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Using Alternative Fungicides and Fertilizers for Management Root Rot and Wilt Diseases and Their Effect on Productivity of Rosemary Plant

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ROOT rot and wilt are considering the most important diseases, causing serious yield losses of rosemary plant. The aim of this work was to investigate the occurrence and distribution of root rot and wilt diseases of *R. officinalis* and identification of their causal pathogens. Also, develop control safe means to management these diseases.

Root rot and wilt rosemary plants symptoms were collected from Qalubyia and Giza Governorates during season 2017. The pathogenicity tests of the isolated fungi, *i.e.* (*Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium debaryanum, Rhizoctonia solani* and *Sclerotinia sclerotiorum*) revealed the ability of these fungi to cause infection. Application of some safe alternatives fungicide and fertilizers, *i.e.* potassium silicate, bioproduct (Plant Guard) and NPK fertilizer compared with systemic fungicide, Carbendazim 50% WP, against the isolated fungi were performed in the greenhouse.

All the tested treatments significantly reduced disease incidence under greenhouse conditions. While, the treatment (NPK + Potassium silicate + Plant Guard) was more efficient in reducing infection of root rot and wilt diseases in the field during 2018 & 2019 and superiority increasing of growth characteristics. Gas chromatographic analysis of rosemary essential oil showed that, Camphor was found as a major component (33.18%), followed by Eucalyptol (1,8-Cineole) with (NPK + Potassium silicate + Plant Guard) treatment. Also, the total phenols and flavonoids content as antioxidants improved with the same treatment in comparison with control.

Keywords: Rosemary, *Rosmarinus officinalis*, Plant Guard, NPK, Carbendazim, Potassium silicate, *Sclerotinia sclerotiorum*, *Fusarium oxysporum*.

Introduction

Rosmarinus officinalis L. (Fam. Lamiaceae) is an important medicinal and aromatic plant commonly used as spice and flavouring agent in foods industry, as preservative or aromatic herb. Among the pharmacologically validated medicinal uses of rosemary are antibacterial, antioxidant and anticancer **(**Bozin et al., 2007 and Yesil-Celiktas et al., 2010**)**. Rosemary (*Rosmarinus officinalis* L.) plant exerts a great number of biochemical and pharmacological properties, namely hepatoprotective (Ponce et al., 2008). Essential oils of *R. officinalis* have been used in aromatherapy, with effect on central nervous system, increasing of breathing rate and blood pressure on human (Hongratanaworakit*,* 2009), as well as antifungal activity**,** (Özcan and Chalchat, 2008) and anti-inflammatory action (Juhás et al., 2009). The main compounds responsible for these biological activities are α-pinene, camphor, 1,8-cineole and bornyl acetate, (Daferera et al.,

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2000, 2003 and Pintore et al., 2002). Root-rot and wilt symptoms have been observed in rosemary plantations in Egypt which resulted in reduction of plant stand, vegetative growth, and consequently low essential oil yield. The causal pathogens of root-rot and wilt diseases are mainly soil-borne diseases. Percentage of occurrence of these diseases always increased in the absence of the control measures in nursery or field conditions. Soilborne fungal diseases, especially root rot and/or wilt, caused by various pathogens, are amongst the most hazardous and destructive disorders on rosemary all over the world. Conway et al. (1997) mentioned that, aerial blight caused by *Rhizoctonia solani* AG-4, was identified as a major disease of greenhouse mist-produced rosemary *(R. officinalis)* cuttings. Putnam (2004) reported that, the isolated *Sclerotinia sclerotiorum* fungus showed on the young rosemary plants growing in a glasshouse in Oregon, USA exhibited branch dieback with grey lesions on the lower stem, wilt of the foliage and entire plant necrosis. All tested antagonists which coating fennel seeds at the rate of 5g/kg seeds reduced the incidence of pre-, post-emergence damping off and root rot diseases. *Trichoderma harzianum*, *T. viride* and Bio Zeid "*T.album* were the most effective antagonists as shown by the highest plants survival and the best fennel yield under field conditions. Moreover application of these antagonists recorded the highest increase in oil amount and oil components as compared with the control. (Ahmed et al., 2017).

The purpose of this study is to investigate the occurrence and distribution of root-rot and wilt diseases of *R. officinalis* and identification of their causal pathogens. Also, develop control safe means to management these diseases. Therefore, the present study expands to evaluate some alternative fungicide means and fertilizers comparison with a recommended fungicide and their effects on plant growth parameters, essential oil content and yield. Moreover, chemical composition of oil and total flavonoids and phenolic content of rosemary plant extract.

Materials and Methods

Laboratory experiments

Isolation, purification and identification of the causal organisms and their frequency

Naturally infected rosemary plants showing symptoms of root rot and wilt disease were collected from Qalubyia and Giza Governorates during 2017 season. Infected roots and stems were cut into small fragments, thoroughly washed with a tap water, then surface sterilized with 2.5 % v/v sodium hypochlorite for two minutes, rinsed several times in sterilized water, and dried between two sterilized filter papers. Plant segments were placed on PDA plates, incubated at 27 ˚C and examined during one week. Counts of fungal colonies grown on PDA were recorded. Purification of the isolated fungi was carried using of hyphal tip or single spore techniques. Fungi were identified depending on their morphological features according to description of Booth (1971), Domsch et al. (1980) and Niternik and Vandler (1981) and the identification was confirmed by the staff member of Department of Mycology, Plant Disease Survey and Plant Pathology Research Institute, ARC, Giza, Egypt. Pure cultures were transferred into PDA slants and kept at low temperature (5˚C) for further studies.

Pathogenicity tests

Pathogenicity test was conducted to all the isolated fungi, *i.e.* (*Fusarium oxysporum, F. solani*, *M. phaseolina, P. debaryanum*, *R. solani* and *S. sclerotiorum*). Soil (1 sand , 1 peatmoss , 1 clay, w,w,w) was sterilized by 5 % formalin solution for one week, then left to dry for two weeks before use. The tested fungi were cultured on autoclaved sorghum grains medium (100 g. corn + 50 g. washed sand + 100 ml. water) and incubated at 27˚C for 15 days, except of *S. sclerotiorum* was incubated at 18±2 ˚C. Soil infestation with above isolated fungi was applied at the rate of 1 % w/w, which thoroughly mixed with the soil of 20 cm except for the control treatment without infection plastic pots were watered one week before planting to enhance colonization of fungi. Each pot was planted with five seedlings. Each treatment was replicated three times. Disease incidence was recorded 30 and 60 days after planting.

Percentage of plants infected with root rot and wilt was recorded as disease percentage using the following formula according to Ahmed, 2013,

$$\%\ \text{Disease incidence} = \frac{\text{Number of infected plants}}{\text{Total plant numbers}} \times 100$$

Fungi were re-isolated from diseased plants and compared with the original isolates.

Greenhouse experiments

Under greenhouse experiment, 30cm plastic pots filled with formalin sterilized mixture sand, peatmoss and clay soil (1, 1, 1 w, w, w) were used for planting. Uniform rooted cuttings about 10 to 12 cm length bearing 10 to 12 leaves (thirty days old) of *Rosmarinus officinalis* L., provided from Medicinal and Aromatic Plants Section at El-Kanater El-Khairia, Qalubia Governorate,Horticulture Research Institute, A.R.C. 5 seedlings/pot were planted for each treatment. Fungicide of Carbendazim 50% WP (2 g/l water), elicitor (potassium silicate) at rate 4 g/l (Nada et al., 2014), bioproduct (Plant Guard) at rate of 4 g/l, fertilizer (NPK) at rate of 5 g/l, [Potassium silicate (4 g/l) + Plant Guard (4 g/l)], [NPK (5 g/l) + Potassium silicate (4 g/l)], [Plant Guard (4 g/l) + NPK (5 g/l)] and [NPK (5 g/l) + Potassium silicate (4 g/l) + Plant Guard (4 g/l)] were used throughout these experiments. Seedlings were dipped in the previously prepared treatments or in water only as control for 30 minutes and planted in the infested soil. A set of 3 plastic pots were used for each particular treatment and control. Each treatment was applied as drench after 15 days from planting. Percentages of infected plants were recorded after 30 and 60 days from transplanting (Ahmed et al., 2017).

Field experiment

A field experiment was carried out at the Faculty of Agriculture farm, Cairo University, Egypt during two successive seasons (2018 and 2019) to evaluate the effect of some safe alternative fungicides and fertilizers *i.e.* potassium silicate, NPK fertilizer and bioproduct (Plant Guard, *Trichoderma harzianum*, 30 × 10^6 cfu/ml) compared with systemic fungicide, Carbendazim 50% WP, in management rosemary wilt and root rot diseases and their effect on productivity. Treatments were arranged in a complete randomized blocks design with 3 replicates. The experimental unit was 6 m^2 (2.4 × 2.5 m) with 4 rows in each plot (10 plants/row). *R. officinalis* L. seedlings were transplanted in the experimental area on March 7*th* in 2018 and 2019 seasons in a naturally infested field with root rot and wilt diseases (This location was chosen because it has a long history of heavy infection).Compost at the rate of 10 m^3/fed was applied to all the experiment area during the preparation of the soil (Ahmed, 2013). The chemical composition of compost which was made from agricultural wastes are obtained from El-Nile Company, Egypt are shown in Table (a),

The following treatments were applied under field experiments

- Potassium silicate (K_2SiO_3) at rate of 4 g/l.
- Bioproduct, Plant Guard (*Trichoderma harzianum*, 30 × 10^6 cfu/ml) at rate of 4 g/l.
- NPK fertilizers were added at the recommended dose of NPK fertilizers (according to the Ministry of Agriculture). as 45 kg/fed calcium super phosphate (15.5%P_2O_5), which was added before transplanting. nitrogen and potassium (48 kg/fed potassium sulphate (48% K_2O, and 60 kg/fed ammonium nitrate (33.5% N), were applied in four equal doses, the 1st one was added before transplanting, the second was applied 45days after transplanting, the third and the fourth were added at monthly intervals.
- Fungicide, Carbendazim 50% WP [Common name, Carbendazim, Chemical composition, 2-(Methoxycarbomylsmino)-benzimidazole) and Manufacture, Agriphar S.A., Belgium.].
- Potassium silicate (4 g/l) + Plant Guard (4 g/l).
- NPK (at the recommended dose) + Potassium silicate (4g/l).
- Plant Guard (4 g/l) + NPK (at the recommended dose).
- NPK (at the recommended dose) + Potassium silicate (4 g/l) + Plant Guard (4 g/l).
- Control (untreated plants).

- Seedlings were dipped in Plant Guard, potassium silicate, Carbendazim 50% WP and water only (as a control) for 30 minutes directly before transplanting and soil drench after planting. The second application as soil drench was 15 days from the first one while, the third and fourth application were applied after the first and second cut in field, respectively using pressurized backpack sprayer and spray wand equipped with one nozzle calibrated to deliver 100 ml/plant.

Data collection and analysis

Disease incidence

The incidence of root rot and wilt diseases of rosemary was determined in field 30 and 60 days after sowing date according to the above mentioned formula .

TABLE (a) The chemical composition of compost.

Characters	
Density	617 kg/m^3
Moisture content	22%
pH (1,15)	7.8
EC (electrical conductivity)(1,15)	6.45 dS/m
Total nitrogen	1.56%
Ammonium-N	1297 mg/kg
Nitrate-N	102 mg/kg
Organic matter	40.49%
Organic carbon	23.48%
Ashe	59.51%
Carbon, nitrogen ratio (C/N ratio)	1,15
Total Phosphorus	2.67%
Total potassium	1.38%
Calcium	1.52%
Magnesium	0.80%
Iron	178 mg/kg
Manganese	469 mg/kg
Cupper	48 mg/kg
Zinc	523 mg/kg
Nematode	Nil
Herbs seeds	Nil
Parasites	Nil

The above mentioned analysis was carried out at The Integrated System of Agricultural Residues Recycling Unit, The Soils, Water and Environment Research institute (SWERI), ARC. Egypt.

Vegetative growth and yield traits

Two cuts were harvested, the first one was 20*th* July and the second on 20*th* *September* in both seasons. Plant samples were randomly collected from all treatments. Plant growth parameters for the two cuts were recorded as plant height (cm), number of branches per plant, fresh and dry weights of herb (g/plant).

Essential Oil Percentage, Yield and constituents

Essential oil was isolated from treated dried herbs of *R. officinalis* by hydrodistillation, according to the standard procedure reported in the Council of Europe. European Pharmacopoeia, (2019). The essential oils were stored in a dark sealed vial at −4°C for further studies. The essential oil yield was calculated in both seasons.

The Gas chromatography analysis of the essential oil samples was carried out using Ds Chrom 6200 Gas Chromatograph apparatus, fitted with capillary column BPX-5, 5 phenyl (equiv.) polysillphenylene-siloxane 30 x 0.25 mm ID x 0.25µ film. The temperature program varied in the range of 70-200 °C, at a rate of 10 °C/min. Flow rates of gases were nitrogen at 1 ml/min, hydrogen at 30 ml/min and 330 ml/min for air. Detector and injector temperatures were 300 °C and 250 °C, respectively.

Total flavonoids content (TFC)

TFC were analyzed using aluminium chloride colorimetric method as mentioned by Miliauskas et al. (2004) with few modifications as 2 ml of plant extract was mixed with the same volume of 2% aluminium chloride solution (Al Cl_3). The mixture was held for 30 min at room temperature. Absorbance measurement was realized at 415 nm against a blank sample, consisting of a 5 ml aluminium chloride without sample, using UV–visible spectrophotometer. The total flavonoids contents were determined using a quercetin (0-50mg/l) as the standard. TFC was presented in milligrams of quercetin equivalents (QE)/100g of dry weight.

Total phenolic content

Total phenolic content was measured using Folin-Ciocalteu procedure (Pothitirat et al., 2009). A volume of 1 ml of plant extract (ethanolic extract) was mixed with 5 ml of the Folin-Ciocalteu reagent and 4 mL of sodium bicarbonate solution [7% (w/v)]. The obtained solution was maintained for 2 h in dark at room temperature. A UV–visible spectrophotometer was used to record absorbance at 765 nm. The result was defined as grams of gallic acid equivalents (GAE) /100 g of dry weight.

Statistical Analysis

This experiment was arranged in a completely randomized design with 9 treatments, 3 replicates in each. The collected data were statistically analyzed by using MSTAT statistical software and the treatments means were compared by using Least significant Difference test (LSD) at 0.5 level of probability according to Snedecor and Cochran (1989).

Results

Isolation, identification and frequency percentages of the isolated fungi

Five fungal genera were isolated from infected samples of rosemary seedlings (60-days-old) and mature plants. Samples showed root rot and/or wilt symptoms were obtained from different nurseries and fields in Giza and Qalubiya Governorates during 2017 season (Fig. 1). The isolated fungi were identified to species as, *Fusarium oxysporum* Schlect., *F. solani* (Mart) Sacc., *Macrophomina phaseolina* (Tassi) Goid., *Pythium debaryanum* Hesse, *Rhizoctonia solani* Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary (Table 1).

As regards percentages of occurrence of the isolated fungi, data presented in Table 1 demonstrated that *F. oxysporum* (35.96%) and *R. solani* (16.28%) were the most frequently isolated

Control (A) (B) (C)

Fig. 1. Naturally infected of rosemary plants, with root rot and wilt symptoms (A) (uninfected plant), B and C (infected plants).

TABLE 1. Frequency percentages of fungi isolated from rosemary plants showing root rot and wilt symptoms, during season 2017 in two governorates, Egypt.

Isolated fungi	**Giza**		**Qalubyia**		**Mean %**
	No. of isolates	**Frequency (%)$^{+}$**	**No. of isolates**	**Frequency (%)$^{+}$**	
Fusarium oxysporum	81	35.37	87	36.55	35.96
Fusarium solani	16	6.99	21	8.82	7.91
Macrophomina phaseolina	26	11.35	29	12.18	11.77
Pythium debaryanum	35	15.28	31	13.03	14.16
Rhizoctonia solani	37	16.16	39	16.39	16.28
Sclerotinia sclerotiorum	34	14.85	31	13.03	13.94
Total No.	229	100	238	100	-

$^{+}$Frequency (%) = (Number of isolates of each fungi / Total number of isolates of all fungi) x100.

fungi followed by *Pythium debaryanum* (14.16 %) and *Sclerotinia sclerotiorum* (13.94%). On the contrary, *Fusarium solani* recorded the lowest mean percentages of occurrence(7.91%), Whereas, *Macrophomina phaseolina* (11.77%) gave the intermediate frequency percentages in isolation trials, in Qalubiya and Giza Governorates.

Pathogenicity tests

Percentages of infected rosemary plants throughout pathogenicity trials under greenhouse conditions were determined (Table 2 and Fig. 2). All fungi tested were significantly pathogenic to rosemary plants causing root rot or wilt diseases in different degrees compared with the control treatment. Moreover, percentages of infection increased as growing plants progressed in age from 30 to 60 days after transplanting. *Macrophomina phaseolina* followed by *Sclerotinia sclerotiorum* were the most virulent fungi with significant differences between each other as they recorded the highest percentages of reduction in survival plants (40.0 & 53.3%, respectively). On the contrary, *F. solani* and *Pythium debaryanum* (26.7%) were the least pathogenic fungi resulting in infected plants after 60 days from transplanting, subsequently, fungi recorded the highest percentage of healthy survivals plants (73.3%). Symptoms of artificially infection by the pathogenic fungi on rosemary plants.

TABLE 2. Pathogenicity of the six isolated fungi on seedlings of rosemary plants after 30 and 60 days, grown in infested soil under greenhouse conditions.

Fungi	(%) Infection after 30 days	(%) Infection after 60 days	Survivals (%)
usarium oxysoprum	6.7	33.3	66.7
F. solani	13.3	26.7	73.3
Macrophomina phaseolina	26.7	60.0	40.0
Pythium debaryanum	20.0	26.7	73.3
Rhizoctonia solani	20.0	40.0	60.0
Sclerotinia sclerotiorum	20.0	46.7	53.3
Control (without fungus)	0.00	0.00	100
L.S.D. at 5%	2.13	1.82	-

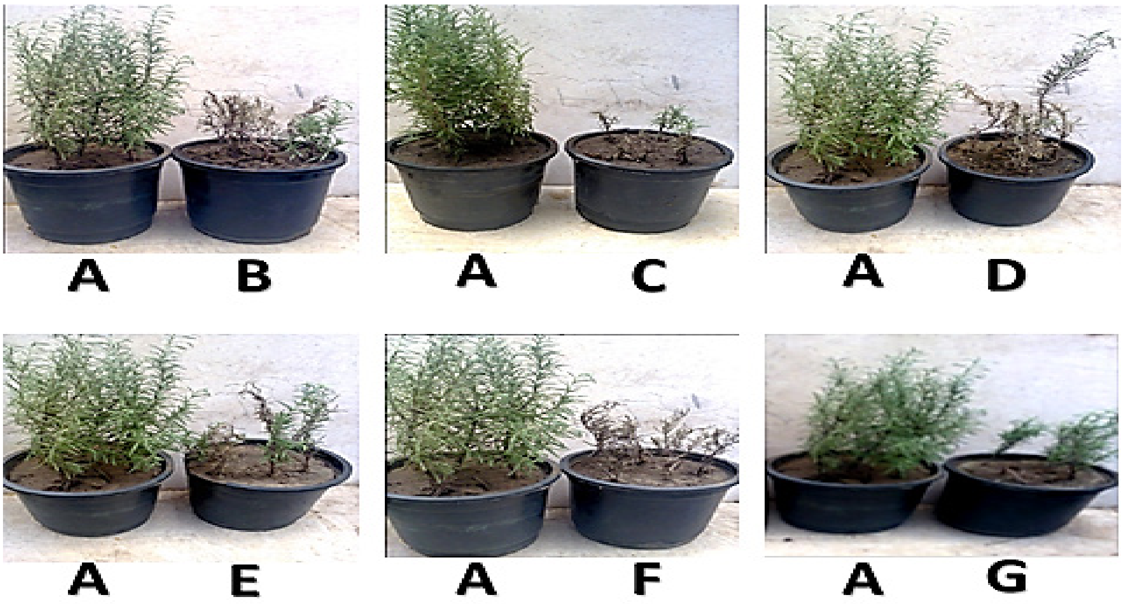

Fig. 2. Rosemary plants grown in soil infested with, *M. phaseolina* (B), *Sclerotinia sclerotiorum* (C), *R. solani* (D), *F. oxysporum* (E) *Pythium* sp. (F) and *F. solani* (G) showing root rot and wilt diseases symptoms on plant growth compared with the healthy plants (A).

Greenhouse experiments

Effect of various control treatments on Disease incidence (%)

Data in Table 3 indicated that, percentages of fungal infections were decreased with all tested treatments. The fungicide (Carbendazime 50% WP), followed by [NPK + Potassium silicate + Plant Guard], [Plant Guard + NPK] and [NPK + Potassium silicate] were the most effective treatments, since they gave the highest significant decreases in disease infection than the controls with all tested fungi after 30 and 60 days from planting. In contrast, NPK treatment recorded the lowest percentage reduction of infection after 60 days from planting with all tested fungi. Followed by, potassium silicate treatment in case of *M. phaseolina*.

Carbendazime 50% WP and [NPK + Potassium silicate + Plant Guard] completely prevented infection of rosemary in case of *F. oxysporum* and *M. phaseolina* after 30 and 60 days from planting, Carbendazime 50% WP in case of *R. solani* and [Plant Guard + NPK], [NPK + Potassium silicate] in case of *F. oxysporum.*

Field experiment

Effect of various control treatments on Disease incidence (%)

Data in Table 4 indicated that, all the treatments tested significantly decreased the percentages of disease incidence after 30 and 60 days of planting. Carbendazim 50%WP and (NPK + Potassium silicate + Plant Guard) were gave the highest decrease percentages followed by (NPK + Potassium silicate), (Plant Guard + NPK) and (Potassium silicate + Plant Guard) in both seasons, respectively. On the other hand, Plant Guard in the first season and potassium silicate and Plant Guard in the second one were the least effective treatments.

The highest percentages of survival, were recorded in case of Carbendazim 50%WP, (NPK + Potassium silicate + Plant Guard), (NPK + Potassium silicate), (Plant Guard + NPK) and (Potassium silicate + Plant Guard) in the both seasons. Whereas, potassium silicate and plant guard were the least effective treatments *on* increasing survivals in both seasons, respectively.

Plant growth parameters

The obtained data in Tables 5&6 showed that all treatments tested at the first and second cuts during the two seasons significantly increased plant growth parameters, i.e. plant height (cm), number of branches/plant and fresh and dry weight /plant (g) of *R. officinalis* compared to the control. Treatments of dual combination between (NPK + Potassium silicate + Plant Guard), (NPK + Potassium silicate) and (Plant Guard + NPK) recorded obviously increasing in all parameters compared to control in both seasons. In contrast, treatment of Plant Guard was the least effective one in both trial seasons.

TABLE 3. Effect of dipping seedlings roots with various control treatments before transplanting and soil drench 15 days from planting date of rosemary on disease incidence under greenhouse conditions.

Treatments	*F. oxysporum*		*M. phaseolina*		*R. solani*		*S. sclerotiorum*	
	(%) Disease incidence after ,		**(%) Disease incidence after ,**		**(%) Disease incidence after ,**		**(%) Disease incidence after ,**	
	30 days	**60 days**	**30 days**	**60 days**	**30 days**	**60 days**	**30 days**	**60 days**
Plant Guard	6.7	13.3	13.3	26.7	6.7	13.3	13.3	26.7
Potassium silicate	6.7	13.3	20.0	33.3	6.7	13.3	13.3	20.0
NPK	6.7	20.0	26.7	40.0	13.3	20.0	13.3	33.3
Potassium silicate + Plant Guard	0.0	6.7	20.0	26.7	0.0	13.3	6.7	20.0
NPK + Potassium silicate	0.0	0.0	13.3	20.0	0.0	6.7	6.7	13.3
Plant Guard + NPK	0.0	0.0	6.7	20.0	0.0	6.7	0.0	13.3
NPK + Potassium silicate + Plant Guard	0.0	0.0	0.0	0.0	0.0	6.7	6.7	6.7
Carbendazim 50% WP	0.0	0.0	0.0	0.0	0.0	0.0	6.7	6.7
Control	13.3	33.3	40.0	60.0	20.0	33.3	20.0	46.7
L.S.D. at 5%	2.6	3.9	3.6	3.5	2.9	5.6	5.9	4.7

TABLE 4. Effect of alternative fungicides and NPK on disease incidence 30 and 60 days after transplanting of rosemary under naturally infection with root rot and/or wilt, of 2018 & 2019 seasons.

Treatments	2018 season			2019 season		
	% Disease incidence after,		% Survival	% Disease incidence after,		% Survival
	30 days	60 days		30 days	60 days	
Plant Guard	10.0	20.0	80.0	12.5	17.5	82.5
Potassium silicate	12.5	17.5	82.5	12.5	17.5	82.5
NPK	10.0	15.0	85.0	7.5	12.5	87.5
Potassium silicate + Plant Guard	7.5	15.0	85.0	5.0	10.0	90.0
NPK + Potassium silicate	7.5	12.5	87.5	2.5	7.5	92.5
Plant Guard + NPK	7.5	12.5	87.5	2.5	10.0	90.0
NPK + Potassium silicate + Plant Guard	5.0	10.0	90.0	2.5	5.0	95.0
Carbendazim 50%WP	3.3	3.3	96.7	0.0	0.0	100.0
Control(without treatment)	22.5	35.0	65.0	22.5	32.5	67.5
L.S.D. at 5%	5.5	4.2	-	6.4	5.1	-

TABLE 5. Effect of alternative fungicides and NPK on plant height and No. of branch/plant of rosemary plants, grown under naturally infection with root rot and/or wilt, at the two cuts of the seasons of 2018 & 2019.

Treatments	Plant growth parameters,							
	2018 season				2019 season			
	Plant height (cm)		No. of branches/ plant		Plant height (cm)		No. of branches/ plant	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Plant Guard	39.33	32.67	30.67	43.00	43.67	36.00	41.33	60.00
Potassium silicate	40.67	33.67	32.33	54.00	44.67	37.67	45.33	73.33
NPK	45.00	37.33	34.67	60.33	47.33	39.33	59.67	80.00
Potassium silicate + Plant Guard	46.67	39.00	36.67	62.00	49.67	41.67	63.67	82.00
Plant Guard + NPK	48.91	41.00	38.67	64.67	55.33	45.67	68.33	83.33
NPK + Potassium silicate	51.00	43.33	42.00	71.00	66.41	46.62	72.33	85.00
NPK + Potassium silicate + Plant Guard	52.43	46.28	46.00	72.67	69.33	48.00	75.33	86.70
Carbendazim 50%WP	42.67	36.67	33.33	57.00	45.67	38.00	57.00	75.00
Control(without treatment)	34.33	25.67	16.67	25.00	35.67	27.33	33.33	56.00
L.S.D. at 5%	2.7	1.3	1.5	6.3	1.7	1.2	2.5	0.8

Essential oil percentage and oil yield/plant (ml)

Data recorded in Table 7 revealed that, the essential oil percentage and yield per plant of rosemary plants were significantly affected by some safe fungicides alternatives, potassium silicate, bioproduct (Plant Guard, *Trichoderma harzianum*), NPK fertilizer and systemic fungicide (Carbendazim 50% WP). It was also noticed that, the combination treatment of NPK, Potassium silicate with Plant Guard gave the highest values of essential oil percentage (0.383, 0.375, 0.372 and 0.363%) and essential oil yield per plant (0.368, 0.165, 0.494 and 0.383 ml/plant) in both cuts of the first and second season respectively, followed by dual combinations between (NPK + Potassium silicate). In contrast, treatment of Plant Guard recorded the lowest value for essential oil % (0.295, 0.279, 0.302 and 0.282%) and essential oil yield (0.114, 0.070, 0.136 and 0.098 ml/plant) in both trial seasons compared to control. Concerning the effect of NPK fertilizer and potassium silicate, it was found that, significant promotion on the essential oil % in 1st and 2nd cuts in both seasons were due to potassium silicate or NPK fertilizer, but, it was insignificant differences with bioproduct (Plant Guard, *Trichoderma harzianum*). NPK fertilizer treatment was the best treatment in this regard, followed by potassium silicate treatment. Whereas, treatment with bioproduct induced the lowest essential oil % and essential oil yield in the two seasons compared to untreated control.

TABLE 6. Effect of alternative fungicides and NPK on fresh and dry weights of plant, grown under naturally infection with root rot and/or wilt, at the two cuts of the seasons of 2018 & 2019.

Treatments	**Plant growth parameters**							
	2018 season				**2019 season**			
	Plant fresh weight (g)		**Plant dry weight (g)**		**Plant fresh weight (g)**		**Plant dry weight (g)**	
	1st cut	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**
Plant Guard	132	98.67	38.73	25.1	154.00	110.00	45.15	34.70
Potassium silicate	143.33	104.67	41.12	31.05	183.67	125.00	52.89	36.85
NPK	186	115.33	60.11	32.31	231.33	158.00	67.08	43.02
Potassium silicate + Plant Guard	210.67	121	60.98	33.27	240.67	169.00	77.93	45.10
Plant Guard + NPK	225.67	125.33	73.52	33.66	258.33	178.67	83.73	47.92
NPK + Potassium silicate	236	130	75.77	37.29	274.33	180.67	96.56	51.09
NPK + Potassium silicate + Plant Guard	248.67	141.67	96.17	44.19	294.00	185.33	132.84	64.25
Carbendazim 50%WP	157.67	112.67	45.76	31.08	226.33	146.00	65.57	37.23
Control(without treatment)	113.33	70.67	32.1	19.31	124.33	76.33	35.2	20.65
L.S.D. at 5%	5.5	4.3	1.8	2.0	1.7	5.1	0.5	3.7

TABLE 7. Effect of alternative fungicides and NPK on oil percentage and essential oil yield (ml)/plant of rosemary plants, grown under naturally infection with root rot and/or wilt, at the two cuts of the seasons 2018 & 2019.

Treatments	**Essential oil percentage and yield**							
	2018 season				**2019 season**			
	Oil%		**Essential oil yield (ml)/plant**		**Oil%**		**Essential oil yield (ml)/plant**	
	1st cut	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**	**1st cut**	**2nd cut**
Plant Guard	0.295	0.279	0.114	0.070	0.302	0.282	0.136	0.098
Potassium silicate	0.312	0.301	0.128	0.094	0.314	0.303	0.166	0.112
NPK	0.326	0.317	0.196	0.102	0.329	0.321	0.220	0.138
Potassium silicate + Plant Guard	0.330	0.323	0.201	0.108	0.334	0.327	0.260	0.147
NPK +Plant Guard	0.336	0.332	0.247	0.112	0.338	0.333	0.283	0.160
NPK + Potassium silicate	0.343	0.339	0.260	0.126	0.356	0.341	0.344	0.174
NPK + Potassium silicate + Plant Guard	0.383	0.375	0.368	0.165	0.372	0.363	0.494	0.233
Carbendazim 50%WP	0.318	0.308	0.146	0.096	0.319	0.311	0.209	0.116
Control(without treatment)	0.287	0.262	0.092	0.051	0.283	0.265	0.098	0.055
L.S.D. at 5%	0.01	0.02	0.01	0.01	0.02	0.02	0.04	0.01

Essential oil composition

Gas Chromatography analysis of Egyptian rosemary (*Rosmarinus officinalis*) essential oil harvested in July, resulted in identification of 18 compounds, are summarized in Table 8. Camphor was found as a main one followed by eucalyptol (1,8 -Cineole), α-pinene, camphene and borneol. Also, in relatively high concentrations, β-pinene, limonene, bornyl acetate, myrcene, α-terpineol, β-caryophyllene, γ-terpinene and linalool were found in control treatment. On the other hand, in the monoterpene hydrocarbons, α-pinene content improved (16.15%) with potassium silicate treatment compared to other treatments and control. Also, camphor (Oxygenated monoterpenes group) content was improved reached (33.18%) with (NPK + Potassium silicate + Plant Guard), followed by (32.08%) for NPK + Potassium silicate treatment in comparison with (26.72%) in case of control. Meanwhile, 1,8- cineole

TABLE 8. **Percentages of main components of rosemary essential oil resulted from different treatments under field conditions.**

Treatments **Chemical Composition %**	**Control (without treatment)**	**Plant Guard**	**Potassium silicate**	**NPK**	**Potassium silicate + Plant Guard**	**Plant Guard + NPK**	**NPK + Potassium silicate**	**NPK + Potassium silicate + Plant Guard**	**Carbendazim 50%WP**
Monoterpene hydrocarbons,									
α-pinene	12.73	13.90	16.15	13.70	12.34	13.62	13.24	11.81	13.83
Camphene	8.49	6.43	6.58	6.73	6.75	6.45	6.37	6.41	7.57
β-pinene	6.31	2.69	2.99	3.07	4.54	2.75	2.37	2.22	4.73
Myrcene	4.93	6.89	6.58	6.14	5.94	5.73	5.46	5.21	5.44
Limonene	6.19	7.01	6.58	6.38	5.94	5.97	5.51	5.13	6.03
γ-terpinene	1.26	1.52	1.32	1.18	1.28	0.96	1.37	1.06	0.83
Total	39.91	38.43	40.19	37.19	36.79	35.48	34.31	31.84	38.42
Oxygenated monoterpenes,									
Eucalyptol (1,8-Cineole)	14.56	18.34	18.66	18.30	18.39	19.47	19.83	21.05	18.20
Linalool	1.03	0.93	1.08	1.18	1.40	1.08	1.05	1.12	0.95
Camphor	26.72	28.04	28.71	30.58	29.34	31.66	32.08	33.18	29.20
Isoborneol	0.34	0.00	0.12	0.12	0.35	0.00	0.23	0.23	0.12
Borneol	7.22	6.78	6.10	7.56	5.94	7.17	8.02	8.42	8.39
α-terpineol	1.72	1.99	2.15	2.36	2.21	2.51	2.22	2.31	2.01
Bornyl acetate	5.62	0.23	0.72	0.59	3.61	0.36	0.47	0.41	0.71
Total	57.22	56.31	57.54	60.68	61.23	62.25	63.90	66.72	59.57
Sesquiterpene hydrocarbons,									
β-caryophyllene	1.38	3.15	1.20	1.06	0.93	1.08	0.82	0.83	0.95
β-farnesene	0.69	1.64	0.60	0.47	0.47	0.60	0.47	0.13	0.47
α-caryophyllene	0.23	0.12	0.24	0.12	0.12	0.24	0.15	0.12	0.12
γ-muurolene	0.11	0.23	0.12	0.24	0.23	0.12	0.23	0.13	0.24
Total	2.41	5.14	2.15	1.89	1.75	2.03	1.67	1.21	1.77
Sesquiterpene oxygenated,									
Caryophyllene oxide	0.30	0.12	0.12	0.24	0.23	0.24	0.12	0.24	0.24

showed the same trend of camphor as values were (21.05 and 19.83%) in NPK + Potassium silicate + Plant Guard and NPK + Potassium silicate treatment, respectively, and decreased to 14.56% in control. While, β-caryophyllene (3.15%) of the Sesquiterpene hydrocarbons group with Plant Guard and Caryophyllene oxide (0.46%) of the Sesquiterpene oxygenated group were superior with control treatment. In terms of the affiliation of the essential oil constituents to the chemical groups, results showed that monoterpene hydrocarbons (31.84-40.19%), oxygenated monoterpenes (56.31-66.72%), sesquiterpene hydrocarbons (1.21-5.14%) and sesquiterpene oxygenated (0.12-0.46%) dominated the oil from all treatments.

Total phenolic and total flavonoids content

The obtained data in Table 9 showed that all tested treatments at the first and second cuts increased phenols and flavonoids percentages than the control (without treatment). Differences among these treatments and control were significant. Treatments of dual combination (NPK + Potassium silicate + Plant Guard), Carbendazim 50%WP fungicide, (NPK + Potassium silicate) and (Plant Guard + NPK) in both seasons of 2018 and 2019 recorded superiority in phenols and flavonoids percentages compared to the control. On the other hand, phenols and flavonoids percentages recorded superiority in the first seasons. In contrast, treatment of Plant Guard treatment was the least effective treatment in both trial seasons.

Discussion

Rosmarinus officinalis L. is one of the most economically important medicinal plants. Root rot and wilt diseases have been observed in rosemary plantations which resulted in reduction in plant stand and vegetative growth and consequently low essential oil yield of harvested plants. Samples showed root rot and/or wilt symptoms were obtained from different nurseries and fields in Giza and Qalubiya Governorates during 2017 season. *F. oxysporum* and *R. solani* were the most frequently isolated fungi followed by *Pythium debaryanum* and *Sclerotinia sclerotiorum*. On the contrary, *Fusarium solani* recorded the lowest mean percentages of occurrence, Whereas, *Macrophomina phaseolina* gave the intermediate frequency percentages in isolation trials, in Qalubiya and Giza Governorates. Some of the identified fungi in the present study were previously reported on rosemary. Cacciola et al. (1997), Conway et al. (1997), Putnam (2004) and Alvarez et al. (2007), who studied soilborne diseases of rosemary and their causal fungal pathogens.

TABLE 9 Effect of alternative fungicides and NPK on phenols percentages and flavonoids of rosemary plants, grown under naturally infection with root rot and/or wilt, at the two cuts of the seasons 2018 & 2019.

Treatments	Phenols and flavonoids percentages,							
	2018 season				2019 season			
	Phenols %		Flavonoids %		Phenols %		Flavonoids %	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Plant Guard	10.10	8.58	0.093	0.065	10.62	9.03	0.105	0.073
Potassium silicate	11.16	9.49	0.104	0.073	11.49	9.77	0.115	0.081
NPK	11.58	9.84	0.130	0.091	11.83	10.05	0.139	0.097
Potassium silicate + Plant Guard	11.27	9.58	0.128	0.089	11.75	9.99	0.135	0.095
Plant Guard + NPK	12.15	10.33	0.136	0.095	12.60	10.71	0.146	0.102
NPK + Potassium silicate	12.70	10.79	0.146	0.102	13.40	11.39	0.155	0.108
NPK + Potassium silicate + Plant Guard	15.34	13.04	0.179	0.125	15.55	13.21	0.184	0.129
Carbendazim 50%WP	14.23	12.10	0.163	0.114	14.77	12.55	0.171	0.120
Control(without treatment)	9.59	8.15	0.074	0.052	9.95	8.46	0.096	0.067
L.S.D. at 5%	0.27	0.42	0.001	0.004	0.44	0.23	0.006	0.004

Pathogenicity of the identified fungi on rosemary seedlings indicated that, *Macrophomina phaseolina* followed by *Sclerotinia sclerotiorum* were the most virulent fungi with significant differences between each other as they recorded the highest percentages of reduction in survival plants. On the contrary, *F. solani* and *Pythium debaryanum* were the least pathogenic fungi resulting in infected plants after 60 days from transplanting, subsequently, fungi recorded the highest percentage of healthy survivals. The aggressiveness of *M. phaseolina* and *S. sclerotiorum* may be due to the presence of melanin pigment. (Polak, 1990) mentioned that, melanin pigment plays a decisive role in the determination of virulence. Also, *S. sclerotiorum* was the major disease in rosemary plants growing in Oregon, USA. (Hassanin, 2007, 2013 and Putnam, 2004).

Under greenhouse conditions, Carbendazim 50% WP, elicitor (potassium silicate), bioproduct (Plant Guard), fertilizer (NPK), [Potassium silicate + Plant Guard, [NPK + Potassium silicate, [Plant Guard + NPK] and [NPK + Potassium silicate + Plant Guard] used as dipping treatment for 30 minutes to roots of cuttings of rosemary gave sufficient control against root-rot and wilt diseases. The fungicide (Carbendazime 50% WP), followed by [NPK + Potassium silicate + Plant Guard], [Plant Guard + NPK] and [NPK + Potassium silicate] were the most effective treatments, since they gave the highest significant decreases in disease infection than the controls with all tested fungi after 30 and 60 days from planting. In contrast, NPK treatment recorded the lowest reduction percentage of infection after 60 days from planting with all tested fungi followed by, potassium silicate treatment in case of *M. phaseolina*, the decrease in the disease incidence by fungicides might be attributed to indirect nontarget effects. Microorganisms are either functionally or nutritionally connected with each other's, and changes in a component of a microbial community may influence the structure of the whole community. This is particularly true for plant-associated microorganisms, which influence on and are influenced by the plant metabolic status (White et al., 2010). Carbendazime 50% WP and [NPK + Potassium silicate + Plant Guard] completely prevented infection of rosemary in case of *F. oxysporum* and *M. phaseolina* after 30 and 60 days from planting, Carbendazime 50% WP in case of *R. solani* and [Plant Guard + NPK], [NPK + Potassium silicate] in case of *F. oxysporum.* Application of NPK fertilizer is not a substitute for fungicides, but an important component in management of plant diseases, allowing reductions in the fungicide doses and, thus, decreasing fungicides and hazardous residues in food crops. In addition, nutrients can affect the development of a disease by affecting plant physiology or by affecting pathogens, or both of them. The level of nutrients can influence the plant growth, which can affect the microclimate, therefore affecting infection and sporulation of the pathogen (Marschner, 1995). Also, the level of nutrients can affect the physiology and biochemistry and especially the integrity of the cell walls, membrane leakage and the chemical composition of the host (Graham and Webb, 1991). Nutrients can affect the growth rate of the host which can enable seedlings to escape/avoid infection when they are at the most susceptible stages. In addition, fertilizers can influence the soil environment and can affect the development of the pathogen. Also, it is evident from this study that potassium silicate is considered one of the resistance inducers that induced resistances for root rot and wilt diseases of rosemary. Many researchers have demonstrated the potential of silicon not only to cell-wall rigidity and reinforcement, it also increases cell-wall elasticity during extension growth (Marschner, 2012). In primary cell walls, silicon interacts with cell-wall constituents such as pectins and polyphenols, which increase cell-wall elasticity during extension growth (Emadian and Newton, 1989). Silicon-enhanced resistance is associated with the density of silicified long and short epidermal cells, the thick layer of silica under the cuticle, the double cuticular layer, the thickened Si-cellulose membrane, formation of papilla, and complexes formed with organic compounds in epidermal cell walls that strengthen plants mechanically. The physical barriers inhibit pathogen penetration and make plant cells less susceptible to enzymatic degradation caused by fungal pathogen invasion (Inanaga et al., 1995, Fauteux et al., 2005, Datnoff et al., 2007 and Van et al., 2013).

The various control treatments tested gave sufficient control against root-rot and wilt diseases affecting rosemary plants under field conditions (naturally infested soil) during two trial. All the treatments tested significantly decreased the percentages of disease incidence after 30 days of planting. Carbendazim 50%WP, (NPK + Potassium silicate + Plant Guard), (NPK + Potassium silicate), (Plant Guard + NPK) and (Potassium silicate + Plant Guard) gave the highest decrease percentages in seasons, respectively. On the other hand, Plant Guard (season, 2018) and Potassium silicate and Plant Guard (season, 2019) were the least effective treatments. As for the efficacy of the treatments tested in decreasing percent of disease incidence after 60 days of transplanting, Carbendazim 50%WP, (NPK + Potassium silicate + Plant Guard), (NPK + Potassium silicate), (Plant Guard + NPK) and (Potassium silicate + Plant Guard) gave the highest decrease percentages in the both seasons, respectively. On the other hand, Plant Guard (season, 2018) and Potassium silicate and Plant Guard (season, 2019) were the least effective treatments. The highest percentages of survivals, however, were recorded in case of Carbendazim 50%WP, (NPK + Potassium silicate + Plant Guard), (NPK + Potassium silicate), (Plant Guard + NPK) and (Potassium silicate + Plant Guard) in the both seasons, 2018 and 2019. Whereas, Potassium silicate and Plant Guard were the least effective treatment in increasing survivals in both seasons. The positive efficacy of these treatments might be due to provide protection to rosemary seedlings against root rot and wilt pathogens after planting of cuttings as a result of their antifungal substances, which partially or completely prevent or delay the fungal infection processes and disease development. There are two types of primary tolerance mechanisms that mineral nutrition can affect either by formation of mechanical barriers, primarily through the development of thicker cell walls, or synthesis of natural defense compounds, such as phytoalexins, antioxidants, and flavanoids, that provide protection against pathogens (Prakash and Verma 2016 and Meena et al., 2016 b). Moreover, addition of nutrients indirectly enhances the pathogen inhibition, thus increasing the yield of crops. As for the role of silicon in potassium silicate, research has shown silicon is most effective for defence, and is most efficiently absorbed, when applied to the roots (Liang et al., 2005), triggering both the biochemical and cell wall physical mechanisms (Dallagnol et al., 2015). Biochemical defence mechanisms involving silicon include local and whole-plant responses to attack. A whole-of-plant response is evident from enhanced resistance to both root and foliar diseases when silicon supply to the roots is increased (Dallagnol et al., 2015). Addition of microorganisms such as fungi (*Trichoderma harzianum*) and any plant growth-promoting organisms can increase nutrient uptake (P, Zn, Mn) by influencing minor element availability through their oxidation-reduction reactions or siderophore release (Huber and McCay-Buis, 1993). *Trichoderma* spp. is known to control pathogens either directly by inhibition of growth and sporulation of the pathogen mechanisms such as mycoparasitism and plant enzyme production, or indirectly by modifying the environmental conditions, or nutrient competition and space, promoting plant growth and enhancing plant defensive mechanisms and antibiosis (Bouhassan et al., 2004).

All treatments tested at the first and second cuts during the two seasons increased plant growth parameters, *i.e.* plant height (cm), number of branches/plant and fresh and dry weight / plant (g) of *R. officinalis* compared to the control. Differences among these treatments and control were significants. Treatments of dual combination between (NPK + Potassium silicate + Plant Guard), (NPK + Potassium silicate) and (Plant Guard + NPK) recorded obviously increasing in all parameters compared to control in both seasons. On contrast, treatment of Plant Guard was the least effective one in both trial seasons. The improvements in plant growth parameters due to soil drench treatments may be attributed to biochemical changing in the stem base tissues. This change includes increasing the activity of peroxidase enzyme, growth hormones and phenol compounds. Also, the beneficial effects of these treatments may be due to the reduction in disease infection and development as well as improving uptake of nutrients. Similar results on various crops, under naturally or artificially infested soil were reported by Hassanin, 2007 & 2013. Potassium is an important macronutrient and the most abundant cation in higher plants. It enhances photosynthesis, maintains cell turgor, reduces respiration, helps in transport of sugars and starches, helps in nitrogen uptake and is essential for protein synthesis. In addition to plant metabolism, Potassium has an important significant role in the growth and development of plants. It activates enzymes such as enzyme of essential oil synthesis, maintains cell turgor, enhances photosynthesis, reduces respiration, helps in transport of sugars and starches, helps in nitrogen uptake and is essential for protein synthesis (Machner, 2001, Silva, 2004). These results are in harmony with Nada et al. (2014), who stated that, Potassium silicate was the most effective treatment in improving plant growth parameters as well as yield and oil yield of coriander. Cherif and Belanger (1992) reported that, supplying the solutions of potassium silicate increased weight root dry of cucumber. Silicon show an improved growth, higher yield, reduced mineral toxicities and better disease and insect resistance (Graham and Webb, 1991, Alvarez and Datnoff, 2001 and Seebold et al., 2000, 2004)**.** Whereas, *Trichoderma* spp. is used as biofertilizers, plant growth promotion, biopesticides and stimulants of natural resistance. Because of the ability of this fungus (*Trichoderma* spp.) to protect plants, enhance vegetative growth and contain pathogen populations under numerous agricultural environments, as well as to act as soil amendments/inoculants for improvement of nutrient ability, decomposition, biodegradation and ability to enhance the physical, chemical and biological properties of the soil (Woo et al., 2014). Also, Plant Guard (*Trichoderma harzianum*) improved the estimated crop parameters compared with the control treatment (Embaby, 2003 and El-Sayed, 2017). Furthermore, to explain the effect of chemical fertilization (NPK) on augmenting the vegetative growth parameters of *Rosmarinus officinalis* plants. It is important to refer to the physiological roles of nitrogen, phosphorus and potassium in plant growth and development. Such three macronutrient elements are the common elements usually included in fertilizers. Which are necessary because the soil is usually in deficient of them due to plant removal leaching or they are not readily available for plants. Therefore, such addition of well-balanced NPK fertilization quantities insured production of high productivity and chemical constituents of rosemary plants. The role of NPK fertilization in promoting vegetative growth characters as well as stimulating the chemical constituents content of rosemary plants could be explained by recognizing their fundamental involvement in the very large number of enzymatic reaction that depend on NPK fertilization. NPK reflected directly on increasing the content of total carbohydrates, total sugars and total free amino acids as well as NPK content in the leaves which were indirectly the cause for enhancing the augmenting of all other vegetative growth traits and chemical constituents of rosemary plants. Therefore, sufficient amount of these nutrients in the plant is necessary for normal growth, in order to obtain satisfactory yield (Cooke, 1982).

Percentage essential oil and essential oil yield per plant of rosemary plants were significantly affected by some safe fungicides alternatives such as, potassium silicate, bioproduct (Plant Guard, *Trichoderma harzianum*), NPK fertilizer and systemic fungicide (Carbendazim 50% WP). It was also noticed that, the combination treatment of NPK, Potassium silicate with Plant Guard gave the highest values of essential oil percentage and essential oil yield per plant in both cuts of the first and second season respectively, followed by dual combinations between (NPK + Potassium silicate). On contrast, treatment of Plant Guard recorded the lowest value for essential oil % and essential oil yield in both trial seasons compared to control. Concerning the effect of NPK fertilizer and potassium silicate, it was found that, significant promotion on the essential oil % in 1st and 2nd cuts in both seasons due to potassium silicate or NPK fertilizer. But, insignificant differences were fond with bioproduct (Plant Guard, *Trichoderma harzianum*). NPK fertilizer treatment was the best treatment in this regard, followed by potassium silicate treatment. Whereas, treatment with bioproduct induced the lowest essential oil % and essential oil yield in the two seasons compared to untreated control. These increases might be attributed to the enhancing effect of biofertilizers (*Trichoderma harzianum*) on vegetative growth, in terms of fresh yield besides increasing uptake of nutrients especially phosphorus element which linked by phosphate bounds in adenosine triphosphate (ATP). In this form, the energy can be undergoing processes such activation uptake and the synthesis of various organic compounds such as essential oil, Heikal (2005).These results were confirmed by Khalifa et al. (2017) which reported that, potassium silicate can be used to make the soil more fertile, enhance growth of plant growth promoting rhizobacteria (PGPR), increase beneficial soil bacterial and maintain soil pH to better yield of crops and oil yield. The application of potassium silicate can improve water-use efficiency and the physiological response of plant leaves as increased the photosynthetic pigments, compatible solutes, and activates enzymes such as enzyme of essential oil synthesis then improve the ability to resist against diseases then increase the yield production (Graham & Webb, 1991, Belanger et al., 1995, Alvarez & Datnoff, 2001, Agarie et al., 1993, Seebold et al., 2000, 2004, Nada et al., 2014 and Nashwa et al., 2015). The effect of different treatments on essential oil yield and constituents may be due to their effect on enzyme activity and metabolism of essential oil production (Burbott and Loomis 1969). These results in harmony with Nada et al. (2014) who stated that, Potassium silicate was the most effective treatment in improving plant growth parameters as well as coriander seed yield and oil yield.

Gas Chromatography analysis of rosemary (*Rosmarinus officinalis*) essential oil, resulted in identification of 18 compounds. Camphor was found as a main ones followed by eucalyptol (1,8 -Cineole), α-pinene, camphene and borneol. Also, in relatively high concentrations, β-pinene, limonene, bornyl acetate, myrcene, α-terpineol, β-caryophyllene, γ-terpinene and linalool were found in control treatment. On the other hand, in the Monoterpene hydrocarbons, α-pinene content improved (16.15%) with Potassium silicate treatment compared to other treatments and control. Also, improved camphor (Oxygenated monoterpenes group) content was improved reached (33.18%) with (NPK + Potassium silicate + Plant Guard), followed by (32.08%) for NPK + Potassium silicate treatment in comparison with (26.72%) in case of control. Meanwhile, 1,8- cineole showed the same trend of camphor the data were (21.05 and 19.83%) in NPK + Potassium silicate + Plant Guard and NPK + Potassium silicate treatment, respectively, and decreased to 14.56% in control. These results are in harmony with those mentioned by Sienkiewicz et al. (2013) and Ayoob et al. (2018) showing camphor oscillated in the range from 18.2 to 28.1 and 1,8-cineole from 6.4 to 18.0%)as a major compound of rosemary oil and classified as light oxygenated compounds. While, β-caryophyllene (3.15%) of the Sesquiterpene hydrocarbons group with Plant Guard and Caryophyllene oxide (0.46%) of the Sesquiterpene oxygenated group were superior with control treatment. In terms of the affiliation of the essential oil constituents to the chemical groups, results showed that monoterpene hydrocarbons (31.84-40.19%), oxygenated monoterpenes (56.31-66.72%), sesquiterpene hydrocarbons (1.21-5.14%) and sesquiterpene oxygenated (0.12-0.46%) dominated the oil from all treatments. Meanwhile, further studies on the alternatives control means by using elicitor, bioproduct and fertilizer may be extended in the future. Furthermore, because of the new strategy of plant protection will consider keeping the environmental conditions free from the harmful pollution a priority of further studies are needed to encourage the use of alternative chemicals control especially with medicinal plants.

All tested treatments at the first and second cuts increased phenols and flavonoids percentages than the control (without treatment). Differences among these treatments and control were significant. Treatments of dual combination (NPK + Potassium silicate + Plant Guard), Carbendazim 50%WP fungicide, (NPK + Potassium silicate) and (Plant Guard + NPK) in both seasons, 2018 and 2019 recorded superiority in phenols and flavonoids percentages compared to the control. On the other hand, phenols and flavonoids percentages recorded superiority in the first seasons. In contrast, treatment of Plant Guard treatment was the least effective treatments in both trial seasons. Application of NPK fertilizer or Potassium silicate may be has an important role in component in formation of mechanical barriers, primarily through the development of thicker cell walls, or synthesis of natural defense compounds, such as Phenols and flavanoids, that provide protection against pathogens (Prakash and Verma, 2016, Meena et al., 2015a, 2016b, Priyadharsini & Muthukumar, 2016 and Kumar et al., 2017). It is believed that silicon creates a physical barrier which can restrict fungal hyphae penetration, or it may induce accumulation of antifungal compounds such as flavonoid and diterpenoid phytoalexins which can degrade fungal cell wall (Alvarez & Datnoff, 2001 and Brescht et al., 2004).

Conclusion

Fungicide alternatives and fertilizers have had a positive effect in management of roots rot and wilt of rosemary plant, improving various plant growth standards and improving the proportion and components of volatile oil.

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Conflict of interest

The authors declare that they have no competing interests.

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استخدام المبيدات الفطرية البديلة والأسمدة فى مكافحة أمراض أعفان الجذور والذبول وتأثيرها على إنتاجية نبات حصالبان

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يعتبر تعفن الجذور والذبول من أهم الأمراض التى تسبب خسائر فادحة في محصول حصالبان. وكان الهدف من ذلك العمل هو التحقق من حدوث وانتشار أمراض تعفن الجذور والذبول على هذا النبات وتحديد مسببات الأمراض المسببة لها. بالإضافة إلى تطوير وسائل المقاومة الآمنة لمكافحة هذه الأمراض.

تم جمع نباتات حصالبان المصابة بأعراض تعفن الجذور والذبول من محافظتي القليوبية والجيزة خلال موسم ٢٠١٧. وكشفت اختبارات العدوى الصناعية للفطريات المعزولة التالية

(*Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium* sp. *Rhizoctonia solani, Sclerotinia sclerotiorum*)

عن قدرة هذه الفطريات في إحداث العدوى. تم تطبيق بعض بدائل المبيدات الفطرية الآمنة, Potassium silicate ، المنتج الحيوي (Plant Guard) والخليط بينهم مع سماد NPKمقارنة بالمبيد الكيماوي (Carbendazim 50% WP) ضد الفطريات المعزولة تحت ظروف الصوبة.

قللت جميع المعاملات المختبرة حدوث الأمراض بشكل ملحوظ تحت ظروف الصوبة مقارنة بالنباتات غير المعاملة. من جهة أخرى، كانت المعاملة (NPK + Potassium silicate + Plant Guard) أكثر كفاءة في الحد من الإصابة بأمراض تعفن الجذور والذبول في الحقل خلال مواسم النمو ٢٠١٨ و ٢٠١٩.

خصائص النمو مثل ارتفاع النبات، عدد الأفرع، والوزن الطازج والجاف للعشب وكذلك الزيت العطري (النسبة المئوية والمحصول). أظهرت المعاملة (NPK + Potassium silicate + Plant Guard) تفوقاً في زيادة مقاييس النمو السابقة، تليها المعاملة (NPK + Potassium silicate) مقارنة بالكونترول في كلا موسمي النمو. أظهر التحليل الكروماتوغرافي للزيت العطري لحصالبان أنه تم العثور على الكامفور كمكوِّن رئيسي (٣٣,١٨٪) ، يليه الأوكاليبتول (١ و ٨-سينيول) وألفا بينين مع المعاملة (NPK + Potassium silicate + Plant Guard). كما تحسن المحتوى الكلي من الفينولات والفلافونويد كمضادات للأكسدة بنفس المعاملة مقارنة بمعاملة الكونترول.

تشير النتائج إلى التأثيرات المحتملة لمعاملة ” NPK + Potassium silicate + Plant Guard“ كمبيد فطري جديد ضد تعفن الجذور والذبول في نباتات حصالبان وتحسين إنتاجية النبات.