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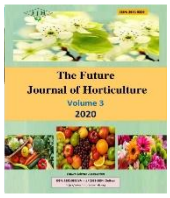

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BIO- AND CHEMICAL CONTROL OF RUST DISEASE AND ITS EFFECT ON SPEARMINT YIELD UNDER SANDY SOIL CONDITIONS

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ABSTRACT: A field experiment was carried out at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt, during the two successive seasons of 2017 and 2018 to study the impact of beneficial microorganisms *Trichoderma harzianum* (fungi), *Streptomyces griseus* (bacteria) and two common chemical fungicides (copper sulphate and difenoconazole) to controlling spearmint rust disease. The vegetative growth, chemical constituents, essential oil production and its components in spearmint (*Mentha viridis* L.) plants were evaluated. The obtained results concluded that the use of copper sulfate as a chemical fungicide and the two bioagents [*Streptomyces griseus* and *Trichoderma harzianum*] reduced the incidence and percentage of rust disease severity in compared to untreated plants (control). Similarly, the level of vegetative growth, essential oil percentage and yield and the percentage of minerals and total carbohydrates in herb as well as carvone content in essential oil were noticeably more in compared to the chemical fungicide (difenoconazole) and untreated plants (control). Thus, reduce the using of chemical fungicides means reducing environmental risks.

Key words: *Mentha viridis,* Rust disease, biological control, *Trichoderma harzianum, Streptomyces griseus,* Copper sulphate, essential oil and carvone.

INTRODUCTION

Spearmint (*Mentha viridis* L.) belongs to family Lamiaceae. It is one of the most important medicinal and aromatic crops in many parts of world as well as in Egypt with local and export value **(Bakeer *et al.*, 2005)**. Spearmint is used in various medicinal and aromatic industries, such as the flavoring of pharmaceutical and other preparation such as toothpaste, mouthwashes and perfumes **(Mahran, 1967)**. Spearmint volatile oil is characterized by high carvone content for 60-70 % **(Lee and Fred, 1998; Elmasta *et al.*, 2006)**. Additionally, Spearmint volatile oil is commonly used as carminative, antimicrobial agent **(Pľuchtová *et al.*, 2018)**. Also, it is added to diarrheal drugs to prevent colics **(Hikal and Omer, 1993)**. Carminative, anti-spasmodic, anti-peptic ulcer agent to treat indigestion, skin diseases, coughs and cold in folk medicine. Different parts of spearmint plants have been reported to possess medicinal properties **(Biswas *et al.*, 2014)**. The major components of essential oils are carvone, limonene and methyl acetate. Therefore, it can be concluded that the active chemical compounds present in *M. viridis* should certainly find a place in the treatment of various bacterial infections **(Balla *et al.*, 2017)**.

The increasing awareness of fungicide-related environmental hazards and the development of fungicide-resistant. Bio- control agents work through different mode of actions. These help bio-control agent to be more durable, more effective and without any chemical residues in human food chain **(Elad *et al.*, 1983).**

Trichoderma spp. is quite known for their abilities in controlling plant pathogens. The use of *Trichoderma* spp. in agriculture can provide numerous advantages, colonization of the root and plant rhizosphere, control several plant pathogens by different mechanisms such as parasitism, antibiosis production and induce resistance stimulation of root growth improvement plant health by promote plant growth. *T. harzianum* Rifai is unique since it can induce systemic resistance to these pathogens **(Atia *et al.*, 2011 and El-Demerdash *et al.*, 2017).** *Trichoderma* spp., and other beneficial rootcolonizing microorganisms, also enhances plant growth and productivity. Intuitively, this might seem counterproductive as most of these species also induce resistance in plants **(Harman *et al.*, 2004)**. The antagonism of *Trichoderma* spp. against rust fungi other than snap bean rust was explained by an antifungal effect of secondary metabolites produced by the *Trichoderma* strain resulting in inhibition of rust spore germination or germ tube elongation. In addition, treatments with bioagents contain growth regulators increase plant nutrients uptake and plant growth **(Govindasamy and Balasubramanian, 1989)**.

Actinomycetes particularly *Streptomyces griseus* enhances soil fertility, against wide range of soil-borne plant pathogens and increased productivity of crops **(Hallmann *et al.*, 1997)**. Actinomycetes promote plant growth mainly by the production of plant growth regulators **(El-Tarabily, 2006)**. Actinomycetes play an important role not only for their plant growth-promoting traits but also for secreting a wide range of antimicrobial products and enzymes that play a critical role in maintaining soil ecology and fertility, about 60% of the new insecticides and herbicides originated from these microorganisms. This growing interest is due to their low toxicity and environmental friendliness; they have a degradable nature while being highly specific and less toxic to non-target organisms **(Flores-Gallegos and Nava-Reyna, 2019)**.

Copper sulphate ($CuSO_4$) is using as a positive control (a commercial fungicide). Copper increases lignin formation, which increases plant defense ability. The divalent copper ion Cu^{++} is liberated and absorbed by the fungus spores until it reaches the lethal dose level. Necessary element in the formation of chlorophyll and has an active role in the process of photosynthesis. Adequate quantity in the plant limits the toxicity of trace elements such as zinc and manganese. It plays a role in the nitrogen reaction inside the plant, as it plays a major role in protein formation. It has an essential role in raising the efficiency of the plant's immune system against many fungal diseases. It neutralizes the toxicity of pesticides because of increasing plant immunity and its ability to cope with stressful conditions **(Pohanish, 2015)**. In addition, copper sulphate a source of Sulphur, which is an essential element in forming several amino acids, proteins, enzymes, vitamins and chlorophyll in plants, also important in photosynthesis **(The Sulphur Institute, 2020)**.

Therefore, the present work was designed to reduce the extensive usage of chemical fungicides in agriculture process and find out the most suitable non-chemical method to protect spearmint plants against rust disease by using and alternative and eco-friendly two effective bioagents (*Trichoderma harzianum* and *Streptomyces griseus*) with commercial chemical fungicides (Copper sulphate and Difenoconazole) to control spearmint rust disease under field conditions. In addition, their effects on growth parameters, production and essential oil percentage and its composition as well as chemical constituents of spearmint (*Mentha viridis* L.) plants.

MATERIALS AND METHODS

Two experiments were carried out at the experimental farm of Ismailia Agricultural Research Station, Ismailia Governorate, Egypt, during the two consecutive seasons of 2017 and 2018. Spearmint (*Mentha viridis* L.) seedlings were obtained from Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, ARC, Egypt. The seedlings were transplanted in the experimental field on 15th March in both seasons. The plot area was 12 m^2 (3x4 m) with 5 rows, 60 cm apart and 25 cm between seedlings within the row (80 plant/plot).

Soil properties

The experimental soil was sandy and its physical chemical properties were: 92% sand, 2% silt, 6% clay, 7.90 pH, 0.39% organic matter, 1.4% calcium carbonate, 18 mg/kg available N, 21 mg/kg available P and 60 mg/kg available K.

Isolation and preparation of Pathogenic Fungi (*Puccinia menthae Pers.)*

Infected plants washed using tap water to remove soil particles, cut into small pieces and surface sterilized in 1% sodium hypochlorite solution for 2 minutes. The sterilized parts washed twice in sterilized distilled water and dried between two sterilized filter papers. The sterilized parts placed onto plain agar medium and incubated at 27°C for 7 days. All the developed fungi were purified using hyphal tip and \ or single spore techniques (Dhingra and Sinclair, 1995). The purified fungi were transferred to slant medium and kept at 5°C for further studies. The isolated fungi were microscopically identified according to their morphological characteristics using the description by **Burnett and Hunter (2003)**.

Experiment design and treatments.

Two microbial bio-agents *Trichoderma harzianum* (fungi)and *Streptomyces griseus* (bacteria) in addition to two different commercial fungicides (Copper sulphate and Difenoconazole) were used to evaluate their effect on mint rust disease, growth parameters, production and of essential oil% and its composition as well as chemical contents of spearmint plants. The two microbial bio-control agents were kindly obtained from Central Laboratory of Organic Agriculture, ARC. Giza. The tow bioagents were formulated as suspension-using method developed by **Abd-El-Moity (1985)** and prepared suspension contains 30 x 106 cfu / ml. Copper sulphate was purchased from the local market and Difenoconazole 25% EC as triazole fungicide produced by Tagros Chemicals India. Ltd. (contains 250 g/kg of Difenoconazole) were used as commercial fungicides.

The experiment was conducted in a randomized complete block design (RCBD) with three replicates and was included five treatments as follow:

1- Untreated plants (control).
2- Trichoderma harzianum at 10 ml/l.
3- Streptomyces griseus at 10 ml/l.
4- Copper sulphate as a commercial fungicide at 2.5 ml/l.
5- Difenoconazole as a chemical commercial fungicide at 0.5 ml/l.

The plants were sprayed with the treatments four times during the growing season, the first one was applied after one month from the transplanting, and the other three sprays were applied at one-month interval. All transplanted plants were received the agricultural practices recommendation

Data Recorded

A. Vegetative growth parameters and herb yield

Two cuts were taken; on the 15^{th} of June and August during both seasons and the vegetative growth parameters were recorded as follows:

1- Plant height (cm).

2- Fresh and dry weights (g/plant).

3- Yield of fresh and dry weights (kg/fed./season).

B. Chemical analysis in dry herb

Essential oil percentage and oil yield

The essential oil of dry herb was extracted by hydro-distillation using a Clevenger type apparatus according to **Guenther (1961)**. Essential oil percentage was calculated as dry weight of dry herb.

Chemical analysis

N, P, K and total carbohydrates percentages were determined in the dry herb according to **A.O.A.C. (2005)**.

Gas chromatography analysis of the essential oil.

The gas chromatography (GC) analysis of the essential oil samples was carried out in the Laboratory of Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, ARC using Ds Chrom 6200 Gas Chromatograph apparatus, fitted with a 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 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. The identification of the compounds was done by matching their retention times with those of authentic samples injected under the same conditions.

Statistical analysis

Simple experiments were designed at a randomized complete block design (RCBD) in three replicates. The collected data were computed and statistically analyzed with analysis of variance according to **Mead *et al.* (1993)** by using means of "MSTAT-C" computer software package. The differences between the means of treatments were tested by L.S.D test at 0.05 probability level.

RESULTS AND DISCUSSION

Disease incidence and severity

The obtained data presented in Table (1) indicate that all treatments of bioagents and the commercial fungicides led to significant effect on the incidence and severity percentage of rust disease compared to untreated plants (control). The lowest percentage of disease incidence and severity were recorded by applying copper sulphate (9.33 and 11.83 % &13.33 and 11.33 % for disease incidence and severity in the 1^{st} and 2^{nd} cuts for both seasons, respectively) with significant differences compared to control and bioagents treatments. While the untreated plants recorded the highest rust incidence and severity as 28.10 and 38.22 % & 44.44 and 42.11% in the 1^{st} and 2^{nd} cuts in both seasons, respectively. Most importantly, it was *Streptomyces griseus* treatment significantly reduced disease incidence and severity percentage compared to *Trichoderma harzianum* treatment and control. These results are in line with these were reported on snap bean rust disease by **Mandour and Metwaly 2015,** damping off in squash by **Metwaly and Elbaz 2013** and basil damping-off disease by **Metwaly and Abd- El Sayed 2018**.

Copper sulphate ($CuSO_4$) as a commercial chemical fungicide. Copper increases the formation of lignin, which increases the ability of plants to defend and the divalent copper ion Cu^{++} is liberated and absorbed by fungicide until it reaches the lethal dose level also the copper ion destroys the protoplasmic structure of the fungal cells and causes their death **(Pohanish, 2015)**. *Trichoderma* spp. controls several plant pathogens by different mechanisms such as parasitism, antibiosis production and induce resistance stimulation of root growth improvement plant health by promote plant growth. *T. harzianum* Rifai is unique since it can induce systemic resistance to these pathogens. **(Atia *et al.*, 2011 and El-Demerdash *et al.*, 2017)**. Actinomycetes play an important role for secreting a wide range of antimicrobial products and enzymes that play a critical role in maintaining soil ecology and fertility, it has low toxicity and environmental friendliness; they have a degradable nature while being highly specific and less toxic to nontarget organisms **(Flores-Gallegos and Nava-Reyna 2019).**

Table 1. Effect of bioagents, copper sulphate and difenoconazole on incidence and severity percentage of spearmint rust disease during 2017and 2018 seasons.

Treatments	Disease incidence %		Disease severity %	
	1st season	2nd season	1st season	2nd season
Untreated plants (control)	28.10	38.22	44.44	42.11
Trichoderma harzianum	23.07	16.81	32.48	32.57
Streptomyces griseus	15.57	16.70	24.66	15.89
Copper sulphate	09.33	11.83	13.33	11.33
Difenoconazole	13.70	12.00	15.00	12.49
LSD at 5%	4.36	1.99	2.89	3.24

Vegetative growth parameters

Data regarding the vegetative growth parameters were recorded in Table (2). Plant height, fresh and dry weights of spearmint herb /plant were significantly increased by spraying both the applied bioagents and commercial fungicides compared to untreated plants (control). Copper sulphate, *Streptomyces griseus* and *Trichoderma harzianum* gave more vegetative growth than difenoconazole fungicide in the first and second cuts in the two seasons. The tallest plants were recorded by spraying copper sulphate in the first and second cuts for the two seasons they recorded the first season to two cuts (63 cm -59.8 cm) respectively and (54 cm – 51 cm) in the second season for the two cuts while control gave the lowest value (51.2 cm - 50 cm)in the first season to both cuts respectively and (44 cm - 44.1 cm) for the second one . The highest weights of fresh and dry herb were obtained with using *Streptomyces griseus* and copper sulphate in most cases in the two seasons. The highest value of fresh weight /plant in the first season (95.4 g -87.7 g) in the two cuts and 99.1 g-106.9 g in the second season the lowest one was 63.9g – 54.2 g in the first season of the second respectively and 57.3 g – 65.2 g in the second one. The highest dry weight/plant in the first season was 31.6 g -37 g in the two cuts resp. and 27.4 g -38.7g in the second season for the two cuts while the lowest one was the control treatment (23.1 g – 24.1 g) the first season to the two cuts, (18.7 g – 27.8 g) for the second season.

Data in table (3) indicated that copper sulphate recorded high amount of yield fresh weight of herb/ fed in the second season (2403.9-2979.1)kg/fed in two cuts and total yield of fresh weight of fed./ seasons (4891.4-6019.1)kg/fed in two seasons respectively .The high dry weight of herb yield was obtained from plants which treated with copper sulphate (1718.1 – 2112.9) kg/ fed/ season respectively the lowest one was the control (1203.5-1484.6) kg/fed/season. Generally, a non-significant difference was showed between Copper sulphate, *Streptomyces griseus* and *Trichoderma harzianum* treatments in herb weighs in the most cases. Similar results regarding bioagents effects on plant growth were showed by **Mandour and Metwaly, 2015** on snap bean, **Metwaly and Elbaz, 2013** on squash and **Metwaly and Abd- El Sayed, 2018** on basil plants.

The incensement in the vegetative growth parameters with copper sulphate, *Streptomyces griseus* and *Trichoderma harzianum* may be backed to that the element copper itself is commonly involved in the formation of some redox enzymes, and its necessary role in the formation of chlorophyll and has an active role in the process of photosynthesis, nitrogen reaction inside the plant, as well as plays a major role in protein formation **(Pohanish, 2015)**. In addition, sulpher is an essential element in forming several amino acids, proteins, enzymes, vitamins and chlorophyll in plants, important role in photosynthesis **(The Sulphur Institute 2020)**. Different reports indicated that treatment with *Streptomyces griseus* enhances soil fertility and increased productivity of crops **(Hallmann *et al.*, 1997)**; also promote plant growth mainly by the production of plant growth regulators **(El-Tarabily, 2006)**. In addition, treatment with *Trichoderma harzianum* increase the nutrient uptake and the efficiency of nitrogen use and can solubilize nutrients in the soil, also treatments with bioagents contain growth regulators that increase plant nutrients uptake and plant growth **(Govindasamy and Balasubramanian 1989)**.

Table 2. Effect of bio agents, copper sulphate and difenoconazole on vegetative growth parameters of spearmint during 2017and 2018 seasons.

Treatments	Plant height/ plant (cm)				Fresh weight of herb/plant / plant (g)				Dry weight of herb/ plant (g)			
	1st season		2nd season		1st season		2nd season		1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Untreated plants (control)	51.2	50.0	44.0	44.1	63.9	54.2	57.3	65.2	23.1	24.1	18.7	27.8
Trichoderma harzianum	56.3	53.8	48.8	49.9	65.8	73.6	87.1	102.2	23.8	33.7	24.2	36.6
Streptomyces griseus	56.4	59.0	47.6	48.3	95.4	75.7	96.7	106.9	31.6	37.0	25.5	33.5
Copper sulphate	63.0	59.8	54.4	51.0	82.3	87.7	99.1	106.7	30.7	34.3	27.4	38.7
Difenoconazole	55.6	56.2	46.6	45.5	74.9	72.1	83.4	79.3	25.6	31.5	26.4	29.9
LSD at 5%	4.7	3.5	2.3	2.0	6.8	9.2	11.9	10.2	5.2	3.8	2.9	6.0

Table 3. Effect of bioagents, copper sulphate and difenoconazole on yield of fresh and dry weights of herb (kg) per cut and per feddan of spearmint during 2017 and 2018 seasons.

Treatments	Yield of fresh weight of herb (kg)/fed./cut				Yield of fresh weight of herb (kg)/fed/season		Yield of dry weight of herb (kg)/fed./cut				Yield of dry weight of herb (kg)/fed/season	
	1st season		2nd season		1st season	2nd season	1st season		2nd season		1st season	2nd season
	1st cut	2nd cut	1st cut	2nd cut			1st cut	2nd cut	1st cut	2nd cut		
Untreated plants (control)	1880.2	1620.8	1632.2	1837.1	3512.4	3457.8	674.1	677.6	529.4	807.0	1203.5	1484.6
Trichoderma harzianum	1820.4	2395.6	2300.8	2768.8	4121.2	5164.4	655.6	933.0	752.7	1049.6	1408.3	1982.6
Streptomyces griseus	2573.6	2656.1	2123.8	2850.4	4697.5	5506.5	969.8	1021.6	713.7	965.2	1683.5	1986.7
Copper sulphate	2487.6	3040.1	2403.9	2979.1	4891.4	6019.1	845.6	1033.9	872.4	1079.0	1718.1	2112.9
Difenoconazole	2340.9	2331.5	2214.4	2212.1	4555.4	4543.6	717.1	876.8	780.0	837.9	1497.2	1714.7
LSD at 5%	357.4	458.2	406.7	555.2	611.1	730.5	146.1	98.6	108.5	197.7	188.6	258.3

Essential oil percentage and yield

Data obtained on the essential oil percentage and yield of *Mentha viridis* herb as affected by the different treatments are presented in Table (4). Essential oil percentage in dry herb ranged from 1.19% to 2.67 % in the first season and from 1.19 to 3.03 % in the second one. In addition, plant yield of essential oil ranged from 0.28 ml to 0.66 ml in the first season while 0.35 ml - 1.14 ml for the second season. Essential oil yield (L)/fed./cut recorded that the large amount was obtained from plants which treated by *Streptomyces griseus* in the first cut in the first season 17.48 L/fed. and 18.34 L/fed. treat by copper sulphate in the second cut, the second season ranged by10.66-31.88L/fed/cut. Essential oil yield per fed./season ranged from 14.33-35.63 L/fed/season for first season and 32.9-58.57 L/fed/season in the second one. Copper sulphate scored the highest percentage in essential oil content, while *Trichoderma harzianum* treatment gave the highest essential oil percentage in the second one for the two seasons with significant differences compared to other treatments. The lowest essential oil percentages and yields were recorded with control and difenoconazole treatments in first and second cuts for the two seasons as well, respectively. Furthermore, copper sulphate, *Streptomyces griseus* and *Trichoderma harzianum* treatments recorded significant incensement in essential oil yields per plant and feddan in comparing with control and difenoconazole treatments in both cuts for the two seasons. **Metwaly and Abd- El Sayed, 2018** used the same bioagents on sweet basil reported giving similar increases in essential oil percentage and the yield as well.

Their effects on the formation of some redox enzymes, chlorophylls, amino acids, enzymes, vitamins and proteins can explain the positive effect of copper sulphate, *Streptomyces griseus* and *Trichoderma harzianum* treatments on essential oil yields. In addition, the active role in the process of photosynthesis, nitrogen reaction inside the plant, enhancing soil fertility; promote plant growth mainly by the production of plant growth regulators and increase plant nutrients uptake due to enhancing plant growth as shown in this work **(Metwaly and Abd- El Sayed, 2018)**

Table 4. Effect of bioagents, copper sulphate and difenoconazole on essential oil percentage and essential oil yields of spearmint dry herb during 2017 and 2018 seasons

Treatments	Essential oil %				Essential oil yield/ plant (ml)				Essential oil yield/ fed./cut (L)				Essential oil yield/ fed./ season (L)	
	1st season		2nd season		1st season		2nd season		1st season		2nd season		1st season	2nd season
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut		
Untreated plants (control)	1.19	2.21	1.19	2.21	0.28	0.23	0,54	0.64	8.01	6.31	15.02	17.88	14.33	32.90
Trichoderma harzianum	1.91	2.85	1.92	3.03	0.45	0.52	0.95	1.14	12.53	14.48	26.70	31.88	27.03	58.57
Streptomyces griseus	1.77	2.69	2.07	2.49	0.63	0.53	0.99	0.86	17.48	14.81	27.59	24.16	32.30	51.77
Copper sulphate	2.04	2.67	2.09	2.71	0.62	0.66	0.99	1.05	17.29	18.34	27.65	29.27	35.63	56.93
Difenoconazole	1.83	1.21	1.99	1.16	0.47	0.55	0.38	0.35	13.21	15.57	10.66	9.73	28.77	20.40
LSD at 5%	0.18	0.06	0.07	0.09	0.14	0.09	0.12	0.21	3.76	2.61	3.41	5.99	5.03	8.42

Essential oil composition

Data illustrated in Table (5) revealed that the main identified components of essential oil were carvone, limonene, 1,8 cineole, β-caryophyllene, Pulegone, Myrcene, Dihydrocarveol acetate and α-pinene. The major components were carvone arranged from 43.89 to 56.63% and limonene from 13.98 to 20.35%. The highest content of carvone 56.63% was recorded by the treatment of copper sulphate, while chemical fungicide (difenoconazole) recorded the lowest content 43.89% in the first season. Copper sulphate and control treatments recorded higher contents of carvone than all other treatments for both seasons. While control plants recorded the lowest content of limonene13.98%, difenoconazole treatment gave the highest content 20.35% in the first season; in addition, copper sulphate, *Streptomyces griseus* and *Trichoderma harzianum* treatment gave higher contents of limonene than control plants for the two seasons. *Trichoderma harzianum* and *Streptomyces griseus* treatments recorded the highest contents of 1,8 cineole as 7.49 & 7.99% and 6.42 & 7.49% compared to control and fungicides treatments for first and second seasons, respectively. Moreover, *Trichoderma harzianum* treatment gave the highest contents of pulegone component in the two seasons 5.18 & 5.33% in compared to all other treatments. Those findings agreement with that obtained by **Metwaly and Abd- El Sayed (2018)** using the same bioagents on sweet basil plants.

Chemical analysis of herb

Data regarding the N, P, K and total carbohydrates percentage in dry herb of *Mentha viridis* during the two seasons were recorded in Table (6). The percentage of nitrogen was significantly increased with using both bioagents under study compared to all other treatments and *Streptomyces griseus* was significantly superior in this respect in the two seasons. Significantly, control treatment recorded the lowest percentage of nitrogen and phosphorus for both seasons, while the highest percentage of phosphorus was obtained by using difenoconazole fungicide with significant differences compared to all other treatments for both seasons. Copper sulphate gave the highest percentage of both potassium and total carbohydrates in the two seasons with significant differences compared to all other treatments; in addition, the lowest percentage of potassium and total carbohydrates was recorded with *Streptomyces griseus* treatment for both seasons. While *Trichoderma harzianum* recorded significant increases in potassium and total carbohydrates percentage compared to control. **Metwaly and Abd-El Sayed (2018)** using microbial bioagents on sweet basil plants found similar results regarding total carbohydrates content.

Table 5. Effect of bioagents, copper sulphate and difenoconazole on essential oil composition of spearmint dry herb during the second cut of 2017 and 2018 seasons

Compound %	Treatments									
	Untreated plants (control)		*Trichoderma harzianum*		*Streptomyces griseus*		Copper sulphate		Difenoconazole	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
α-pinene	1.85	1.43	1.43	0.87	2.40	2.36	2.56	2.82	2.91	2.56
Myrcene	2.93	2.41	1.87	3.67	3.41	3.74	3.51	3.98	4.02	2.01
p-cymene	0.91	1.02	0.90	2.63	1.05	1.25	1.13	1.17	1.21	1.37
Limonene	13.98	19.34	18.96	14.60	18.66	19.28	19.63	19.38	20.35	18.90
1,8 Cineole	5.48	4.30	7.49	7.99	6.32	7.49	6.16	6.92	6.42	5.99
β-ocimene	0.60	0.53	0.52	0.39	0.48	0.42	0.51	0.42	0.43	0.60
Dihydrocarveol	0.52	1.53	1.38	1.34	0.53	1.03	1.03	0.33	1.13	1.11
Trans-carveol	0.73	2.28	1.28	3.07	2.37	2.44	1.44	1.79	2.51	2.00
Pulegone	4.62	2.80	5.18	5.33	2.17	5.21	2.30	4.79	1.67	3.70
Carvone	54.94	56.25	53.25	53.83	53.87	52.67	56.63	54.05	43.89	53.40
Dihydrocarveol acetate	3.43	0.92	3.77	1.42	1.88	1.38	2.16	1.84	1.47	1.36
β-caryophyllene	8.31	1.49	1.88	1.10	1.82	0.53	2.39	1.88	9.24	1.51
Caryophellene oxide	0.73	0.66	0.72	0.77	0.59	0.50	0.57	0.65	0.61	0.97
Identified compounds	99.03	94.97	98.62	97.00	95.55	98.30	100.00	100.00	95.86	95.50

Table 6. Effect of bioagents, copper sulphate and difenoconazole on N, P, K and total carbohydrates percentage of spearmint dry herb during 2017and 2018 seasons.

Treatments	Nitrogen %		Phosphorus %		Potassium %		Total carbohydrates %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Untreated plants (control)	1.393	1.403	0.134	0.135	3.890	3.900	37.89	37.90
Trichoderma harzianum	1.527	1.547	0.141	0.143	4.147	4.167	37.36	37.38
Streptomyces griseus	1.607	1.597	0.147	0.148	3.873	3.863	36.52	36.51
Copper sulphate	1.467	1.477	0.157	0.156	5.247	5.267	39.20	39.21
Difenoconazole	1.460	1.470	0.169	0.171	4.993	4.983	38.08	38.10
LSD at 5%	0.007	0.009	0.008	0.010	0.008	0.010	0.03	0.03

CONCLUSION

Accordingly, it can be concluded that using copper sulphate as a common chemical fungicide and the two microbial biological agents (*Streptomyces griseus* and *Trichoderma harzianum*) reduces the incidence and severity of rust disease compared to untreated plants (control). Moreover, it increased vegetative growth, essential oil yield, and the ratio of minerals and total carbohydrates in spearmint (*Mentha viridis* L.) herb as well as carvone content in the essential oil compared to the chemical fungicide (difenoconazole) and untreated plants (control). Thus, reducing the use of chemical fungicides and reducing environmental risks.

RECOMODATION

Use the biological treatments (*Trichoderma harziaum* and *Streptomyces griseus)* to control rust disease in spearmint as it is easy prepare and low costs. They gave high quantity, quality safety products.

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