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Using Different Bioagents to Control Root Rot on *Nigella Sativa* L. Plant

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Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2687-8151

Online ISSN: 2687-8216

DOI:
10.37229/fsa.fja.2024.03.20

Received: 1 February 2024
Accepted: 5 March 2024
Published: 20 March 2024

Publisher's Note: FA stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: A field study was conducted to evaluate the efficacy of biocontrol agents on controlling root rot on *Nigella sativa L.* plants in two successive seasons 2018/2019 and 2019/2020 in the Experimental Farm of South Tahrir Horticulture Research Station, at Ali Mubarak farm, El- Behira Governorate, Horticulture Research Institute (HRI), Agriculture Research Center (ARC), Egypt. Ten isolates of *Bacillus* spp. and *Streptomyces* spp. (5 Bacillus and 5 Streptomyces) were evaluated for their effectiveness against *Rhizoctonia solani*. *B. subtilis, B. licheniformis* and *S. rochei* were used *in vivo* in two forms powder and suspension to suppress *R. solani* root rot. The results showed that *Streptomyces rochei* (OP164572), *Bacillus subtilis* (MT110640) and *Bacillus licheniformis* (OP164574) gave the highest reduction on growth of *R. solani* (55.47%, 60.17% and 59.53%) respectively, compared with control. Correspondingly, when biologically treated plants were compared to untreated control plants, physiological and biochemical indicators were likewise significantly elevated. Under field condition, our results revealed that used suspension form was more effective than powder form for management of root rot disease in *Nigella sativa L.*, plants. Results showed that, when compared to untreated plants, all biocontrol agents decreased the frequency of root rot and improved vegetative growth, seed yield, volatile oil production, and fixed oil. The treatment with *B. subtilis* suspension produced the highest percentage of survived plants (98% vs. 14% in the control), as well as the highest growth, seed yield, volatile oil, and fixed oil content followed by *S. rochei* suspension, while treated by *B. licheniformis* was the less effective. The effect of bioagenst against *R. solani* was examined under fixed camera light microscope. *B. subtilis, B. licheniformis*, and *S. rochei* suspensions can be recommended as fungicides alternatives for controlling black cumin root rot disease in greenhouse and field environments.

Key words: Biological control, Black cumin, *R. solani*, *Bacillus* spp., *Streptomyces* spp., *Bacillus licheniformis*, root rot, seed volatile oil and fixed oil.

1. Introduction

Black cumin *(Nigella sativa L.*) plants Family: Ranunculaceae seeds have great economic importance as an export crop. It has special importance in the Middle and Upper Egypt, especially in Giza, Fayoum,

BeniSuef, El-Minya, and Assiut Governorates **(FAO, 2016).** It is an annual herb with therapeutic properties; the seeds are used globally in folk herbal medicine to prevent many diseases **(Chevallier, 1996).** This plant's seeds have been used to treat a variety of diseases, including skin infections, as well as a spice and food preservative. **(Schleicher and Saleh, 2000).** Black cumin seeds antimicrobial effects against different pathogenic microbes were investigated **(Abu-Al-Basal, 2009 and Abou-Zied, 2011)**. In folklore, *Nigella sativa L.* seeds and oil are used in a variety of foods and medications **(Khalid and Shedeed, 2016**). Moreover, *Nigella sativa L.* have a pharmacological property as antithypertensive, antidiarrhreal livertonics, and antimicrobial **(Ahmad *et al.*, 2013).**

Black cumin seeds are extensively used in both medicine and food formulations **(Babayan *et al*., 1978 and Takruri and Dameh, 1998).** The seeds' extract has been proven to have anti-cancer properties and to be highly effective against Dalton's lymphoma ascites cells. **(Salomi *et al*., 1989)**. Furthermore, black seed oil is widely used to treat paralysis, especially facial paralysis, back pain, and rheumatism. It has also been shown in experiments to stimulate the flow of bile. **(Riaz *et al*., 1996 and Saeed *et al*., 1996)**.

Root rot disease is the most destructive disease attack *N. sativa L.* plants **(Srivastava and Chandra, 1983; Hashmi, 1988; Dubey, 1995; Sinha and Singh, 1994 and Baiuomy and Shalaby, 2006)**. These diseases are brought on by soil borne fungi and may be transmitted by seeds **(El-Wakil and Ghoneem, 1999)**. Percentages of occurrence of these diseases increased in the absence of control measurements in field and replanting the crop in the same location for several years. The symptoms, which begin with yellowing and drying of leaves, cause the plants to dry out too soon, significantly lowering the yield. The disease was recorded as early as by **(Kamal, 1980)** in India and by **(Hilal *et al*., 1994)** in Egypt. Root rot caused by a *Rhizoctonia and Fusarium compl*ex, symptoms start with yellowing and drying of leaves, result in premature drying of plants, which drastically reduces the *Nigella sativa L.*, yield **(Peter, 2004)**. Furthermore, **(Baiuomy and Shalaby, 2006)** carry a survey on *Nigella sativa L.* crop plantations in Egypt during (2003 & 2004) and isolated different root-rot pathogens. Also, pathogenicity test was carried out, they found that *F. oxysporum, M. phaseolina* and *R. solani* were the most harmful fungi.

An alternative to chemical control for soil-borne diseases is biological control using bioagents (**Abo-Elyousr *et al*., 2019).** It provides an efficient method of controlling infections without endangering humans, animals, plants, or the environment. **(Sallam *et al*., 2013)**. Various *Bacillus* spp have been employed as potential biocontrol agents against different *Fusarium spp.* In many nations around the world, using plant growth-promoting rhizobacteria (PGPR) has become common practice. **(Abo-Elyousr *et al.*, 2009 and Alamri *et al*., 2019).** Biological control, as an effective mean to protect cultivated crop, especially those grow under organic agriculture conditions, is considered the most important method to produce fresh, healthy high quality and quantity of crops free of chemical residues **(Abd-El-Moity, 2001 and Rafik *et al.*, 2014).**

In addition to the biological control agents described above, actinobacteria (mainly *Streptomyces spp*.) are presently considered another potential alternative for the biocontrol of plant diseases. Indeed, actinobacteria may be one of the main tactics in integrated crop management since they have a wide diversity of beneficial effects, increased availability of main plant mineral nutrients through production of extracellular enzymes, phytohormones siderophores and release of enzymes such as cellulases, glucanases, xylanases, chitinases, lipases, and proteases (**Díaz-Díaz *et al*., 2022; Mitra *et al*., 2022).** Streptomyces *spp.* produced volatiles caused resistance in rice against *Rhizoctonia solani* **(Wan *et al*., 2008)**. Regarding this, in legumes and herbaceous crops, *Streptomyces spp*. prove to be the most efficient BCAs against soil-borne diseases, including fungal species from the genera Rhizoctonia, Phytophthora, Fusarium, and Pythium **(Vurukonda *et al*., 2021).**

The present research was carried out to study black cumin root-rot disease and their causal pathogens, studying different bioagent for controlling these diseases under field conditions, to avoid the bad effect of synthetic fungicides.

2. Materials and Methods

2.1. Isolation, purification and identification of the causal pathogens

Plants showing typical symptoms of root-rot of Black cumin (*Nigella sativa L.,)* were collected from different governorates, *i.e*. Minya, Fayoum and Giza, during the growing seasons of 2018/2019 and used in this study. The infected roots were washed carefully with tap water, and then cut into small pieces. These small pieces of infected roots, then surface sterilized using 2% sodium hypochlorite solution for 2 minutes. Then transferred into sterilized Petri dishes contained PDA medium. Inoculated plates were incubated at 25°C for 4 days and examined periodically. The obtained colonies were purified using the hyphal tip technique **(Hawker, 1960)** or single spore method. The isolated fungi were recognized based on their morphological traits and properties under the using the explanation provided by **(Sneh *et al*., 1992)** for *R. solani*. **(Booth, 1971)** for the genus of *Fusarium*. The fungal isolates were maintained on sterilized PDA slants and kept in a refrigerator at 5 °C for further studies.

2.2. Isolation and purification of the biological control agents

Two biocontrol agents *(Bacillus subtilis* (MT110640) and *Streptomyces rochei* (OP164572)) were previously Isolated and identified. Soil extract agar medium was used for isolating antagonistic bacteria. Separated bacterial colonies were transferred to slants of nutrient glucose agar medium (NGA) according to **(Dowson, 1957)** for purification and identified. Four different *Bacillus* spp. were isolated and tested for their antagonistic effect against the *R. solani* plus *Bacillus subtilis*. Using a molecular approach that involved DNA extraction, amplification (PCR), and sequencing of the 16S rDNA gene amplification, the most effective isolate was identified. Streptomyces was isolated from soil according to **(Hamada, 2006).**

The colonies of streptomyces were transferred in to Petri dishes containing starch nitrate agar medium (StNA) medium supplemented with sodium propionate to suppress any bacterial or fungal contamination, then incubated at 30°C. After 3-5 days, pure individual colonies were transferred to slant tubes containing (StNA) medium and were incubated at 30°C for 5 days. The antagonistic effect of four isolated Streptomyces and *Streptomyces rochei* was studied.

2.3. Pathogenicity

Greenhouse experiment was carried out in a greenhouse belongs to Central Lab of Organic Agriculture, ARC, Giza, Egypt. Plastic pots (25 cm) contained 2.7 kg of clean peat moss. *R. solani* was the most frequently isolated so it used in further experiments. Ten black cumin seeds/ pot were sown. Five *R. solani* isolates were tested for causing root rot; Five replicates/ treatment as 50 seeds/each treatment.

2.4. Inoculum preparation and soil infestation

The five *Rhizoctonia solani* isolates were tested for their virulence in causing damping-off on black cumin plants.

R. solani inocula were created in sterilized bottles with 250 g of corn sand meal medium plus 0.2% peptone solution **(Paulitz and Schroeder 2005)**. Bottles were separately inoculated using equal agar disks (0.5 cm diameter) taken from the culture of *R. solani*. Inoculated bottles were incubated at 25 ^{o}C. After 10 days, the number of colony forming unit (c.f.u)/g of each isolate was determined. Plastic pots were infested with *R. solani* at a rate of 10g of inoculated adjusted ($7x10^6$ c.f.u) corn sand meal/kg soil. Infestation process was carried out 5 days before sowing black cumin seeds. Pots contained non-infested soil were used as control. Five replicates were used for each treatment. All pots received the same treatments regarding, irrigation and nutrition regime. Normal open greenhouse conditions were used to maintain planted pots.

2.5. Disease assessment

The average percentage of pre-, post-emergence and survive plants was recorded after 15 and 30 days after sowing date, respectively, while the survive plants were recorded 45 days after sowing.

The following formula was used to determine all disease assessment percentages **(El-Helaly *et al.*, 1970)**:

% Pre-emergence = Number of non-germinated seeds X 100.

Total number of sown seeds

% Post-emergence = Number of dead seedlings X 100.

Total number of sown seeds

% Survived plants = Number of survived plants X 100.

Total number of sown seeds.

2.6. The Antagonistic effect of the different bioagents against *Rhizoctonia solani*

Ten isolates of bacteria and Streptomyces were tested using the dual culture plate method for their antagonistic potential against *R. solani* **(Siddiqui *et al.*, 2001).** A loop full of 48 hrs old culture of Bacillus spp. and a loop full of 7 days old culture of *Streptomyces* spp. were streaked on one side of Petri plate contained NGA medium for Bacillus and StNA for Streptomyces. The other side of each plate was inoculated with a disk (0.5 cm in diameter) of the tested pathogen of 4 days-old colony grown on PDA medium. Plates inoculated with *R. solani* alone was used as control treatment.

Three replicates were used for each treatment. The plates were incubated at 28ºC±2. when the *R. solani* mycelium covered all the medium surface of control plates, the percentage of reduction in the mycelial growth of the pathogenic fungi was recorded and calculated with the following equation: Growth reduction % = 100 - [G1 / G2 x 100]

Where;

G1= growth diameter of the pathogenic fungus in treated plates (mm)

G2= is the growth diameter of the pathogenic fungus in the check plates (mm).

Two investigate the morphological changes in *R. solani* mycelia induced by *Bacillus subtilis* or *Streptomyces rochei* camera fixed light microscope (40x) was used.

2.7. Using different bioagents for controlling *R. solani* as powder or suspension under field condition

Using the method developed by **(Abd-El-Moity, 1985)**, two isolates of Bacillus (*Bacillus subtilis* and *Bacillus licheniformis*) and *Streptomyces rochei* were prepared as a powder or suspension.

The different *Bacillus spp*. were grown separately on liquid NG medium for 2 days, at 28ºC ±2. *Streptomyces rochei* isolate was grown on liquid starch medium for 7 days at 30ºC ±2.

The cultures were adjusted and then combined with sterilized talc powder at a rate of 1:10 (v/w), carboxymethyl cellulose, and calcium carbonate to bring the pH down to 7.0. The mixture was then allowed to dry at room temperature. The adjusted antagonistic cultures were also prepared as suspensions with water at a rate of 1:10 (v/v) then mixed with 0.5 % potassium soap and 1% Arabic gum to increase distribution and adhesive of bio-agent on the surface of treated plants. Two Bacillus isolates or *Streptomyces rochei* were adjusted to contain 10^7 c.f.u./g or ml. Both powder and suspension formulas were used as seed treatments. Ten ml or gm of each biocontrol agents were used for each replicate.

2.8. Field experiments

2.81. Plant materials and agricultural practices

This study was carried out at the Experimental Farm of South Tahrir Horticulture Research Station, at Ali Mubarak farm, El- Behira Governorate, Horticulture Research Institute (HRI), Agriculture Research Center, (ARC), Egypt, during the two successive seasons of 2018/2019 and 2019/2020. The seeds of *Nigella sativa L.* were obtained from the Experimental Farm of Medicinal and Aromatic Plants Research Department, El- Kanater El- Khairia. and were sown in the trays on 10th October in the first season and 15th October in the second season at distances of 25 cm between hills

and 75 cm between rows. The experimental area divided in three plots each plot contains seven irrigation lines. For the period of the experiment, a drip irrigating system was used, using droppers (4 L/h) every three days for an hour. Two plants were thinned in each hill. Ammonium sulfate (20.6% N), calcium superphosphate (15.5% P2 O5), and potassium sulfate (48% K2O) were added as chemical fertilizers (NPK) at the recommended level as well as cultural practices until the harvest date. Black cumin plants were harvested on 10th may in the first season and 17th may in the second season.

The experiment included 7 treatments as follow:

There were seven treatments in the experiment; each treatment was replicated three times, with 25 plants in each replicate.

1. Control.
2. *Bacillus subtilit*s (powder).
3. *Bacillus licheniformis* (powder).
4. *Streptomyces rochei* (powder)
5. *Bacillus subtilits* (suspension).
6. *Bacillus licheniformis* (suspension).
7. *Streptomyces rochei* (suspension).

The treatments were conducted in three times, the first after 30 days from sowing and repeated at 30 days intervals for the 2nd and 3rd in each season.

The physical and chemical characteristics of the soil: The experimental field's soil's physical and chemical properties were ascertained using **(Jackson, 1973)** and are shown in Table (1).

Table (1). physical and chemical analysis of the experimental soil

Chemical and physical characteristics			**Sandy soil**	
			1st season	**2nd season**
Soluble Cations (meg./100gm soil)	**Soluble Cations**	Ca+2	0.64	0.65
		Mg+2	0.30	0.32
		Na+	0.38	0.41
		K+	0.03	0.03
	Soluble Anions	Co3-	…….	…….
		Hco3-	0.54	0.50
		Cl-	0.38	0.39
		So4	0.40	0.40
pH			8	7
ds/m(E.C).			0.26	0.26
% sand			93	92
% Silt			2.70	2.73
% Clay			2.92	2.90
Texture Class			Sandy	Sandy

2.8.2. Data recorded

Plant height (cm). number of branches/plant, seed yield/plant (g), seed yield kg/fed., volatile oil percentage was determined according to **(British Pharmacopeia, 1963).** Chemical composition of volatile oil: As mentioned by **(Hoftman, 1967 and Bunzen, *et al.*, 1969)**, the components of the

volatile oil extracted from *Nigella sativa L*. seeds were identified by subjecting oil samples from the second season to gas liquid chromatography (GLC) analysis**.,** and fixed oil percentage was determined according to **(A.O.C.S., 1964).**

2.9. Statistical analysis

Complete randomized blocks with seven treatments were used in the experiment's design; each treatment was duplicated three times, with each replication having 25 plants. Statistical analysis was performed using analysis of variance (ANOVA) and L.S.D. at 5% to compare the treatment means **(Snedecor and Cochran, 1980).**

3. Results and Discussion

3.1. Isolation, purification and identification of the causal pathogen of *Nigella sativa L.*, plants root-rot

Different pathogenic fungi were found at the Black cumin plants showing root-rot symptoms. Data in Table (2) showed that two fungal genera, i.e. *Rhizoctonia* spp and *Fusarium* spp. were isolated from infected samples of black cumin seedlings (40-days-old).

The *Rhizoctonia* isolates were more frequent compare with *Fusarium* isolates. For that, the research was completed and made the pathogenicity test on the five isolates of *Rhizoctonia* spp. An important soil-borne pathogen that leads a necrotrophic lifestyle is *R. solani* **(Anderson, 1982)**. This fungus has the potential to seriously damage numerous commercially important trees, horticulture and agricultural crops **(Kühn *et al*., 2009; Jabnoun- Khiareddine *et al*., 2015 and Anees *et al*., 2016)**.

Table (2). Frequency percentages of root-rot pathogens isolated from *Nigella sativa L.*, plants showing root rot symptoms from Giza, Fayoum and Manya governorates

Isolated fungi governorates	No. of *R. solani* isolates	No. of *F. solani* isolates
Minya	2	1
Fayoum	1	0
Giza	2	1

3.2. Identification of the biological control agents

Different bio-agents were isolated and evaluated their ability in controlling *R. solani*. Some of them belonging to *Bacillus* spp. and others belonging to *Streptomyces* spp. They were identified according to their morphological characteristic under light microscope according to (**Waksman, 1959 and Schleifer, 2009).**

The *Bacillus* sp. which given the highest antagonistic effect were identified with DNA, the sequences of the isolate was submitted to NCBI Gen Bank and gave an accession number (OP164574) for *Bacillus licheniformis*. Also, *Bacillus subtilis* (MT110640) and *S. rochi* strain (OP164572) were previously isolated and identified.

3.3. Pathogenicity test

Results in Table (3) showed that all *R. solani* isolates could cause damping-off to black cumin plants at both pre- and post-emergence stages. Also, percentage of survival plants was affected. The pathogenic isolates varied significantly in their ability to cause damping-off. *R. solani* isolate No. 2 was the most aggressive one since it caused 52.66% pre-emergence damping-off, followed by isolate No. 3 which caused 41.3%, whereas isolate No.1 occupied the third rank, respectively. On contrary, when the pathogenic isolates were compared for post-emergence damping-off, the percentage of infection caused by isolate No. 1 was 53.3% followed by isolate No. 3 being 25.3%. A comparable pattern was discovered when the isolates were compared in respect of survival plants. The recorded percentage of survived plants were 12.01%, 33.3 and 40 % for isolates No. 1, 3 and 2, respectively. **(Abdel-Sattar *et al*., 2017)**

stated that, the differences in R. solani Anastomosis Groups (AGs) are responsible for the virulence of isolates in pathogenicity. Rhizoctonia isolates differed among themselves in severity of the infection, although similar conditions, this demonstrates the part in various virulence **(Ali, 2021)**.

Table (3). Pathogenic ability of different isolates of *Rhizoctonia solani* on damping-off incidence of *Nigella sativa L.*, plants

Fungal isolates	Damping-off incidence (%)		Survival plants (%)
	Pre-emergence	Post-emergence	
***R. solani*. 1**	34.66	53.33	12.01
***R. solani*. 2**	52.66	7.33	40.01
***R. solani*. 3**	41.33	25.33	33.34
***R. solani* 4**	15.33	12.66	72.01
***R. solani* 5**	14.66	7.33	78.01
Control	0.0	0.0	100.0
LSD 0.05	0.94	0.73	1.02

3.4. Laboratory studies

In a preliminary trial, different biocontrol isolates were assessed for their antagonistic effect against *R. solani* using dual culture technique. Out of Five Bacillus isolates and five isolates of *Streptomyces* proved to be potential biocontrol agents against *R. solani*. Data on Table (4) indicated that four isolates of Bacillus spp. significantly decreased growth of the harmful fungus in comparison to the control treatment. Percentages of reduction of *R. solani* ranged between 31.40 to 60.17 %. *Bacillus subtilis* (1) showed the highest antagonistic effects followed by *Bacillus licheniformis* (2). The most effective isolate among *Streptomyces spp. was S. rochei* (1) which given 55.47% reduction in growth of *R. solani*.

Data in Fig (1) showed that, the greatest percentage of decrease in mycelial growth in dual culture technique in *Bacillus spp*. group was *B. subtilis* and in *Streptomyces spp.* was *S. rochei* gave 60.17% and 55.47%, respectively, against *R. solani* compared with control.

Data in Fig (2) showed suppression and malformation as an effect of *S. rochei* and *B. subtilis* on the mycelial growth of *R. solani* under light microscope. It seemed to change the structure of *R. solani* mycelia by lysing the pathogen's hyphal tips, causing swelling, pores, an uneven membrane border, and shrinking the pathogen's hyphal tip. **(Hashem *et al.*, 2019**) found that, *Bacillus subtilis* can be used to induce systemic resistance in plants against root rot pathogens. Additionally, it is capable of synthesizing a wide range of secondary metabolites, antioxidants, and enzymes that break down cell walls to help plants prevent root rot. In addition, Bacillus spp., such as B. *subtilis*, are hostile to bacteria and fungi that cause plant pathogenicity, *bacillus spp.* generates a minimum of 66 various antibiotic compounds **(Ferreira *et al*., 1991). (Anitha and Rebeeth, 2009)** reported that, In the medium, the chitinase enzyme was generated by *Streptomyces griseus*, *Rhizoctonia solani* increased the amount of this enzyme, and crude chitinase enzyme extraction revealed a zone of inhibition on pathogen-inoculated PDA plates at all tested concentrations. According to **(Evangelista-Martínez *et al*., 2020)**, there was a 63% reduction in *Rhizoctonia solani* when the antagonistic activity of the Streptomyces isolate CACIS-1.5CA was tested. Many investigators stated that *Streptomyces spp*. caused a reduction in different pathogenic fungi due to antifungal substances produced by Streptomyces isolates as enzymes and antibiotics **(Baharlouei *et al.*, 2013; Adegboye & Babalola 2012 and Mingma *et al*. 2014).** These

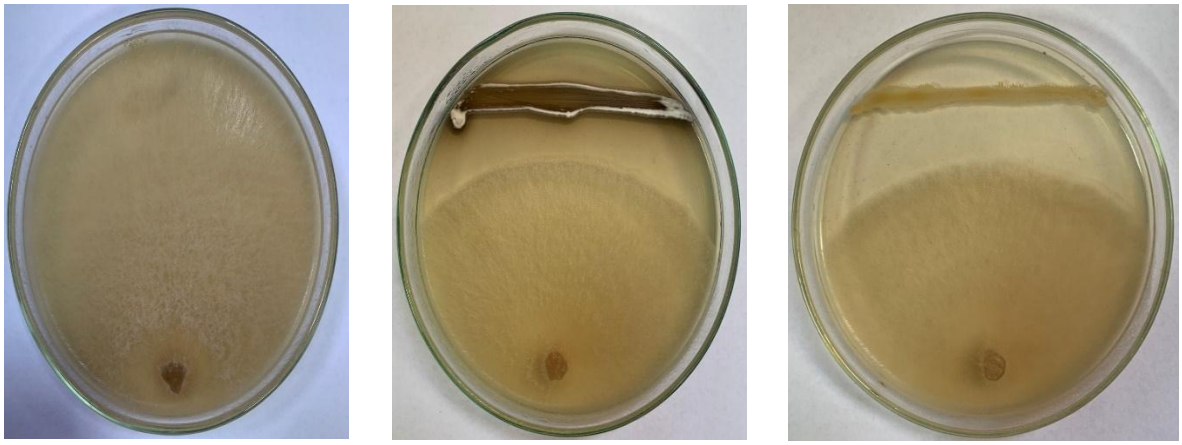
outcomes are in agreement with those reported by **(Liu *et al.*, 2019)** who found that *Streptomyces spp.* significantly inhibited *Rhizoctonia solani*'s mycelial growth.

In comparison with other bacterial species, *B. subtilis* had considerable antifungal activity and significantly reduced the growth of *R. solani*, caused malformation and decay of the pathogenic mycelium of *R. solani* and also cause a complete cell wall destruction due to the antagonistic activities as production of organic acid, lytic enzymes, cyanogens and solubilized various sources of organic and inorganic phosphates **(El-Nashar *et al.*, 2001 and Kumar *et al.*, 2012).**

Table (4). Evaluation of the bioagent activity against *R. solani*

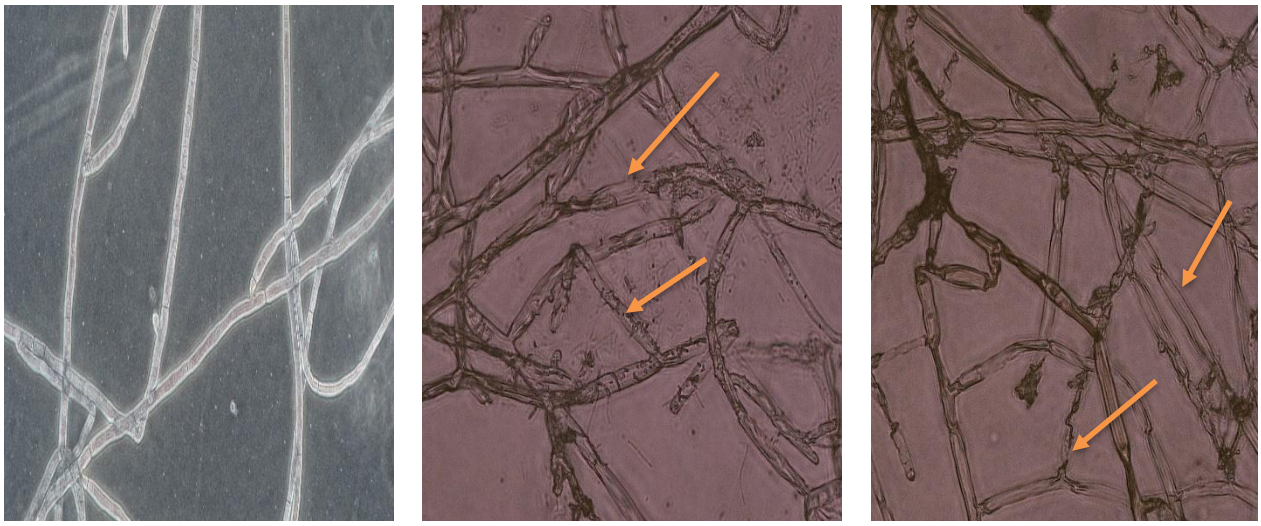
Bioagent isolates	**Reduction in the linear growth of *R. solani* (%)***
Bacillus subtilis (1)	60.17
Bacillus licheniformis (2)	59.53
Bacillus sp. (3)	31.40
Bacillus sp. (4)	37.40
Bacillus sp. (5)	0.00
Streptomyces rochi. (1)	55.47
Streptomyces sp. (2)	0.00
Streptomyces sp. (3)	42.80
Streptomyces sp. (4)	34.30
Streptomyces sp. (5)	40.00
Control	0.00
LSD 0.05	0.59

*Reduction according to the control treatment.



Control *S. rochei* + *R. solani* *B. subtilis* + *R. solani*

Fig. (1). *In vitro* antagonistic effect of *B. subtilis* and *Streptomyces rochei* against *R. solani*



Control
Untreated mycelium of *R. solani*

S. rochei + *R. solani*
Smaller and thinner *S. rochei* mycelium, broken, and distorted of *R. solani*

B. subtilis + *R. solani*

Fig. (2). Light micrograph shows the colonization of *B. subtilis* and *S. rochei* on *R. solani* and thinner *S. rochei* mycelium, broken, and distorted of *R. solani* mycelium

3.5. Field experiments

Evaluation of different bio-agents for controlling damping-off *Nigella sativa L.*, plants

Isolates of either *B. subtilis*, *B. licheniformis* or *S. rochei* were formulated as powder or suspension and tested two years to study its effect on black cumin damping-off disease under the field conditions. Results present in Table (5) indicated that all treatments of black cumin seeds with either *B. subtilis*, *B. licheniformis* or *S. rochei* caused a significant reduction in pre- and post-emergence damping-off in the two seasons and increased survival plants compared with the control treatment. All isolates in both powder and suspension formulas achieved a significant effect on percentage of survival plants. Data also revealed that seed treatments with the two isolates of Bacillus and Streptomyces as suspension are the highest effect than powder treatment. *B. subtilis* either as suspension or powder showed the highest effect in all treatments. It reduced pre-emergence damping-off with (2.0%), and increased the percentage of survived plants with (98.0%) in season 2019 compared with only 14% in the control treatment. *S. rochei* as suspension occupied the second rank and gave 94.7% of survival. The pre and post emergence in *B. licheniformis* treatment was decreased by 19.3 and 8.2%, respectively, as compared to control.

B. subtilis, *B. licheniformis* or *S. rochei* proved their efficacy in controlling root rot in *Nigella sativa* caused by *R. solani*. These isolates' potential to create a wide range of antifungal chemicals, such as volatiles, enzymes, lipopeptides, and many short peptides, may be the reason for their capacity to control diseases **(Romero, *et al.*, 2007).** *Bacillus spp*. produces lipopeptid antifungal antibiotic iturin A in the soil which could suppress the disease. **(Szczech and Shoda, 2006).** Also, B. subtilis excretes phytohormone as indole-3-acetic acid, auxins, cytokinins and gibberellins that helps to improve seed germination. (**Bakonyi, *et al.*, 2013)**. According to Kanini et al. (2013), *Streptomyces rochei* has demonstrated promise as a producer of metabolites with a wide range of antibacterial activity.

Streptomyces rochei is a member of the Actinomycetales order, which is significant because its members provide valuable bioactive secondary metabolites for commercial and industrial uses **(El-Tarabily *et al.*, 2000; Bressan and Figueiredo, 2005)**. Thus far, several types of antibiotics derived from Streptomyces have been identified as effective against fungi infections **(Kim *et al.*, 1999; Hwang *et al.*, 2001; Rodríguez *et al.*, 2002; Remsing *et al.*, 2003).**

Table (5). Effect of different bioagents, used as powder or suspension against damping-off disease and survived *Nigella sativa L.*, plants under greenhouse conditions seasons 2018 and 2019

Forms of preparation	Treatments	Pre-emergence (%)		Post emergence (%)		Survived plants (%)	
		2018	2019	2018	2019	2018	2019
	Control	57.3	58.6	25.3	27.4	17.4	14.0
Powder	*B. licheniformis*	20.6	22.6	11.6	13.1	67.8	64.3
	Streptomyces rochei	16.6	14.6	7.1	4.6	76.3	80.8
	B. subtilis	8.6	10.0	2.8	2.2	88.6	87.8
Suspension	*B. licheniformis*	18.0	19.3	4.0	8.2	78.0	72.5
	Streptomyces rochei	4.6	5.3	1.3	0.0	94.1	94.7
	B. subtilis	2.6	2.0	0.0	0.0	94.4	98.0
LSD 0.05		1.08	0.74	0.50	0.82	1.24	1.16

Effect of different biocontrol agents on vegetative growth

Plant height cm/plant and number of branches/plant

Data in Table (6) showed that, treated by all biocontrol agents used *(Bacillus licheniformis, Streptomyces rochei, Bacillus subtilits)* in both forms powder or suspension caused significant increase in plant height and number of branches/plant than in the untreated plants in the two seasons.

In powder forms, it was observed that, the plants which were treated by *B. subtilits* recorded significant increase in the plant height and number of branches (48.90 and 49.18 cm/plant) and (10.73 and 11.15 branches/plant) followed by those plants treated by *S. rochei. (*47.27 and 47.56 cm/plant) and (9.47 and 11.08 branches/plant) compared to the control (42.03 and 43.20 cm/plant) and (6.57and 8.00 branches/plant) or treated by *B. licheniformis* (43.87 and 44.91 cm/plant) and (7.72 and 9.60 branches/plant) in both seasons respectively.

As shown in Table (6) the effect of biocontrol agents as suspension form on plant height and number of branches gave significant effect than control and powder form in both seasons. Treated with suspension of *B. subtilits* recorded the highest increased in plant height and number of branches (52.64 and 54.91cm/plant) and (12.78 and13.42 branches/plant) followed by suspension *S. rochei.* (50.00 and53.83 cm/plant) and (11.50 and12.73 branches/plant) compared to control (42.03 and 43.20 cm/plant) and (6.57and 8.00 branches/plant) or treated by suspension *B. licheniformis* (45.67and 46.88cm/plant) and (8.97and 10.07 branches/plant) in both seasons respectively.

Table (6). Effect of different bio agents on growth characters of *Nigella sativa. L.*, plants during 2018/ 2019 seasons

Growth characters					
Forms	Treatments	1st season		2nd season	
		Plant height (cm)	Number of branches /plant	Plant height (cm)	Number of branches /plant
Powder	Control	42.03	6.57	43.20	8.00
	Bacillus licheniformis	43.87	7.72	44.91	9.60
	Streptomyces rochei	47.27	9.47	47.56	11.08
	Bacillus subtilits	48.90	10.73	49.18	11.15
Suspension	*Bacillus licheniformis*	45.67	8.97	46.88	10.07
	Streptomyces rochei	50.00	11.50	53.83	12.73
	Bacillus subtilits	52.64	12.78	54.91	13.42
LSD 0.05		0.77	0.58	0.48	0.36

Effect of different biocontrol agents on seed yield g/plant and seed yield kg/fed.

Data in Table (7) indicated that, plants treated with all bio control agents in both forms powder or suspension significantly enhanced seed yield g/plant and seed yield kg/fed. compared with untreated plants in both seasons**.**

Among the bio agents used in this study, *Bacillus subtilits* in suspension form recorded the highest seed yield gm/plant and seed yield/fed. (26.02 g/plant and 572.48 kg/fed.), followed by those plants treated with *Streptomyces rochei* in suspension form (23.74 g/plant and 522.32 kg/fed.) in the first season, the same trend were obtained in the second season highest seed yield gm/plant and seed yield/fed. were recorded with *Bacillus subtilits* in suspension form (29.87 g/plant and 657.10 kg/fed.) followed by the same case of *Streptomyces rochei* (28.00 g/plant and 616.07 kg/fed.) compared to control and other bio agents used in this study.

In general, *Bacillus spp.* and *Streptomyces spp*. have a great strong antagonistic effect as amendment against root rot diseases, that their active metabolites such as siderophore, hydrogen cyanide, indole acitic acid and salicylic acid play an important role in prevention of plant diseases, also their ability to prompting growth and yield of black cumin seed plants, these results came in harmony with those obtained by **(Mhmoud, 2015; Wang *et al*., 2016; Awad and Fayyadh, 2018; Aljawasim *et al*., 2020 and Fasusi *et al*., 2021).** Also, **(El-Mogy *et al*., 2012)** reported that the *Bacillus spp.* and *Streptomyces spp*. as bio agents against root rot fungi and promoting the growth and yield of some vegetables such as cucumber, tomato, pepper and cantaloupe. **(Yao *et al*., 2021)** said that *Streptomyces spp* controlling rot of cucumber plants due to their antifungal and bio control activity. **(Zhang *et al*., 2020 and Chen *et al*., 2013)** reported that root rot diseases can be controlled by *Bacillus spp*. and *Streptomyces spp.* due to their antibiotics effect, in addition to promoting the growth. On bean plants **(Adhilakshmi *et al*., 2014)** studied that *Streptomyces spp.* promoting the growth and yield due to its effect against root rot diseases. **(Gopalakrishnan *et al*., 2012)** found that, Streptomyces strains significantly enhanced the panicle length, panicle weight, 1000 seed weight, total dry matter, root dry weight (16-24%), grain yield (9-11%) over the control. It is clearly that, using suspension form were more effective than powder form for management of root rot disease in black cumin plants, this consistent with **(Al-Sman *et al*., 2019)** They discovered that Bacillus spp. function as plant growth-promoting agents and that, when produced as a suspension, they significantly reduced the severity of the root rot disease and enhanced the quantity of fixed and volatile oils in black cumin, as well as its vegetative development and seed yield. **(Sreevidya *et al*., 2016)** evaluated four isolates of actinomycetes for their plant growth-promotion properties under field conditions on chickpea, all exhibited increase in weight and yield, and these isolates were found to produce chitinase, lipase, cellulase, protease, siderophore, and indole acetic acid, the actinomycetes treated plots improved total N, available P and organic content compared to un-inoculated control.

Table (7). Effect of different bio agents on seed yield g/plant and kg/ fed. of *Nigella sativa. L.*, plants during 2018/ 2019 and 2019/2020 seasons

Forms	Treatments	1st season		2nd season	
		Seed yield (g)/ plant	seed yield kg)/ fed.	Seed yield (g)/ plant	seed yield kg)/ fed.
Powder	Control	15.01	330.14	16.65	366.19
	Bacillus licheniformis	16.99	373.82	21.37	470.22
	Streptomyces rochei	18.70	411.36	24.73	544.03
	Bacillus subtilits	23.48	516.45	27.00	593.89
Suspension	*Bacillus licheniformis*	17.09	376.02	24.29	534.36
	Streptomyces rochei	23.74	522.32	28.00	616.07
	Bacillus subtilits	26.02	572.48	29.87	657.10
LSD 0.05		2.06	45.46	2.63	57.98

Effect of different biocontrol agents on oil production

Volatile oil percentage

Table (8) depicted the results of volatile oil of black cumin (*Nigella sativa.L)* plant. Highly significant variations were observed with different treatments bio control agents. Volatile oil percentage recorded an increase with all treatments *(Bacillus licheniformis, Streptomyces rochei* and *Bacillus subtilits)* in both forms powder or suspension compared to plants that have not been treated in two seasons. In powder case the greatest volatile oil percentage was obtained from *Bacillus subtilits* which gave (0.149 and 0.160 %) compared to the control (0.123 and 0.114%) and (0.130 and 0.125%) with *Bacillus licheniformis* and (0.142 and 0.155%) with *Streptomyces rochei* treatment in the two seasons, respectively. The application of suspension form gave the highest volatile oil content in plants treated by *Bacillus subtilits* suspension the values were (0.180 and 0.179%) followed by *Streptomyces rochei* suspension (0.176 and 0.175%) in the 1st and 2nd seasons, respectively.

Table (8). Effect of different bioagents on volatile oil % of *Nigella sativa. L.*, plants during 2018/ 2019 and 2019/2020 seasons

Forms	Treatments	Volatile oil %	
		1st season	2nd season
Powder	**Control**	0.123	0.114
	Bacillus licheniformis	0.130	0.125
	Streptomyces rochei	0.142	0.155
	Bacillus subtilits	0.149	0.160
Suspension	***Bacillus licheniformis***	0.136	0.127
	Streptomyces rochei	0.176	0.175
	Bacillus subtilits	0.180	0.179
LSD 0.05		0.003	0.007

Volatile oil components

The contents of volatile oil components in black cumin plants during the second season were shown in Table (9) the main constituent of volatile oil was limonene, and thymoquinone was the second main compound. All treatments of bio control agents increased limonene and thymoquinon contents compared to control. The highest concentration of limonene and thymoquinone (18.99 and 14.35%) were recorded in form of *Bacillus subtilits* suspension followed (17.87 and 13.85%) by treated *Streptomyces rochei* suspension. The lowest contents of limonene and thymoquinon were obtained in the control (8.96 and 7.62%), respectively. The outcomes were consistent with those reported by (**Abdel-Aziez, 2014)** who discovered that, in comparison to the control, bio fertilizers increased the amount of limonene components in black cumin oil. Furthermore, the productivity of *Pimpinella anisum* and *Cuminum cyminum* plants, as well as their oil yield content, were markedly increased by inoculation with diazotrophs and phosphate dissolving bacteria **(Abdel-Aziez, 2006).**

Fixed oil percentage

It was found in Table (10) all bio control agents significantly increased fixed oil% compared to that of contol untreated plants. In powder form bio control agents, the highest fixed oil percentage were (28.45 and 29.73%) with powder *Bacillus subtilits* compared to (18.47 and 19.36%) in the control, (22.52 and 24.17%) with *Bacillus licheniformis* and (27.38 and 28.35%) with *Streptomyces rochei* in both seasons, respectively. Also, in suspension form of biocontrol agents, the highest fixed oil content in black cumin plants were obtained with suspension of *Bacillus subtilits* (32.26 and 33.04%) followed

by (29.53 and 30.55%) with *Streptomyces rochei* compared to (26.84 and 27.33%) with suspension of *Bacillus licheniformis* in the two seasons, respectively,

Generally, it could be concluded that, the treatments of bio control agents in suspension form were more effective than those treatments in the powder form. These findings were in agreement with **(Al-Sman *et al*., 2019)** found that, bio control agents when used as a suspension forms enhanced the vegetative development, seed productivity, fixed and volatile oils in black cumin plants.

Table (9). Effect of different bio agents on volatile oil components of *Nigella sativa L*., plants during 2018/ 2019 and 2019/2020 seasons

Components	Treatments						
		Powder form			Suspension form		
	control	*Bacillus licheniformis*	*Streptomyces rochei*	*Bacillus subtilits*	*Bacillus licheniformis*	*Streptomyces rochei*	*Bacillus subtilits*
αPinene	9.36	8.25	9.60	8.22	7.96	6.25	6.98
βPinene	3.69	8.05	8.08	4.93	8.02	6.25	7.45
Myrcene	2.96	5.67	3.83	4.85	5.25	2.90	3.14
P-Cymene	6.62	4.07	6.05	5.94	4.95	6.35	5.56
Limonene	8.96	13.75	13.98	17.53	16.05	17.87	18.99
γTerpinene	2.35	3.70	4.45	5.90	6.28	7..45	8.15
Thymoquinone	7.62	11.08	12.15	13.63	10.75	13.85	14.35
Terpinene-4-ol	6.43	8.07	7.53	9.65	10.41	6.24	10.35
Carvacrol	7.55	5.92	6.15	6.15	7.11	6.15	5.08
Trans-anethol	2.28	5.26	5.55	6.65	7.35	7.90	8.15
Unkown							

Table (10). Effect of different bio agents on fixed oil (%) of *Nigella sativa. L*., plants during 2018/ 2019 and 2019/2020 seasons

Forms	Treatments	Fixed oil (%)	
		1st season	2nd season
Powder	Control	18.47	19.36
	Bacillus licheniformis	22.52	24.17
	Streptomyces rochei	27.38	28.35
	Bacillus subtilits	28.45	29.73
Suspension	*Bacillus licheniformis*	26.84	27.33
	Streptomyces rochei	29.53	30.55
	Bacillus subtilits	32.26	33.04
LSD 0.05		2.68	1.98

4. Conclusion

The results of this study demonstrated that a group of compatible, agriculturally important microorganisms with a range of biological roles could be a useful tool for the long-term control of *Nigella sativa L*. rhizoctonia root rot. Using *Bacillus subtilits* and *Streptomyces rochei* in suspension form in field conditions have a greater effect on root rot disease, gave the highest growth, seed yield, volatile and fixed oil content. It could be concluded that, bio control agent application reduced rot root disease of (*Nigella sativa L.)* plant and enhanced the vegetative growth, seed yield, volatile oil production and fixed oil.

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