Biological Control of *Mentha Viridis* Root Rot Caused by *Fusarium Solani* by Using Mycorrhizal Fungi and Silicate Dissolving Bacterium

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ABSTRACT: This work was carried out in Experimental Farm of (PPRI) during the season of 2012 and 2013.The aim of the study was to investigate the effect of the inoculation of spearmint (*Mentha viridis*) plants with silicate dissolving bacterium *(Microbacterium trichotecenolyticum*) and or vesicular arbuscular mycorrhizal fungi (VAM) on the incidence of root rot disease, plant growth and volatile oil percentage. The obtained results are summarized as follows: in all cuts in the two seasons, treatment applied with pathogenic fungus alone all plants died (control) . The inoculation with silicate dissolving bacterium plus mycorrhizal fungi reduced growth of tested fungus and at same time spearmint plants were significantly taller than other treatments in both seasons. The same trend was observed in the branching of spearmint plants except in the second cut of the first season, the differences between treatments were not significant. The combination between silicate dissolving bacterium and mycorrhizal fungi (VAM) significantly increased dry weight/ plant in the second cut at the first season, also the highest volatile oil percentage was achieved with this treatment. In addition GLC analysis revealed that the highest percentage of carvone (the main component of the volatile oil) was produced with that exact treatment.
Keywords: Biological control, *Mentha viridis* root rot disease, *Fusarium solani*, Mycorrhizal fungi, Silicate dissolving bacterium.

INTRODUCTION

Spearmint (*Mentha viridis*,L.) belongs to family Lamiaceae (Labiatae).It is one of the most important medicinal and aromatic crops in many parts of world as well as in Egypt for local use and exportation (Bakeer *et al*., 2005). It is used in various medicinal and aromatic industries, such as the flavoring of pharmaceutical and other preparation such as tooth paste, mouth washes and perfumes (Mahran,1967).Spearmint volatile oil is characterized by a high carvone content for (60-70)% (Lee and Fred,1998; Elmasta *et al.,* 2006). Spearmint volatile oil is used as carminative, antimicrobial, also it is added to diarrheal drugs to prevent colics (Hikal and Omer,1993).

The complex disease, root rot caused by (*Fusarium oxysporium* , *Rhizoctonia solani, Macrophomina phaseolina* (Bakeer *et at.,* 2005) are considered as one of the most destructive soil borne disease attacking crops.

Arbuscular mycorrhizal fungi (VAM) are ubiquitous soil fungi that form obligate associations with plants roots. The potential benefit of mycorrhizal to agriculture is more apparent than even before because of the need to increase food, fiber and fuel production to keep pace with the increase in world population is crucial, especially in the lesser developed areas of the world. Hypotheses proposed to explain VAM fungal effects on soil borne plant pathogens generally have been considered to have either a physical or physiological basis. It is already known that VAM fungi have been shown to affect root growth, nutrient absorption and host physiological responses to environmental stresses. However, VAM fungi have not been shown to interact directly with pathogens through antagonism, antibiosis or predation. The indirectly effect of the host-pathogen relationship by physiologically altering the host or by competing for space or host resources (Kulkarni *et al*., 1997).

Silicon (Si) is known as a beneficial element for plants the direct and indirect benefits of the element for crops (especially grasses) are related to resistance to diseases, pest and drought. The importance of Si on plant nutrition is set greater in the case of organic farming, since the use of biocides (insecticide, fungicides, nematicides, etc.) (Buck *et al*., 2008).

Supplying Si through fertilization can contribute for the increase in production and protection of organically grown crops. (Epstein, 2001) cited some scientifically proven examples of beneficial effects that Si promotes in the plants, such as resistance to plant pathogen attack, better structure of plant architecture, resistance to herbivore of insects, mitigation of heavy metal phytotoxicity. Traditionally it is suggested that an accumulation of Si in the epidermal tissue of the plant main mechanism which provide defense against insect and fungal attacks. Steel slag was considered as an adequate source of Si and registered in 1955 in Japan as a fertilizer (Kingston, 2008).Numerous studies demonstrated the high agronomic benefits of steel slag mainly due to it is high soluble Si (Ferreirex *et al*.,2008).

MATERIALS AND METHODS

This study was carried out at Experimental Farm of Mycology Research & Diseases Survey Dept., PPRI, ARC, in two successive seasons 2012 and 2013.The aim of this study was to investigate the effect of applying VAM fungi and / or silicate dissolving bacterium on the incidence of spearmint plants root rot.

1.Source of the pathogenic fungus and plant material

Pathogenic fungal isolate of *Fusarium solani*, the causal agent of spearmint root rot disease, was obtained from Mycology Research & Diseases Survey Dept., PPRI , ARC. This isolate proved to be aggressive in previous studies.VAM fungi inoculum containing spores of *Glomus mosseae*, *Glomus intraradices, G.faculatum* and *Gigaspora* sp. was also obtained from Mycology Research & Plant Diseases Survey Dept, PPRI, ARC. It was applied at the rate 10 g / kg soil (consisted of soil, extra material hyphal fragments and infected sudan grass root fragments).

2.Source of silicate dissolving bacterium

Domestic isolate was kindly provided by Department of Microbiology/ SWERI / ARC.

3.Experimental procedures

A pathogenic isolate of *Fusarium solani* was grown on sand –corn medium (1:1W:W and 40% water) for 15 days at 25 ± 2 ºC. Pots (30 cm diameter) containing sterilized sandy loam soil were artificially infested separately with prepared fungal inoculum at the rate of 3% of soil weight. The inoculum was mixed thoroughly with the upper layer of the soil then irrigated every other day and left for 7 days to ensure the distribution of the inoculum. Each treatment was replicated three times every replicate consisted of nine pots (1 plant / pot) and nine ones left without infestation to serve as control. Prior to cultivation, electric furnace steel slag (by product of Ezz –Dekheilah steel company)was air dried, ground at < 60 mesh and mixed in the soil pots at rate equivalent to 250 kg/fed. At planting date and 15 days later, strain of silicate dissolving bacterium was applied in liquid form to the steel slag treated soil at rates equivalent to 5 L / fed. Spearmint rhizomes (12-15 cm in length) were obtained from the Farm of El Kanater El- Khairia and planted in the inoculated plastic pots (30 cm diameter) on 15 th February, 2012 and 2013 in the first and second seasons, respectively.

4. Experiment layout

The experiment layout was designed in complete randomized blocks included five treatments each treatment was replicated three times and every replicate consisted of nine pots (1plant /pot), the recorded data were statistically analyzed according to Snedecor and Conchran (1968), using L.S.D at 5% .

5.Chemical fertilization

The sources of chemical fertilizers (NPK) were ammonium sulphate (20.6 % N), calcium superphosphate (15.5% P_2O_5 and potassium sulphate 48% K_2O). (NPK) fertilizers were added at the recommended level in five doses, the 1st was for all phosphorous amount which was added during soil preparation, the rest (NK) were applied in two equal doses for each cut, on 29 th March and 28 th April for the1st cut and on 20 th May (after the1st cut) and 22 th June for the 2^{ed}one in the two seasons. The plants were harvested twice, the first cut was conducted on 17 th May and the second one on 18 th July in both seasons.

6.Treatments

1.(Control) *Fusarium solani* (all plants died and were not subject to statistical analysis) .
2.*Fusarium solani* + Mycorrhizal fungi (VAM).
3.*Fusarium solani* +.silicate dissolving bacterium
4.*Fusarium solani*+ + Mycorrhizal fungi (VAM) + silicate dissolving bacterium.
5.Spearmint plants without any infection (normal).

7.Data recorded

The following data were recorded:
1.Incidence of root rot disease
2.Plant height and number of branches/ plant.
3.Herb fresh and dry weights (g/ plant).
4.Volatile oil percentage in dry herb according to British Pharmacopeia (1963).

8.Volatile oil components

Samples taken from the oil obtained in the first cut of the first season were analyzed using gas liquid chromatography (GLC), to determine their main constituents. The use of GLC in the quantitative determinations was performed using the methods described by (Bunzen *et al*., 1969 and Hoftman, 1967).

RESULS AND DISCUSSION

1. Incidence of root rot disease

Results presented in Table (1) showed that spearmint plants response to inoculation with the tested bio agents (VAM and silicate dissolving bacterium) . It is obvious that they significantly decreased the incidence of root rot disease. Spearmint plants were protected from early infection with *Fusarium solani* .The best results were obtained from inoculation with silicate dissolving bacterium + Mycorrhizal fungi (VAM) in the first and second season respectively. Such effect may be due to that Si deposited on the tissue surface act as physical barrier .It prevents physical penetration and /or makes the plant cells less susceptible to enzymatic degradation by fungal pathogens. This mechanism is supported by the positive correlation between the Si content and the degree of suppression of diseases and pests. Also, Si function as a signal to induce the production of phytoalexin (Cherif *et al*.,1994).Si application to cucumber resulted in the stimulation of the chitinase activity and rapid activation of peroxidases and polyphenoloxidases after infection with *Pythium* spp. Glycosidically bound phenolics extracted from Si treated plant when subjected to acid or beta glucosidase hydrolysis displayed a strong fungistatic activity. However, in oat attacked by *Blumeria yraminis*, Si deficiency promoted the synthesis of phenolic compounds(Carver *et al*.,1998).The phenylalanine ammonia – lyase activity was enhanced by Si deficiency.(Harlpur *et al.*, 1990) showed that simultaneous inoculation of VAM fungi with the pathogen reduced the incidence of disease caused by *Sclerotium rolfsii* [*Corticium rolfsii*] on wheat in pot trials. Also (Rabie, 1998) revealed the effectiveness of *G. mosseae* against *Botrytis faba* of broad bean. It was suggested that mycorrhizal fungi increase the resistance of plants against pathogen attack by increasing the nutritional needs of plants, and other factors that cause the unavailability of nutrients for the pathogen, reducing environmental stress on plants and soil microorganisms in improving rhizosphere. Mycorrhizal infection may alter the host plant metabolism through certain chemical, physiological and morphological inductions (Mosse, 1973)..Mycorrhizal fungi (VAM) inoculated plants have higher levels of orginine that is inhibitory to chlamydospores of disease causing pathogen (Amarantus, 2001) reported that VAM can cause unavailability of nutrients for pathogen, that claim is supported by (Turk *et al*., 2006) who said that arbuscular ,vesicles and the network of intracellular hyphae cause the unavailability of space to colonize and reduce pathogens carbohydrate supply in the plant roots .Mycorrhizal fungi (VAM) may improve response in the root system of plants root against pathogens. It is speculated that mycorrhizal chickpea seedlings that survived might have benefited from some or all of these modifications. These results are in harmony with those obtained by (Harplur *et al*.,1990) on wheat, (Liu *et al*., 1995) on cotton , (Rabie, 1998) on broad bean and (Moussa and Daoud, 2013) on corn.

Table 1. Effect of mycorrhizal fungi and silicate dissolving bacterium on incidence of root rot disease of spearmint plants during 2012 and 2013 seasons

Treatments	% root rot	
	1 st season(2012)	2 ed season (2013)
Control (*Fusarium solani*)	100.00	100.00
F.solani+VAM	66.67	33.33
F.solani + silicate dissolving bacterium	50.00	50.00
F.solani+ silicate dissolving bacterium+ VAM	16.67	0.00
Spearmint plants without any infection (normal)	50.00	50.00
LSD at 5%	40.63	23.48

VAM =mycorrhizal fungi

2.Vegetative growth

2.1. Plant height and number of branches/ plant

From data in Table (2) it can be noticed that inoculation of the plants with mycorrhizal fungi (VAM) and or silicate dissolving bacterium significantly increased plant height in both seasons. The tallest plants in the first and second seasons at the two cuts were recorded when the plants inoculated with silicate dissolving bacterium + Mycorrhizal fungi (VAM) giving 54.78 and 47.09 cm at first and second cuts, respectively. The same trend was observed in the second season giving 46.56 and 37.88 cm. Regarding the effect of resistance agents on number of branches, data showed that all studied parameters in Table (2) significantly increased except in the second cut of the first season. The most effective treatment was *Fusarium solani* +silicate dissolving bacterium + Mycorrhizal fungi (VAM), which recorded 42.69 and 32.65 at first and second cuts, respectively. The same trend was observed in the second season giving 28.17 and 24.17. These results are in agreement with that obtained by (Rabie,1998) on broad bean.

2.2. Herb fresh and dry weights (g /plant)

Data in Table (3) indicated that there were insignificant differences in herb fresh weight / plant due to all treatments in concerning the effect of antistress agents. As for herb dry weight, inoculation of spearmint plants with silicate dissolving bacterium+ Mycorrhizal fungi (VAM) had no significant effect on herb dry weight in the two season except in the second cut of the first season. The inoculation of spearmint plants with silicate dissolving bacterium+ Mycorrhizal fungi (VAM) gave the highest dry weight /plant in the first season at the second cut (50.45 g / plant).These results are in harmony with those obtained by (Harlpur *et al.,* 1990) on wheat, (Cherif *et al.,* 1994) on cucumber and (Rabie, 1998) on broad bean. This behavior may be due to the positive role of silicate dissolving bacterium which dissolving Si from steel slag by product protect plants from unfavorable conditions and biotic stresses (Ferreirex *et al*.,2008).

Table 2. Effect of mycorrhizal fungi and silicate dissolving bacterium on plant height and number of branches of spearmint plants during 2012 and 2013 seasons

	1st season		2ed season	
Treatments	**1 st cut**	**2 ed cut**	**1 st cut**	**2 ed cut**
Plant height				
Control (*Fusarium solani*)	0.0	0.0	0.0	0.0
F.solani+ VAM	46.00	42.67	35.78	30.78
F.solani+silicate dissolving bacterium	49.11	44.02	37.67	33.56
F.solani+ silicate dissolving bacterium+ VAM	54.78	47.09	46.56	37.88
spearmint without any infection (normal)	52.22	50.03	42.11	35.90
LSD at5%	4.20	4.02	2.95	3.97
Number of branches				
Control (*Fusarium solani*)	0.0	0.0	0.0	0.0
F.solani+VAM	27.50	21.77	18.00	15.10
F.solani+ silicate dissolving bacterium	29.83	23.77	23.72	16.33
F.solani+ silicate dissolving bacterium+ VAM	42.69	32.65	28.17	24.17
spearmint without any infection (normal)	33.73	25.49	25.49	20.33
LSD at 5%	2.38	NS	5.33	5.70

VAM =mycorrhizal fungi

Table 3. Effect of mycorrhizal fungi and silicate dissolving bacterium on herb fresh and dry weights/plant (g) ofspearmint plants during 2012 and 2013 seasons

Treatments	1 st cut	2 ed cut	1 st cut	2 ed cut
	herb fresh weight /plant(g)			
	1st season		**2ed season**	
Control (*Fusarium solani*)	0.0	0.0	0.0	0.0
F.solani+VAM	65.00	59.00	47.32	43.73
F.solani+ silicate dissolving bacterium	81.00	62.52	55.92	52.62
F.solani+ silicate dissolving bacterium+VAM	98.00	93.53	71.21	65.27
Spearmint without any infection	87.15	76.39	63.98	59.29
LSD at 5%	NS	NS	NS	NS
	herb dry weight /plant(g)			
Control (*Fusarium solani*)	0.0	0.0	0.0	0.0
F.solani+VAM	23.03	24.48	18.35	43.73
F.solani+ silicate dissolving bacterium	28.11	28.69	21.31	52.62
F.solani+ silicate dissolving bacterium+VAM	52.94	50.45	28.12	65.27
Spearmint without any infection (normal)	39.09	37.86	25.18	59.29
LSD at 5%	NS	18.47	NS	NS

VAM=mycorrhizal fungi

3.Volatile oil percentage

Essential oil percentage of spearmint dry herb were significantly responded to the inoculation with silicate dissolving bacterium and or VAM as recorded in Table(4). Regarding the effect of resistance agents, data revealed that, treatment applied with *Fusarium solani* alone all plants were died at the same time the combination between silicate dissolving bacterium and mycorrhizal fungi achieved the highest essential oil percentage of spearmint dry herb, in both season. These values represented 3.94% and 3.76% at two cuts in the first season, while in the second season the values were (2.99 and 2.84) at the first and second cuts respectively. The enhancement of essential oil percentage of spearmint dry herb by the inoculation with silicate dissolving bacterium + mycorrhizal fungi (VAM) may be due to the role of mycorrhizal fungi (VAM) in making phosphorus, manganese, zinc, in available form transfer of metabolite compounds and the efficiency of its root system leading to more absorption of water and nutrients, which led to increasing the rate of physiological processes and finally gave better yield (Liu *et al*., 1995). Also, silicon (Si) is essential for normal growth and development of plants, as well as in improving host plant resistance to pathogens attack (Nxumalo *et al.,* 2008).

4. GLC analysis of essential oil

The GLC analysis were carried out on the essential oil of spearmint plants of two treatments in the first cut of the first season, normal plants (without any infection) and mycorrhizal fungi (VAM) plus silicate dissolving bacterium. Data were recorded in Table (5) and Figures (1-2) revealed that, carvone and D-Limonene were the main components. It was observed that carvone content tended to increase up to 61.84 % in case of mycorrhizal fungi (VAM) plus silicate dissolving bacterium. Also data emphasized that D-Limonene content tended to increase up to 26.38% with the same treatment.

Table 4. Effect of mycorrhizal fungi and silicate dissolving bacterium on volatile oil percentage of spearmint plants during 2012 and 2013 seasons

Volatile oil percentage (in dry herb)				
	1st season		2ed season	
Treatments	1 st cut	2 ed cut	1 st cut	2 ed cut
Control (*Fusarium solani*)	0.0	0.0	0.0	0.0
F.solani+VAM	2.22	2.20	2.04	2.03
F.solani+ silicate dissolving bacterium	2.77	2.55	2.14	2.09
F.solani+silicate dissolving bacterium+VAM	3.94	3.76	2.99	2.84
Spearmint without any infection (normal)	3.77	3.67	2.88	2.80
LSD at 5%	0.44	0.52	0.40	0.53

VAM=mycorrhizal fungi

Table 5. Effect of mycorrhizal fungi (VAM) and silicate dissolving bacterium on volatile oil components % of spearmint plants in the 1St Season (1St cut)

Treatments / Volatile oil components	normal plants (without any infection)%	silicate dissolving bacterium + mycorrhizal fungi (VAM)%
α-Pinene	0.98	0.87
β-Pinene	3.25	1.26
D-Limonene	24.52	26.38
ρ-Cymene	1.16	0.58
1,8- Cineole	0.89	0.53
γ –Terpinone	3.62	3.07
Carvone	61.60	61.84
α-Terpinone	0.38	0.34
β-Caryophyllene	0.71	0.94
Eugenol	1.04	0.55

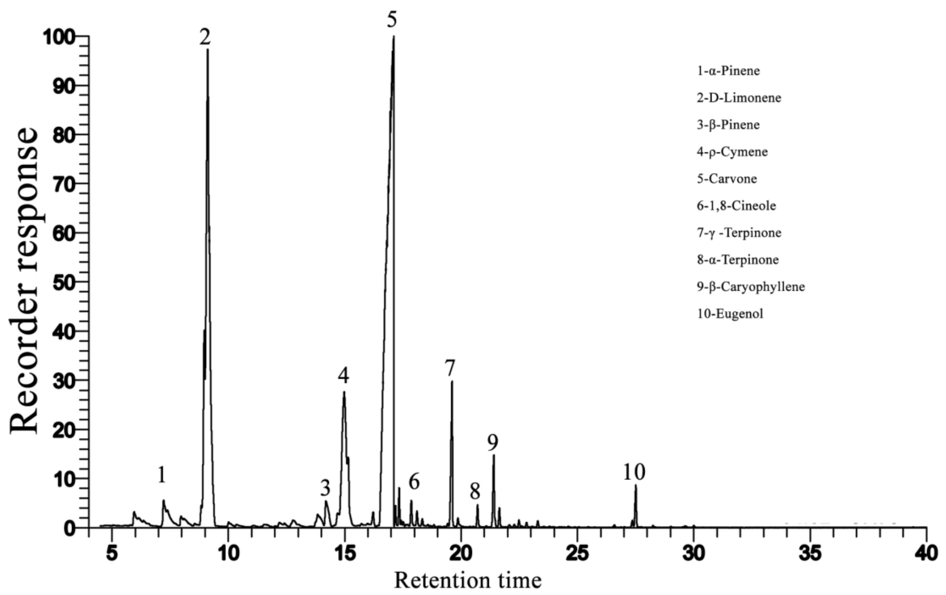

Figure 1. Chromatogram of spearmint volatile oil distilled from normal plants (without any infection).

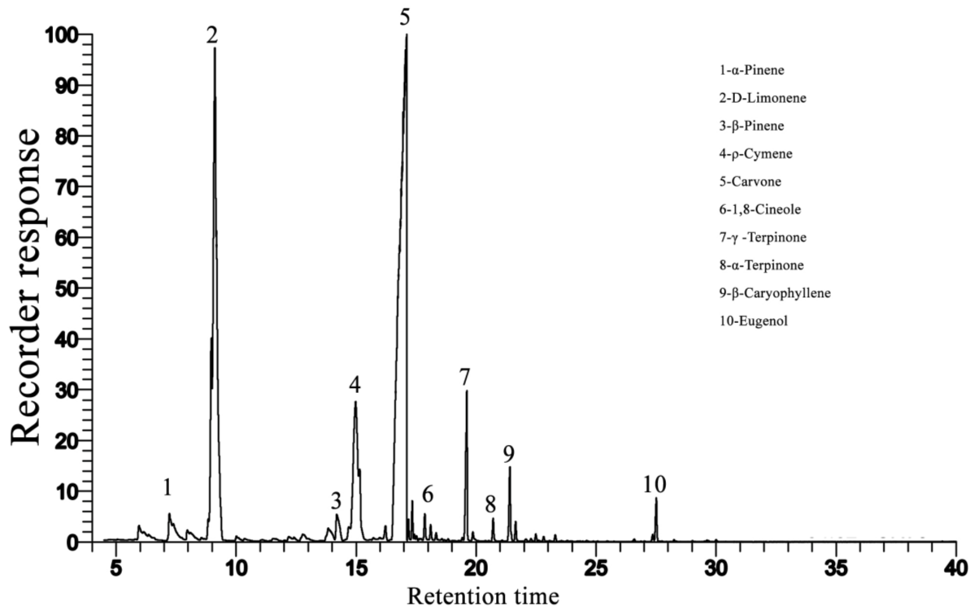

Figure 2. Chromatogram of spearmint volatile oil distilled from plants inoculated with mycorrhizal fungi (VAM) plus silicate dissolving bacterium.

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الملخص العربي

المكافحة الحيوية لعفن جذور النعناع البلدى المتسبب عن فطر فيوزاريم سولانى باستخدام فطريات الميكوريزا والبكتريا المذيبة للسليكات

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اجريت هذه التجربة فى مزرعة معهد بحوث امراض النبات خلال موسمى 2012– 2013لدراسة تأثير التلقيح الميكروبى لنباتات النعناع البلدى بالبكتريا المذيبة للسليكات وفطريات الميكوريزا مجتعمين او كلامنهما على حدة على مرض عفن الجذور فى نباتات النعناع البلدي وكذلك النمو والنسبة المئوية للزيت الطيار ومكوناتة وكانت اهم النتائج كما يلى :ادى التفاعل بين التلقيح الميكروبى لنباتات النعناع البلدى بالبكتريا المذيبة للسليكات وكذلك فطريات الميكوريزا الى ايقاف نموونشاط الفطر الممرض وكذلك الحصول على اعلى النباتات ارتفاعا فى كلا الموسمين وكذلك زيادة عدد الافرع على النباتات ماعدا فى الحشة الثانية للموسم الاول حيث لم يكن هناك فروق معنوية بين المعاملات .كما ادى التفاعل بين البكتريا المذيبة للسليكات وفطريات الميكوريزا الى زيادة الوزن الجاف للعشب زيادة معنوية فى الحشة الثانية للموسم الاول . اما بالنسبة لنسبة الزيت فقد تم الحصول على اعلى نسبة للزيت من التفاعل بين التلقيح الميكروبى بالبكتريا المذيبة للسليكات وفطريات الميكوريزا ذلك فى كلا الموسمين. وفيما يتعلق بتحليل مكونات الزيت فقد ادى التلقيح الميكروبى لنباتات النعناع البلدى بفطريات الميكوريزا بالاضافة الى البكتريا المذيبة للسليكات الى الحصول على اعلى نسبة للكارفون (Carvone) المكون الرئيسى فى الزيت.