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ESSENTIAL OIL COMPOSITION OF SWEET BASIL (*OCIMUM BASILICUM* L.) IN SYMBIOTIC RELATIONSHIP WITH *PIRIFORMOSPORA INDICA* AND PACLOBUTRAZOL APPLICATION UNDER SALT STRESS

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Essential oil content and oil composition of paclobutrazol treated sweet basil (*Ocimum basilicum* L.) plant inoculated with *Piriformospora indica* under salt stress were investigated by GC-MS. The results show a slight increase in essential oil content when basil plants subjected to moderate salinity stress (3 dS m^{-1} of NaCl). It decreased significantly with increasing salinity level to 9 dS m^{-1}. The findings revealed that leaf area, above ground and leaf dry weights, essential oil content and yield were significantly affected by *P. indica* inoculation, however paclobutrazol application significantly influenced essential oil yield but not content. Fungal symbiosis as well as paclobutrazol application ameliorated the negative effects of salinity on dry matter and essential oil yield. The main constituents found in the volatile oil of *O. basilicum* in control treatment were Geranial (26.03%), Neral (24.88%) and Estragole (24.78%). The compounds concentrations showed some differences in *P. indica* and paclobutrazol treatments. The results demonstrate that micorrhiza-like fungi concomitantly increase essential oil production and biomass in sweet basil, a medicinal herb rich in commercially valuable essential oils.

Keywords: Essential oil – GC-MS – *Ocimum basilicum* – paclobutrazol – *Piriformospora indica* – salinity

INTRODUCTION

Essential oils are the most important raw materials of the fragrance and cosmetic [26], food and pharmaceutical industries due to their therapeutic, antimicrobial and antioxidant activities [14, 27]. It has also been proven that they have biological activities that make them suitable to be used as herbicides, pesticides and anticancer compounds [20]. In this regard, common basil (*Ocimum basilicum* L.) is one of the most important essential oil crops which are cultivated commercially in many countries. The essential oil of *O. basilicum* is used as perfumery [7], and medicinal plant in folk medicine [27]. Also, antiviral and antimicrobial activities of basil essential oil have been reported [14].

In recent years, researchers are looking for new strategies to deal with salinity in order to minimize its negative effects. One of these methods is application of benefi-

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cial microorganisms (such as symbiotic fungi, bacteria, etc.) to improve growth and increase plant performance. Beneficial fungi improve plant growth and performance as well as enhance host plant tolerance to environmental stresses. They are also involved in phytoremediation, food safety and sustainable production of agricultural products [3].

Piriformospora indica, is one of these beneficial fungi, a plant root endophytic fungi which was isolated from the desert soil of northwest India in the state of Rajasthan [23]. It co-exists with roots of many plant species and promotes their growth and performance [18]. It increases plant resistance to biotic and abiotic environmental stresses such as diseases [11], heavy metals [29], drought [21] and salinity [3, 5]. The symbiotic interaction of endophytic fungi resulted into higher biomass production of the aerial parts of the plant along with floral parts which can be utilized in pharmaceutical industries for medicinally important chemical production. Its metabolic interaction may also favor the synthesis of biologically active secondary metabolites [13].

On the other hand, besides the application of biotic factors in order to alleviate the negative effects of environmental stresses, in recent years, the use of materials with plant growth regulator (PGR) properties has increased in agriculture. Paclobutrazol (PBZ), a triazole derivative which has been used to mitigate environmental stress in some crops. PBZ-mediated stress protection is often explained in terms of hormonal changes [16].

This study was carried out to elucidate the *P. indica* fungi influence on performance of *O. basilicum* plants, especially in saline condition. We comprehensively investigated the alleviative effects of *P. indica* or paclobutrazol on basil plants under salt stress.

MATERIALS AND METHODS

P. indica *inoculum preparation*

The endomycorrhizal fungus *P. indica* culture was kindly gifted by Prof. Kogel, Head of IPAZ institute, University of Giessen, Germany. *P. indica* was cultured in liquid Kafer's medium [15] at 24 °C in 2 weeks.

Root colonization assay

Roots of inoculated basil were washed thoroughly under running tap water and cut into 1 cm pieces. Segments were stained following the method described by Vierheilig et al. [24]. The root-pieces were examined under light microscope at the magnification of ×10–40 (Fig. 1).

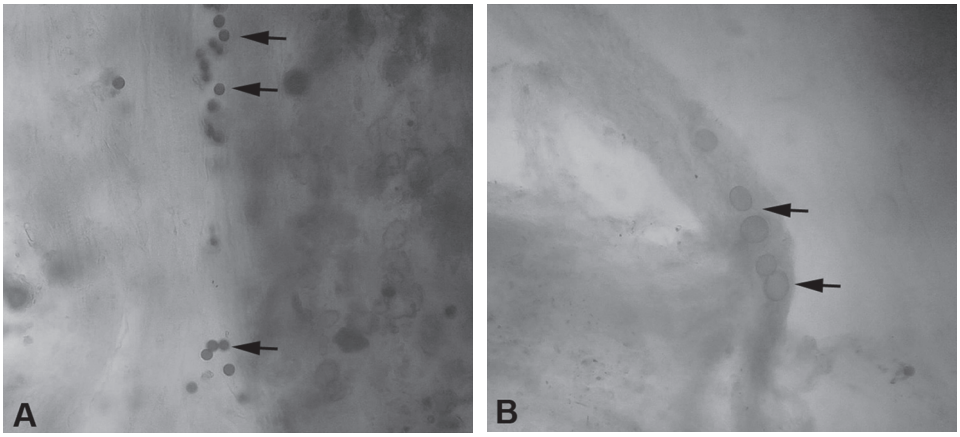

Fig. 1. Development of *P. indica* (arrows) in root cells of 3 weeks old basil plants. A: ×10; B: ×40

Plant materials

A field experiment with factorial arrangement based on randomized complete block design with three replications was conducted. Treatments were fungi inoculation (inoculated and uninoculated control); application of four different salinity levels (0, 3, 6, 9 dS m^{-1} of NaCl), and paclobutrazol application (0, 20, 40 mg L^{-1}).

Local sweet basil seeds were surface sterilized by dipping in 10 percent sodium hypochlorite solution for 5 minutes, then rinsed with sterilized distilled water. The seeds were divided into two parts: one without inoculation and another unit inoculated by dipping in suspension for 3 hours. Then the seeds were sown in farm with sandy-loam soil texture, pH = 7.26 and E.C. = 0.63 dS m^{-1}. Paclobutrazol foliar spraying was performed on the six-leaf-stage of the plants, and one week later, salinity treatment started. Sampling for growth parameters started at early flowering stage.

Essential oil extraction

Aerial parts of cultivated *O. basilicum* at flowering stage were collected in summer (June 2015) during daytime (10–12 a.m.). They were dried in shades, and leaves were then separated and collected. Essential oil was extracted using hydro-distillation in a Clevenger apparatus. Each sample (50 g) was placed in a 2 liter round bottomed flask containing 1.5 L of water and refluxed for 3 h. After completion and recording of the volume (mL), the oil was collected in amber flasks and stored in freezer at –20 °C until GC/MS analysis.

Analysis of the essential oil composition by GC/MS

Essential oil composition was analyzed by GC/MS using Agilent Technologies 7890 equipped with a HP-5MS fused silica column (30 m × 0.25 mm; film thickness 0.25 μm), in the following conditions: helium as carrier gas at 1 mL min^{-1}; injector split at 250 °C (split ratio 1:100); detector at 280 °C, column temperature program was 60 °C for 3 min, followed by 4 °C/min to 240 °C, then 10 °C/min to 270 °C, ending with a 3 min isothermal at 270 °C. The mass spectra were taken at 70 eV. The identification of the constituents was done by computerized matching of the acquired mass spectra (MS5975) with those stored in NIST21 and wailly07 mass spectral libraries of the GC/MS data system and other published mass spectra [1].

Statistical analysis

Data were analyzed using SAS (9.1) statistical program and means were compared using an LSD test ($p < 0.05$).

RESULTS

Essential oil content

The findings of this study show that *O. basilicum* essential oil content and essential oil yield decreased significantly with salinity increase (Table 1). However, there was a slight increase (3% compared to the control) in essential oil content when basil plants were subjected to moderate salinity stress (3 dS m^{-1} NaCl). Compared to the control, 8% reduction was recorded in essential oil content when salinity was increased to 6 dS m^{-1}. This decline was more noticeable when salinity reached to 9 dS m^{-1} and almost decreased to the half (52% reduction) (Table 1).

P. indica symbiosis with basil improved essential oil content by 2%, compared to the uninoculated control. Hence, essential oil yield significantly increased in fungi-inoculated treatments (51% higher than after control treatment). On the other hand, PBZ foliar spray increased essential oil content, however, the increase was not significant. Inoculation with *P. indica* and application of PBZ ameliorate the negative effects of salinity on basil dry matter and essential oil yield (Table 2).

Plant leaf and aerial dry weight

As expected, the results showed that increased salinity significantly reduced plant leaf and aerial dry weight (Table 1). Leaf and aerial dry weight reached to the lowest level in 9 dS m^{-1} of salinity (45% and 60%, respectively, compared to the control). However,

Table 1
Effect of *Piriformospora indica* symbiosis, paclobutrazol foliar spray and soil salinity on *Ocimum basilicum* plants

		Leaf area (cm^2 $plant^{-1}$)	Aerial dry weight (g $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)	Essential oil content (%)	Essential oil yield (L ha^{-1})
Fungi	–Pi	513.94b	8.388b	3.03b	0.716b	9.4122b
	+Pi	614.48a	9.703a	4.773a	0.733a	14.253a
LSD at 0.05		*16.805*	*0.388*	*0.1742*	*0.0054*	*0.5905*
Paclobutrazol (mg l^{-1})	0	567.43a	9.119ab	4.228a	0.721b	12.945a
	20	565.69a	8.691b	3.792b	0.724ab	11.471b
	40	559.51a	9.329a	3.684b	0.728a	11.082b
LSD at 0.05		*20.581*	*0.4752*	*0.2134*	*0.0066*	*0.7232*
Salinity (dS m^{-1} NaCl)	0	774.01a	12.531a	5.21a	0.846b	17.629a
	3	636.96b	10.488b	4.104b	0.869a	14.313b
	6	496.56c	8.168c	3.434c	0.778c	10.699c
	9	349.32d	4.998d	2.857d	0.405d	4.689d
LSD at 0.05		*23.765*	*0.5487*	*0.2464*	*0.0076*	*0.8351*

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

symbiosis relationship with *P. indica* helped basil to ameliorate the negative effect of salinity on dry matter. Compared to the control the most positive effect of symbiosis was observed in medium and high levels of salinity with 77% and 15% (6 dS m^{-1}) 187% and 75% (9 dS m^{-1}) leaf and plant aerial dry weight, respectively, improvement compared to the control (Table 2). PBZ application remarkably decreased basil dry matters in control treatments. However, under salinity condition, PBZ significantly enhanced basil leaf and plant aerial dry weight, especially in medium and high levels of salinity. The highest increase was observed in 40 mg L^{-1} of PBZ (Table 2).

Leaf area

Salt stress significantly reduced basil leaf area. The findings show that inoculation with *P. indica* significantly ameliorate the negative effect of salt stress on leaf area, the improvement was more significant in high (9 dS m^{-1}) level of salinity (59% higher compared to uninoculated control, whereas the improvement in the 0 dS m^{-1} of salinity was only 10%). In normal condition, PBZ application reduced basil leaf area but the reduction was not significant, while under salinity condition, PBZ foliar spray significantly alleviated the negative effects of salt stress on basil leaf area (in 9 dS m^{-1} level of salinity, 14% in 20 mg L^{-1} and 39% in 40 mg L^{-1} PBZ compared to the control).

Table 2
The interaction effect of *Piriformospora indica* symbiosis and paclobutrazol foliar spray with soil salinity on *Ocimum basilicum* plants

Fungi	Salinity (dS m^{-1})	Leaf area (cm^2 $plant^{-1}$)	Aerial dry weight (g $plant^{-1}$)	Leaf dry weight (g $plant^{-1}$)	Essential oil content (%)	Essential oil yield (L ha^{-1})
–Pi	S0	735.88b	12.225a	4.796b	0.838c	16.069b
	S3	607.42d	10.085c	3.371d	0.857b	11.542d
	S6	442.94f	7.604e	2.480e	0.777d	7.709e
	S9	269.54g	3.640g	1.475f	0.394f	2.329f
+Pi	S0	812.15a	12.836a	5.624a	0.853b	19.19 a
	S3	666.50c	10.890b	4.838b	0.882a	17.084b
	S6	550.17e	8.731d	4.388c	0.780d	13.689c
	S9	429.09f	6.356f	4.239c	0.416e	7.049e
LSD at 0.05		*33.609*	*0.7759*	*0.3484*	*0.0108*	*1.181*
Paclobutrazol						
0 $mg \cdot l^{-1}$	S0	827.48a	13.573a	6.050a	0.840b	20.345a
	S3	706.45c	11.351b	4.713c	0.868a	16.422bc
	S6	439.32f	7.661e	3.382ef	0.775c	10.492g
	S9	296.48h	3.891g	2.765h	0.402d	4.522h
20 $mg \cdot l^{-1}$	S0	760.16b	12.177b	5.161b	0.845b	17.465b
	S3	652.94d	10.107c	3.914d	0.868a	13.642de
	S6	511.09e	7.918e	3.243fg	0.778c	10.103g
	S9	338.57g	4.561g	2.850gh	0.405d	4.673h
40 $mg \cdot l^{-1}$	S0	734.39bc	11.843b	4.420c	0.852b	15.078cd
	S3	551.50e	10.005c	3.686de	0.872a	12.877ef
	S6	539.27e	8.924d	3.677de	0.782c	11.502fg
	S9	412.90f	6.543f	2.957fgh	0.408d	4.872h
LSD at 0.05		*41.163*	*0.9503*	*0.4267*	*0.0132*	*1.4465*

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$.

Essential oil components

The essential oils of basil after control treatment were geranial (26.03%) as the major component, neral (24.88%) as the second main component, and estragole (24.78%) as the third component (Table 3).

As expected, oil composition was slightly different after salinity, inoculation and PBZ spraying treatments under normal and stressful condition. It can be seen that the three samples represented in Table 3 (control, *P. indica* and paclobutrazol under normal condition) contained a significant amount of geranial (26.03%, 27.32% and 26.49%, respectively), estragole (24.78%, 24.86% and 24.12%, respectively) and neral (24.88%, 24.98% and 24.16% respectively). The results in Table 3 show that inoculation resulted in the highest content of (6-methyl-5-hepten-2-one; D-limonene; eucalyptol; estragole; neral; geranial; 2,6-Octadien-1-ol, 3,7-dimethyl-, and geraniol) and PBZ treatment gave the highest content of (γ-terpinene; cyclohexanone 5-methyl-2-(1-methylethenyl); 1-tert-butyl-3,3-dimethylcyclopentene; ethenyl-cyclohexane; eugenol and caryophyllene; caryophyllene oxide) in basil plant essential oil composition under in normal condition. However, essential oil composition showed some differences under increased salinity condition, especially when the plant was treated with *P. indica* and PBZ (data not shown).

Table 3
Effect of *P. indica* inoculation and paclobutrazol application on percentage composition of *Ocimum basilicum* essential oils in normal condition

No	Compound	Concentration (%)			
		Rt	Control	Inoculation with *P. indica*	Paclobutrazol (40 mg · l^{-1})
1	α-Pinene	4.91	0.07	0.11	0.10
2	ß-Pinene	6.05	0.04	0.07	0.06
3	1-Octen-3-ol	6.15	–	0.06	0.06
4	6-Methyl-5-hepten-2-one	6.37	0.72	0.94	0.81
5	ß-Myrcene	6.48	–	0.10	0.09
6	Octanal	6.85	–	0.07	0.07
7	D-Limonene	7.61	0.48	0.60	0.39
8	Eucalyptol	7.68	0.18	0.35	0.22
9	Benzeneacetaldehyde	8.11	–	0.11	0.14
10	β-Ocimene	8.26	–	0.09	0.08
11	γ-Terpinene	8.58	–	0.11	0.23
12	Camphor	9.54	0.13	0.14	0.14
13	Benzenemethanol, 4-(1-methylethyl)-	9.89	–	0.20	0.19
14	Linalool	9.99	0.22	0.16	0.18
15	Photocitral B	10.05	0.11	0.14	0.15

Table 3. (continued)

No	Compound	Concentration (%)			
		Rt	Control	Inoculation with *P. indica*	Paclobutrazol (40 mg · l^{-1})
16	±-4-Acetyl-1-methylcyclohexene	11.01	0.14	0.06	0.10
17	Methyl ethyl cyclopentene	11.26	0.28	0.37	0.39
18	1,5-Heptadiene, 2,6-dimethyl-	11.51	0.17	0.29	0.28
19	3,3-Dimethyl-hepta-4,5-dien-2-one	11.64	0.51	0.66	0.69
20	Cyclohexanone, 5-methyl-2-(1-metylethy)-	11.77	0.11	0.68	1.02
21	Photonerol B	12.18	0.11	–	–
22	Menthofuran	12.09	–	0.11	0.16
23	1-Tert-butyl-3,3-dimethylcyclopentene	12.20	0.77	1.28	1.31
24	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	12.44	0.14	0.69	1.08
25	Cyclohexanecarboxylic acid, 2-oxo-, ethyl ester	12.56	0.37	0.51	0.50
26	Ethenyl-cyclohexane	12.84	1.23	1.69	1.73
27	α-Terpineol	13.11	–	0.10	0.11
28	Methyl chavicol (estragole)	13.46	24.78	24.86	24.12
29	2,6-Octadien-1-ol, 3,7-dimethyl-	14.51	1.21	1.49	1.22
30	Neral (citral B)	15.03	24.88	24.98	24.16
31	Geraniol	15.42	0.29	0.44	0.28
32	Geranial (citral A)	16.08	26.03	27.42	26.49
33	Camphene	16.22	–	0.07	–
34	Carane	16.62	–	–	0.11
35	Santolina triene	16.70	0.12	0.08	0.10
36	Phenol, 2-methyl-5-(1-methylethyl)-	16.85	0.09	0.14	0.20
37	2,6-Octadienoic acid, 3,7-dimethyl, methyl ester	17.56	0.15	0.12	0.14
38	α-Cubebene	18.31	0.06	0.07	0.08
39	Eugenol	18.59	0.12	0.14	0.16
40	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester	18.86	0.15	0.16	0.15
41	Copaene	19.13	0.53	0.20	0.26
42	ß-Bourbonene	19.41	0.11	0.10	0.11
43	Hexanoic acid, 4-hexen-1-yl ester	19.56	0.63	0.52	0.56
44	Benzene, 1,2-dimethoxy-4-(2-propenyl)-	20.14	1.15	0.96	1.03
45	Caryophyllene	20.50	1.95	2.17	2.19

Table 3. (continued)

No	Compound	Concentration (%)			
		Rt	Control	Inoculation with *P. indica*	Paclobutrazol (40 mg · l^{-1})
46	Bicyclo[3.1.1]hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	21.03	0.81	0.57	0.55
47	α.-Caryophyllene	21.54	0.85	0.92	1.03
48	1,6,10-Dodecatriene,7,11-dimethyl-3-methylene-	21.72	0.26	0.26	0.26
49	Germacrene D	22.39	1.27	1.18	1.22
50	Naphthalene	22.51	0.27	0.14	0.18
51	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-	23.24	0.11	0.09	0.09
52	α-Bergamotene	23.66	–	0.17	0.17
53	cis-α-Bisabolene	24.27	1.68	1.33	1.33
54	Caryophyllene oxide	25.35	0.77	0.83	0.92
55	12-Oxabicyclo[9.1.0]dodeca-3,7-diene,	26.10	0.60	0.30	0.38
56	γ-Cadinene	26.99	0.22	0.08	0.15
57	α-Bisabolol	28.20	0.13	0.10	0.12
58	4,6-*bis*(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-carbaldehyde	38.64	0.46	0.26	0.24
59	1,10-Phenanthroline	55.99	3.03	–	–
	Total		98.49	99.84	98.28

Rt: Retention.

DISCUSSION

Similar to previous studies published by other researchers, our investigation show that plants treated with *P. indica* were superior in development to control plants. Colonization of roots by fungi has various positive effects on host performance through higher chlorophyll content [30], higher relative water content (RWC) along with greater absorption of water and mineral nutrients [10], moreover increasing the production of growth-promoting substances [4, 10], like synthesis of phytohormones [22].

Our results show that endophytic fungi helped basil to ameliorate the negative effects of salt stress, which was more obvious at higher levels of salinity. This could indicate that *P. indica* is more efficient water higher salinity and intensive salt stress. *P. indica* probably causes accumulation of sodium ions in roots and inhibits their entrance to aerial parts of plants through activation of unknown physiologic or molecular mechanisms, leading to alleviation of negative effects of salt stress [19].

Paclobutrazol decreases cell division in meristematic plates by lower GA levels, followed by the reduction in leaf area [28]. In our experiments, high percentage of essential oil content under PBZ treatments can be explained by the reduced leaf area leading to the increase of oil gland density. On the other hand, an increase in oil content in some of the salt-stressed plants might be attributed to the decline in the primary metabolites due to the effects of salinity, causing intermediary products to become available for secondary metabolites synthesis [17]. However, under higher salinity levels, only a few numbers of plants reached to the flowering stage. Given that the highest amount of basil essential oil is achieved in the full bloom stage [8], so not reaching to flowering stage together with few number of full bloom plants could be the cause of low essential oil content in basil plants of 6 and 9 (ds m^{-1}) salinity level treatments.

It should be noted that despite the fact that application of PBZ decreased growth parameters of basil, it was effective in ameliorating the negative effects of salinity on essential oil yield. It could be argued that PBZ-treated plants had an improved quality of growth, with less foliar necrosis, milder symptom expression, less defoliation [6] and ameliorated dehydration through osmotic balance [12].

In our study, essential oil content and following that, essential oil yield was significantly increased in basil plants inoculated with *P. indica* compared to control plants. The observed modification in the synthesis of essential oils can be considered as a defense response to fungal colonization. Considering the fungicide properties of several essential oils [25] it may be that such a relation exists in the case of the fungal symbiosis. The increased in essential oils in fungi treated *O. basilicum* plants could also be related to the increased number of peltate glands, the structures responsible for oil production [9]. The higher number of glands can be related to alterations in the hormonal profile of the plants as higher levels of auxins, cytokinins, and gibberellins were recorded in inoculated plants [2]. In addition to all mentioned above, significant increase of basil dry weight and leaf area in inoculation treatments (Tables 1 and 2) can be the most effective factor of the modification of essential oil content and essential oil yield. This was more evident when these plants were under salinity stress. However, generally, oil composition differences in all treatments were not significant in our study. Moreover, the differences were not observed in important compounds such as esteragole, geranial and neral (two isomers of citral). High quantities of esteragole and citral in the oil samples make them most important in an economic point of view.

Besides the effects of salinity, endophytic fungi symbiosis and PBZ application on essential oil contents, other factors, like environmental and genetic factors and different chemocultivars, can also influence the oil composition. *P. indica* potentially represent an alternative way of promoting growth of the medicinal herb, sweet basil, especially under environmental stress conditions, as natural ways of growing such crops are currently highly sought after in the herbal industry.

REFERENCES

1. Adams, R. P. (2007) *Identification of essential oil components by gas chromatography/mass spectrometry.* Allured Publ. Corp., San Diego.
2. Allen, M. F., Moore, Jr. T. S. Christensen, M. (1980) Phytohormone changes in *Bouteloua gracilis* infected by vesicular-arbuscular mycorrhizae: I. Cytokinin increases in the host plant. *Can. J. Bot. 58,* 371–374.
3. Ansari, M. W., Trivedi, D. K., Sahoo, R. K., Gill, S. S., Tuteja, N. (2013) A critical review on fungi mediated plant responses with special emphasis to *Piriformospora indica* on improved production and protection of crops. *Plant Physiol. Biochem. 70,* 403–410.
4. Ashoori, M., Ashraf, S., Alipour, Z. T. (2015) Investigating the effect of two species of mycorrhiza fungi and salinity on growth, function and chlorophyll content on *Ocimum basilicum*. *Intl. J. Agri. Crop Sci. 8,* 503–509.
5. Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., Kogel, K. H., Schäfer, P., Schwarczinger, I., Zuccaro, A. (2008) Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytol. 180,* 501–510.
6. Bañón, S., González, A., Cano, E. A., Franco, J. A., Fernández, J. A. (2002) Growth, development and colour response of potted Dianthus caryophyllus cv. Mondriaan to paclobutrazol treatment. *Sci. Hortic. 94,* 371–377.
7. Bauer, K., Garbe, D., Surburg, H. (2008) *Common fragrance and flavor materials: preparation, properties and uses.* Wiley-VCH Verlag, Weinheim, Germany.
8. Carvalho Filho, J. L., Blank, A. F., Alves, P. B., Ehlert, P. A., Melo, A. S., Cavalcanti, S. C., Arrigoni-Blank, M. D., Silva-Mann, R. (2006) Influence of the harvesting time, temperature and drying period on basil (*Ocimum basilicum* L.) essential oil. *Rev. Bras. Farmacogn. 16,* 24–30.
9. Copetta, A., Lingua, G., Berta, G. (2006) Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. *Mycorrhiza 16,* 485–494.
10. Dolatabadi, H. K., Goltapeh, E. M., Moieni, A., Jaimand, K., Sardrood, B. P., Varma, A. (2011) Effect of *Piriformospora indica* and *Sebacina vermifera* on plant growth and essential oil yield in *Thymus vulgaris* in vitro and in vivo experiments. *Symbiosis 53,* 29–35.
11. Ghahfarokhi, R. M., Goltapeh, M. E. (2010) Potential of the root endophytic fungus *Piriformospora indica*; *Sebacina vermifera* and Trichoderma species in biocontrol of take-all disease of wheat *Gaeumannomyces graminis* var. tritici in vitro. *J. Agric. Technol. 6,* 11–18.
12. Hajihashemi, S., Kiarostami, K., Enteshari, S., Saboora, A. (2006) The effects of salt stress and paclobutrazol on some physiological parameters of two salt tolerant and salt sensitive cultivars of wheat. *Pak. J. Biol. Sci. 9,* 1370–1374.
13. Haque, M. A., Hossain, M. S., Rahman, M. Z., Rahman, M. R., Hossain, M. S., Mosihuzzaman, M., Nahar, N., Khan, S. I. (2005) Isolation of bioactive secondary metabolites from the endophytic fungus of *Ocimum basilicum*. *J. Pharm. Sci. 4,* 127–130.
14. Hussain, A. I., Anwar, F., Sherazi, S. T., Przybylski, R. (2008) Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. *Food. Chem. 108,* 986–995.
15. Käfer, E. (1977) Meiotic and mitotic recombination in Aspergillus and its chromosomal aberrations. *Adv. Genet. 19,* 33–131.
16. Manivannan, P., Jaleel, C. A., Kishorekumar, A., Sankar, B., Somasundaram, R., Panneerselvam, R. (2008) Protection of *Vigna unguiculata* (L.) Walp. plants from salt stress by paclobutrazol. *Colloids Surf. B Biointerfaces, 61,* 315–318.
17. Morales, C., Cusido, R. M., Palazon, J., Bonfill, M. (1993) Tolerance of mint plants to soil salinity. *J. Indian Soc. Soil Sci. 44,* 184–186.

18. Oelmüller, R., Sherameti, I., Tripathi, S., Varma, A. (2009) *Piriformospora indica*, a cultivable root endophyte with multiple biotechnological applications. *Symbiosis 49,* 1–7.
19. Sepahri, M., Saleh Rastin, N., Hossieni Salkedeh, G., Khayam Nekouie, M. (2009) Effect of endophytic fungus, *Piriformospora indica*, on growth and resistance of *Hordeum vulgare* L. to salinity stress. *Rangeland 3,* 508–518 (In Persian).
20. Sharafzadeh, S., Zare, M. (2011) Influence of growth regulators on growth and secondary metabolites of some medicinal plants from Lamiaceae family. *Adv. Environ. Biol. 1,* 2296–2303.
21. Sun, C., Johnson, J. M., Cai, D., Sherameti, I., Oelmüller, R., Lou, B. (2010) *Piriformospora indica* confers drought tolerance in Chinese cabbage leaves by stimulating antioxidant enzymes, the expression of drought-related genes and the plastid-localized CAS protein. *J. Plant. Physiol. 167,* 1009–1017.
22. Vadassery, J., Ritter, C., Venus, Y., Camehl, I., Varma, A., Shahollari, B., Novák, O., Strnad, M., Ludwig-Müller, J., Oelmüller, R. (2008) The role of auxins and cytokinins in the mutualistic interaction between Arabidopsis and *Piriformospora indica*. *Mol. Plant-Microbe Interact. 21,* 1371–1383.
23. Varma, A., Verma, S., Sahay, N., Bütehorn, B., Franken, P. (1999) *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. *Appl. Environ. Microbiol. 65,* 2741–2744.
24. Vierheilig, H., Coughlan, A. P., Wyss, U., Piché, Y. (1998) Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Appl. Environ. Microbiol. 64,* 5004–5007.
25. Vos, C. M., Yang, Y., De Coninck, B., Cammue, B. P. (2014) Fungal (-like) biocontrol organisms in tomato disease control. *Biol. Control, 74,* 65–81.
26. Wogiatzi, E., Papachatzis, A., Kalorizou, H., Chouliara, A., Chouliaras, N. (2011) Evaluation of essential oil yield and chemical components of selected basil cultivars. *Biotechnol. Equip. 25,* 2525–2527.
27. World Health Organization. (2011) *Traditional medicine fact sheet No 134.* December, 2008.
28. Yeshitela, T., Robbertse, P. J., Stassen, P. J. (2004) Paclobutrazol suppressed vegetative growth and improved yield as well as fruit quality of 'Tommy Atkins' mango (*Mangifera indica*) in Ethiopia. *N. Z. J. Crop Hortic. Sci. 32,* 281–293.
29. Zhang, H. W., Song, Y. C., Tan, R. X. (2006) Biology and chemistry of endophytes. *Nat. Prod. Rep. 23,* 753–771.
30. Zuccarini, P., Okurowska, P. (2008) Effects of mycorrhizal colonization and fertilization on growth and photosynthesis of sweet basil under salt stress. *J. Plant Nutr. 31,* 497–513.