012; Onder *et al.* 2015).

Potato production occupies a very important place in Syria, with more than 25,000 ha of planted area and an average marketable yield of about 19.7 t/ha for various potato varieties grown in different climatic conditions, soils, and cropping systems (Annual Agricultural Statistics 2011). Traditionally, sprinkle and furrow irrigation methods are the most common irrigation practices used in potato production in the Mediterranean cropping system. Generally, drip irrigation method is not widely adopted for potato production in Syria because of the high initial investment compared to other used irrigation methods. Currently, because of water scarcity, growers have been encouraged to adopt drip irrigation by Syrian government in order to enhance water productivity. Although the cost of its installation has relatively dropped recently, drip irrigation is still uncommon and its use has seen a moderate increase only.

In this context, the objective of the study reported herein was to assess the effects of various irrigation methods, including drip irrigation, on two potato varieties widely grown in Syria. The obtained results may encourage the introduction of alternative and more practical irrigation methods that would sustain potato productivity while using less water in the context of water savings and environmental protection.

MATERIAL AND METHODS

Site description and field procedure

Field experiments were conducted at Teezeen's Irrigation Research Station, near Hama, Syria (35°48′ N, 36°27′ E), for two consecutive growing seasons 2010 and 2011. The area is characterized by the dry Mediterranean type climate. The total annual rainfall is about 350 mm, on average. Some climatic data collected during the course of those experiments are shown in Table 1.

Representative soil samples were taken up to a depth of 90 cm in 15 cm increments prior to planting. The soil type is most likely Inceptisols (classified as Calcixerollic Xerochrept), had a clayey texture throughout the 90 cm soil profile. Some selected soil properties are shown in Table 2. The experimental field was conventionally prepared, i.e., disked, ploughed, and bedded into 0.75 m hills before planting. Two spring potato varieties Spunta and Marfona were planted on the 23rd Feb and 11th Feb of the year 2010 and 2011, respectively, spaced 0.75 × 0.20 m apart, giving about 67,000 plants/ha.

Irrigation treatments

The experiment was a split plot design with a 4 × 2 factorial type. Treatments consisted of four irrigation methods as main-plot treatments, and two spring potato varieties as subplot treatments, in three replicates.

The 1st irrigation method was a set system of sprinkle irrigation, including 12 sprinklers spaced at 9 × 12 m, covering an experimental plot of 12 × 60 m. The 2nd and 3rd methods were two regimes of drip irrigation with two different modes of dripper spacing/dripper flow: the 1st was with 30 cm at 4 l/h (Drip30) and the 2nd with 60 cm at 8 l/h (Drip60). In both drip methods, amounts of irrigation water per unit area were identical. An experimental plot of 24 × 60 m was used for drip-irrigated potato. Each crop row had its own dripline. The 4th irrigation method was furrow irrigation, with an experimental plot of 9 × 60 m. A minimum spacing of 5.0 m was maintained between plots to minimize water intervention among treatments. Under each irrigation method conditions, the plot was further divided into six subplots (experimental units) for both potato varieties with three replicates. Each experimental unit was of 30 × 3 m with four crop rows spaced 0.75 m.

Soil water content (SWC) was observed using neutron probe technique. Access tubes were installed in the central row of each experimental unit. This technique enabled monitoring SWC status in the root zone and provided feedback data for irrigation scheduling. The active root depths, which were determined by soil moisture depletion curve generated by neutron probe feedback data, were 0.30 m during the period from planting until the middle of tuber initiation, and then reached up to 0.60 m until maturation, under both sprinkle and drip irrigation methods. However, under furrow irrigation method, active root depths were 0.45 and 0.75 m at both periods, respectively. These active root depths were used to calculated irrigation water amounts needed in each irrigation event. Water was applied when SWC in active root zone reached 75 to 80% of field capacity. A reduction factor due to the ground cover percentage was used under drip irrigation methods as 0.5 from planting until the flowering stage, and 0.75 until termination period. Volumes of water applied were monitored by flow meters.

Fertiliser application

In 2010, 46.0 kg of P_2O_5/ha as triple super phosphate (TSP), 48 kg of K_2O/ha as potassium sulphate, and 30.0 kg of N/ha as urea (20% of the total N amount) were broadcasted before planting according to the soil availability index for all experimental units under all irrigation methods. The remaining amount of N (120 kg N/ha) was applied in four equally split applications. In 2011, 78 kg P_2O_5/ha as TSP, 39 kg K_2O/ha as potassium sulphate, and 37 kg N/ha as urea (20% of the total N amount) were applied before planting. The remaining amount of N (142 kg N/ha) was added in three equally split applications. During the growing seasons, the added N-fertilisers were either broadcasted for the sprinkle and furrow irrigated plots or injected through the drip method for the drip-fertigated plots.

T a b l e 1

Climatic data of the experimental site during both studied growing seasons

Year	Variable	February	March	April	May	June
2010	T_{max} [°C]	14.1	18.0	22.9	28.6	33.1
	T_{min} [ºC]	8.6	9.6	12.9	17.6	22.6
	$T_{average}$ [°C]	13.1	15.7	20.3	25.9	30.0
	RH [%]	82.0	73.0	59.3	50.0	47.0
	Bright sunshine [h]	4.3	6.7	9.4	11.3	12.6
	Rainfall [mm]	30.5	8.8	2.4	0.0	0.0
2011	T_{max} [°C]	14.2	16.0	20.2	25.6	31.8
	T_{min} [°C]	8.5	7.6	12.4	16.0	22.0
	$T_{average}$ [°C]	12.6	13.9	18.0	23.1	29.3
	RH [%]	81.0	75.0	68.7	61.3	48.0
	Bright sunshine [h]	5.5	7.9	7.8	10.0	12.1
	Rainfall [mm]	42.7	55.3	26.1	14.8	0.0

T_{max} – maximum temperature; T_{min} – minimum temperature; $T_{average}$ – average temperature; RH – relative air humidity

T a b l e 2

Selected chemical and physical properties of soil at the experimental site

Depth [cm]	pH (1:5)	EC [ds/m]	Avail-P [ppm]	Total N [%]	OM [%]	Soil texture
0–15	8.4	0.30	12.8	0.079	0.77	Clay
15–30	8.5	0.30	10.3	0.078	0.75	Clay
30–45	8.3	0.32	7.1	0.076	0.75	Clay
45–60	8.6	0.33	4.4	0.063	0.78	Clay
60–75	8.3	0.34	4.2	0.054	0.63	Clay
75–90	8.3	0.29	4.5	0.046	0.70	Clay

EC – electrical conductivity; OM – organic matter

In each experimental unit under all irrigation methods, 1.0 m^2 labelled subplot was established at the end of the central row, and fertilised with a 2% atom excess ^{15}N-labeled urea at the same rate as the specified N applications.

Plant sampling and analysis

At the tuber bulking stage, two whole plant samples from the labelled subplots of all experimental units were collected. The samples were separated into two parts, tubers and aboveground vegetative part. The two parts were weighed and then oven dried at 65°C, weighed again for dry matter yield determination (TDM), then ground and analyzed for ^{15}N atom excess (a.e %) and total N content percentage (Zapata 1990). Isotopic ^{15}N (a.e %) was determined by emission spectrometry using Jasco-N 150 emission spectrometer. Nitrogen use efficiency (NUE) was calculated according to Zapata (1990). Harvest index (HI) at bulking stage was calculated by dividing the tuber biomass by the whole plant bio-mass (the sum of tuber and aboveground biomass) of the collected plants from the labelled subplots of all experimental units. At harvest (mid-June for both seasons), tuber yield was determined from the yield subplot (the whole plot except the labelled subplot). Yield was sorted into marketable (MY) and non-marketable yield according to a local grade.

Water productivity and irrigation water use efficiency

Potato evapotranspiration (ETc) was calculated using the water balance equation:

$$ETc = 1 + P - Dp - Ro \pm \Delta(SWC) \qquad (1)$$

where: I is the amount of irrigation water applied [mm], P is the precipitation [mm], Dp is the deep percolation [mm], and Ro is the amount of runoff [mm], Δ(SWC) is the change in soil water content [mm] in the specified soil profile. Since the amount of irrigation water was controlled, runoff was assumed to be zero. Observing SWC showed that the deep percolation was negligible below 0.60 m in depth. Water productivity (WP) and irrigation water use efficiency (IWUE) were calculated using equations [2] and [3], and were expressed as kg per m^3 of water. WP was calculated as the relationship between fresh marketable yield (MY, t/ha) and seasonal evapotranspiration (ETc). Whereas, IWUE was calculated as the relationship between MY and the total amount of irrigation water applied [m^3/ha].

$$WP = \frac{MY}{ETc} \qquad (2)$$

$$IWUE = \frac{MY}{I} \qquad (3)$$

Statistical analysis

The measured variables, i.e., fresh marketable yield (MY) at harvest, total dry matter yield (TDM) at the tuber bulking stage, water productivity (WP), irrigation water use efficiency (IWUE), nitrogen use efficiency (NUE), and harvest index (HI) were subjected to a two-way analysis of variance using the DSAASTAT add-in version 2011 (Onofri 2007). A combined analysis of data over the two studied years was performed to identify spring potato variety and irrigation methods whose average effect over years is stable and high (Gomez & Gomez 1984). Mean comparison was made only for data after combined analysis using the least significant difference test (*LSD*) at the 5% level of significance.

RESULTS AND DISCUSSION

Marketable tuber yield (MY) and total dry matter (TDM)

In 2010 growing season, marketable yield (MY) was affected by both tested factors (potato variety and irrigation method); while in 2011 the same trend was not observed, which might be attributed to the seasonal effect, manifested by lower average temperature and higher precipitation in 2011 compared with in 2010 (Table 1). However, the combined analysis over the two years showed no significant differences between both varieties, nor between tested irrigation methods, nor by their interaction (Tables 3 and 4).

The mean marketable yields were 27.9 and 25.8 t/ha,for Spunta and Marfona varieties, respectively. Although not significant, Spunta MY was 8% higher than Marfona MY, regardless of the irrigation method used. The mean MY for both potato varieties were 25.8, 26.2, 28.8, and 26.5 t/ha, under drip (Drip30 and Drip60), sprinkle, and furrow irrigation methods, respectively. The average MY of both potato varieties under sprinkle method was 10% higher, although not significant, than MY under the other irrigation methods (Table 4). Similar results were reported which showed that the drip and furrow irrigation methods had no significant impact on tuber yield over years (Ati *et al.* 2012; Onder *et al.* 2015).

TDM at bulking stage was also not affected by either potato variety or irrigation method, in both years (Table 3). The mean TDM of Spunta variety was 10.9 t/ha which was about 11% lower, although not significant, than the mean TDM of Marfona (12.1 t/ha) regardless of the irrigation method. The mean TDMs for both potato varieties were 9.6, 11.9, 13.5, and 11.0 t/ha, under Drip30, Drip60, sprinkle, and furrow irrigation methods, respectively. Although not significant, the percentage of increase in TDM yield under sprinkle method was 28.7, 12.0, and 18.5% compared with corresponding TDM under Drip30, Drip60, and furrow methods, respectively (Table 4).

Regression analysis revealed that aboveground biomass and tuber yield at the bulking stage were not good parameter for estimating the fresh marketable tuber yield at harvest under both drip and furrow-irrigation methods (Table 5). This result is in agreement with the findings of Janat (2007). However, a linear regression was found to be significant under sprinkle irrigation conditions (r = 0.62 with $p < 0.05$).

Harvest index (HI)

The combined analysis over the two years showed that the harvest index was not affected neither by the tested factors nor by their interaction (Table 3). So, no significant differences were found between both varieties, or between irrigation methods (Table 4). This finding could be explained by

Table 3

Analysis of variance for marketable tuber yield (MY), total dry matter (TDM), harvest index (HI), water productivity (WP), irrigation water use efficiency (IWUE), and nitrogen use efficiency (NUE), as affected by years, varieties, and irrigation methods (significance of *F-test* values)

Source of variance	*df*	MY	TDM	HI	WP	IWUE	NUE
2010							
Irrigation methods (I)	3	++	ns	ns	++	++	++
Varieties (V)	1	++	ns	ns	++	++	ns
I × V	3	ns	ns	ns	ns	ns	ns
Error	8	–	–	–	–	–	–
2011							
Irrigation methods (I)	3	ns	ns	ns	++	++	ns
Varieties (V)	1	ns	ns	ns	ns	ns	ns
I × V	3	ns	ns	ns	ns	ns	ns
Error	8	–	–	–	–	–	–
Combined analysis 2010–2011							
Irrigation methods (I)	3	ns	ns	ns	++	++	++
Varieties (V)	1	ns	ns	ns	ns	ns	ns
Interactions							
I × V	3	ns	ns	ns	ns	ns	ns
Year × I	3	ns	ns	ns	ns	ns	ns
Year × V	1	ns	ns	ns	ns	ns	ns
Year × I × V	3	ns	ns	ns	ns	ns	ns
Error	16	–	–	–	–	–	

++ – significant at 1% level; ns – non-significant at 5% level; *df* – degree of freedom the fact that MY and TDM were comparable under the different irrigation methods. Since the harvest index was calculated by dividing the tuber biomass by the whole plant biomass, and because there were no significant differences between tuber and whole plant biomass, the differences in harvest index values were very close for both potato varieties and irrigation methods. The lack of HI response might also be attributed to the uniformity of the lengths of growing season regardless of the irrigation method used, since all treatments were planted and harvested at the same time. This finding is in agreement with Janat (2007). The harvest index values reported in this study ranged between 0.71 and 0.78, although differences were non-significant.

Water productivity and irrigation water use efficiency (WP and IWUE)

During the growing season (mid-February to mid-June), the mean values of the maximum (T_{max}), minimum (T_{min}), overall average ($T_{average}$) temperatures, to which the plants were exposed in 2010 were, respectively, 7.0, 6.3, and 7.6% warmer than in 2011. Concerning the relative air humidity (RH), the air was also 6.5% drier in 2010 than in 2011. Spring potato plants received a total amount of 41.7 mm of rain in 2010, while in 2011 they received 138.9 mm (Table 1). Due to the relative differences between both years concerning climatic conditions (seasonal effects), different amounts of irrigation water were applied. The irrigation water

T a b l e 4

Mean values of the marketable yield (MY) at harvest, total dry matter yield (TDM) at the tuber bulking stage, harvest index (HI), water productivity (WP), irrigation water use efficiency (IWUE), and nitrogen use efficiency (NUE) as affected by years, varieties, and irrigation methods

Yield parameters										
Irrigation method	Variety	MY [t/ha]*			TDM [t/ha]*			HI*		
		Year			Year			Year		
		2010	2011	Av.**	2010	2011	Av.**	2010	2011	Av.**
Drip30	Sp	31.6	20.7	25.8[a]	8.1	9.2	9.60[a]	0.78	0.75	0.77[a]
	Ma	28.5	22.4		12.0	9.0		0.68	0.85	
Drip60	Sp	32.0	23.8	26.2[a]	10.3	9.4	11.9[a]	0.75	0.83	0.78[a]
	Ma	30.7	18.4		15.9	11.7		0.71	0.84	
Sprinkle	Sp	36.9	23.3	28.8[a]	18.7	9.1	13.5[a]	0.69	0.69	0.71[a]
	Ma	32.2	22.6		16.8	9.3		0.72	0.74	
Furrow	Sp	31.1	23.7	26.5[a]	9.7	12.4	11.0[a]	0.67	0.84	0.78[a]
	Ma	28.0	23.3		9.9	12.0		0.77	0.84	

Water and fertiliser use parameters										
Irrigation method	Variety	WP [kg/m³]*			IWUE [kg/m³]*			NUE [%]*		
		Year			Year			Year		
		2010	2011	Av.**	2010	2011	Av.**	2010	2011	Av.**
Drip30	Sp	7.38	6.84	7.07[a]	7.62	8.28	7.94[a]	14.0	31.7	24.8[b]
	Ma	6.66	7.41		6.87	8.97		21.5	32.1	
Drip60	Sp	7.48	7.86	7.15[a]	7.72	9.52	8.00[a]	17.9	23.9	23.2[b]
	Ma	7.17	6.10		7.40	7.38		19.8	31.3	
Sprinkle	Sp	6.16	6.16	5.92[b]	6.26	7.39	6.57[b]	35.9	65.6	56.7[a]
	Ma	5.37	5.99		5.46	7.18		62.5	62.9	
Furrow	Sp	3.90	4.01	3.84[c]	3.98	4.46	4.11[c]	17.8	34.3	28.6[b]
	Ma	3.51	3.95		3.58	4.40		26.6	35.6	

*Average of three replications
**Within a column, means followed by the same letter are not significantly different at 5% level according to *LSD* test

applied to drip, sprinkle, and furrow methods were, 4,146; 5,899; and 7,808 m^3/ha in the 2010 season, and 2,500; 3,147; and 5,303 m^3/ha in the 2011 season, respectively. As expected, the drip-irrigated potato crop needed less water than the other methods. The seasonal evapotranspiration (ETc), as calculated using Eq.[1], was 428, 599, and 797 mm under drip, sprinkle, and furrow methods conditions in the 2010 season, respectively; whereas, in the 2011 season they were 303, 377, and 591 mm, respectively. Similar studies found that seasonal ETc for potato crop ranged from 350 to 800 mm under various environmental conditions (Ati *et al.* 2010; Badr *et al.* 2012; Cantore *et al.* 2014; Onder *et al.* 2015)

Analysis of variance revealed that both WP and IWUE were highly significantly influenced by both tested factors (potato variety and irrigation method) in 2010; while in 2011 WP and IWUE were affected only by the irrigation factor. Nevertheless, the combined analysis of data over the two years confirmed that both WP and IWUE were significantly affected by the irrigation method factor at 1% level (Table 3). As none of the interaction effects involving irrigation methods in the combined analysis was significant at the 5% level, the data was, therefore, averaged over the two years and both potato varieties as can be seen in Table 4 (Gomez & Gomez 1984).

Although no significant differences were observed in marketable tuber yield between irrigation methods, both modes of drip irrigation significantly increased WP and IWUE relative to sprinkle and furrow irrigation methods. The highest values of WP and IWUE were 7.15 and 8.00 kg/m^3, respectively. While the lowest values of WP and IWUE were 3.84 and 4.11 kg/m^3, respectively, which were obtained under furrow irrigation. The percentages of increase in WP and IWUE values under drip irrigation were about 20% compared to sprinkle, and about 95% relative to furrow irrigation. This indicates the potential of drip irrigation in saving water and improving WP and IWUE of potato crop. Onder *et al.* (2015) concluded that if water is in short supply, the drip irrigation method offers higher water productivity compared to the surface method. Other studies have also reported increased WP and IWUE under drip irrigation (Janat 2007; Ati *et al.* 2012; Badr *et al.* 2012; Cantore *et al.* 2014).

T a b l e 5

Regression analysis of yield at harvest vs. the whole plant biomass (tuber and aboveground biomass) at the bulking stage (r = coefficient of correlation)

r – values	Whole plant biomass at the bulking stage
Yield at harvest under Drip30	0.22^{ns}
Yield at harvest under Drip60	0.51^{ns}
Yield at harvest under sprinkle	0.62^{+}
Yield at harvest under furrow	0.39^{ns}

$^{+}$ – significant at 5% level; ^{ns} – non-significant at 5% level

Nitrogen use efficiency (NUE)

Nitrogen use efficiency was assessed from the ^{15}N labelled subplots for the whole plant (tubers + aboveground) at bulking stage. The combined analysis of data over the two years confirmed the main effect of irrigation methods on NUE, but none of the interactions were significant at the 5% level (Table 3). Thus, data was averaged over both years and potato varieties (Table 4). Noticeable increase in nitrogen use efficiency was observed under sprinkle-irrigated potato (56.7%) relative to drip- and furrow-irrigated potato. The value of NUE under sprinkle irrigation was as twice as those values of the other irrigation methods.

In general, NUE values under drip fertigation and furrow irrigation observed in the present study were relatively low. This could be explained by the fact that in this study NUE values were calculated up to the bulking stage only. A relatively large part of the applied nitrogen fertiliser could be recovered by the plant at a later stage due to translocation into the tubers (Westermann *et al.* 1988; Janat 2007; El-Mokh *et al.* 2015). Lower NUE under drip and furrow irrigation could also be attributed to the lateral movement of ^{14}N from its subplots to the adjacent ^{15}N subplots and vice versa. This movement could be facilitated by water movement which is bi-dimensional under drip and furrow conditions relative to the mono-dimensional movement of water under sprinkle irrigation. On the other hand, furrow irrigation may induce N fertiliser leaching beyond the root zone, resulting in reduced NUE (Janat 2007).

CONCLUSIONS

The following conclusions can be obtained from the agro-pedo-climatic context of the experiments:

- both studied factors (potato variety and irrigation method) performed consistently, and were not influenced by environmental conditions between years,
- non-significant differences were observed between the two tested spring potato varieties.
- marketable tuber and total dry matter yields were not affected by irrigation methods,
- the highest value of nitrogen use efficiency was obtained in sprinkle-irrigated potato relative to drip- and furrow-irrigated ones,
- Seasonal evapotranspiration was considerably reduced and water productivity and irrigation water use efficiency were significantly enhanced under drip-irrigated potato crop, relative to the other irrigation methods. So, adopting drip irrigation can effectively address water shortage and its consequences, for sustainable potato crop production, in the dry Mediterranean region.

Acknowledgements. The authors would like to thank the Atomic Energy Commission of Syria for the financial support.

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Received: December 10, 2017

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 64, 2018 (2): 65−79

Original paper DOI: 10.2478/agri-2018-0007

MAIN SULPHUR CONTENT IN ESSENTIAL OIL OF *ERUCA SATIVA* AS AFFECTED BY NANO IRON AND NANO ZINC MIXED WITH ORGANIC MANURE

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MAHMOUD, A.W.M. – TAHA, S.: Main sulphur content in essential oil of *Eruca sativa* as affected by nano iron and nano zinc mixed with organic manure. Ariculture (Poľnohospodárstvo), vol. *64*, 2018, no. 2, pp. 65–79.

Profitable prospective of rocket plant is progressively growing recently. Hence an experiment was conducted in open field to evaluate the effect of chicken manure, nano iron (Fe), nano zinc (Zn) and combination of them on morphological, fresh weight and seed yield, photosynthetic rate, transpiration rate, water use efficiency and chemicals constituents represented in macro and micro elements, plant pigments, total phenolics, total carbohydrate, alkaloids, tannins, flavonoids, ascorbic acid, crude protein, total fatty acids, indole acetic acid (IAA) and abscisic acid (ABA) hormones, oil seed yield and methylthiobutyl-isothiocyanate as main sulphur content in essential oil of *Eruca sativa* Mill. compared to chemical fertilisers (NPK) as control. Results revealed that, nano Fe and Zn treatments either alone or in combination with manure had the upper hand, where significantly increased almost all parameters under study in comparison with control. The outcomes of present research gave emphasis to global warning about pollution of chemical fertilisers and safety production.

Key words: chemicals constituents, nano Fe and Zn, oil yield, photosynthetic rate, rocket plant

Rocket salad (*Eruca sativa* Mill.) is a well known fresh vegetable that belonged to the Brassica family. The consumption of vegetables as fresh salad has been increased worldwide because of the health consciousness. In Egypt, about 5,000 fadden (one fadden equal 4,200 m^2) are cultivated with rocket plants (FAO 2000). Leaves of rocket are mainly used as an astringent, antiphlogistic, diuretic, tonic, depurative, emollient, laxative, digestive, stimulant, and rubefacient (Yaniv *et al*. 1998). Besides its importance as green salad available all over the year, rocket seeds contain oil which is promising to be medical oil (Khoobchandani *et al*. 2010). Similar to other Brassica crops, rocket salad is known for various phytochemical metabolites such as polyphenols, vitamin C (Kim *et al.* 2006; Martınez-Ballestra *et al*. 2008). It is rich in iron, potassium and sulfur also contains elevated levels of proteins and vitamins A (Porto *et al.* 2013).

Chemical fertilisation has met with success only in some situations, and negative effects of chemical fertilisers application such as a high probability of leaching, spray drift (wind), volatilization, and high energy consumption for their creation*,* the risk of toxic build up of chemicals such as arsenic, cadmium, and uranium in the soil. Long-term use of chemical fertiliser can change the soil pH, hurt helpful microbial ecosystems, enhance pests, stimulation of the vegetative growth and reduction of soil water storage on plant growth in semi-arid region have been reported (Rai *et al.* 2015).

Several reports state the possibility of improving nutritional quality, polyphenols, antioxidants, anthocyanins, vitamins, and minerals in vegetables

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by the organic fertilisers (Peck *et al*. 2006). Among agronomical practices, the advantages of the utilization of organic manure for improving the physicochemical properties of soil have been documented (Rudrappa *et al.* 2006). Numerous researchers showed the importance of manure on increasing crop production. Manure is loaded in nutrients and can provide all macronutrients (N, P, K, Ca, Mg and S) essential for plant growth, as well as micronutrients, furthermore, it creates suitable environment for the activity of soil microorganisms (Fageria 2012).

Micronutrients are nutrients required by plants in small quantities throughout their life to organize a range of physiological functions. Although these elements are used in very small amounts, they are important to plant development and profitable crop production as the macronutrients. The soil in semi-arid regions is characterized by poor structure, low organic carbon content, high pH, low cation exchange capacity and salinity/alkalinity problems, and these conditions increase micronutrient deficiencies (Marschner & Rengel 2012). Among the micronutrients iron and zinc are essential for plant growth and food production. Fe is involved in the production of chlorophyll, photosynthesis, mitochondrial respiration, hormone biosynthesis (ethylene, gibberellic acid and jasmonic acid) production (Marschner & Rengel 2012). Besides, zinc is a necessary component of various enzyme systems for energy production, protein synthesis; it maintains the structural integrity of biomembranes and growth regulation (Alloway 2008; Marschner & Rengel 2012).

In recent years, some researchers tried to examine the potential of nanotechnology to improve efficiency of micronutrients uptake that result in the development of efficient new nano fertiliser delivery systems for field application (Naderi & Danesh-Shahraki 2013; Rameshaiah & Jpallavi 2015). Nano-formulated fertilisers can release nutrients more slowly in cooperation with other fertilisers which may lead to improvement of nutrient use efficiency. The application of zinc nano fertiliser on pearl millet improved some properties such as shoot length, root length, chlorophyll content, total soluble leaf proteins and plant dry biomass. The grain yield at crop maturity was improved by 37.7% due to application of zinc nano-fertiliser in pearl millet (Tarafdar *et al.* 2014). Faizan *et al.* (2017) concluded that presence of nano Zn enhanced the antioxidant systems and improved photosynthetic efficiency in tomato. The best fertiliser treatment for obtaining high seed yield was identified in sulfur plus nano zinc on chickpea (Sabaghnia & Janmohammadi 2017). In *Brassica napus* (Zakerin *et al.* 2014) showed that, the maximum grain yield and oil yield were obtained from nano Zn + Fe foliar application on barley.

The present research aims to investigate alternative source of chemical fertilisers using application of manure, and combination of manure fertiliser with foliar application of nano-iron and zinc on morphological, yield, chemical compositions represented in Methyl-thiobutyl-isothiocyanate (molecular weight: 161.3, $C_6 H_{11} N S_2$) as a main component of essential oil and has been used in variety of medical (particularly breast and testis cancer), industry and cosmetic fields, beside fixed oil constitutes from seed yield of rocket plants.

MATERIAL AND METHODS

Present investigation was done at Kafr Tuhurmis, El-Giza Governorate, with the latitude of 30.021074 and the longitude is 31.173145. Category with the Gps of 30° 1' 15.8664'' N and 31° 10' 23.3220'' E. Elevation is a 24 meters height, Egypt. For four continues repeated seasons during 2015 and 2016, every two cuts represented as one season. Mechanical and chemical analyses of soil were performed at Soil & Water and Environment Research Institute, Agriculture Research Center (A.R.C) according to Richards (1954) and Jackson (1973) shown in Table1.

Plant material, transplant and harvest dates

Seeds of (*Eruca sativa* Mill.) were obtained from experimental farm of Faculty of Pharmacy, Cairo University, and planted in an open area with a distance of 40 cm between rows, and 30 cm spacing between plants in plots with 3 × 5 m^2. The seeds were planted on February 5th and the first cut was taken on March 14th, while the second cut was taken on April 15th 2015, both two cuts considered as the first season. The seeds were planted again on February 5th, the first cut was taken on March 15th, whereas the second one was taken on April 16th 2016; both two cuts considered as the second season.

T a b l e 1

Some physical and chemical characteristics of clay soil used for growing *Eruca sativa* plant

Parameter	Value
Physical characteristics	
Texture	Clay
Clay [%]	40.50
Silt [%]	35.10
Fine sand [%]	21.00
Coarse sand [%]	3.40
pH	7.88
EC [dS/m]	1.63
Organic matter [%]	1.70
Chemical characteristics	
Soluble cations [meq/l]	
Ca^{++}	7.22
Mg^{++}	2.98
K^{+}	0.33
Na^{+}	6.22
Soluble anions [meq/l]	
Cl^{-}	3.50
SO_4^{-}	2.45
Available N [ppm]	30.10
Available P [ppm]	22.50

Land Preparation

Before planting, the soil was first mechanically ploughed and planked twice till the soil surface has been settled, then plots established.

T a b l e 2

Some chemical analysis of used chicken manure used

Parameter	Value
Total macronutrients [%]	
N	2.60
P	0.70
K	1.15
Total micronutrients [ppm]	
Fe	26.5
Zn	34.3
Mn	16.0
Cu	28.5
pH	7.60
C/N ratio	19.7
Organic matter [%]	62.4

Chemical fertilisers added

Chemical fertilisers as recommended dose according to Ministry of Agriculture and Land Reclamation were added at the rate of 50 kg/fed. (fadden equal 4,200 m^2) of calcium superphosphate (15.5%) after 10 days from planting, while 50 kg/fed. ammonium sulphate (20.5%) and 20 kg/fed. of potassium sulphate (40%) were added after every cut.

Chicken manure added

As Ministry of Agriculture and Land Reclamation recommended 5 tons/fed. chicken manure was added to the soil one week before plating.

Nano Fe and Zn preparation and addition

Nano iron was prepared with little modify from magnetite (Fe_3O_4) of Fe^{3+} and Fe^{2+} at a molar ratio of 3:2 by reduction–precipitation in aqueous ammonia (0.3 mol/L) and coated tetramethyl ammonium hydroxide under vigorous stirring for 2 h using magnetic stirring, precipitate was then separated by magnetic filtration using a permanent magnet, and washed with distilled water until a neutral pH was obtained according to Qu *et al*. (1999). While nano zinc was prepared from aqueous solution of zinc sulfate and sodium hydroxide solution was added slowly in a molar ratio of 1:2 under vigorous stirring for 8 h. The precipitate obtained was filtered and washed thoroughly with ionized water. The precipitate was dried in an oven at 100°C, then expose to 15 psi of pressure for 6 hours (Daneshvar *et al*. 2007).

TEM (transmission electronic microscope) Figure 1 (a & b) was done in TEM lab (FA-CURP) Faculty of Agriculture Cairo University Research Park to determine the nano size.

Both Fe and Zn nano particles at concentrations 20 and 40 ppm were applied to *Eruca sativa* plants as foliar at 10, 17 and 24 days after planting in both seasons.

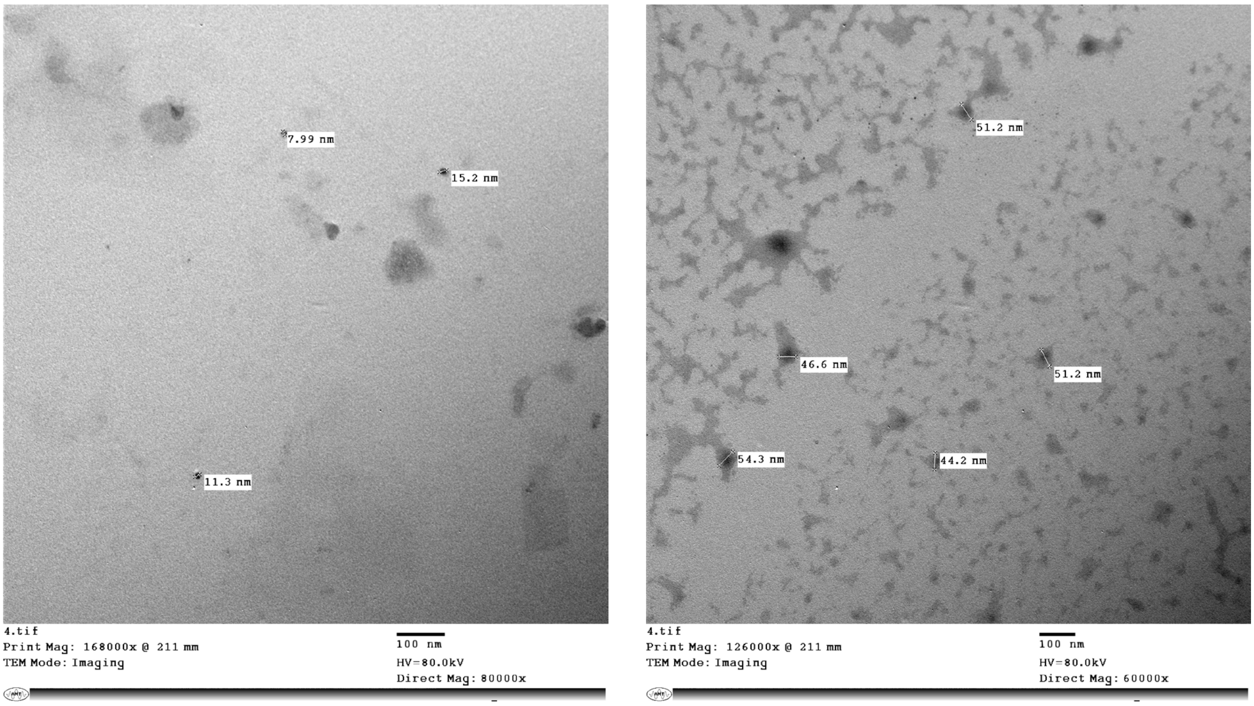
Treatments

- NPK fertilisers (recommended doses) as control,
- Chicken Manure,
- Nano Fe and Zn,
- Nano Fe and Zn + chicken manure.

Data recorded

A. Plant growth parameters upon cuts

- Plant height [cm],



(a) (b)

Figure 1. Zinc nano particles (a) – Iron nano particles (b)

- Leaf area [m^2],
- Plant fresh weight [g],
- Plant dry weight [g],
- Yield fresh weight [t/fadden],
- Seed yield [kg].

Ash percentage was determined quantitatively by grinding 2 g of leaves sample and combusting the material in a muffle furnace at 550°C to constant weight. The total ash content was calculated using the following formula:

$$\% = \frac{A \times 100}{B}$$

where: A – weight of the ash in gram, B – weight of the anhydrous raw material in gram.

Moisture content was determined according to Tsand & Frutani (1989).

B. Chemical analysis

The total nitrogen content of the dried leaves was determined by using the modified- micro-Kjeldahel method as described by Helrich (1990). The nitrogen percentage was multiplied by 6.25 to estimate the crude protein percentages in leaves.

Phosphorus in leaves was determined calorimetrically by using the chloro-stannous molybdophosphoric blue color method in sulphuric acid according to Jackson (1973).

Potassium concentrations in leaves were determined by using the flame photometer apparatus (CORNING M 410, Germany).

Concentration of Ca, Mg, Fe, Mn, Cu, Ni, Pb and Zn were determined using Atomic Absorption Spectrophotometer with air-acetylene fuel (Pye Unicam, model SP-1900, US).

Plant pigments – total chlorophylls and carotenoids content were measured by spectrophotometer and calculated according to the equation described by Moran (1982).

Total carbohydrates in plant leaves were determined by phosphor molybdic acid method according to Helrich (1990).

Total phenolic contents of the extracts were determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi 1965).

Vitamin – C content as ascorbic acid [mg] was estimated in leaves fresh weight according to Helrich (1990) method using 2,6-dichloro phenolindophenol.

The sugars were separated on a chromatographic column filled with cation exchange resin (sulfonated polystyrene-divinylbenzene copolymer in the form of Ca^{2+}). The mobile phase consisted of an aqueous solution of calcium disodium ethylene diamine tetraacetate. The eluted constituents were detected by a refractometric detector and determined by the external standard method.

Total flavonoids were determined using the method of Meda *et al.* (2005).

Tannin contents were determined using Folin-Ciocalteu reagent method as described by Chahardehi *et al*. (2009)

Endogenous phytohormones – the analysis was performed according to Fales and Jaouni (1973) for the determination of indole-acetic acid (IAA) and abscisic acid (ABA). The quantification of the endogenous phytohormones was carried out with Ati-Unicumgas– liquid chromatography, 610 Series, equipped with flame ionization detector according to the method described by Vogel (1975).

Net photosynthesis, stomatal conductance and water use efficiency

Measurements of net photosynthesis on an area basis [µmol CO_2 m^2/s], leaf stomatal conductance [mol H_2O m^2/s], and water use efficiency of five different leaves per treatment was monitored using a LICOR 6400 (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically active radiation, PAR) within the sampling chamber was set at 1,500 µmol m^2/s, using a Li-6400-02B LED light source (LI-COR). The CO_2 flow into the chamber was maintained at a concentration of 400 µmol/mol using an LI-6400-01 CO_2 mixer (LI-COR).

Essential oil in leaves

The leaves from *E. sativa* were hydro-distilled for 3 hours with a Likens–Nickerson-type apparatus, using diethyl ether to get yellowish oil yield.

Gas chromatography

GC–MS was carried out with a Hewlett-Packard with a flame ionization detector (FID) on a capillary column (TC-WAX FFS fused silica 60 m × 0.25 mm i.d). The column temperature was programmed from 60°C to 240°C at a rate of 3°C/min and held at 240°C. The injector and detector temperatures were 250°C and 280°C, respectively.

Fixed oil content [% of the seeds]

Fixed oil was extracted from seeds by using a Soxhlet apparatus. The oil percentage was determined according to the methylation (change fixed oil into fatty acid) and GLC analysis was also recorded by GC according to Kinsella *et al.* (1977).

GLC of fatty acid methyl esters

Separation of fatty acid methyl esters was carried out using capillary column, which contained 15% diethyl glycol succinate (DEGS). The injector port and flame ionization detector were set at 240°C. The flow rate of carrier gas, nitrogen, was 10 ml/min. The gas chromatograph (Perkin-Elemar model 8310) had a temperature program from 100 to 190°C with interment rate of 7°C/minute. The initial and final time were identified according to their retention time compared to those of authentic samples.

Statistical analysis

The experimental design was randomized complete blocks design with ten replicates. The data were analysed using ANOVA at 5% significance level, the difference between treatments, then analysed using DMRT (Duncan Multiple Range Test) at level 5% (Duncan 1955).

RESULTS AND DISCUSSION

Growth parameters and yield

Data related to growth parameters of the rocket plants using different types of fertilisers presented in Table 3 showed that, nano Fe and Zn combined with manure treatments had significantly stimulated growth characteristics with the highest values compared to all other treatments. The integrative nano Fe + Zn + manure application significantly increased plant height, leaf area, plant fresh and dry weight, moisture and ash percentage over either control (recommended dose of NPK fertilisers) or other treatments, and consequently the yield of rocket plants during both seasons.

According to Table 3, it is clear that mixed nano Fe and Zn with manure would not only result in high production of both fresh weight and seed yield but also improved the other desirable agronomic traits for market processes (plant height, leaf area, fresh weight and moisture). Moreover, the combination treatment significantly exceeded the growth parameter, and consequently the seed yield of rocket plants. This integrative addition exceeded seed yield by 26.6 and 14% as compared to NPK fertiliser in both seasons, respectively.

Results of the current study show that vegetative growth parameters were considerably improved by both manure and nano-micronutrient fertilisers. The association of manure and nano Fe + Zn fertilisers may complement the lack of some nutrients of manure fertiliser. Organic manures applied in integration with the inorganic fertilisers gave higher yield than sole chemical fertilizers (Badawi *et al.* 2005; Sarwar *et al*. 2008). The beneficial effects of these treatments on rocket plant are due to nutrients availability to be absorbed by plant roots and improvement of soil's physical, chemical and biological properties as evident by higher water retention, decreased soil pH, and increased soil organic.

The findings of present research are consistent with those of El-Ghamry *et al.* (2009) who found that, farmyard manure plays an important role in supplying some essential plant nutrients. Bala *et al.* (2014) reported that the beneficial role of nano-fertilisers on growth of chickpea had significant effects compared to control without nano-fertilisers. Furthermore, Amirnia *et al.* (2014) have emphasized the positive effects of some micronutrient and macronutrients nano-fertilisers (iron, phosphorus and potassium) on saffron (*Crocus sativus* L.) production. Liu *et al.* (2006) reported that, nano-particles application was safe for wheat production and has some economic benefits and has also shown that nanoparticles get into plant cells through either sto-

T a b l e 3

Effect of NPK, manure and nano Fe and Zn on growth characters of rocket plants during two seasons

Treatment	Plant height [cm]		Leaf area [cm^2]		Fresh weight /plant [g]		Dry weight [g/plant]	
	F1	F2	F1	F2	F1	F2	F1	F2
NPK	24.3^a	22.5^b	67.16^b	69.43^b	30.79^b	32.85^b	7.32^b	8.41^b
Manure	21.1^b	23.1^b	66.85^b	70.20^b	31.58^b	33.62^b	8.61^b	8.55^b
Nano Fe + Zn	20.7^b	22.2^b	68.02^b	70.13^b	32.45^b	33.92^b	7.12^b	8.68^b
Nano Fe + Zn + manure	25.3^a	30.3^a	71.11^a	75.42^a	40.35^a	43.77^a	10.36^a	12.36^a
Treatment	Moisture [% dry matter]		Ash [% dry matter]		Yield F. W. [t/fed.]		Seed yield [kg/fed.]	
	F1	F2	F1	F2	F1	F2	F1	F2
NPK	6.14^b	7.12^b	16.22^b	17.31^b	11.12^b	12.14^b	310.2^c	364.5^c
Manure	7.24^b	7.15^b	17.25^b	18.28^b	11.51^b	12.73^b	340.2^b	369.7^b
Nano Fe + Zn	6.18^b	7.14^b	16.19^b	17.44^b	11.14^b	12.79^b	308.5^c	365.2^c
Nano Fe + Zn + manure	8.15^a	9.23^a	21.15^a	23.45^a	14.29^a	15.30^a	392.8^a	416.5^a

Means with the same letter in a column are not significantly different by DMRT at level 5%
F1 – first season; F2 – second season

matal or vascular system. Our results also showed that the highest seed yield was related to nano Zn + Fe manure as compared to manure fertiliser alone. Nanoparticles with small size and large surface area are expected to be the ideal material for use as a fertiliser in plants. When materials are transformed to a nano-scale, their physical, chemical and biological characteristics as well as catalytic properties even more increased (Mazaherinia *et al.* 2010). In this subject Eichert *et al.* (2008) suggested that, stomatal pathway is highly capacitive because of its large size exclusion limit and its high transport velocity. These scientific reports support the present hypothesis of nanoparticle penetration plant cell through stomatal opening and natural nano-pore which may enhance plant cell metabolic activities hence, lead to higher plant production (Mosanna & Behrozyar 2015).

Plant pigments, carbohydrates, phenols, alkaloids, tannins and flavonoids concentrations

It is evident from Table 4, that manure fertiliser combined with nano Fe + Zn, lead to significant increases in the mean values of chlorophyll, carotenoids, carbohydrates, phenols, alkaloids, tannins and flavonoids concentrations compared with the NPK treatment (control). Total chlorophyll statistically increased by (29.5 and 25.4%) over control during both seasons, respectively, while the increase was bigger than double in concentration of carotenoids. It reached (128 and 120%) during both seasons compared with control. For the carbohydrates and phenols percentage, the presented data revealed that the combined treatment of nano Fe + Zn + manure enhanced the accumulation by (30.3–27.8%) and (31.9–30.4%), respectively as compared to control plants during the two seasons. The same trend was found for alkaloids, tannins and flavonoids percentage.

Previous researches indicated that, fertilisation type had an influence on the phyto-nutritional quality of crops. Chemical fertilisers are believed to reduce the antioxidant levels while organic fertilisers were proven to enhance the antioxidant content in plants (Dumas *et al.* 2003). In tomato, the presence of zinc oxide nano-particles improved the antioxidant systems and photosynthetic efficiency as well (Faizan *et al.* 2017). The increase in total carbohydrates may be due to the increase of photosynthesis (Figure 2) as a result of increase in photosynthetic pigments content in leaves (Table 4). Manure appears to be a source of a number of essential elements that may play an important role in plant metabolism, notably the most significant function would appear to involve carbohydrate metabolism and photosynthesis (Tisdale & Nelson 1975). These results are in agreement with those obtained from Leclerc *et al.*

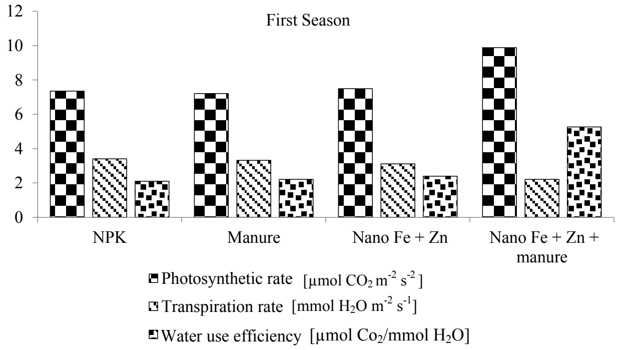
T a b l e 4

Effect of NPK, manure and nano Fe and Zn on chemical characters of rocket plants during two seasons

Treatment	Total chlorophyll F.W. [mg/g]		Total carotenoids F.W. [mg/g]		Total carbohydrates [%]		Total phenols [%]		Total alkaloids [%]		Tannins [%]		Total flavonoids [%]	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
NPK	28.42^{c}	30.51^{c}	3.03^{c}	3.58^{c}	17.43^{b}	19.42^{c}	58.29^{c}	61.58^{c}	10.43^{c}	12.46^{c}	51.42^{b}	48.11^{c}	20.52^{b}	18.41^{c}
Manure	30.58^{b}	32.71b	4.88b	5.61^{b}	18.39^{b}	21.52^{b}	63.29^{b}	66.41^{b}	12.82^{b}	13.71^{b}	47.33^{c}	50.30^{b}	20.39^{b}	22.58^{b}
Nano Fe + Zn	34.21^{b}	37.08^{a}	4.57^{b}	5.49^{b}	17.58^{b}	21.17^{b}	60.72^{c}	62.35^{c}	12.24^{b}	12.44^{c}	45.97^{c}	51.21^{b}	22.68^{a}	22.79^{b}
Nano Fe + Zn + manure	36.79^{a}	38.26^{a}	6.92^{a}	7.89^{a}	22.75^{a}	24.83^{a}	76.91^{a}	80.31^{a}	14.09^{a}	14.85^{a}	52.67^{a}	53.27^{a}	23.95^{a}	25.78^{a}

Means with the same letter in a column are not significantly different by DMRT at level 5%
F1 – first season; F2 – second season

(1991) on carrot and Soliman & Mahmoud (2013) on *Adansonia digitata* L. Zn application in wheat could alleviate the oxidative stress of wheat through transcriptional regulation of multiple defense pathways, such as antioxidant enzymes and flavonoid secondary metabolism (Ma *et al.* 2017). Organic fertilisation has a stimulatory effect on accumulation of phenolics in fennel; it is well known that, the higher concentrations of phenolics can be explained by the role of organic fertilisers inducing the acetate shikimate pathway, resulting in higher production of flavonoids and phenolics (Sousa *et al.* 2008). Furthermore, the chlorophyll concentration noticeably responded to nutrients management and the highest value was obtained by combined application of manure and nano Fe plus Zn. There is a close relationship between the capacity of photosynthetic carbon dioxide fixation and the chlorophyll concentration. Therefore, the increase in the chlorophyll concentration can be considered equivalent to the increased source strength represented in the export rate of a photo-assimilates from the source tissue (White *et al*. 2016).



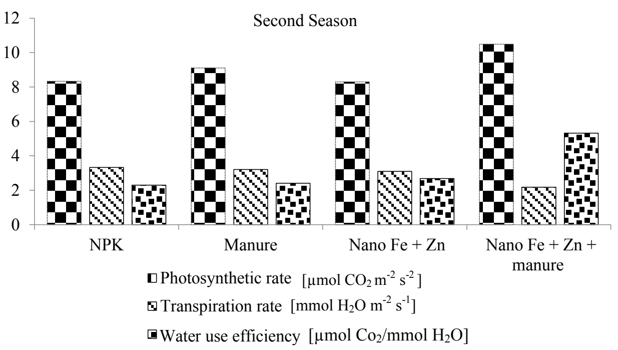


Figure 2. Effect of NPK, manure and nano Fe, nano Zn treatments on photosynthetic rate, transpiration rate and water use efficiency of *Eruca sativa* plant during two seasons

Photosynthetic rate, transpiration rate and water use efficiency

Data of photosynthetic rate, transpiration rate and water use efficiency of the rocket plants fertilised with NPK, manure fertiliser or nano Fe + Zn individually and in combination with manure are presented in Figure 2. It was cleared that application of nano Fe + nano Zn fertilisers mixed with manure fertiliser significantly increased photosynthetic rate and water use efficiency (WUE) as compared to control in both seasons. Although, the application of NPK or manure treatments or nano Fe plus nano Zn individually were observed to show in significant differences for photosynthetic rate and water use efficiency. Application of nano Fe + nano Zn mixed with manure fertiliser significantly increased WUE which reached 5.26 and 5.32 μmol CO_2/mmol H_2O as compared with control (2.10 and 2.3 μmol CO_2/mmol H_2O) in both seasons, respectively.

This might be due to the greater yield obtained where the capacity of the plant to store the moisture would also increase its water use and converting into the yield per unit of water applied (Beheshti & Fard 2010). The inorganic amendments were also found to have greater WUE over the control. Karasahin (2015) reported that, greater water use efficiency by the use of inorganic fertilisers than the organic manures. Nitrogen fertiliser increases the efficiency of water use by wheat (Deng *et al*. 2004). Positive effect on WUE was reported with Subhan *et al*. (2017) who found that, the inorganic fertilisers gave significant higher total dry matter, grain and straw yield and also due to the greater grain yield. The calculated WUE was greater in the NPK treatment as compared with organic manure; it's worth mentioning that, WUE is important parameters in the water scarce areas.

Enhancement values in photosynthetic rate 34.3 and 25.8% (Figure 2) with same previous mixed application may be due to increasing of leaf area (5.9 and 8.6%) (Table 3) and chlorophyll content (29.5 and 25.4%) over control (Table 4). Meanwhile, leaf numbers and leaf area are noticeably affected by both organic and inorganic fertilisers. The leaves are the most important photosynthetic organ and assimilate supplying source that play critical roles in light interception, evapotranspiration and significant response to fertilisers (Pandey & Singh 2011). Increasing leaf area can directly affect the rate of photosynthetic (Evans & Sadler 2007). The chlorophyll contents were enhanced by (20.71%), and the net photosynthetic rate was promoted by (31.87%) as compared with the control in spinach plants (Gao *et al.* 2006).

Macro and micro nutrients concentrations

When investigated Tables 5 and 6, we could found that a significant increase in N, P, and K concentrations were recorded as a result of nano Fe + nano Zn + manure application over control (NPK). In both seasons, combination treatment showed an increase in N (61 and 35%), P (33 and 55%), K (55 and 49%), respectively compared to control treatments. Also the same treatment showed an increase concentrations of N (29.7 and 27.6%), P (71 and 65.5%), and K (113.6 and 106.8%), respectively compared to manure treatment alone. In addition to these results, with combination treatment Mg content was increased by 25 and 20% as compared to NPK treatment. Generally, addition of nano Fe + nano Zn alone led to reduce the accumulation of all macro-elements in comparison with other treatments. The results of combined treatment application as well as individual treatment of manure or NPK were increased Ca content significantly than the results of addition of nano Fe + nano Zn alone. Meanwhile, Mn, Fe, Cu, Zn, Pb and Ni exhibited an increasing trend in response to manure application (Table 6).

Several recent articles have articulated the benefits of nano-scale micronutrients to crop yield enhancement as well as the accumulation of nutrients. For example, Nasri *et al.* (2010) stated that, the use of iron and zinc nano-nutrients caused 12% increment in amount of nitrogen in corn grain, indicating the role of these elements in increasing nitrogen percentage. Raliyaan & Tarafdar (2013) and Raliya *et al*. (2016) reported that increases in plant growth, yield, and nutrient content upon treatment with nano ZnO. Similarly, Subbaiah *et al*. (2016) demonstrated that, nano ZnO increased growth, yield, and Zn content of maize. Still, the agronomic effectiveness of nano practical compared to non-nano forms of micronutrients has yet to be fully resolved (Mahmoud *et al.* 2017).

On the contrary, according to Trinchera *et al.* (2008) chemical fertilisation is able to supply greater amounts of nitrogen to plants in a brief period to improve better metabolism without needing for additional activity of enzymes, however, in case of plants receiving the organic fertilisers, may face a condition of slowly releasing the available nitrogen. This slow release of N may be considered as adverse environmental conditions due to nutrient deficiency.

Main sulphur content, glucose, fructose, ascorbic and crude protein contents

Going with main sulphur content represented in

T a b l e 5

Effect of NPK, manure and nano Fe and Zn on macro-elements of rocket plants during two seasons

Treatment	N [%]		P [%]		K [%]		Mg [%]		Ca [%]	
	F1	F2	F1	F2	F1	F1	F2	F1	F2	F1
NPK	2.33^{b}	2.97^{c}	0.27^{b}	0.31^{b}	3.30^{b}	3.61^{b}	0.12^{b}	0.15^{b}	1.25^{a}	1.36^{a}
Manure	2.89^{b}	3.15^{b}	0.21^{c}	0.29^{b}	2.19^{c}	2.78^{c}	0.15a	0.17a	1.26a	1.38a
Nano Fe + Zn	1.94^{c}	2.85^{c}	0.19^{c}	0.25^{c}	2.17^{c}	2.58^{c}	0.10^{b}	0.13^{c}	1.24^{b}	1.35^{b}
Nano Fe + Zn + manure	3.75^{a}	4.02^{a}	0.36^{a}	0.48^{a}	4.68^{a}	5.75^{a}	0.15^{a}	0.18^{a}	1.34^{a}	1.49^{a}

Means with the same letter in a column are not significantly different by DMRT at level 5%
F1– first season; F2 – second season

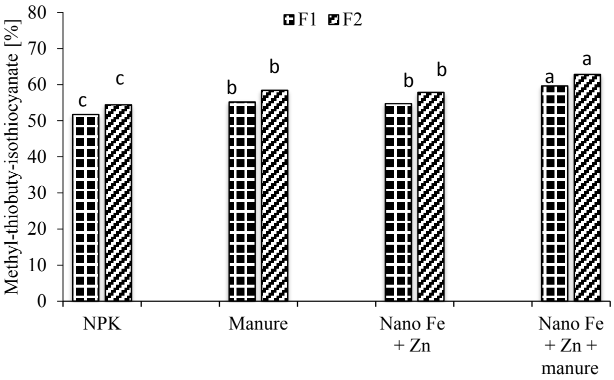


Figure 3. Effect of NPK, manure and nano Fe and Zn treatments on main sulphur content
F1 – first season; F2 – second season

methyl-thiobutyl-isothiocyanate (Figure 3), glucose and fructose contents (Table 7), significantly increased due to application of either manure fertiliser alone or nano Fe + Zn or combination treatment over control. It was found that, combination treatment had a profound effect on methyl-thiobutyl-isothiocyanate (15.3 and 15.5%) during both seasons, respectively in comparison to control plants. Also the maximum improvements in ascorbic and crude protein were observed due to manure fertiliser and combination treatment applied of manure and nano Fe + Zn over both control and nano Fe + Zn individually. The integrative treatment increased crude protein, glucose and fructose contents (61 and 36%), (31 and 30%), (90 and 98.5%), respec-

T a b l e 6

Effect of NPK, manure and nano Fe and Zn on micro-elements of rocket plants during two seasons

Treatment	Mn [ppm]		Fe [ppm]		Cu [ppm]		Zn [ppm]		Pb [ppm]		Ni [ppm]	
	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2	F1	F2
NPK	103.1^{c}	107.3^{c}	90.2^{c}	99.4^{c}	22.6^{a}	19.3^{a}	28.3^{c}	30.5^{c}	9.33^{b}	7.21^{b}	0.12^{b}	0.11^{b}
Manure	110.8^{b}	111.5^{b}	112.3^{b}	114.1^{b}	20.8^{b}	23.3^{a}	31.7^{b}	33.4^{b}	11.7^{a}	13.5^{a}	0.21a	0.13a
Nano Fe + Zn	104.4^{c}	104.7^{d}	110.2^{b}	113.3^{b}	22.1^{a}	20.7^{a}	34.5^{a}	36.4^{a}	7.12^{c}	6.09^{b}	0.10^{c}	0.09^{c}
Nano Fe + Zn + manure	112.4^{a}	118.7^{a}	114.8^{a}	117.9^{a}	24.9^{a}	24.8^{a}	35.4^{a}	38.7^{a}	12.5^{a}	13.2^{a}	0.19^{a}	0.14^{a}

Means with the same letter in a column are not significantly different by DMRT at level 5%
F1 – first season; F2 – second season

T a b l e 7

Effect of NPK, manure and nano Fe and Zn on chemical constituents of rocket plants during two seasons

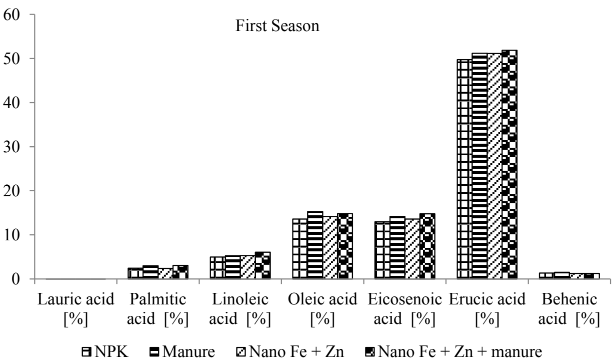
Treatment	Ascorbic acid [mg/100 g]		Crude protein [%]		Glucose [%] [g/100 g F.W.]		Fructose [%] [g/100 g F.W.]	
	F1	F2	F1	F2	F1	F2	F1	F2
NPK	48.55^{c}	51.82^{c}	14.56^{c}	18.50^{c}	1.16^{c}	1.24^{c}	1.26^{c}	1.4^{c}
Manure	50.11^{b}	53.71^{b}	18.06^{b}	20.1^{b}	2.2^{b}	2.46^{b}	1.96^{b}	2.24^{b}
Nano Fe + Zn	47.38^{c}	50.94^{c}	12.12^{d}	17.81^{c}	4.4^{a}	3.68^{b}	2.14^{a}	1.76^{c}
Nano Fe + Zn + manure	54.92^{a}	56.38^{a}	23.43^{a}	25.12^{a}	4.74^{a}	5.02^{a}	2.56^{a}	2.78^{a}

Means with the same letter in a column are not significantly different by DMRT at level 5%
F1– first season; F2 – second season

tively compared with NPK for both seasons.

The increase in sulphur, ascorbic, crude protein, glucose and fructose contents may be due to advantageous effects of the manure and nano Fe + Zn resulting in extra release of nutrients in an accessible form for plant uptake, which resulted in higher efficiency of the photosynthesis process, increasing synthesis of afore mentioned chemical constituents.

Results in Figure 4 revealed that, manure fertilisation had a great impact on formation of free fatty acids content, meanwhile application of either manure only or nano Fe + Zn + manure resulted in a significant increase in free fatty acids content of rocket seed as compared to NPK treatment during the two seasons. The highest lauric, oleic and behenic acids contents were observed with both manure fertiliser alone and nano Fe + Zn + manure in 1st and 2nd seasons as compared with NPK. While, the highest accumulation of palmitic, eicosenoic and erucic acids were obtained with combination



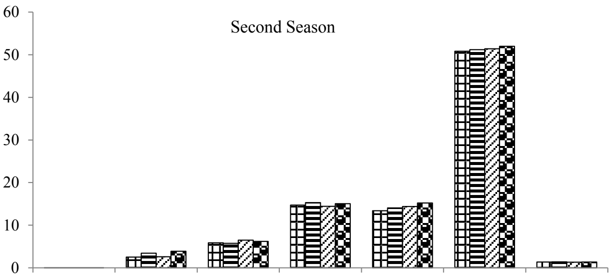


Figure 4. Effect of NPK, manure and treatments on percentage of some fatty acids content of seed oil of rocket plants during two seasons

treatment compared with other treatments. As for the individual treatment of nano Fe + nano Zn, produced the highest percentage of linoleic acid in the second season, whereas the combination treatment produced the best amount in the first season.

The increase in essential oil productivity with manure alone or combination treatment compared with control treatment could be explained on the basis of available elements, vitamins, hormone-like substances, amino acids and sugars, which have a significant effect on the physiological or biochemical processes within the plant and consequently boost essential oil yield. These results coincide with those obtained by Mahmoud (2012) on yarrow plant *(Achillea millefolium*) who stated that application of compost mixed with zeolite, humic acids and biofertilisers increased oil yield. In sunflowers under non-manure conditions, the application of the nanofertilisers could not affect the oil content, while the application of nano Zn and Fe under manure conditions significantly improved oil yield (Janmohammadi *et al.* 2016). Other researchers found that combination of the organic sources with inorganic nutrients gives higher oil content in rapeseed and Indian mustard (Tripathi *et al*. 2011). It has been revealed that nutrient management can affect the fatty acids biosynthesis pathway in oil crops and it seems that under nutritional imbalance a large proportion of photosynthates are diverted to protein formation. Deficiency of carbohydrates may stimulate the degradation fatty acids to acetyl co-enzyme A and it leads to decreased oil content (Marschner & Rengel 2012). Also, Mahmoud *et al*. (2017) concluded that application of organic fertilisers, natural soil amendments and chemical fertilisers (nano form) produced higher growth characteristics, chemical composition and oil yield in comparison with results derived from chemical fertilisers NPK on caraway plant.

Phytohormones concentrations

The results of hormonal analysis represented in abscisic acid (ABA) and indole acetic acid (IAA) in rocket plant as affected by different treatments are displayed in Figures 5 and 6. The increase of growth parameter was associated with high level of growth promoter (IAA) and low level of ABA. Hence it was found that nano Fe + nano Zn under manure fertiliser treatment gave significantly the highest content of IAA over all other treatments including control (NPK) in both season. The increases were mainly (52 and 92.7%), respectively over control in the 1st and 2nd seasons.

It was established that, zinc was considered as an essential micronutrient for normal growth, development, and health of plants and human. Zinc enhances cation-exchange capacity of the roots, which in turn enhances absorption of essential nutrients, especially nitrogen which is responsible for higher protein content. Zinc plays a vital role in carbohydrate and proteins metabolism as well as it controls plant growth hormone IAA. Zn is also an essential component of dehydrogenase, proteinase, and peptides enzymes as well as promotes starch formation, seed maturation and production (Fageria *et al.* 2002). Besides, it controls the synthesis of indole acetic acid, a phytohormone which intensely regulates the plant growth, as well as necessary for chlorophyll synthesis and carbohydrate formation (Vitosh *et al*. 1994). With reference to manure that may contains beneficial microorganisms which increase crop growth by secretion and synthesized phytohormones (Yamada & Xu 2001).

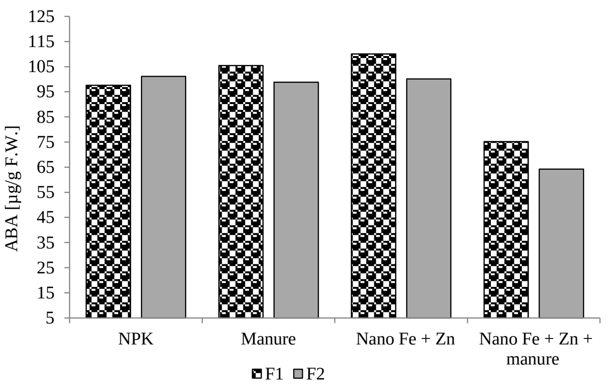


Figure 5. Effect of NPK, manure and nano Fe and Zn on ABA concentration of rocket plants during two seasons
F1 – first season; F2 – second season

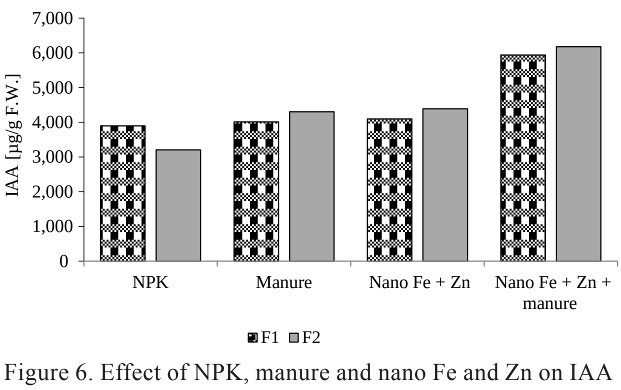


Figure 6. Effect of NPK, manure and nano Fe and Zn on IAA concentration of rocket plants during two seasons
F1 – first season; F2 – second season

Concerning ABA content, in the first season, both nano Fe + Zn under manure fertiliser treatment and control treatment (NPK) significantly resulted in the lowest ABA content compared with manure fertiliser alone and nano Fe + Zn alone. In contrast, in the second season, it was clear that nano Fe + Zn mixed with manure significantly resulted in the lowest ABA in comparison with all other treatments. The above-mentioned results are in consonance with those obtained by Solimanan and Mahmoud (2013) on *Adansonia digitata* L. The stimulated effects of mixed treatment may be attributed to the production of hormones like substances from manure fertiliser. As mentioned by Mehnaz *et al*. (2001), who reported that IAA produced by rhizobacteria as plant growth promoting is believed to increase root growth and root length, resulting in better nutrient uptake present in organic substances.

Marek and Skorupska (2001) provided evidence that *Bacillus* sp. produced hormones, which effectively reverse a chemical-induced inhibition of stem growth, working together in the presence of organic fertilisers.

CONCLUSIONS

The achieved results from this investigation are sturdily verify that mixture of nano Zn and Fe with manure were boosted growth characters, yield whether seed oil or leaves fresh weight and chemical constituents with concern on main sulphur methyl-thiobutyl-isothiocyanate compound of rocket plant besides antioxidant compounds (ascorbic acid, total phenolics, total flavonoids and carotenoids) as compared to commercial fertilisers NPK. Particularly if we took into consideration that *Eruca sativa* plant enters variety of high economic pharmaceutical and cosmetics industries. Consequently, it could conclude that nano and organic fertilisers could replace chemical for improving the quality of the produced crops in addition to decreasing the production costs and environmental pollution.

Acknowledgements. We would like to show our appreciation and express our deepest gratitude towards Cairo University, Faculty of Agriculture, Research Park [FA-CURP] and plant physiology department for samples analyses facilities.

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Received: January 4, 2018

Agriculture (Poľnohospodárstvo), 64, 2018 (2): 80−86
DOI: 10.2478/agri-2018-0008 Original paper

WHEAT GRAIN ENRICHMENT WITH ZINC THROUGH USING ZINC FERTILISER AND PRECEDING PLANT RESIDUES INCORPORATION

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ARANI, A.B. – MANDARI, A. – NAZARLI, H.: Wheat grain enrichment with zinc through using zinc fertiliser and preceding plant residues incorporation. Agriculture (Poľnohospodárstvo), vol. *64*, 2018, no. 2, pp. 80–86.

Micronutrients and particularly zinc (Zn) deficiency affects crops productivity and human health, therefore improving Zn concentration within plant tissues might be regarded as an aim of sustainable agriculture. In this respect, a field experiment was carried out to examine the potential influence of preceding crop residues including bean and wheat incorporation into the soil as a way to improve zinc accumulation within subsequent wheat (*Triticum aestivum* L.) grain. The experiment was initiated at autumn 2014 and in the first year, a piece of farm land in Dehaghan-Isfahan-Iran was divided into two equal parts devoted to wheat and bean cultivation. At the end of the harvest season, aboveground plant residues were incorporated to 0–30 cm layer of soil. In the next year, the entire farm devoted to wheat production. The applied treatments included: control, zinc sulfate (60 kg/ha), wheat residues, bean (*Phaseolus vulgaris* L.) residues, wheat residues + zinc sulfate and bean residue + zinc sulfate. The results of analysis of variance showed the highly significant differences between treatments in terms of grain zinc, protein, phytic acid to zinc molar ratio (PA/Zn), quantitative yield and soil electrical conductivity (EC). Soil pH and organic carbon (OC) were not affected by treatments while soil EC significantly increased by using plant residues. The highest grain yield (3.8 t/ha), grain protein (10.3 mg/kg) and zinc concentrations (36 mg/kg) were obtained by using bean residues plus $ZnSO_4$ while the lowest quantities were related to control treatment. The treatments had no significant impact on grain acid phytic concentration but phytic acid / zinc molar ratio was affected by treatments and the lowest ratio (which is a positive attribute) was measured from plots containing bean residues plus $ZnSO_4$ while the highest occurred in control plots.

Key words: biofortification, grain protein, micronutrients malnutrition, phytic acid, quantitative and qualitative yield, zinc sulfate

Micronutrients and especially zinc deficiency is rampant in soils under cereals cultivation throughout the world and it considerably reduces either quantitative or qualitative yield. The areas with Zn-deficient soils are mainly areas where Zn deficiency in human is also rampant and particularly located in South and West Asia (Hotz & Brown 2004; Alloway 2004). Zinc deficiency is particularly widespread in populations of developing countries where people's diet is cereal based as staple food (Cakmak 2008). Based on an estimation, micronutrient malnutrition, and especially zinc, iron deficit affects about three billion people around the world (Bouis 2007) and it has been estimated that over two billion people are at risk of Zn deficiency disorders (World Health Organization 2002; Hotz & Brown 2004). However, it should also be mentioned that excessive zinc as a heavy metal has severe toxic effects on plants, animals, and human health (Ullah *et al.* 2015).

About 50 percent of cereals-growing lands around the globe encounter with different levels of zinc deficiency (Zhao & McGrath 2009). Zinc

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deficiency in soils is caused by both primary and secondary deficiencies. In the case of primary deficiency, the total zinc concentration of the soil is limiting factor for plant requirements which mainly occurs in areas with sandy or strongly leached tropical soils (Cakmak *et al*. 2010). The range of total zinc concentrations in soils reported in the literature tends to show an overall mean total concentration of around 55 mg Zn/kg (Kiekens 1995) however the zinc concentration in sandy soils of arid/semi-arid areas of South West Asia such as Iran (including the location of experiment) is considerably lower than global average (Table 1). Adequate zinc concentration in soil is a crucial factor to achieve high crop yields. In this respect, it is reported that in China, high wheat yields need 29.4 mg Zn per kg in shoots and 1.98 mg DTPA-Zn/kg in soil (Liu *et al.* 2017).

Soils affected by secondary zinc deficiency have enough zinc concentration to cover plant nutritional requirements yet the amount of zinc uptake is not enough to meet plant needs. High soil pH and high concentrations of calcium carbonate, bicarbonate, phosphate, calcium, magnesium and sodium in soils are the main factors which cause secondary zinc deficiency (Alloway 2009). Since agricultural soils in arid and semi-arid areas such as Iran are predominantly alkaline and taking into account crops needs, zinc and iron are considered as the most yield-limiting micronutrient in these areas.

Cereals and more precisely wheat and rice contain the main source of energy in developing countries especially South and West Asian countries (Cakmak 2008). Wheat grain generally has a low potential to accumulate Zn particularly when the content of Zn in soil is not sufficient. In addition, the amount of phytic acid which declines Zn availability in human body, is high within wheat grain. So, using agronomic approaches in order to enhance Zn accumulation within wheat grain is necessary. Biofortification means producing cereals which are enriched of micro nutrients and biofortified grains have higher bioavailability of these elements. Biofortification includes both agronomic and genetic approaches (Ghandilyan *et al*. 2006; Distelfeld *et al*. 2007). Producing cereals with enhanced content of micro-nutrients (biofortification) especially zinc through agronomic or genetic approaches and improving their bioavailability have been regarded as a cost-effective way to alleviate micro-nutrients malnutrition (Peleg *et al.* 2008).

Using organic materials like residues of preceding crop as agricultural inputs is a useful management strategy which on the one hand may improve soil structure and on the other hand provides minerals required by plants (Baldi & Toselli 2014; Hueso-González *et al*. 2014). Local organic materials such as manure farmyard and plant residues are environment-friendly sources of micro-nutrients (Tejada & Benítez 2014). Applying plant residues from pervious cultivation has been regarded as one of the agronomic biofortification approaches to enhance wheat grain quality (Khoshgoftarmanesh *et al*. 2017).

The current investigation was conducted in a region with a chronic Zn deficiency with the following goal: improving soil Zn bioavailability by employing agronomic approaches including using the plant residues of previous cultivation (bean and wheat) accompanied with chemical fertiliser ($ZnSO_4$). In this regard, the influence of mentioned inputs on quantitative and qualitative attributes of wheat grain where investigated.

MATERIAL AND METHODS

Experimental, soil sampling and applied treatments

The investigation was conducted as a field experiment in Dehaghan-Esfahan, Iran (31°56'N, 51°39'E). In the first year, a piece of farm land was divided into two equal parts, in one part, wheat was cultivated at November 2014 and another part was devoted to bean cultivation at early March 2015. Each experimental plot area was 12 m^2 (4 m in length, 3 m in width). The densities for wheat and bean were 250, 50 plants/m^2 respectively. After harvesting at summer, the harvested aboveground residues of both products were crushed into pieces of 1–3 cm, then mixed, and incorporated into the 0–30 cm layer of the farm soil. The chemical properties of each crop residues are presented in Table 2. In middle autumn 2015 (28 October), all experimental plots were devoted to bread wheat (Sepahan cultivar) cultivation. So the rotations during two years were bean-wheat and consecutive wheat cultiva-

tion. The experiment was conducted as a randomized complete block design with three replications with six treatments, including using bean residues, wheat residues, zinc sulfate, wheat residues + zinc sulfate, bean residues + zinc sulfate and control (without plant residues of preceding crop and chemical fertiliser). The crops were irrigated to keep soil moisture at approximately 70% field capacity, using the basin irrigation method. Irrigation rates were based on evapotranspiration data collected at the local weather station of Dehaghan. During the experimental period, the medium temperature of day and night was 10 and 25°C and the relative humidity was about 35%. During the growing period, the necessary agricultural cares like irrigation as plot and weeds control was done both mechanically and chemically (using 2,4-D). Finally, the wheat plants
were harvested at June 2016.

The soil chemical and physical properties before performing experiment at autumn 2014 are presented in Table 1. The chemical zinc fertiliser for certain treatments was applied to soil in form of $ZnSO_4$ at 60 kg/ha based on what Iranian Soil and Water Institute had recommended (Milani *et al.* 1998). Soil properties including pH, organic carbon and electrical conductivity were measured at the end of the experiment in summer 2016 to determine the influence of treatments.

Analysis of grain zinc, acid phytic (PA), PA/Zn molar ratio and protein concentration

In order to quantify the grain Zn content, in brief, samples were extracted by HNO_3 and H_2O_2 in a microwave and the concentration of Zn within extracts measured by atomic absorption method microwave (Prasad 1999). To determine Phytic acid (PA) concentration in grains, the method of Makower (1970) was performed. In order to measure grain's protein concentration, first the concentration of nitrogen within grain was determined by using the well-known Kjeldahl method then to convert grain nitrogen to protein, based on what Tkachuk (1969) proposed, a specific factor (5.7) was used.

Statistical analysis

The data statistical analysis was done by SAS statistical software. To ensure residual normality, univariate procedure was used. The analysis of variance conducted through GLM procedure. The means comparison was also done by using *LSD* test ($P \leq 0.05$).

T a b l e 1

Physical and chemical properties of soil (0–30 cm)

Soil texture	Mn	Zn	Fe	K	P	N	Lime	Organic matter	pH	EC
	[mg/kg]					[%]				[dS/m]
Loam-clay	8.5	0.3	4.8	235.2	9.1	1.51	35	1.1	7.4	1.1

T a b l e 2

The properties of applied plant residues

Plant residues	C:N	Proteins [g/kg]	Cu	Mn	Zn	Fe	K	P	N	pH	EC [dS/m]
			[mg/kg]				[%]				
Bean residual	15.4	10.7	20	40	10	270	1.2	1.90	1.7	5.7	5.0
Wheat residual	22.7	3.8	6	27	4	200	1.38	0.70	0.6	5.7	4.1

RESULTS AND DISCUSSION

Response of soil EC, pH, organic carbon to applied treatments

The results of analysis of variance are summarized in Table 3. The means comparison showed that the highest soil EC was related to using wheat residues with or without chemical fertiliser while the lowest EC was observed in the treatment with $ZnSO_4$ without residues (Table 4). On the opposite side with the current results, Nash and Baligay (1974) expressed that incorporating plant residues of preceding crop to soil by causing increase in biological activity and fungal, bacterial secretion, gradually reduces alkaline soil's pH while increases acidic soil's pH. However the changes in soil pH caused by decaying residues are temporary and soil buffer property prevent marked changes in pH. In current study, soil pH and organic carbon didn't respond to plant residues and Zn fertiliser. In fact, adding plant residues to soil increases organic matters yet the process is time consuming and the changes will occur during long

T a b l e 3

Analysis of variance of soil properties, qualitative and quantitative yield in response to incorporating plant residues and $ZnSO_4$ fertiliser

Source of variation	df	pH	EC	O.C	Grain phytic acid	Phytic acid to zinc molar ratio	Grain Zn concentration	Grain protein	Grain yield
Block	2	0.005 ^{ns}	0.009 ^{ns}	0.001 ^{ns}	0.002 ^{ns}	11.04 ^{ns}	58.57 ^{ns}	0.38 ^{ns}	0.005 ^{ns}
Treatment	5	0.003 ^{ns}	0.023 $^{++}$	0.016 ^{ns}	0.032 ^{ns}	40.12^{++}	78.57^{+}	4.53^{++}	0.488^{++}
Error	10	0.010	0.002	0.010	0.019	4.00	25.94	0.24	0.010
C.V [%]	–	1.31	9.89	6.13	20.13	9.62	16.24	5.49	3.11

EC – electrical conductivity; O.C – organic carbon; ns – not significant at the 0.05 probability level
$^{+}$Significant at the 0.05 probability levels; $^{++}$Significant at the 0.01 probability levels

T a b l e 4

Means comparison for soil properties, wheat qualitative and quantitative yield in response to incorporating plant residues and $ZnSO_4$ fertiliser

Treatment	pH	EC [dS/m]	O.C [%]	Grain PA [gr 100 gr/grain]	Grain PA/Zn [mg/100/grain]	Grain Zn [mg/kg]	Grain protein [mg/kg]	Grain yield [t/ha]
Control	7.33^{a}	0.83^{c}	1.19^{b}	0.68^{a}	25.00^{a}	26.00^{d}	7.54^{c}	2.80^{f}
$ZnSO_4$	7.36^{a}	0.81^{c}	1.18^{b}	0.62^{ab}	19.00^{bc}	33.00^{ab}	7.92^{c}	3.10^{d}
Wheat residues	7.26^{a}	0.99^{a}	1.72^{a}	0.70^{a}	24.60^{a}	28.00^{c}	8.20^{bc}	3.00^{e}
Bean residues	7.22^{a}	0.92^{b}	1.64^{a}	0.6^{ab}	20.20^{b}	30.00^{b}	8.51^{bc}	3.40^{b}
Wheat residues + $ZnSO_4$	7.36^{a}	0.97^{a}	1.64^{a}	0.69^{a}	19.00^{bc}	35.00^{a}	9.91^{b}	3.20^{c}
Bean residues + $ZnSO_4$	7.35^{a}	0.92^{b}	1.64^{a}	0.58^{b}	16.00^{c}	36.00^{a}	10.30^{a}	3.80^{a}

EC – electrical conductivity; O.C – organic carbon; PA – phytic acid
Similar letters within each columns show that the means are not significantly different based on *LSD* test results at $P \leq 0.05$

term periods. As mentioned above, plant residues particularly of preceding wheat caused significant increase in soil EC. In this respect other researchers also reported that using organic compounds as input may increase soil EC since these compounds contain high amount of nutrients (Wanchez-Monedaro *et al*. 2001; Eghbal *et al*. 2004).

Grain zinc concentration

Using plant residues had significant effect on grain zinc concentration (Table 3). $ZnSO_4$ had substantial impact on grain Zn concentration as the greatest quantities were obtained when this fertiliser was used either with plant residues (bean or wheat) or without them (Table 4). In absence of $ZnSO_4$, bean residues caused significant improvement in grain Zn compared with wheat residues and control treatment (Table 4). It was already expressed that adding plant residues increases the organic carbon soluble in the soil which may absorb zinc element and improve Zn level within wheat grain (Schulin *et al*. 2008). Also, a significant correlation between soil zinc and zinc in grain has been reported although, in all the study treatments the grain zinc concentration was less than 35–40 mg/kg that is the critical level for grain quality (Cakmak 2008). Probably, not reaching to this critical level is caused by lack of primary soil zinc concentration. In fact, the most important factor regarding grain zinc concentration is the primary concentration of this element within soil (Calvino & Sandra's 2003; Wissuma *et al*. 2007). Increasing Zn fertiliser ($ZnSO_4.7H_2O$) rate from 0 to 1,500 kg/ha markedly improves Zn content within wheat grain and contributes in zinc-biofortification (Wang *et al*. 2015). In this regard, promising results about the possibility of using Zn fertilisers in order to improve wheat grain Zn concentration has been achieved (Cakmak 2008). Since the potential of wheat new varieties to accumulate greater content of Zn within grain depends on the available Zn in the soil, breeding and agronomic biofortification strategies seem to be complementary (Joy *et al*. 2015). The effect of Zn fertilisation on grain Zn biofortification depends on soil Zn content. The soil in current study suffered from severe Zn deficiency and as a result grain Zn accumulation positively responded to Zn fertilisation. Soil Zn application is effective in terms of grain Zn biofortification provided Zn content in soil is lower than appropriate (Cakmak *et al*. 2010).

Grain phytic acid (PA) content and phytic acid to zinc (PA/Zn) molar ratio

As shown in Table 3, the impact of treatments on grain acid phytic content was insignificant. In contrast, the differences between treatments in terms of phytic acid to zinc (PA/Zn) molar ratio (Table 3) were significant. Based on the result of means comparison, adding bean residues decreased the ratio and bean residues compared with wheat residues affected (reductive) the ratio more effectively. Furthermore, $ZnSO_4$ fertiliser affected the ratio significantly as PA/Zn ratio was lower in all treatments which included $ZnSO_4$. This subject was also reported by researchers (Dorostcar *et al*. 2013). The PA/Zn molar ratio is considered as an index of zinc bioavailability in human diet (Weaver & Kannan 2002). In general, it is proposed that Zn absorption will be markedly limited when the PA/Zn molar ratio is over 15 (Gargari *et al*. 2007) and Zn bioavailability is considered as insufficient when the ratio is below 15 (Ryan *et al*. 2008). In this respect, Wang *et al*. (2015) reported that using high rates of Zn fertilisers to soil rapidly increases zinc concentrations while at the same time reduces PA concentrations in wheat grains and as a result the molar ratio of PA/Zn declines.

Grain protein concentration

The results of analysis of variance in terms of grain protein are presented in Table 3. Using plant residues without chemical fertiliser and vice versa caused no significant improvement in grain protein (Table 4). When bean residues and zinc fertiliser applied together, it led to significant increase in grain protein as the greatest quantity was obtained by using bean residues and $ZnSO_4$ (Table 4). There are other reports that express wheat rotation with other plants specially legumes, increases the wheat grain protein (Peck *et al*. 2008; Marschner 1995). Proteins are considered as a factor for improving zinc absorption in nutritive regimes. Greater content of grain protein improves element zinc absorption. Zinc consumption can be effective in curing the lack of zinc in human, increasing the protein and the zinc concentration in grain (Marschner 1995).

Grain yield

Bean residues incorporation into the soil, increased the grain yield by 18 percent compared to control (Table 4). The highest grain yield was obtained when the bean residues with zinc sulfate were added to the soil together and it led to 26 percent increase in grain yield compared to control. As shown in Table 2, the bean residues contained higher content of zinc and protein. So the greater grain yield by using bean residues might be caused of more nitrogen and zinc in bean residues. It is reported that using 23 kg of zinc fertiliser can increase wheat grain yield significantly (Cakmak 2008). The promoting impact of preceding legumes on following cereal's growth, may be caused by the nutrients release during decomposition of legume residues (Theuerl & Buscot 2010) that consequently are utilized by cereal crop. In addition to well-known ability of legumes regarding nitrogen biological fixation, these crops also may mobilize P in soils and thereby often contain greater amount of P within plant organs. This source of P subsequently will become available during decomposition of residues (Nuruzzaman *et al*. 2005).

CONCLUSIONS

This experiment showed that the using plant residues particularly legume species (in this case bean) might be a useful way to improve wheat quantitative and qualitative yield through grain enrichment of zinc and protein. Furthermore, since soil suffered from severe zinc deficiency, applying 60 kg/ha of Zn chemical fertiliser ($ZnSO_4$) accompanied with plant residues caused significant improvement in grain PA/Zn ratio (lower is better), grain Zn and grain yield. Also, the results of the experiment showed that residues with lower C/N ratio such as bean may have a more effective role in growth and yield of succeeding wheat. Using plant residues with high degradation might be more useful for crops with low growth period. In addition since using plant residues caused increase in soil EC, the amount of residues must be applied precisely to prevent soil salinization. At the end, it must be mentioned that in current investigation the data were gathered only for one year so the obtained results are preliminary.

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Received: January 13, 2018

DE GRUYTER OPEN

Agriculture (Poľnohospodárstvo), 64, 2018 (2): 87−93

Original paper DOI: 10.2478/agri-2018-0009

AGRONOMIC AND ECONOMIC PERFORMANCE OF GENETICALLY MODIFIED AND CONVENTIONAL MAIZE

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HRČKOVÁ, K. – MIHALČÍK, P. – ŽÁK, Š. – HAŠANA, R. – ONDREIČKOVÁ, K. – KRAIC, J.: Agronomic and economic performance of genetically modified and conventional maize. Agriculture (Poľnohospodárstvo), vol. *64*, 2018, no. 2, pp. 87–93.

The parameters determining the agronomic and economic performance of genetically modified maize hybrid MON 89034 × NK603 and conventional hybrids were compared under conditions that eliminated the herbicide tolerance in GM hybrid as well as the use of insecticides in conventional hybrids. The GM hybrid confirmed ability to protect itself against the European corn borer and its average grain yield was higher by 6.36–14.42% (i.e. 0.82–1.86 t/ha) in comparison with conventional hybrids. The year of cultivation statistically significant influenced agronomic parameters and the financial income of maize production. The maize genotype did not statistically significantly affected any evaluated parameter. The final income was statistically significant ($P < 0.05$) negatively influenced by all observed agronomic parameters with the exception of the seed price. The price of maize grains on the market was the only one factor that statistically significant ($P < 0.05$) influenced financial income of the maize production.

Key words: maize, genetically modified hybrid, grain production, economic benefit

The genetically modified (GM) maize (*Zea mays* L.) has been commercially grown since the year 1996 and it still belongs to four GM "megacrops". Nevertheless, farmers in the European Union (EU) may not cultivate GM maize with any genetic modification except of the MON 810 resistant to European corn borer [ECB, *Ostrinia nubilalis* (Hübner)]. The genetic potential, agronomic traits and economic benefits of others GM maize cultivars and hybrids are unknown to them. However, conventional hybrids without genetic modifications are also able to provide high yield of grains and phytomass, but they need efficient protection against weeds, pests, and pathogens, usually by chemical pesticides. Reports on the economic performance of GM maize resistant against the ECB by virtue of the expression of transgene encoding the *Bt*-toxin from *Bacillus thuringiensis* have been published. According to Lauer and Wedberg (1999) and Marra *et al*. (2003) the yield of *Bt*-hybrids was unaffected by the ECB infestation and under the natural infestation was similar to conventional hybrids. Moreover, economic effects were very variable due to the large differences in geographical incidences of the ECB. Graeber *et al*. (1999) concluded that under low infestation of ECB the *Bt*-hybrids had similar yield and agronomic performance to their non-*Bt* near-isolines. On the other side, under high pressure of the ECB, the *Bt*-hy-

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brids had a 9% yield advantage over non-*Bt* hybrids (Traore *et al*. 2000). Ma and Subedi (2005) reported that some of tested *Bt*-hybrids produced a similar or up to 12% lower grain yields in comparison with their non-*Bt* counterpart, also when the stalk lodging and breakage of the non-*Bt* counterpart by the ECB was low to moderate. Field experiments performed in South Africa (Gouse *et al*. 2005) and Spain (Gómez-Barbero *et al*. 2008) reported that yield advantage of the *Bt*-maize was associated with reduced pesticide costs and increased income per hectare.

The ECB resistant and glyphosate tolerant GM maize hybrid MON 89034 × NK603 should provide agronomic benefit, especially under ECB natural infestation as well as the higher flexibility in weed management during the growing season. Its advantages under lower infestation of the ECB and modest requirements for weed elimination should be limited.

The aim of our field study was to compare performance of conventional maize hybrids with the GM hybrid MON 89034 × NK603 in the main parameters determining the economic effect of grain production, but under conditions eliminating herbicide tolerance of the GM hybrid as well as insecticide applications in conventional hybrids.

MATERIAL AND METHODS

Field trials

The field experiments were performed at the experimental station in Borovce (Research Institute of Plant Production, Slovakia) in years 2011–2012. The soil type in the locality is Luvi-haplic Chernozem, the pH range is 5.5–7.2, the depth of topsoil is 0.24–0.28 m, and content of humus is 1.8–2.0%.

The GM hybrid MON 89034 × NK603 (trade name: Genuity® VT Double Pro™, Monsanto Company, St. Louis, USA) tolerant to herbicide glyphosate and resistant against lepidopteran insects and conventional hybrids PR36V52 (DuPont Pioneer, Johnston, USA), LG 3475 (Limagrain, Saint Beauzire, France), NK Columbia (Syngenta, Basel, Switzerland), DKC 5143 (Monsanto Company, St. Louis, USA) were sown for the comparison study.

The harvested area of each experimental plot was 600 m^2. Plots were organized by the method of random sub-blocks in four repetitions. Soil was managed conventionally with ploughing in autumn and fertilization before sowing by Polidap® (200 kg/ha) containing mainly ammonium hydrogen phosphate (Grupa Azoty Zakłady Chemiczne "Police" S.A., Poland) and urea (130 kg/ha). Only the herbicide Guardian® Tetra [214 g/l Terbuthylazine, 450 g/l Acetochlor] (DuPont Pioneer, Johnston, USA) (3.5 l/ha) or a tank mix of MaisTer® power [31.5 g/l Foramsulfuron, 1.0 g/l Iodosulfuron, 10 g/l Thiencarbazone, 15 g/l Cyprosulfamide] (Bayer AG, Leverkusen, Germany) (150 g/ha) with Istroekol (Duslo a.s., Šaľa, Slovakia) (2.0 l/ha) were applied pre-emergently. Grains were harvested by the Claas Lexion 550 (Class GmbH & Co. KGaA, Harsewinkel, Germany) and after the harvest were deteriorated by squeezer press machine and ploughed into the soil. The grain yields were calculated at standard moisture content 14%.

Phenological and morphological traits, resistance against the ECB, and economical parameters of production were evaluated. Economic parameters included the price of seeds (according to the valid price lists at that time), the yield of grains, and the price of grain production sold (according to the market prices quoted on the Commodity Exchange Bratislava on Dec 13, 2011 and Dec 18, 2012, respectively).

Obtained data were processed by the analysis of variance (ANOVA) and correlation analysis with Spearman rank correlation using the software Statgraphics® Centurion XVII (Statpoint Technologies Inc., The Plains, USA). The Fisher's least significant differences (*LSD*) were estimated for $\alpha = 0.05$ and $\alpha = 0.01$.

RESULTS AND DISCUSSION

Hybrids were tested during two years with different climatic conditions (air temperature, total precipitation, and distribution of precipitation during growing season, Figure 1). Differences in number of germinated plants, number of sterile plants, full flowering of male flowers, length of vegetative period, plant height, perimeter of the main stalk, yield of phytomass, length of cob, number of rows in cob, number of grains per row, length of corridors in stalk per plant, number of larvae and pupae per plant, and rate of damaged stalks were not statistically significant, neither between years, nor between maize genotypes. There were evaluated parameters the most related to grain yield as the number of cobs per hectare, thousand grains weight (TGW), grain yield, and resistance against ECB (number of corridors in the stalk, number of plants infested by the ECB, frequency of broken plants, and a number of larvae and pupae in stalks per 100 plants).

Number of cobs per hectare

This parameter has been observed before grain harvest. The range in number of cobs per hectare was 62,913–75,102 and GM hybrid had the lowest value in both years. Different years affected this parameter statistically significant ($P < 0.05$) however, maize genotypes affected the number of cobs per hectare insignificantly.

Thousand grains weight

TGW ranged during two years from 240.9 g to 334.6 g. The GM hybrid had the highest TGW in both years. Better climatic conditions for development of this parameter were in the year 2011 (average TGW 318.4 g), worse in 2012 (average TGW 249.8 g). TGW was affected statistically significant ($P < 0.05$) by years, differences among maize genotypes not.

Yield of grains

The highest two-year average yield had GM hybrid (12.90 t/ha), conventional hybrids were in the range 11.04–12.08 t/ha. The years of testing were statistically significant different ($P < 0.05$) and also statistically significantly ($P < 0.05$) affected the grain yield of tested maize genotypes. On the contrary, all maize hybrids responded to climate conditions almost uniformly and differences in grain yield between them were statistically insignificant. The GM hybrid behaved as equivalent of conventional hybrids in the grain yield, even though it could not express its genetic potential to tolerate herbicide glyphosate.

Resistance against the ECB

The presence of the ECB was found at testing locality in both years. Tested hybrids were infested, except of the resistant GM hybrid. Number of corridors in stalk per plant varied from zero (only in GM hybrid) to 2.4. Differences in number of corridors in stalk per plant were not statistically significant ($P <$ 0.05) between years as well as between hybrids. The GM hybrid plants were infested by the ECB in frequency 0–1.7% (measured as frequency of holes on the stalks and cobs). Level of infestation in conventional hybrids was in the range 28.3–72.5%. Higher infestation (60.8–72.5%) was in the growing season 2011, lower in 2012 (28.3–37.5%) however, differences between years as well as between hybrids in

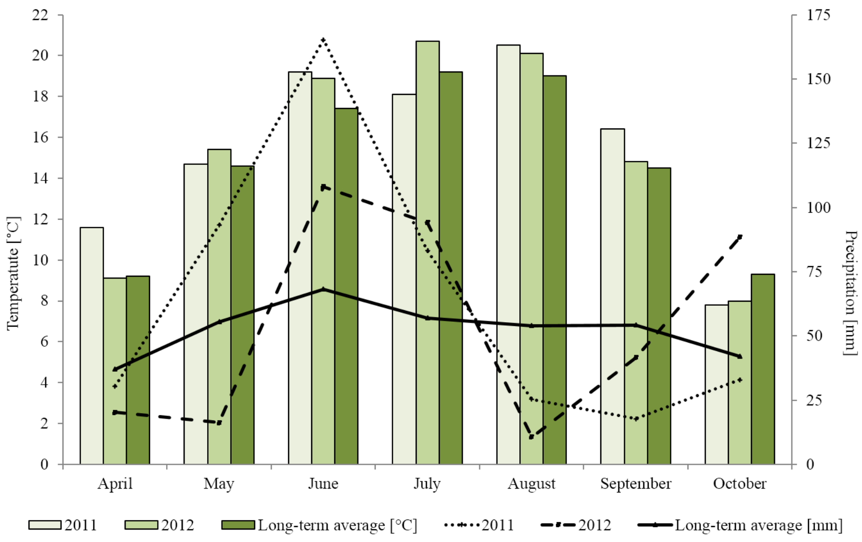

Figure 1. Average monthly temperature and precipitation in testing locality during both vegetation seasons

this parameter were not statistically significant (P < 0.05). Attack of the ECB has been manifested also by the breakage of plant stalk. Conventional hybrids had 1.7–13.3% of plants with stalks broken under the cob, the GM hybrid had no broken stalk. Differences between years in this parameter were statistically significant (P < 0.05), but between maize genotypes were statistically insignificant. The average abundance of larvae and pupae in stalks per 100 plants of conventional hybrids was high in the year 2011 (64.8%), lower in the year 2012 (32.3%), in GM hybrid was none. The GM hybrid confirmed its ability to protect itself against ECB. Frequency of infestation by the ECB, number of corridors created by the larvae, and relevant number of broken stalks, statistically insignificantly influenced the average grain yield due to different abundance of the ECB in both years at the experimental location.

Economic assessment

The economic evaluation was based on the grain production. Both, the two-year average grain yield and income were the highest in the GM hybrid (Figure 2, 3). Differences in income between two years were statistically significant (P < 0.05), differences between hybrids not. The final income was in significant negative relationship (P < 0.05, P < 0.01) to the all observed agronomic parameters with the ex-

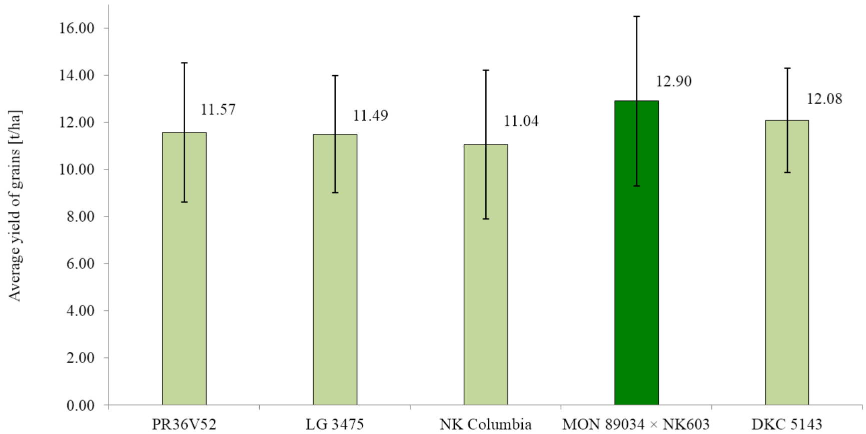

Figure 2. The average grain yield [t/ha] from both years of GM hybrid (dark column) and conventional hybrids (light columns). The values represent the means (± standard error). Differences were statistically insignificant (P > 0.05)

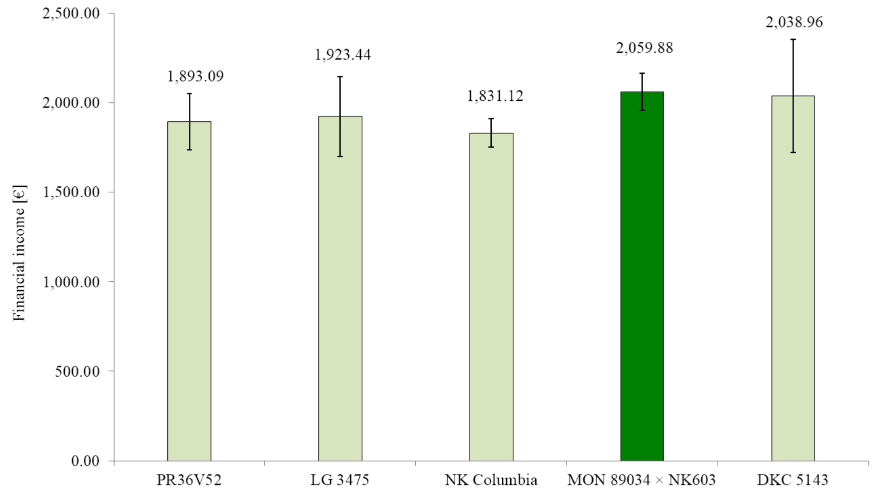

Figure 3. The average financial income (€) from both years of GM hybrid (dark column) and conventional hybrids (light columns) seeds sold (± standard error). Differences were statistically insignificant (P > 0.05)

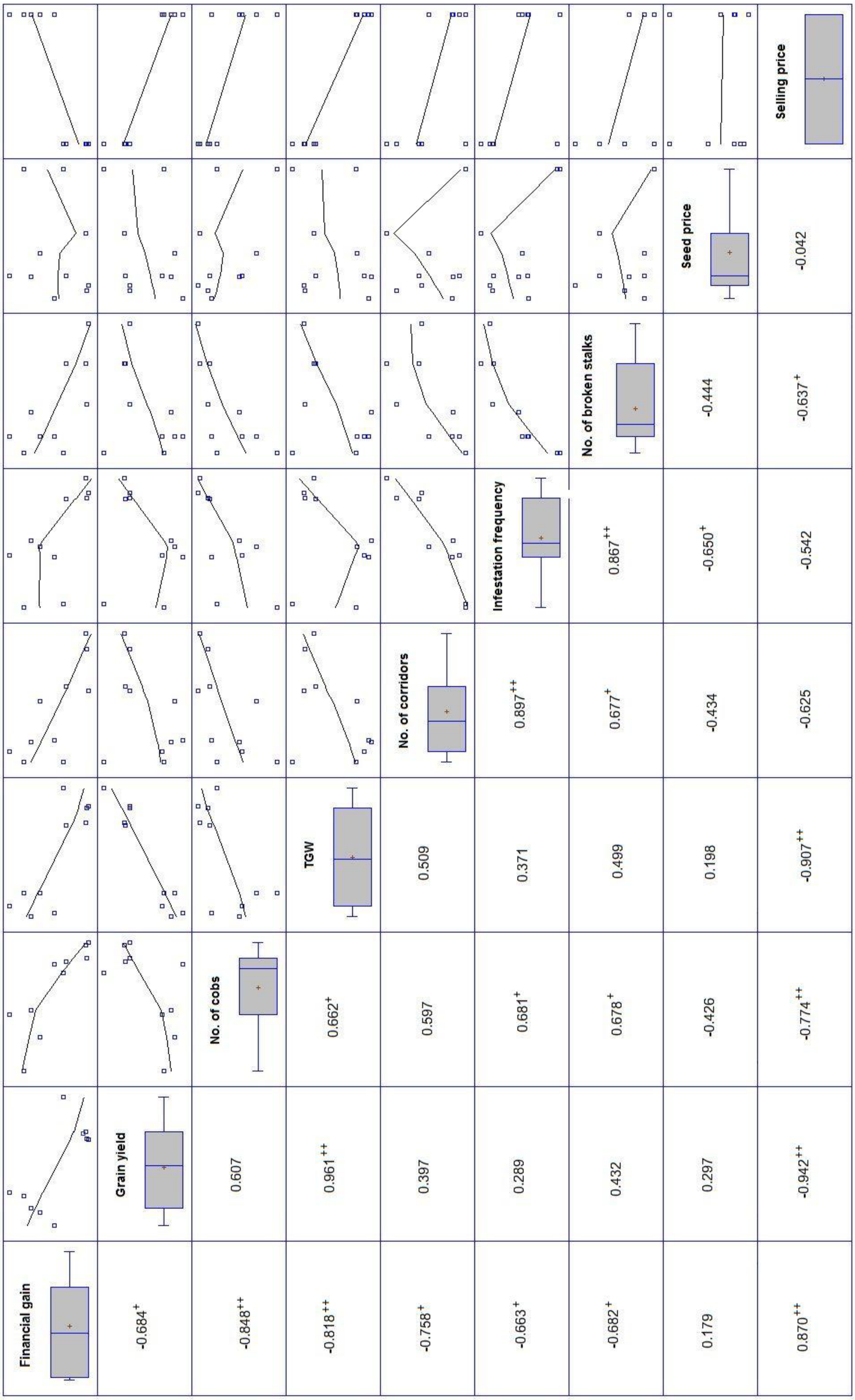

Figure 4. The scatterplot matrix (points represent the values of 5 hybrids in two years and lines represent running means; above the diagonal line) and Spearman's correlation coefficients and level of significance of agronomical and economical parameters of tested conventional and GM hybrids ($^{+}P < 0.05$; $^{++}P < 0.01$; below the diagonal line)

ception of seed price (Figure 4). The most expensive were the seeds of the GM hybrid, with price higher by 18.3–50.4% in comparison with conventional hybrids. It was not proven by our results that that higher seed price of GM hybrid is the main reason why to do not pay to grow them. This initial investment was not statistically significant from the point of view of the final income. Neither the year, nor the hybrid determined the income gained from the grain production and its sale. The only factor that influenced positively and significantly ($P < 0.05$) the financial income was the price of commodity (i.e. maize grains) on the market (Figure 4).

Tested GM hybrid MON 89034 × NK603 has behaved in the field conditions as an equivalent to conventional hybrids. Its resistance against the ECB and yield parameters were similar to *Bt*-maize MON 810 tested previously in Poland, Czech Republic, and Slovakia (Bereś 2010; Kocourek & Stará 2012; Mihalčík *et al*. 2012), as well as to other maize *Bt*-hybrids tested in Canada (Yanni *et al*. 2011). The average abundance of the ECB at our testing location was not very high. Therefore, the resistance against ECB could not very distinctly affect yield of grains (Mungai *et al*. 2005). An economic return should be realized only when the density of ECB is enough to cause economic loss greater than the premium paid for the transgenic seeds (Rice & Pilcher 1998). Both of these conclusions have been reflected in our results. However, there are also others emergent effects of *Bt*-maize growing as association with area – wide suppression of ECB population and related savings for conventional maize growers (Hutchinson *et al*. 2010). Moreover, significantly lower concentrations of mycotoxins produced by *Fusarium* spp. are found in *Bt*-hybrids (Papst *et al*. 2004; Ostry *et al*. 2010). The stacked GM hybrid MON 89034 × NK603 possess also tolerance to glyphosate, but the effect of this transgenic trait on economic performance was not analyzed in our study, although it is known that the application of glyphosate in MON 89034 × NK603 can result to higher grain yield by around 37% (Ravisankar *et al*. 2011). Díaz *et al*. (2017) reported that the GM hybrid MON 89034 × NK603 itself provides economic gain to farmers through higher yields, reduction of production cost for pest control as well as benefit for the environment. Our results present that limits for growing of GM hybrids are not in terms of their grain yield, the quality of production, and economic efficiency. Xu *et al*. (2013) concluded that adoption of genetically engineered traits had a strong positive impact on maize yield in the Central Corn Belt in the USA generally. According to Jones *et al*. (2017) adoption of GM crops could make a positive contribution to competitiveness in any EU country that adopts them. However, this is strongly limited by strict and complicated legislation in the EU that is the main reason for their non-adoption by agricultural practice.

CONCLUSIONS

The GM maize hybrid MON 89034 × NK603 demonstrated equivalency with conventional hybrids in all agronomic traits and economic parameter (income) of grain production. It expressed effectively self-resistance against the ECB. Climatically different growing seasons significantly affected the yield of grain, but all hybrids, including the GM one, responded almost uniformly and differences in grain yield between them were insignificant. Neither the year of cultivation and the genotype, nor the grain yield affected the final economic income, of maize grain production, in contrast to the selling price of maize grains on the market.

Acknowledgements. Funding of this study has been provided by the project ITMS 26210120039 “Systems biology for protection, reproduction and use of plant resources of Slovakia” from the Operational Programme Research and Development co-financed by the European Regional Development Fund.

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Received: January 24, 2018