**Scientific J. Flowers & Ornamental Plants**
**www.ssfop.com/journal**
**ISSN: 2356-7864** **doi: 10.21608/sjfop.2018.12814**

# SUPPRESSION OF DAMPING-OFF DISEASE BY SOME MICROORGANISMS ON SWEET BASIL (*OCIMUM BASILICUM* L.) PLANTS UNDER FIELD CONDITIONS

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***Scientific J. Flowers & Ornamental Plants, 5(1):15-29 (2018).***

**Received:**
5/3/2018
**Accepted:**
22/3/2018

**ABSTRACT:** This study was carried out at the Experimental Farm of Medicinal and Aromatic Plants Research Department in El Kanater El Khairia, HRI, ARC, during the two successive seasons of 2016 and 2017 to study the effect of some microorganisms (*Bacillus subtilis*, *Trichoderma harzianum*, *Pseudomonas fluoroscens* and *Streptomyces griseus*) on suppressing of damping-off disease of basil seedling, vegetative growth, oil production and chemical constituents of sweet basil (*Ocimum basilicum* L.) plants. The plants treated with any of the bioagents, showed highest effect in controlling damping-off and increased basil productivity compared with control plans. The results showed that, all bioagents treatments significantly increased vegetative growth (plant height, number of branches, fresh and dry weights (g/plant), oil percentage, oil yield, chemical constituents (chlorophyll (a), (b) and carotenoids contents as well as total carbohydrates percentage) compared with control plants. *Streptomyces griseus* gave the best effect against damping-off disease and all plant characteristics compared with control plants in the first season while, in the second season, *Pseudomonas fluorescens* increased plant protection and all plant characteristics. GC analysis of sweet basil essential oil of all treatments identified nine components. The major component of the essential oils was Linalool.

**Key words:** damping-off disease, biological control, essential oil, sweet basil plants.

## INTRODUCTION

Sweet basil (*Ocimum basilicum* L.) belongs to family Lamiaceae and is known with its highly aromatic leaves utilized either fresh or dried for culinary and is one of the leading herb crops. It is considered economically useful because of their basic natural characteristics as essential oil producers (Lawrence, 1993). Sweet basil is a popular culinary herb used in food and oral care products (Machale *et al.*, 1997). Also, basil is well known as a plant of a folk medicinal used as carminative, galactogogue, stomachic and antispasmodic tonic and vermifugem. In addition, basil tea taken hot is good for treating nausea, flatulence and dysentery. Basil is used in pharmaceutically as diuretic and stimulating properties, in perfumes and cosmetics for its smell; in fact, it is a part of many fragrance compositions (Khatri *et al.*, 1995). Medicinal and aromatic plants have a major role in agriculture and industry. They are the main source for safe drugs and raw substances used in manufacturing of pharmaceuticals. Some of their components are nucleus to the chemical biosynthesis (Dudai *et al.*, 2002).

Although chemical control, some time, achieves considerable results but it causes negative impact to environment and human health. Biological control is one of the most promising and safe measure in this respect. Biological control agents work through different mode of actions. These help biocontrol agent to be more durable, more effective and without any chemical residues in human food chain (Abd El-Moity, 1981 and Elad *et al.,* 1983). Biological control of plant diseases using microorganisms is a very promising alternative to the use of fungicides. Biological control more cheap and has no accumulating effects as chemical pesticides. *Trichoderma* spp. are 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 rhizosphere of plant, 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 (Harman *et al.,* 2004).

*Bacillus subtilis* Cohn. is known as effective antagonist bacteria against several plant pathogens. These antagonist acts through antibiosis, secretion of volatile toxic metabolites, destructive enzymes and competition for space and nutrition. (Intana *et al*., 2008) found that applications of *B. subtilis* are an important tool not only for organic growers, but also for conventional growers. They are using *B. subtilis* in integrated pest management programs allow reducing the risk of both the development of strains resistant and reduce toxic residues in the final product.

*Pseudomonas fluorescens* is considered as an important group of the antagonistic bacteria where it was effective against several soil borne pathogens in field and greenhouse trails (Jayashree *et al.,* 2000). Moreover, *Pseudomonas* spp. received great attention as biocontrol agent because of their catabolic versatility, excellent root-colonizing abilities and production of broad range antifungal metabolites such as 2,4 diacetyl-phloroglucinoal (DAPG), pyoluteorin, pyrrolnitrin and phenazines (Raaijmaker *et al.,* 2002). Bacteria identified as plant growth promoting rhizobacteria and biocontrol strains often belong to the following genera *i.e. Bacillus* (Nair *et al.,* 2002) and *Pseudomonas* (Mark *et al.,* 2006). Reasonable control result was obtaining *Pseudomonas fluorescens* antagonizes pathogenic fungi through certain mode of action, in addition to production of some pigments and antibiotics (Jaryaraj *et al*., 2007) .This mode of action of *Pseudomonas fluorescens* on chelating all available ferrous in the court of infection (Samavat *et al*., 2014).

Soil actinomycetes particularly *Streptomyces spp*. (*Streptomyces griseus* Waksman) enhances soil fertility and has antagonistic activity against wide range of soil-borne plant pathogens (Dulaney, 1953 and Hallmann *et al.,* 1997) to provide an effective method to increase productivity of crops (Emmert and Handelsman, 1999). The mechanisms by which actinomycetes promote plant growth include mainly the production of plant growth regulators PGRs (El-Tarabily, 2006).

The present work was designed to reduce using chemicals in agriculture process and find out the most suitable non-chemical strategy to protect basil plants against damping- off disease. The main objectives of this research were to study the efficacy of some bioagents and number of applications on incidence of damping- off disease in basil plants under field conditions, their influence on growth parameters, oil production and chemical constituents of sweet basil plants.

## MATERIALS AND METHODS

A field experiment was carried out at the Experimental Farm of Medicinal and Aromatic Plants Research Department in El Kanater El Khairia, HRI, ARC in Egypt, during two successive seasons of 2016 and 2017. The aim of this study was to evaluate the effect of some bioagents (*Bacillus*

*subtilis, Trichoderma harzianum, Streptomyces griseus* and *Pseudomonas flouresens*) on the percentage of damping-off , growth, oil production and chemical composition of sweet basil plants.

The physical and chemical characteristics of the experiments field soil were determined according to Jackson (1973) and are shown in Table (a).

**Table a. Soil properties of the experimental farm of Medicinal and Aromatic Plants Research Department in El-Kanater El-Khairia.**

| Soil properties | 2015/2016 | 2016/2017 |
|---|---|---|
| **Physical properties** | | |
| **Clay %** | 50.3 | 54.5 |
| **Silt %** | 19.5 | 19.72 |
| **Sand %** | 21.3 | 22.13 |
| **Texture** | Clay | Clay |
| **Chemical properties** | | |
| **E.C. (mmhos/cm)** | 0.44 | 0.65 |
| **pH** | 7.3 | 7.8 |
| **Organic matter (%)** | 1.29 | 1.45 |
| **Available N (ppm)** | 32.0 | 38.0 |
| **Available P (ppm)** | 26.45 | 26.75 |
| **Available K (ppm)** | 0.81 | 0.93 |

**The experimental layout:**

The layout of the experiment was designed in complete blocks design with thirteen treatments, each treatment replicated three times. Each replicate contained 27 plants (plot).

The experiment included 13 treatments:

1. Untreated plants (control)
2. *Bacillus subtilis*-1
3. *Bacillus subtilis*-2
4. *Bacillus subtilis*-3
5. *Trichoderma harzianum*-1
6. *Trichoderma harzianum*-2
7. *Trichoderma harzianum*-3
8. *Streptomyces griseus*-1
9. *Streptomyces griseus*-2
10. *Streptomyces griseus*-3
11. *Pseudomonas flouresens*-1
12. *Pseudomonas flouresens*-2
13. *Pseudomonas flouresens*-3

Number of applications: 1= One times, 2= Two times, 3= Three times.

Seeds were sown on 6$^{th}$ and 8$^{th}$ February in the first and second seasons, respectively in peatmoss medium in nursery beds; germination was occurred during 8-10 days after sowing for the two tested seasons, when seedling were about 10-15 cm in height and bearing 6-8 leaves, they were transplanted in plots (1.80 x 2.5 m$^2$), each had 3 rows with distance of 60 cm between rows and 30cm between plants within the row, Each plot included (27 plant/plot).

The experimental soil was naturally infested with both *M. phaseolina*, *F. solani* and *R. solani* which all these pathogenic fungi isolated in laboratory conditions and examined to pathogenisity tests on basil plants cultivar grandvert under greenhouse conditions. To reach the optimum number of applications needed to obtain the highest percentage of plant protection, different bioagents were used as one, two and three applications with interval 15 days between applications. These adding times were at transplanting, fifteen days after transplanting and one month after transplanting. Biocontrol agents were used in liquid form and the first applied as seedlings soaking in the suspension for 20 minutes, where the second and third treatments were applied as soil drench, at five liters of each bioagent/plot. Control plants were treated with water.

All plants were examined and percentage of damping-off were calculated to study effect of repeating treatment on damping- off disease. In all field experiments, the normal agricultural practices and irrigation were used as normal. Chemical fertilizers (NPK) were added as ammonium sulphate (20.6% N), calcium superphosphate (15.5% $P_2O_5$) and potassium sulphate (48% $K_2O$) at the recommended doses.

**Data recorded:**

Sweet basil plants were harvested two times by cutting the vegetative parts 10-15

cm above the soil surface. The first and second cuts were done on 5th June and 24th July at the first season and at 6th June and 26th July in the second season for the first and second cuts, respectively. Data recorded as follows:

1- Vegetative growth (plant height (cm), branches number/plant, herb fresh and dry weights per plant (g).

2- Essential oil percentage was determined in fresh herb by British Pharmacopoeia (1963).

3- Essential oil composition (essential oil samples of the 2nd cut during the 2nd season were subjected to gas liquid chromatography (GLC) according to the methods of (Hoftman 1967 and Bunzen *et al.*, 1969).

4- Chemical analysis: Total carbohydrates were estimated using the method described by (Michel *et al*., 1956). The estimation contents of chlorophyll a, b, and carotenoids contents were determined in fresh leaf (mg/g fresh matter) according to (Saric *et al.*, 1967).

**Statistical analysis:**

The experiment layout was designed in complete randomized blocks included thirteen treatments each treatment was replicated three times .The recorded data were statistically analyzed according to (Snedecor and Conchran, 1980) mean of the treatments were compared using LSD at 5%.

## RESULTS AND DISCUSSION

**Disease incidence:**

Number of application was studied to figure out the optimum number of treatments can produce considerable control of damping-off disease .The main target of this study was to improve quality and quantity of basil in field. Different field experiments were carried out to confirm results were obtained from laboratory and green house experiments in addition to find out the most effective biocontrol agent, the proper number of applications which must be adding. To determine the suitable number of adding of different antagonist to get the highest effect, different bioagents were added at three different times. These adding times were at planting time, fifteen days after planting and one month after planting.

Obtained data indicated that, *Streptomyces griseus* show high effect on plant protection and percentage of surviving plants reached (81.67%) which recorded (18.33 %) on post damping- off disease. This is due to, that *Streptomyces spp*. works effectively under alkaline soil condition. Egyptian soil is alkaline so effective of *Streptomyces spp*. and bioagents showed good results under this pH value condition.

Data obtained from these studies revealed that *Streptomyces griseus* gave better result regarding disease control compared with control treatment on the one time application than any number of applications. Adding any antagonist one time application before planting resulted in good control of pathogens and increased percentage of surviving plants compared with using the same antagonist two times after two weeks from planting time or three number of application after 30 days from planting time at the first season. This is due to, treating with different bioagents one time number of applications before planting gives chance for antagonists treatments to establish and speared out in court of infection surrounding healthy tissues and preventing pathogens to attack new developed roots (Abd El-Moity *et al*., 1991 and Sallam *et al*., 2008).

Effect of *Streptomyces griseus* against pathogens can be explained by Golinska and Dahm (2013), they stated that *Streptomyces* strains produce expolysaccharide; this compound has effect against *Fusarium spp*., so presence of this antagonist in court of infection lead to presence a concentration of this compound causing depression for *Fusarium* consequently cannot cause any disease symptom for treated plant, with *Streptomyces* spp. (El-Tarabily and Sivasithamparam, 2006).

**Table 1. Effect of some bioagents at different applications number on the percentage of damping -off disease incidence on basil plants during 2016 and 2017 seasons.**

| Treatments | Percentage of disease incidence of damping-off | | | |
|---|---|---|---|---|
| | Season 2016 | | Season 2017 | |
| | Post | Survival | Post | Survival |
| **Control** | 63.33 | 36.67 | 61.67 | 38.33 |
| ***Bacillus subtilis*-1** | 41.67 | 58.33 | 50.00 | 50.00 |
| ***Bacillus subtilis*-2** | 50.00 | 50.00 | 50.00 | 50.00 |
| ***Bacillus subtilis*-3** | 63.33 | 36.67 | 48.33 | 51.67 |
| ***Trichoderma harzianum*-1** | 25.00 | 75.00 | 53.33 | 46.67 |
| ***Trichodermaharzianum*-2** | 35.00 | 61.67 | 48.33 | 51.67 |
| ***Trichoderma harzianum*-3** | 36.67 | 63.33 | 33.33 | 66.67 |
| ***Streptomyces griseus*-1** | 18.33 | 81.67 | 40.00 | 60.00 |
| ***Streptomyces griseus*-2** | 23.33 | 76.66 | 35.00 | 65.00 |
| ***Streptomyces griseus*-3** | 21.67 | 78.33 | 26.67 | 73.33 |
| ***Pseudomonas flouresens*-1** | 38.33 | 61.67 | 36.67 | 63.33 |
| ***Pseudomonas flouresens*-2** | 50.00 | 50.00 | 33.33 | 66.67 |
| ***Pseudomonas flouresens*-3** | 43.33 | 56.67 | 25.00 | 75.00 |
| **LSD at 5 %** | 6.98 | 7.36 | 8.60 | 8.19 |

**Number of applications: 1=One times, 2= Two times, 3= Three times.**

Actinomycetes produce chemically diverse metabolites many of which exhibit antibiotic activity and plant growth regulatory activity. In addition, they produce lytic enzymes which not only facilitate their survival by degrading diverse substrates but which can inhibit the growth of phytopathogens. This is due to, that *Streptomyces spp*. works effectively under alkaline soil condition. Egyptian soil is alkaline so effective *Streptomyces* spp. bioagents showed good results under this pH value condition (Hoster *et al.*, 2005).

On the contrary, at second season, adding antagonist fifteen days and one month after planting this means that, the developed roots before this period, already attacked by pathogens which negatively affect plant growth and developing (Mannanov and Sattararova 2009). Data also indicated that increase number of application lead to increase efficacy of the treatment. This due to, increase number of application means increase number of bioagent on the surface of treated plants. *P. fluorescens* applied three times was the most effective in controlling basil damping -off disease which recorded (25%) on post damping- off and 75% on seedling survivals. This effect might be due to that *P. fluorescens* can inhibit disease through more than one mode of action and cleat iron and prevent other microorganisms to utilize this element. As a result to iron starvation, the pathogen cannot grow, penetrate and cause disease (Loper, 1988 and Becker and Cook, 1988), Also, *P. fluorescens* produced some antifungal substances, *i.e.* pyrrolintrin, pyoluteorin and 2, 4 diacetyl ploroglucinol (Sarniguet *et al*., 1995, Karunanithi *et al*., 2000 and Jayaraj *et al*., 2007). In a competitive environment, such as soil, it is certainly more advantageous to use efficient chelators produced by its neighbors rather than to produce its own siderophores, especially if they are less effective and metabolically costly, as demonstrated previously. In addition, since desferrioxamines are synthesized by many soil organisms, in this way, *P. fluorescens* could obtain iron at a low energy cost in many competitive situations. Interestingly, it should be noted that purified desferrioxamine added to the culture medium had a positive effect on the growth of *P. fluorecens*. In contrast, the production of desferrioxamines seems to be conserved throughout streptomycetes *S.*

*ambofaciens* negatively impacted the growth because of the secretion of secondary metabolites and/or through nutrient competition (Dumas *et al.,* 2013).

**Vegetative growth:**

Data in Table (2) revealed that all bioagents treatments (*Bacillus subtilis, Trichoderma harzianum*, *Pseudomonas fluoroscens* and *Streptomyces griseus*) with different application numbers (one, two and three time) significantly increased vegetative growth (plant height, number of branches, fresh and dry weights (g/plant) compared with control plants in all cuts during two seasons. In the first season, the highest effective biocontrol treatment *Streptomyces griseus* were obtained from the three application numbers, and showed also the highest values of plant height (cm), number of branches/plant, fresh and dry weights were obtained when used at the second cut than the first cut at two seasons which gave (108.46 cm and 106.13 cm) and the number of branches (36.78 and 34.27). Herb fresh and dry weights increased gradually with increasing the application numbers. The heaviest fresh and dry weights were obtained from these plants applied with *Streptomyces griseus* in the first seasons. The values of herb fresh weight was (544.76 and 636.4 g/plant) and dry weight (157.37 and 162.55g/plant) for the first and second cuts, respectively. On the contrary the lowest values compared with any other biological treatments were recorded when *Bacillus subtilis* was used in two cuts at first season. While, in the second season, the plants treated with three applications for *Pseudomonas fluoroscens* gave the heaviest herb fresh weights (653.93 and 733.23 g/plant) and herb dry weight (165.15 and 183.46 g/plant), for the first and second cuts, respectively. *Trichoderma harzianum* was recorded the least bioagents effect on vegetative growth in the first cut but *Bacillus subtilis* in second cut at second seasons. Plant height, number of branches, in addition to fresh and dry weights show significant increase in plants treated with different bioagents when compared with control treatments these increases in growth characteristics can be due to the growth regulators produced by them. In addition to these growth regulators, also protect plants against disease, consequently improve performance of plant in photosynthesis which leads to increase yield (Elad *et al*., 1980 and Lamb and Rosskopf, 2002). Soil actinomycetes particularly *Streptomyces spp*. (*Streptomyces griseus* Waksman) enhances soil fertility and has antagonistic activity against wide range of soil-borne plant pathogens (Dulaney, 1953 and Hallmann *et al.,* 1997) to provide an effective method to increase productivity of crops (Emmert and Handelsman 1999). The mechanisms by which actinomycetes promote plant growth include mainly the production of plant growth regulators (PGRs) such as gibberellin-like substance and auxines (Katznelson and Cole 1965 and El-Tarabily and Sivasithamparam, 2003 ). It has ability to produce active compounds, such as antifungal and antibacterial antibiotics or plant growth regulators that have been developed for agricultural uses (Gopalakrishnan *et al.*, 2011). Healthy root system can absorb high amount of nutrient substances which reflect in good healthy foliage system eventually this healthy foliage system gives good inflorescences, healthy seeds and high yield (Abd El-Moity *et al*., 1991; and Mosa *et al*., 2013). In addition to effect of different bioagents on size and health of root systems, these bioagents also produce some plant growth promoting (PGP) materials which improve plant growth. *Pseudomonas fluorescens* also used as biocontrol agent. *Pseudomonas fluorescens* antagonizes pathogenic fungi through certain mode of action. This mode of action rely on cleating all available ferrous in the court of infection (Naureen *et al*., 2009) in addition to production of some pigments and antibiotics (Jaryaraj *et al*., 2007). Healthy plants, as obtained data, have bigger plant with more branches this bigger green area carry out more photosynthesis more sugar consequently more oil. These increases are

**Table 2. Effect of some bioagents at different applications number on vegetative growth of sweet basil plants during 2016 and 2017seasons.**

| Treatments | Plant height (cm) | | No. of branches | | Herb fresh weight (g/plant) | | Herb dry weight (g/plant) | |
|---|---|---|---|---|---|---|---|---|
| | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| **First season 2016** | | | | | | | | |
| **Control** | 62.55 | 52.94 | 11.48 | 10.81 | 146.53 | 156.52 | 36.54 | 39.13 |
| ***Bacillus subtilis*-1** | 102.12 | 101.83 | 30.24 | 31.60 | 425.42 | 602.24 | 130.26 | 151.72 |
| ***Bacillus subtilis*-2** | 102.99 | 103.65 | 32.63 | 34.26 | 503.94 | 606.68 | 142.35 | 153.58 |
| ***Bacillus subtilis*-3** | 102.14 | 104.14 | 33.40 | 34.25 | 515.61 | 617.28 | 147.03 | 155.44 |
| ***Trichoderma harzianum*-1** | 103.05 | 102.70 | 31.40 | 30.53 | 493.14 | 536.70 | 135.81 | 135.04 |
| ***Trichodermaharzianum*-2** | 103.09 | 105.73 | 32.52 | 34.20 | 504.97 | 554.71 | 143.22 | 141.95 |
| ***Trichoderma harzianum*-3** | 103.60 | 105.77 | 32.36 | 34.42 | 513.77 | 603.43 | 144.19 | 152.89 |
| ***Streptomyces griseus*-1** | 102.49 | 102.49 | 32.00 | 34.72 | 500.56 | 600.67 | 141.55 | 151.98 |
| ***Streptomyces griseus*-2** | 105.41 | 107.66 | 33.87 | 35.56 | 540.52 | 608.48 | 155.29 | 153.55 |
| ***Streptomyces griseus*-3** | 106.13 | 108.46 | 34.27 | 36.78 | 544.76 | 636.14 | 157.37 | 162.55 |
| ***Pseudomonas flouresens*-1** | 100.44 | 101.44 | 31.36 | 34.63 | 515.89 | 596.60 | 144.40 | 145.12 |
| ***Pseudomonas flouresens*-2** | 104.81 | 104.20 | 33.82 | 34.41 | 530.72 | 602.14 | 147.94 | 149.53 |
| ***Pseudomonas flouresens*-3** | 105.09 | 105.88 | 34.00 | 34.88 | 538.31 | 611.99 | 148.58 | 152.96 |
| **LSD at 5 %** | 2.78 | 1.84 | 1.96 | 1.74 | 8.80 | 6.16 | 4.36 | 5.33 |
| **Second season 2017** | | | | | | | | |
| **Control** | 50.97 | 73.61 | 12.54 | 14.09 | 169.11 | 207.86 | 42.74 | 51.47 |
| ***Bacillus subtilis*-1** | 101.72 | 103.64 | 31.74 | 32.24 | 593.07 | 610.11 | 151.89 | 153.63 |
| ***Bacillus subtilis*-2** | 102.74 | 105.84 | 31.76 | 32.89 | 614.90 | 617.52 | 155.00 | 155.72 |
| ***Bacillus subtilis*-3** | 102.81 | 108.17 | 32.75 | 34.56 | 641.02 | 632.16 | 158.45 | 160.77 |
| ***Trichoderma harzianum*-1** | 100.73 | 107.79 | 30.87 | 33.27 | 615.56 | 634.58 | 152.93 | 158.46 |
| ***Trichodermaharzianum*-2** | 101.70 | 111.72 | 31.17 | 35.12 | 620.40 | 653.90 | 154.45 | 163.93 |
| ***Trichoderma harzianum*-3** | 102.44 | 112.90 | 31.93 | 35.55 | 638.15 | 673.00 | 158.46 | 167.74 |
| ***Streptomyces griseus*-1** | 103.80 | 113.49 | 29.02 | 31.95 | 605.11 | 641.06 | 154.59 | 162.60 |
| ***Streptomyces griseus*-2** | 105.49 | 115.31 | 30.82 | 35.66 | 623.08 | 659.79 | 156.55 | 164.49 |
| ***Streptomyces griseus*-3** | 107.99 | 116.24 | 31.94 | 35.88 | 642.20 | 683.76 | 160.24 | 172.60 |
| ***Pseudomonas flouresens*-1** | 102.77 | 109.29 | 31.00 | 32.07 | 592.51 | 714.03 | 150.94 | 178.86 |
| ***Pseudomonas flouresens*-2** | 104.88 | 112.68 | 32.47 | 34.04 | 638.92 | 731.79 | 157.53 | 181.12 |
| ***Pseudomonas flouresens*-3** | 105.53 | 115.45 | 33.13 | 37.62 | 653.93 | 733.27 | 165.15 | 183.46 |
| **LSD at 5 %** | 2.56 | 2.82 | 1.62 | 1.46 | 6.26 | 4.27 | 3.64 | 3.02 |

**Number of applications: 1=One times, 2= Two times, 3= Three times.**

due to effect of these bioagents on plant protection against different diseases. Healthy vigor plants produce more green leaves which carry out photosynthesis. This photosynthesis leads to high amount of branches and yield (Abdel-Aziz, 2010). Data also showed that adding antagonists as soil drench showed good results in protecting plants and productivity. This is due to adding antagonists in liquid form allow antagonists to spread in court of infection very fast which take little time to germinate and spread in court of infection preventing pathogens to come close and attack plant tissue, Abd El-Moity (1976).

**Oil yield:**

Data presented in Table (3) revealed that, essential oil percentage and oil yield ml/plant were significantly affected by different treatments of bioagents as compared with control. It was evident that all

**Table 3. Effect of some bioagents at different applications number on oil percentage and oil yield/plant of sweet basil plants during 2016 and 2017 seasons.**

| Treatments | Essential oil (%) | | Essential oil yield/ plant (ml) | |
|---|---|---|---|---|
| | 1st cut | 2nd cut | 1st cut | 2nd cut |
| **First season 2016** | | | | |
| **Control** | 0.13 | 0.15 | 0.19 | 0.23 |
| ***Bacillus subtilis*-1** | 0.23 | 0.27 | 0.98 | 1.63 |
| ***Bacillus subtilis*-2** | 0.24 | 0.31 | 1.21 | 1.88 |
| ***Bacillus subtilis*-3** | 0.24 | 0.32 | 1.24 | 1.98 |
| ***Trichoderma harzianum*-1** | 0.24 | 0.27 | 1.18 | 1.45 |
| ***Trichodermaharzianum*-2** | 0.25 | 0.32 | 1.26 | 1.78 |
| ***Trichoderma harzianum*-3** | 0.26 | 0.32 | 1.34 | 1.93 |
| ***Streptomyces griseus*-1** | 0.27 | 0.33 | 1.35 | 1.98 |
| ***Streptomyces griseus*-2** | 0.30 | 0.37 | 1.62 | 2.25 |
| ***Streptomyces griseus*-3** | 0.31 | 0.38 | 1.69 | 2.42 |
| ***Pseudomonas flouresens*-1** | 0.24 | 0.32 | 1.24 | 1.91 |
| ***Pseudomonas flouresens*-2** | 0.26 | 0.34 | 1.38 | 2.05 |
| ***Pseudomonas flouresens*-3** | 0.26 | 0.34 | 1.40 | 2.08 |
| **LSD at 5 %** | 0.25 | 0.31 | 1.24 | 1.81 |
| **Second season 2017** | | | | |
| **Control** | 0.14 | 0.17 | 0.24 | 0.35 |
| ***Bacillus subtilis*-1** | 0.22 | 0.30 | 1.30 | 1.83 |
| ***Bacillus subtilis*-2** | 0.24 | 0.32 | 1.48 | 1.98 |
| ***Bacillus subtilis*-3** | 0.24 | 0.33 | 1.54 | 2.09 |
| ***Trichoderma harzianum*-1** | 0.23 | 0.31 | 1.42 | 1.97 |
| ***Trichodermaharzianum*-2** | 0.24 | 0.33 | 1.49 | 2.16 |
| ***Trichoderma harzianum*-3** | 0.24 | 0.33 | 1.53 | 2.22 |
| ***Streptomyces griseus*-1** | 0.24 | 0.36 | 1.45 | 2.31 |
| ***Streptomyces griseus*-2** | 0.25 | 0.37 | 1.56 | 2.44 |
| ***Streptomyces griseus*-3** | 0.25 | 0.37 | 1.61 | 2.53 |
| ***Pseudomonas flouresens*-1** | 0.23 | 0.35 | 1.36 | 2.50 |
| ***Pseudomonas flouresens*-2** | 0.25 | 0.38 | 1.60 | 2.78 |
| ***Pseudomonas flouresens*-3** | 0.26 | 0.39 | 1.70 | 2.86 |
| **LSD at 5 %** | 0.24 | 0.33 | 1.41 | 2.16 |

**Number of applications: 1=One times, 2= Two times, 3= Three times.**

bioagents in most treatments have positive effect on oil percentage and oil yield ml/plant in all applications number at two seasons. Data obtained in Table (3) revealed that *Streptomyces griseus* was the most bioagents which increased oil percentage and oil yield ml/plant with three applications in the two cuts of the first season, which recorded (0.31 and 0.38 %) and oil yield /plant (1.69 and 2.42 ml/plant). While, in the second season, plants treated with three applications number of *Pseudomonas fluoroscens* gave the highest values of essential oil percentage and oil yield ml/plant (0.26 and 0.39%) and (1.70 and 2.86 ml/plant) in the first and second cuts, respectively. *Streptomyces griseus* led to increase oil percentage and oil yield. These increases in size of plants were correlated with increase in essential oil due to that potassium element is responsible for oil formation (Mahfouz and Sharaf-Eldin, 2007). In addition to action of potassium Subramaniam *et al.* (2012) explained that

increase size and weight of panicle rice treated with *Streptomyces sp*. is due to plant growth promoting (PGP) produce by adding antagonists, this lead to improve potassium absorbing consequently increase percentage of oil (Mahfouz and Sharaf-Eldin, 2007 and Gebily, 2015). Actinomycetales are especially useful as biological agents for reducing phytopathogen infection in that they produce over 60% of the approximately 5,000 known antibiotics, some strains synthesizing 30 or more including many with fungicidal activity. They produce an enormous diversity of hydrolytic enzymes including enzymes that degrade fungal cell wall components, such as chitinases, cellulases, and glucanases. They are heterotrophically diverse, as they are evolutionarily adapted for growth in the soil or in close proximity to plant roots utilizing a wide range of carbon sources. They produce spores under environmental deleterious conditions. They grow vegetative as mycelia, thus allowing root colonization and translocation of nutrients over relatively large distances.

**Essential oil component:**

The composition of sweet basil essential oil, hydro distilled from the fresh herb from different treatments at the second cut of the second season, were analyzed by GC. In our work, Data showed that essential oil constituents were also affected by presence of different used biocontrol agents. The chemical composition regarding major compounds present in the essential oils of sweet basil (*Ocimum basilicum* L.) plants are presented in Table (4).

Linalool was the most abundant compound in all analyzed oils, followed by Terpinole, β- Caryophyllene, 1, 8 Cineol, Campher, Methyl chavicol, Geranyl acetate, α-pinene and β-pinene.

**Table 4. Effect of some bioagents at different applications number on essential oil composition of sweet basil in the first cut second season 2017.**

| Treatments | Essential oil component | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | α -Pinene | β -Pinene | 1,8 Cineol | linalool | Campher | Terpinole | Geranyl acetate | Methyl chavicol | β- Caryophylle |
| **Control** | 1.38 | 0.12 | 8.14 | 35.00 | 3.33 | 31.74 | 3.65 | 4.15 | 10.25 |
| ***Bacillus subtilis*-1** | 1.03 | 0.03 | 4.18 | 27.03 | 3.25 | 21.04 | 5.64 | 3.61 | 11.52 |
| ***Bacillus subtilis*-2** | 0.51 | 0.63 | 4.02 | 41.63 | 5.00 | 22.26 | 4.28 | 2.02 | 15.26 |
| ***Bacillus subtilis*-3** | 0.39 | 0.42 | 1.13 | 42.15 | 5.16 | 30.50 | 2.96 | 3.71 | 11.32 |
| ***Trichoderma harzianum*-1** | 0.63 | 0.54 | 6.42 | 36.81 | 2.93 | 20.90 | 3.78 | 2.22 | 10.18 |
| ***Trichodermaharzianum*-2** | 0.72 | 0.55 | 6.73 | 42.84 | 3.47 | 23.01 | 4.40 | 1.78 | 11.39 |
| ***Trichoderma harzianum*-3** | 0.67 | 0.44 | 6.88 | 42.91 | 3.65 | 24.65 | 5.11 | 2.09 | 11.53 |
| ***Streptomyces griseus*-1** | 1.52 | 0.19 | 6.64 | 37.39 | 3.88 | 18.14 | 4.07 | 2.82 | 10.21 |
| ***Streptomyces griseus*-2** | 0.75 | 0.05 | 5.12 | 40.38 | 5.27 | 21.52 | 4.77 | 2.32 | 10.11 |
| ***Streptomyces griseus*-3** | 0.66 | 0.73 | 5.95 | 43.51 | 4.94 | 24.40 | 4.84 | 0.80 | 10.52 |
| ***Pseudomonas flouresens*-1** | 0.40 | 0.38 | 3.43 | 35.35 | 4.54 | 21.35 | 4.52 | 4.13 | 8.64 |
| ***Pseudomonas flouresens*-2** | 0.38 | 1.63 | 5.42 | 40.64 | 5.12 | 24.03 | 4.63 | 4.08 | 10.11 |
| ***Pseudomonas flouresens*-3** | 0.28 | 0.86 | 4.69 | 39.69 | 5.33 | 25.34 | 5.31 | 3.34 | 10.35 |

**Number of applications: 1=One times, 2= Two times, 3= Three times.**

All treatments resulted in increased the main component (linalool) compared to untreated plants (35 %), except plants treated with one applications of *Bacillus subtilis* decreased it (27.03%). While, when plants treated with the three applications of *Streptomyces griseus* gave the highest value of linalool (43.51%). Data showed that, all treatments of bioagents (*Bacillus subtilis* , *Trichoderma harzianum*, *Pseudomonas fluoroscens* and *Streptomyces griseus*) with different application numbers (one, two or three time) decreased Methyl chavicol compared with control (4.15%). These results can explained in the light of the fact that plants, some time, produce toxic substance as mechanism of acquired resistance (Abd El-Moniem, 2001). *T. harzianum* belong to family Moniliaceae which contains a lot of pathogens, so that *T. harzianum* stimulate this phenomena and percentage of oil was increased in treated basil plants compare with control or other of *B. subtilis* and *Streptomyces griseus*. *B. subtilis* or *streptomyces griseus* did not estimate the same effect because no relating members to these genera can cause any disease for basil (Hader *et al.,* 1992 and Gebily, 2015).The fluctuation within this margin is controlled according to some physiological factors. One of these factors can be explained through the work of (Mahmoud *et al*., 1995 and Abd El-Moniem, 2001) they stated that treating plants with fungal spores led to production of some antifungal substance, which create acquired resistance.

**Pigments and total carbohydrates contents:**

Data presented in Table (5) showed that, application of different biological treatments at different application numbers led to changes in some chemical components compared with control treatment. Data also indicate that, positive correlation between increase of total carotenoids and percentage of total carbohydrates and increase the number of applications when used bioagent was noticed in first and second cuts at two seasons. Using *Streptomyces griseus* either two or three times application recorded the same results in the two cuts of the two seasons, showed the highest values on the chlorophyll (a) and (b) compared with any other treatment. On the contrary, *Streptomyces griseus* when used in three times application, the highest values on carotenoids and total carbohydrates were recorded compare with any other biological treatment in first season. While, in the second season, when plants treated with the three applications of *Pseudomonas fluoroscens* gave the highest values of chlorophyll (a), (b) and carotenoids contents. *Streptomyces griseus* occupied the first rank after three application numbers which recorded the highest total carbohydrates percentage in the two cuts compared with any treatments or control plants. A remarkable diversity of metabolites with antibiotic activity is produced by *Pseudomonas*, *Bacillus*, and *Streptomycete*s strains. Some of these microorganisms could produce simultaneously more than one compound (for example, *P. fluorescens* strains CHAO and Pf-5 and/or act by more than one mechanism (e.g., antibiosis and competition for nutrients). These bioagents could act as bio-fertilizers and as biopesticides. In this way, PGPR could constitute a group of bacteria of great importance. In addition to the nitrogen supply Rhizo-bio-promote growth of the plant as a symbiotic partner in several ways, such as mobilization of nutrients, enhancement in stress resistance, solubilization of phosphates, production of phytohormones and siderophores. Siderophores act as iron source for the plant under iron depleted conditions (Mingma *et al*., 2015). Among bioactive compound producers, the genus *Streptomyces* is dominant, and produces compounds such as ivermectin, tetracycline, streptomycin, nystatin, etc.. (Ser *et al.,* 2015).

**Table 5. Effect of some bioagents at different applications number on chemical constituents in sweet basil plants during 2016 and 2017 seasons.**

| Treatments | chlorophyll (a) (mg/gm) | | chlorophyll (b) (mg/gm) | | Carotenoids (mg/gm) | | (%) Total carbohydrates | |
|---|---|---|---|---|---|---|---|---|
| | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut | 1st cut | 2nd cut |
| **First season 2016** | | | | | | | | |
| **Control** | 0.224 | 0.213 | 0.224 | 0.213 | 0.708 | 0.730 | 22.85 | 23.18 |
| ***Bacillus subtilis*-1** | 0.274 | 0.271 | 0.274 | 0.271 | 1.046 | 1.062 | 30.24 | 33.24 |
| ***Bacillus subtilis*-2** | 0.283 | 0.277 | 0.283 | 0.277 | 1.076 | 1.085 | 33.56 | 33.56 |
| ***Bacillus subtilis*-3** | 0.284 | 0.286 | 0.284 | 0.286 | 1.177 | 1.175 | 34.71 | 37.05 |
| ***Trichoderma harzianum*-1** | 0.291 | 0.289 | 0.291 | 0.289 | 1.077 | 1.073 | 33.77 | 35.77 |
| ***Trichodermaharzianum*-2** | 0.293 | 0.296 | 0.293 | 0.296 | 1.167 | 1.161 | 35.63 | 37.63 |
| ***Trichoderma harzianum*-3** | 0.292 | 0.295 | 0.292 | 0.295 | 1.174 | 1.171 | 36.01 | 37.60 |
| ***Streptomyces griseus*-1** | 0.283 | 0.290 | 0.283 | 0.290 | 1.074 | 1.088 | 32.22 | 35.89 |
| ***Streptomyces griseus*-2** | 0.296 | 0.302 | 0.296 | 0.302 | 1.187 | 1.122 | 35.31 | 37.98 |
| ***Streptomyces griseus*-3** | 0.296 | 0.302 | 0.296 | 0.302 | 1.302 | 1.302 | 36.64 | 38.31 |
| ***Pseudomonas flouresens*-1** | 0.287 | 0.278 | 0.287 | 0.278 | 1.034 | 1.025 | 30.24 | 33.58 |
| ***Pseudomonas flouresens*-2** | 0.285 | 0.286 | 0.285 | 0.286 | 1.084 | 1.067 | 32.49 | 34.83 |
| ***Pseudomonas flouresens*-3** | 0.287 | 0.291 | 0.287 | 0.291 | 1.164 | 1.164 | 34.51 | 35.84 |
| **LSD at 5 %** | 0.016 | 0.011 | 0.051 | 0.131 | 0.021 | 0.011 | 2.30 | 2.35 |
| **Second season 2017** | | | | | | | | |
| **Control** | 0.208 | 0.217 | 0.208 | 0.217 | 0.675 | 0.700 | 20.18 | 19.18 |
| ***Bacillus subtilis*-1** | 0.274 | 0.281 | 0.274 | 0.281 | 1.043 | 1.041 | 25.24 | 24.24 |
| ***Bacillus subtilis*-2** | 0.281 | 0.283 | 0.281 | 0.283 | 1.065 | 1.058 | 28.56 | 27.56 |
| ***Bacillus subtilis*-3** | 0.282 | 0.285 | 0.282 | 0.285 | 1.149 | 1.129 | 30.38 | 29.71 |
| ***Trichoderma harzianum*-1** | 0.289 | 0.290 | 0.289 | 0.290 | 1.078 | 1.070 | 29.44 | 28.11 |
| ***Trichodermaharzianum*-2** | 0.290 | 0.291 | 0.290 | 0.291 | 1.164 | 1.162 | 30.30 | 29.63 |
| ***Trichoderma harzianum*-3** | 0.290 | 0.292 | 0.290 | 0.292 | 1.174 | 1.162 | 31.01 | 30.51 |
| ***Streptomyces griseus*-1** | 0.278 | 0.279 | 0.278 | 0.279 | 1.078 | 1.082 | 30.22 | 31.22 |
| ***Streptomyces griseus*-2** | 0.280 | 0.293 | 0.280 | 0.293 | 1.092 | 1.094 | 33.98 | 35.31 |
| ***Streptomyces griseus*-3** | 0.285 | 0.297 | 0.285 | 0.297 | 1.229 | 1.211 | 34.64 | 35.98 |
| ***Pseudomonas flouresens*-1** | 0.289 | 0.300 | 0.289 | 0.300 | 1.029 | 1.021 | 28.58 | 29.58 |
| ***Pseudomonas flouresens*-2** | 0.294 | 0.305 | 0.294 | 0.305 | 1.078 | 1.080 | 31.16 | 32.83 |
| ***Pseudomonas flouresens*-3** | 0.295 | 0.308 | 0.295 | 0.308 | 1.173 | 1.160 | 31.84 | 35.18 |
| **LSD at 5 %** | 0.015 | 0.017 | 0.202 | 0.151 | 1.043 | 1.041 | 1.85 | 2.45 |

**Number of applications: 1=One times, 2= Two times, 3= Three times.**

## CONCLUSION

It could be concluded that the application of all bioagents are safety and effective method against soil borne fungi. Also, it gave the highest percentage of survive plants, growth, yield and oil production and the highest percentage of main component of the essential oil. Among soil microorganisms, bacteria and fungi and to a lesser extent actinomycetes, have received considerable attention as biocontrol agents of soil-borne fungal plant pathogens and as plant growth promoters. Within actinomycetes, *Streptomyces griseus* was found to be more effective in this respect. Strains of *Streptomyces griseus* promote plant growth by producing plant growth regulators. Enhancement of plant growth by

the antagonists is considered to help the host by producing compensatory roots that mask the impact of root diseases. So, in order to obtain good results in basil plantations under field conditions, treating basil plants with different bioagents at three number applications during the growing seasons could be recommended.

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## مقاومة مرض موت البادرات باستخدام بعض الكائنات الحية الدقيقة علي نباتات الريحان الحلو تحت ظروف الحقل

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أجريت هذه الدراسة في قسم بحوث النباتات الطبية والعطرية في القناطر الخيرية، مركز البحوث الزراعية، خلال الموسمين التاليين لعامي ٢٠١٦ و ٢٠١٧ لدراسة تأثير بعض الكائنات الحية الدقيقة *Bacillus subtilis*، *Trichoderma harzianum*، *Pseudomonas fluoroscens* و *Streptomyces griseus* على مقاومة مرض موت البادرات في الريحان، النمو الخضري، إنتاج الزيت والمكونات الكيميائية لنبات الريحان الحلو ( *Ocimum basilicum* L.). أوضحت النتائج ان النباتات المعاملة بكل من الكائنات الحية الدقيقة أعلى تأثير في التحكم في مرض موت البادرات وزيادة إنتاجية ومحصول الريحان مقارنة بالنباتات الغير معاملة. أوضحت النتائج أن جميع النباتات المعاملة بالكائنات الحيوية أحدثت زيادة معنوية كبيرة في صفات النمو الخضري (ارتفاع النبات، عدد الافرع، الأوزان الجافة والجافة (جم)، نسبة الزيت، محصول الزيت، المكونات الكيميائية (الكلوروفيل (أ) ، (ب) ومحتويات الكاروتينات بالإضافة إلى نسبة الكربوهيدرات الكلية) مقارنة بالنباتات الغير معاملة. أعطت *Streptomyces griseus* أفضل تأثير ضد موت البادرات وجميع الخصائص النباتية في الموسم الأول، بينما في الموسم الثاني، زادت *Pseudomonas fluorescens* من حماية النبات وجميع خصائص النبات. وعند تقدير المكون الاساسي للزيت باستخدام جهاز التحليل الكروماتوجرافي تم التعرف علي تسع انواع من المكونات الاساسية للزيت وكان اكثرهم نسبة اللينالول.