Management Approaches of Sage Root Rot Disease and plant Productivity by Biological Control under Organic Farming System

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ABSTRACT

Sage is one of the most appreciated plants for its plethora biologically active. But, root rot disease, a prevalent fungal disease that can afflict a variety of plants, including sage, leads to the death of plants and a decrease in yield, which has prompted the exploration of alternative strategies for disease control. The current research was conducted over the consecutive growing seasons of 2022 and 2023 at the Al-Quassassin Research Station, Agricultural Research Center (ARC), Ismailia Governorate, Egypt. The objective was to investigate the impact of different treatments, *Trichoderma harzianum*, *Bacillus subtilis*, angel yeast, humic acid, compost tea and their combinations, on the management of sage root rot disease and its influence on productivity within an organic farming system. In greenhouse study results, *Rhizoctonia solani* proved to be the most aggressive soil borne disease, causing the highest incidence rates at 40.0%, 35.0% and 20.0% for pre-, post-emergence damping-off and root rot, respectively. *Sclerotium rolfsii* followed closely, with incidence rates of 35.0%, 30.0%, and 12% for these respective stages. The antagonists' isolates exhibited significant variations in their capacity to inhibit the *in vitro* linear growth of both tested pathogenic fungi. *T. harzianum* demonstrated the most substantial reduction in mycelial growth, with an impressive 80.95% decrease, closely trailed by *T. album* at 80.05%, *B. subtilis* at 74.30%, and *B. megaterium* 64.37%, on average. In the field experiment, all treatments resulted in significant reduction of the disease incidence and severity of sage root rot and promoted the growth parameters, oil and crop yield of sage plants in both seasons. *T. harzianum* combined with Compost tea demonstrated the highest efficacy in controlling root rot of plants, resulting in the highest yield and quality of sage.

Keywords: Sage, Root rot, Bacillus subtilis, Trichoderma harzianum & yield.

INTRODUCTION

Sage (*Salvia officinalis* L.) stands as the most well-known species within the *Labiatae* (*Lamiacae*) family genus. It is found primarily in the Mediterranean Basin, as well as parts of Southeast Africa and South America (Abd-Rabbu *et al.*, 2021). It is grown in many countries for the production of dried leaves, which are used as raw materials in medicine, scent and the food sector. It is an useful herb known for its resilient, earthy flavor that is commonly used in the culinary realm to enhance the flavors of meats, poultry, and stuffing (Habib *et al.*, 2017; Greco *et al.*, 2021 and Valkovszki *et al.*, 2023). *S. officinalis* is famous for having the highest content of this priceless oil among the numerous Salvia species (Yaldiz and Camlica, 2023). Its long history in traditional medicine suggests that it may have anti-inflammatory, antioxidant, and antibacterial properties, and it is widely used to treat sore throats and digestive disorders (Bakhtiari *et al.*, 2023).

Maintaining soil productivity while using sustainable and organic farming techniques requires the presence of flourishing and vigorous soil microbial populations. Crop rotation, cover crops, and the use of organic amendments like manures and composts are just a few of the tactics that growers use. These techniques significantly raise the amount of soil organic matter (SOM), which improves soil biology and soil quality as an entirety (Ahmed *et al.*, 2023 and Anand *et al.*, 2023). Sage crops are exposed to a variety of diseases, including root rot caused by *Fusarium solani* and *Rhizoctonia solani*. *Phytophthora cryptogea* L. is also responsible for sage root and crown rot, offers a big risk and resulting in significant losses. Unluckily, there is little evidence known about the diseases' association with sage and appropriate management techniques (Çakır *et al.*, 2017).

Instead of depending completely on chemical pesticides, biological treatments such as specific bacteria or fungi, which may surpass or control the pathogens responsible for root rot (Abdel-Latif and Zakarya, 2023). Furthermore, enhancing soil health and plant resistance through appropriate cultivation

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techniques can also help prevent root rot (Munir *et al.,* 2023). These alternatives offer more environmentally friendly and sustainable approaches to disease management, reducing the impact on ecosystems and potentially improving long-term crop health (Đurović *et al.,* 2022 and Abdel-Latif and Zakarya, 2023). The practical implementation of biocontrol agents such as *Trichoderma harzianum* Rifai KRL-AG2 in the management of *Fusarium* spp.-caused sage root rot has proved moderate efficiency in controlling disease incidence (57.85%) *in vivo* conditions, as well as its ability to induce resistance in treated plants against specific pathogens and adaptability due to a wide range of environmental conditions and shown significance in certain yield and quality criteria but fell short of the expected disease suppression (Ahmed *et al.,* 2016 and Đurović *et al.,* 2022).

Trichoderma spp. antagonistic activity was examined in a wide range of applications including *in vitro*, pot research, and *in vivo* and their significance in increasing disease resistance enzymes such as chitinase, peroxidase, and polyphenoloxidase was observed. Additionally, it was discovered that *Bacillus subtilis* BN1 establishes a defense against sage root rot pathogens by colonizing the plant's root system, produces antibiotic substances, lytic enzymes such chitinase and beta-1,3-glucanase, which are recognized for their capacity to break down hyphae and consume parts of pathogenic fungi's cell walls (Abdel-Latif & Zakarya, 2023; Egamberdieva *et al.,* 2023 and Uçar *et al.,* 2023).

In fact, compost is an important source of plant nutrition and is essential for preserving soil fertility. It has numerous advantages for the health of the soil and plants, including the addition of humus to improve the soil's physical qualities, increase the soil's capacity for cation exchange, improve water retention, producing safer, flavorful, and chemical-free plants (Eisa *et al.,* 2022 and Elsherpiny *et al.,* 2023).

Compost tea also contains different organic components like bioactive compounds, phenolics, humic acid, soluble mineral nutrients, plant growth regulators, and volatile fatty acids, all of which contribute to its disease-suppressive effects (Gea *et al.,* 2021; Carrascosa *et al.,* 2023 and Pilla *et al.,* 2023).

As an all-natural stimulant, yeast is famous for its nutrient-rich composition, which includes significant amounts of protein and vitamin B components like thiamin, riboflavin, and pyridoxine. In addition, yeast is well known for producing a variety of molecules, including vitamins, amino acids, hormones, and growth-regulating proteins resulting in higher plant growth and production (El-Sherpiny *et al.,* 2022; Goncharuk *et al.,* 2022 and Al-Fraihat *et al.,* 2023).

Humic acid, a commercially available substance, possesses numerous functional groups along its carbon chain and is categorized into three types: humic acid, fulvic acid, and humin. These components play a pivotal role in enhancing the physical, chemical, and biological properties of soil (Elhindi *et al*., 2023). The functional groups within the carbon chain may exhibit acidic characteristics (e.g., carboxylic acid and phenol), alkaline traits (e.g., amine, imines), or remain neutral (e.g., alcohol, aldehyde, ketone, ether, ester, and amide). All of these contribute collectively to the improvement of plant growth (Rekaby *et al.,* 2023).

Consequently, this study's primary objective was to assess the impact of various antagonists, whether used individually or in combination with natural compounds, on the growth and productivity of sage plants under field conditions. The study also aimed to optimize both the quantity and quality of sage herb, along with achieving an optimal oil yield from sage.

MATERIALS AND METHODS

Isolation, purification and identification of fungi associated with rotted roots of sage

Root-rotted sage plant samples were obtained from a private organic farm situated at Al-Quassassin Research Station, Agricultural Research Center (ARC), El-Quassassin City, Ismailia Governorate, Egypt. Isolation of these samples followed the method outlined by Ahmed *et al*. (2016). Subsequently, the identification of isolated fungi was conducted at the Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Egypt. This identification process relied on cultural, morphological, and microscopic characteristics, with reference to descriptions provided by Gilman (1957); Barnett and Hunter (1987) and Singh (1982).

Antagonistic microorganisms

Trichoderma album, T. harzianum, Bacillus subtilis and *B. megaterium* (30×10^6) were kindly obtained from CLOA and used at the rate of 1 lit/100 lit water/fed.

Pathogenicity tests

Pathogenicity testing was carried out in potted soils within a greenhouse setting at CLOA carried out pathogenicity testing in potted soils within a greenhouse setting, employing the method and technique delineated by Ahmed (2005) and Ahmed (2013). The pathogenic transplant fungi were subsequently re-isolated from the infected plants, and the application of Koch's postulates was followed. Recorded data were expressed as a percentage of disease incidence observed in each treatment.

Disease assessment

The percentages of root rot and healthy survival were calculated at 10, 21, and 45 days after sowing using the formula specified by Ahmed (2005) and Ahmed (2013). Additionally, pathogenic fungi were re-isolated from the infected plants, and the principles of Koch's postulates were adhered to in the investigation.

$$\%\ Pre-emergence = \frac{Number\ of\ non\ germinted\ seeds}{Total\ number\ of\ sown\ seeds} \times 100$$

$$\%\ Post-emergence = \frac{Number\ of\ deed\ seedlings}{Total\ number\ of\ sown\ seeds} \times 100$$

$$\%\ Root\ rottrd\ plants = \frac{Number\ of\ root\ rotted\ plants}{Total\ number\ of\ sown\ seeds} \times 100$$

$$\%\ Survived\ plants = \frac{Number\ of\ survivals}{Total\ number\ of\ sown\ seeds} \times 100$$

***In vitro* experiments**

The effect of several antagonists on the linear growth of sage root rot fungi was studied in the lab setting. Trichoderma harzianum and T. album as antagonistic fungi, in addition,*Bacillus subtilis* and *B. megaterium* as antagonistic bacteria. Unless otherwise specified, autoclaved Gliotoxin Fermentation Agar (GFA) and Nutrient Glucose Agar (NGA) mediums were used to cultivate the antagonistic fungi and bacteria, respectively. In order evaluating the effects of antagonistic fungi and bacteria, 9 cm plates with 10 ml of GFA or NGA media were employed. The antagonistic fungi and bacteria were introduced at a rate of 10% to warm sterilized GFA and NGA media, respectively, and poured into Petri plates (10ml/plate) before solidification. The treated plates were injected in the center with discs collected from the periphery of pathogenic fungus cultures that were 5 days old.

The control treatment consisted of plates with pathogenic fungi inoculated and media devoid of antagonists. In the case of each specific treatment, three plates were utilized. Plates with inoculations were incubated at 25°C. In cases where mycelial mats covered the medium surface in the control treatment, the experiment was deemed complete. All of the plates were checked, and the percentages of pathogenic fungi's mycelial growth that was reduced were estimated using the method proposed by Ahmed (2005) and Ahmed (2013) as following:

$$\%\ Reduction\ in\ Linear\ growth\ of\ pathogenic\ fungi = \frac{G1 - G2}{G1} \times 100$$

Where: G1: growth of pathogenic fungus in control only, G2: growth of pathogen against antagonist.

Field experiment

Preparation of the biocontrol agents

In this experiment, we identified the two most effective biocontrol agents from the *in vitro* trial. Suspensions were then prepared, containing propagules of *Trichoderma harzianum* as antagonistic fungi and *Bacillus subtilis* as antagonistic bacteria. The *T. harzianum* suspension was cultured for 10 days in liquid Gliotoxin fermentation (GF) medium, while the *B. subtilis* suspension was grown for 3 days in Nutrient Glucose (NG) medium, both at a temperature of 25±2ºC and complete darkness conditions. This methodology follows the procedures outlined by Ahmed (2013) and Ahmed *et al.* (2016). After the growth period, all cultures were homogenized in an electric blender for 2 minutes, resulting in suspensions with a concentration of 30×10^6 spores/ml. These suspensions, diluted at a ratio of 1:100, were utilized in the field experiments.

Materials, which used during this study:

Plant materials:

The seeds of *Salvia officinalis* L. utilized in this study were sourced from the Medicinal and Aromatic Plants Department, HRI, Agricultural Research Center (ARC), located in Dokki, Ministry of Agriculture, Giza, Egypt.

Angel Yeast:

Angel Yeast, procured from Egypt for Biotechnology Co. located in Beni Suef Governorate, Egypt, was employed in this study as a foliar application. The application rate was set at 1 liter per 50 liters of water per feddan.

Compost:

Concerning fertilization on this farm, mature compost, consisting of a combination of extensive animal manure and plant residue, was applied on the 1st of March in both the 2022 and 2023 growing seasons. The compost, sourced from the same farm, was applied at a rate of 10 m^3 per feddan. The composting process, lasting between 2.5 to 3 months, aimed to enhance soil with organic matter and enrich fertility. The nutrient content provided by this compost, as analyzed at the Soil, Water, and Environmental Research Institute, ARC, Giza, Egypt, is detailed in Table (1).

Table 1. Main parameters characteristics of mature compost which add to sage soil during 2022 and 2023 growing seasons

Bulk Density (kg/m^3)	Solid Matter (g/L)	Humidity	pH (10:1)	EC (10) dS/m	Soluble N (mg/kg)		O.M (%)	O. C (%)	Total N (%)	C/N	Total P (%)	Total K (%)
					NH_4^+	NO_3^-						
640	85	32	6.50	3.80	41.5	28.9	82.50	15.20	1.40	1.11	1.10	0.9

Compost tea:

To prepare aerated compost tea, one liter of homogenized compost mixture was mixed with 1:10 tap water (sourced from an agricultural well) in a 1:20 handmade compost tea brewer. The actively aerated compost tea was left to brew at room temperature for a four-day incubation period in the 1:20 handmade compost tea brewer equipped with an air pump. After the incubation, the actively aerated compost was filtered through a two-layer 800-micron mesh bag, reducing the likelihood of clogging in application equipment for foliage spray. The application rate for foliage spray was set at 1 liter per 20 liters of water per feddan. The compost tea analysis is presented in Table (2).

Humic acid:

Humic acid (HA), obtained in liquid form as potassium humate (K_2O 12%) from CLOA, was applied to the soil at a rate of 1 liter per 50 liters of water per feddan.

Soil analysis:

A composite sample of clay and sandy soils (1:1 w/w) was collected from Al-Tal Al-Kabeer Center, Ismailia Governorate, Egypt, to represent the soil mixture for a pot experiment. The collected soil was air-dried, crushed, and processed for physical and chemical property determinations, following the procedures outlined by Piper (1966). The physical and chemical analysis of the soil, which was utilized in evaluating the efficacy of various antagonists and organic matter against sage *in vivo* root rot fungi, is detailed in Table (3).

***In vivo* Treatments:**

The treatments were used during this study (*Trichoderma harzianum*, *Bacillus subtilis*, Angel yeast, Humic acid, Compost tea, *T. harzianum* + Angel yeast, *T. harzianum* + Humic acid, *T. harzianum* + Compost tea, *B. subtilis* + Angel yeast, *B. subtilis* + Humic acid, *B. subtilis* + Compost tea, Angel yeast + Compost tea, Angel yeast + Humic acid and Compost tea +Humic acid) to evaluate their efficacy in controlling the sage root rot disease. In additional to control treatment.

Table 2. Main characteristics of the prepared compost tea which add to sage soil during 2022 and 2023 growing seasons

Organic Matter (ppm)	NH_4^+ (ppm)	NO_3 (ppm)	Total N (ppm)	Total P (ppm)	Total K (ppm)
8004.24	4.37	24.76	742.56	11000	12000

Table 3. The soil's physical and chemical characteristics at the experiment site

Properties	Value	Properties	Value
Clay	4.8	Saturation (%)	33
Sand	78.5	pH	7.94
Silt	16.7	E.C (mmohs/cm) (1 soil:5 water)	2.83
Texture	Sandy loam	Soluble cations and anions (mq/l)	
Plant available nutrients contents in the soil (mg kg^{-1} soil)		CO_3^{2-}	0
N	18.02	HCO_3^-	1.5
P	2.18	Cl^-	20.3
K	169	Ca^{2+}	7.2
Mn	1.72	SO_4^{2-}	3.2
Zn	3.38	Mg^{2+}	3.4
Fe	1.72	K^+	0.3
Cu	1.4	Na^+	14.1

Experimental setup:

All field experiments, unless specified otherwise, were conducted at Al-Quassassin Research Station, Agricultural Research Center (ARC), El- Quassassin City, Ismailia Governorate, Egypt, during the 15th of March in the 2022 and 2023 growing seasons. The experimental plot, measuring 10.5 m^2, consisted of 3 rows (3 meters in length x 3.5 meters in width) with approximately 70 cm spacing. Each row was planted with 30 sage seedlings in naturally infested soil. The soil in the area has a light loamy texture and is naturally infested. To prepare the soil, mature compost was applied at a rate of 10 m^3 per feddan before the seedling date. The field conditions were characterized by a temperature of around 22 ± 5o C, with a relative humidity of 60 ± 10 R.H. The area had access to Nile water, and a drip irrigation system, supplied by wells, was utilized. Sage seeds were initially sown in nursery beds with clay soil on the 25th of October in both the 2021 and 2022 growing seasons. The seedlings, with a height ranging from 10 to 15 cm, were transplanted into sandy loam soil on the 15th of March during both the 2022 and 2023 growing seasons. In all experiments, sage seedlings underwent a treatment where they were immersed in a diluted solution (1:100) of the recommended dose of different bioagents, namely *T. harzianum and B. subtilis*. This solution was prepared as a suspension at a concentration of 30×10^6 spores/ml. Alternatively, the seedlings were immersed in a diluted solution (1:50) of humic acid, either separately or in mixtures with the bioagents, for a duration of 30 minutes before the transplanting process. The mixtures were then blended with 5% Arabic gum and Tween 80 at a concentration of 0.3%. As a control group, transplants were soaked in water only for the same duration. The plots underwent periodic irrigation and were subjected to all other standard agricultural practices. After 45 and 90 days from seedlings, compost tea and Angel yeast were applied either separately or in mixtures at the recommended doses mentioned earlier. Sage plants sprayed with water only during the same periods served as the control treatment. Ammonium sulphate (20.5%), calcium superphosphate (15.5%), and potassium sulphate (52%), were applied uniformly to all experimental plots at rates of 300, 250, and 50 kg/fed, respectively. The sage plants were taken out twice for cuttings.

Disease assessment

In all field experiments, the disease incidence of root rot and the percentage of healthy surviving plants were documented after 45 and 90 days from transplanting, following the method outlined by Ahmed and El-Fiki (2017), as follows:

$$\%\ Disease\ incidence\ (DI) = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ transplants} \times 100$$

$$\%\ Survived\ plants = \frac{Number\ of\ survived\ plants}{Total\ number\ of\ transplants} \times 100$$

$$Efficacy\ \% = \frac{Control - Treatment}{Control} \times 100$$

Sage plants were harvested on two cutting dates. The 1St cut on 15th May and the 2nd cut on 6th September from the non-flowering plants in both seasons, respectively. Nine randomly selected surviving plant samples were collected from each experimental unit to assess various vegetable growth parameters, including plant height (cm), number of branches per plant, fresh weight (g), and dry weight (g). Additionally, various analyses were conducted to determine yield components, oil quality, microminerals, protein, carbohydrates, and biochemical components.

According to Lichtenthaler and Wellburn (1983), the third leaf of the plant tip (terminal leaflet) was used to measure the amount of *B*-carotenoids and chlorophyll (mg/g FW).

Chemical components determinations

Determination of essential oil percentage constituents of sage herb:

Using the conventional method described in the Council of Europe, essential oil was extracted from dried herbs of S. *officinalis* that had been processed by hydrodistillation (European Pharmacopoeia, 2019). The resulting crude oil was then dried over pure anhydrous sodium sulfate at a concentration of 120–150 g/l of oil, following the method described by Ahmed *et al.* (2016). In both seasons, the essential oil yield was calculated.

Essential Oil Components:

In the second season, the chemical composition of the volatile oil in the selected sample from the first cut was analyzed at the Laboratory of Medicinal and Aromatic Plants Research Department, ARC, using Ds Chrom 6200 Gas Chromatograph apparatus with BPX-5 capillary column, which is a 30 x 0.25 mm ID x 0.25µ film with 5 phenyl (equiv.) polysillphenylene-siloxane. The temperature programme was adjusted at a rate of 10°C per minute, between 70 and 200°C. Gas flow rates were 1 ml/min for nitrogen, 30 ml/min for hydrogen, and 330 ml/min for air. The temperatures of the injector and detector were 250°C and 300 °C, respectively.

Determination of minerals:

A half gram of samples was digested using a mixture of H_2SO_4 and H_2O_2, following the procedure outlined by Cottenie (1980) and Burns (1984). The extracted samples were then utilized to determine the concentrations of the following minerals:

Nitrogen (N) and crude protein content of *S. officinalis* were determined in the digested solution according to the AOAC (Association of Official Agricultural Chemists) method of 2005.

Phosphorus (P) content (g/100g dry weight) was determined using the method described by Isaac (1990).

Potassium (K) content (g/100g dry weight) was determined against a standard using a flame photometer, following the procedure outlined by Kalra (1998).

Determination of total phenolics content (TPC):

Total phenols for each treatment were evaluated and expressed as milligrams of gallic acid per gram of dry weight (mg GA/g DW). The quantification was done with reference to the gallic acid reference curve, following the method developed by Kosar *et al.* (2008).

Statistical analysis

All the collected data underwent statistical analysis using MSTAT software. Treatment means were compared based on the least significant difference (L.S.D.) at a 0.5 level of probability, following the guidelines outlined by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

RESULTS:

Isolation, purification and identification of the associated microorganisms:

Frequency of fungi isolated from rotted roots:

The fungi isolated from rotted roots of sage plants were purified and identified. The identified fungi include *Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium sp., Rhizoctonia solani, and Sclerotium rolfsii.* According to the data presented in Table (4), *R. solani* and *S. rolfsii* exhibited the highest frequency among the isolated fungi from the rotted sage samples collected in Ismailia Governorate.

Pathogenicity tests:

Table (5) highlights that sage plants exhibited high susceptibility to pre- and post-emergence damping-off, as well as root rot diseases caused by *Fusarium oxysporum, F. solani, Macrophomina phaseolina, Pythium sp., Rhizoctonia solani, and Sclerotium rolfsii.* Among these soilborne pathogens, *Rhizoctonia solani* emerged as the most virulent, exerting the greatest impact on the incidence of pre- and post-emergence damping-off, and root rot at rates of 40.0%, 35.0%, and 20.0%, respectively. Following closely, *S. rolfsii* ranked second with infection rates of 35.0%, 30.0%, and 12% for pre- and post-emergence damping-off, and root rot, respectively.

In contrast, *Pythium spp*. demonstrated the lowest infection rates of 20.0%, 15.0%, and 8.0% for pre- and post-emergence damping-off, and root rot, while registering the highest percentage (57%) for standing plants. Notably, there were no significant differences observed between the treatments involving *F. oxysporum and M. phaseolina*, particularly at the pre-, post-emergence, and root rot disease stages.

Effect of antagonists on the linear growth of the pathogenic fungi:

Data in Table (6) reveal significant variations in the inhibitory impact of antagonistic isolates on the *in vitro* linear growth of each tested pathogenic fungus. *T. harzianum* exhibited the most substantial decrease in mycelial growth, causing an overall reduction of 80.95%. Following closely, *T. album* demonstrated a reduction of 80.05%, while *B. subtilis* showed a 74.30% average reduction. Conversely, *B. megaterium* had the least effect, resulting in an average decrease in pathogen growth of 64.37%. The average reduction in mycelial growth due to the presence of the four antagonists was most pronounced for *F. solani* (79.85%), followed by *R. solani* (75.71%), *S. rolfsii* (73.01%), and *M. phaseolina* (71.13%).

Table 4. Frequency (%) of fungi isolated from the rotten roots of sage collected from Ismailia Governorate during 2022/23 growing season

Isolated fungi	Frequency of isolated fungi	
	No.	**(%)**
Fusarium oxysporum (Schlecht)	5	14.3
F. solani (Marti "Sacc.")	4	11.4
Macrophomina phaseolina (Tassi) Goid. (1947)	3	08.6
Pythium sp. (Pringsheim)	2	05.7
Rhizoctonia solani (Kuhn)	11	31.4
Sclerotium rolfsii (Sacc.)	10	28.6
Total	35	100

Table 5. Effect of artificial inoculation with the tested fungi on the incidence of pre- and post- emergence damping-off of sage under greenhouse conditions

Tested fungi	Pre-emergence (%)	Post-emergence (%)	Root rotted plants %	Plant survival (%)
Fusarium oxysporum	25.0	20.0	10.0	45.0
F. solani	30.0	25.0	09.0	36.0
Rhizoctonia solani	40.0	35.0	20.0	05.0
Macrophomina phaseolina	25.0	20.0	15.0	40.0
Sclerotium rolfsii	35.0	30.0	12.0	23.0
Pythium spp.	20.0	15.0	08.0	57.0
Control "Untreated"	00.00	00.00	00.00	100.00
L.S.D at 5%	1.45	1.42	0.74	2.06

Table 6. Effect of the antagonists on the percentage of reduction in the linear growth of the pathogenic fungi

Antagonists	% Reduction in growth of pathogenic fungi				
	F. solani	***M. phaseolina***	***R. solani***	***S. rolfsii***	**Mean**
T. harzianum	89.10	72.67	82.55	79.6	80.98
T. album	85.30	78.53	79.80	76.55	80.05
B. subtilis	78.80	70.03	74.82	73.55	74.30
B. megaterium	66.20	63.27	65.67	62.33	64.37
Control "Untreated"	00.00	00.00	00.00	00.00	00.00
Mean	79.85	71.13	75.71	73.01	74.92
L.S.D at 5% for					
Pathogenic fungi (P)	= 0.092		Antagonists (A)	= 0.097	
A x P	= 0.289				

***In vivo* experiments**

Effect of some antagonists alone or in combination with natural compound treatments on disease incidence and plant survival (%) of sage plants:

The data presented in Table (7) demonstrates that all tested biological control treatments, either alone or in combination with natural compounds, significantly reduced disease incidence and increased the percentage of sage plants surviving in both the 2022 and 2023 growing seasons. The various antagonistic isolates, either alone or in combination with natural compound treatments, exhibited differing effects against disease incidence. *T. harzianum*, in combination with Compost tea, emerged as the most effective treatment with a disease control efficacy of 90.00% and 88.86% in the two seasons, respectively. Following closely, *B. subtilis,* in combination with Compost tea, showed a disease control efficacy of 87.83% and 86.18%. Conversely, humic acid in combination with Compost tea exhibited the least efficacy, with disease control rates of 30.45% and 36.93% in the two seasons, respectively. When considering biocontrol agents alone, *T. harzianum* demonstrated the highest efficacy (78.70% and 78.86%), followed by B. subtilis (76.11% and 76.15%) in controlling root rot diseases of sage plants during the two successive seasons. In contrast, humic acid as a natural compound exhibited the least effectiveness, with disease control rates of 48.27% and 52.32% in both seasons, respectively.

Effect of some tested antagonists alone or in combination with natural compound treatments on sage plant performance

Growth characteristics (Plant height and No. of branches/plant)

Resulting data in Table (8) presents the impact of the various treatments on specific growth characteristics of sage plants, including plant height and the number of branches per plant across two cuts during the two seasons. The data clearly demonstrate that the most effective treatment for achieving maximum values in terms of plant height (63.67 cm and 97.33 cm for the first and second cuts, respectively, in the first season; and 69.58 cm and 102.33 cm for the first and second cuts, respectively, in the second season) and the number of branches per plant (26 and 27 for the first and second cuts, respectively, in the first season; and 26.67 and 29.33 for the first and second cuts, respectively, in the second season) was the combined application of *Trichoderma harzianum* and compost tea.

The treatment combining *Bacillus subtilis* with compost tea performed as the second-best option, outperforming other treatments. In contrast, the control group exhibited the lowest values for plant height (28 cm and 43.92 cm for the first and second cuts, respectively, in the first season; and 29 cm and 45.33 cm for the first and second cuts, respectively, in the second season) and the number of branches per plant (7.67 and 9 for the first and second cuts, respectively, in the first season; and 8.33 and 10 for the first and second cuts, respectively, in the second season).

Table 7. Effect of some antagonists alone or in combination with natural compound treatments on the average of disease incidence and plant survival (%) of sage 45 and 90-days transplanting under field conditions during 2022 and 2023 growing seasons

Different treatments	2022 growing season			2023 growing season		
	Disease Incidence (%)	Plant survival (%)	Efficacy (%)	Disease incidence (%)	Plant survival (%)	Efficacy (%)
T. harzianum	16.33	83.67	78.70	18.32	81.68	78.86
B. subtilis	18.32	81.68	76.11	20.67	79.33	76.15
Angel Yeast	33.66	66.34	56.10	35.67	64.33	58.85
Humic *acid*	39.67	60.33	48.27	41.33	58.67	52.32
Compost tea	28.99	71.01	62.19	29.22	70.78	66.29
T. harzianum + Angel Yeast	10.22	89.78	86.67	13.33	86.67	84.62
T. harzianum + Humic *acid*	23.32	76.68	69.59	24.99	75.01	71.17
T. harzianum + Compost tea	07.67	92.33	90.00	09.66	90.34	88.86
B. subtilis + Angel Yeast	12.66	87.34	83.49	15.22	84.78	82.44
B. subtilis + Humic *acid*	24.33	75.67	68.27	26.33	73.67	69.62
B. subtilis + Compost tea	09.33	90.67	87.83	11.98	88.02	86.18
Angel Yeast + Compost tea	41.33	58.67	46.10	43.22	56.78	50.14
Angel Yeast + Humic acid	53.33	46.67	30.45	54.67	45.33	36.93
Compost tea + Humic acid	49.99	50.01	34.81	51.66	48.34	40.40
Control	76.68	23.32	00.00	86.68	13.32	00.00
LSD at 5%	1.46	1.62	------	1.49	1.52	------

Table 8. Effect of some antagonists alone or in combination with natural compound treatments on some growth characteristics of sage plants under field conditions during 2022 and 2023 growing seasons

Different treatments	2022 growing season				2023 growing season			
	Plant height (cm)		No. of branches / plant		Plant height(cm)		No. of branches /plant	
	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut
T. harzianum	54.91	84.67	20.33	23.67	60.33	93.33	21.61	24.67
B. subtilis	54.00	82.33	19.61	22.33	59.33	91.67	20.00	23.33
Angel Yeast	47.33	75.33	16.67	19.00	50.00	76.33	17.33	19.67
Humic *acid*	44.67	72.33	16.11	18.00	49.67	74.67	17.11	18.67
Compost tea	49.33	77.67	17.33	19.67	54.33	80.00	18.33	20.33
T. harzianum + Angel Yeast	60.33	91.00	23.00	25.00	62.94	98.67	23.91	26.00
T. harzianum + Humic *acid*	51.89	80.44	18.91	21.67	57.33	86.33	19.91	22.00
T. harzianum + Compost tea	63.67	97.33	26.00	27.00	69.58	102.33	26.67	29.33
B. subtilis + Angel Yeast	57.00	87.33	21.33	24.67	61.42	95.00	22.33	25.11
B. subtilis + Humic *acid*	51.00	80.00	18.00	20.67	54.91	83.00	18.67	21.33
B. subtilis + Compost tea	61.61	95.00	25.67	26.33	67.92	100.33	25.33	27.67
Angel Yeast + Compost tea	43.00	71.12	15.67	17.00	46.33	73.33	16.33	18.33
Angel Yeast + Humic acid	39.67	65.00	13.00	15.00	41.67	68.33	14.11	16.00
Compost tea + Humic acid	41.00	68.00	14.67	16.33	45.33	70.67	15.67	17.67
Control	28.00	43.92	07.67	09.00	29.00	45.33	08.33	10.00
LSD at 5%	8.603	10.59	5.59	3.91	7.09	8.64	4.72	3.28

Herb fresh and dry weights:

Presented data in Table (9) displays the influences of the studied treatments on herb fresh and dry weights of sage plants, over two harvests (two cuts) of the first and second seasons.

The data indicate that the superior treatment for obtaining the highest values of herb fresh weights (322.3 g plant^{-1} and 854.0 g plant^{-1} for the first and second cuts, respectively, in the first season; and 354.3 g plant^{-1} and 936.0 g plant^{-1} for the first and second cuts, respectively, in the second season) and herb dry weights (94.41 g plant^{-1} and 245.6 g plant^{-1} for the first and second cuts, respectively, in the first season; and 102.2 g plant^{-1} and 251.6 g plant^{-1} for the first and second cuts, respectively, in the second season) resulted from the combined application of *T. harzianum* and compost tea.

It was followed by the treatment combining *B. subtilis* with compost tea, surpassing other treatments in terms of herb fresh and dry weights. Conversely, the control group exhibited the lowest values for herb fresh weights (144.7 g plant^{-1} and 338.7 g plant^{-1} for the first and second cuts, respectively, in the first season; and 147 g plant^{-1} and 377.2 g plant^{-1} for the first and second cuts, respectively, in the second season) and herb dry weights (37.26 g plant-1 and 121.4 g plant^{-1} for the first and second cuts, respectively, in the first season; and 43.14 g plant^{-1} and 86.07 g plant^{-1} for the first and second cuts, respectively, in the second season). This consistent trend in growth characteristics persisted throughout both growing seasons in 2022 and 2023.

Chemical constituents (N,P,K):

Data in Table (10) provides information on how different antagonists and/or natural compound treatments influenced the levels of NPK (nitrogen, phosphorus, and potassium) in sage plants. Notably, the combined treatment of *T. harzianum* + compost tea resulted in the highest recorded values for NPK in 2022 and 2023 growing seasons in comparison with control treatment.

Chlorophyll a, b and B-carotene:

Table (11) indicates the effect of the various treatments on chlorophyll a, b and *B*-carotene of sage plants under field conditions during 2022 and 2023 growing seasons. The data clearly demonstrate that the most effective treatment for achieving maximum values in terms of chlorophyll a (1.125 and 1.214 mg/g for the first and second cuts, respectively, in the first season; and 1.566 and 1.611 mg/g for the first and second cuts, respectively, in the second season), the chlorophyll b (0.618 and 0.691 mg/g for the first and second cuts, respectively, in the first season; and 1.175 and 1.461 mg/g for the first and second cuts, respectively, in the second season) and the *B*-carotene (0.958 and 0.992 mg/g for the first and second cuts, respectively, in the first season; and 1.424 and 1.581 mg/g for the first and second cuts, respectively, in the second season) was the combined application of *Trichoderma harzianum* and compost tea. The treatment combining *B. subtilis* with compost tea performed as the second-best option, outperforming other treatments.

Table 9. Effect of some antagonists alone or in combination with natural compound treatments on yield components of sage plants under field conditions during 2022 and 2023 growing seasons

Different treatments	2022 growing season				2023 growing season			
	Herb fresh weight / (g/plant)		Herb dry weight / (g/plant)		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
T. harzianum	209.7	591.3	61.20	162.0	231.0	658.0	76.28	197.5
B. subtilis	191.0	567.0	53.29	153.8	210.7	615.3	66.97	170.0
Angel Yeast	163.7	419.5	46.94	111.6	165.7	472.8	54.02	127.1
Humic *acid*	152.7	337.0	42.25	84.27	158.0	361.4	53.81	85.49
Compost tea	156.0	394.0	48.65	116.1	161.0	469.8	47.29	137.0
T. harzianum + Angel Yeast	297.7	715.3	87.77	192.2	313.3	757.3	109.4	199.5
T. harzianum + Humic *acid*	244.7	599.3	62.2	181.8	278.0	660.7	88.85	209.2
T. harzianum + Compost tea	322.3	854.0	94.41	245.6	354.3	936.0	102.2	251.6
B. subtilis + Angel Yeast	280.3	670.7	76.05	203.0	284.0	721.3	130.2	186.1
B. subtilis + Humic *acid*	222.0	534.0	50.3	158.5	274.6	632.5	71.65	180.3
B. subtilis + Compost tea	309.7	803.0	77.77	205.2	340.7	817.7	91.01	237.2
Angel Yeast + Compost tea	181.3	472.8	54.15	125.6	207.5	540.9	73.34	143.6
Angel Yeast + Humic acid	168.0	437.7	48.64	125.3	177.3	474.7	44.76	145.5
Compost tea + Humic acid	178.7	437.3	40.48	163.5	197.3	505.7	60.09	156.5
Control	144.7	338.7	37.26	121.4	147.0	377.2	43.14	86.07
LSD at 5%	14.18	53.24	14.84	25.02	22.87	77.89	18.58	32.58

Table 10. Effect of some antagonists and/ or in natural compound treatments on NPK of sage plants under field conditions during 2022 and 2023 growing seasons

Different treatments	2022 growing season						2023 growing season					
	N%		P%		K%		N%		P%		K%	
	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut
T. harzianum	2.27	3.07	0.36	0.46	1.09	1.84	2.80	3.25	0.42	0.52	1.11	1.91
B. subtilis	2.24	2.94	0.30	0.44	1.05	1.76	2.65	3.11	0.36	0.49	1.07	1.85
Angel Yeast	1.93	2.56	0.19	0.17	0.83	1.38	2.39	2.72	0.22	0.25	0.86	1.54
Humic *acid*	1.56	1.78	0.14	0.09	0.56	1.24	1.60	2.25	0.14	0.18	0.68	1.32
Compost tea	1.80	2.44	0.16	0.14	0.82	1.46	2.34	2.71	0.19	0.21	0.75	1.39
T. harzianum + Angel Yeast	2.57	3.36	0.53	0.63	1.30	2.09	3.09	3.64	0.59	0.69	1.32	2.15
T. harzianum + Humic *acid*	2.40	3.28	0.45	0.52	1.25	2.02	2.93	3.41	0.51	0.58	1.23	2.04
T. harzianum + Compost tea	3.20	3.55	0.69	0.83	1.45	2.23	3.22	3.83	0.75	0.89	1.41	2.34
B. subtilis + Angel Yeast	2.49	3.32	0.49	0.58	1.27	2.06	3.03	3.57	0.55	0.64	1.28	2.09
B. subtilis + Humic *acid*	2.36	3.15	0.39	0.48	1.14	1.97	2.86	3.36	0.44	0.53	1.19	2.01
B. subtilis + Compost tea	3.11	3.39	0.63	0.69	1.38	2.17	3.20	3.73	0.69	0.75	1.37	2.22
Angel Yeast + Compost tea	2.12	2.75	0.28	0.31	0.94	1.67	2.56	2.91	0.34	0.37	0.98	1.79
Angel Yeast + Humic acid	1.96	2.62	0.20	0.20	0.85	1.50	2.42	2.75	0.25	0.28	0.91	1.65
Compost tea + Humic acid	2.00	2.73	0.26	0.23	0.93	1.57	2.49	2.86	0.28	0.32	0.94	1.71
Control	1.76	2.30	0.12	0.11	0.72	1.15	2.31	2.61	0.16	0.20	0.48	1.25
LSD at 5%	0.074	0.106	0.092	0.075	0.158	0.290	0.330	0.106	0.074	0.075	0.191	0.224

In contrast, the control group exhibited the lowest values for chlorophyll a (0.782 and 0.808 mg/g for the first and second cuts, respectively, in the first season; and 0.491 and 0.497 mg/g for the first and second cuts, respectively, in the second season), the chlorophyll b (0.345 and 0.361 mg/g for the first and second cuts, respectively, in the first season; and 0.375 and 0.428 mg/g for the first and second cuts, respectively, in the second season) and the *B*-carotene (0.527 and 0.547 mg/g for the first and second cuts, respectively, in the first season; and 0.401 and 0.439 mg/g for the first and second cuts, respectively, in the second season).

Table 11. Effect of some antagonists alone or in combination with natural compound treatments on chlorophyll a, b and B-carotene of sage plants under field conditions during 2022 and 2023 growing seasons

Different treatments	Chlorophyll a, mg/g				Chlorophyll b, mg/g				B-carotene, mg/g			
	1st season		2nd season		1st season		2nd season		1st season		2nd season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
T. harzianum	1.026	1.040	1.055	1.080	0.516	0.529	0.692	0.756	0.813	0.818	0.737	0.790
B. subtilis	1.004	1.022	1.015	1.021	0.505	0.518	0.646	0.720	0.795	0.799	0.716	0.727
Angel Yeast	0.910	0.927	0.816	0.821	0.423	0.457	0.551	0.560	0.662	0.679	0.591	0.614
Humic *acid*	0.853	0.870	0.572	0.614	0.370	0.392	0.498	0.516	0.593	0.614	0.512	0.527
Compost tea	0.896	0.904	0.693	0.754	0.411	0.441	0.530	0.540	0.633	0.659	0.523	0.566
T. harzianum + Angel Yeast	1.078	1.146	1.305	1.378	0.556	0.577	0.901	0.955	0.911	0.917	1.011	1.095
T. harzianum + Humic *acid*	1.050	1.080	1.141	1.154	0.530	0.539	0.827	0.860	0.844	0.849	0.852	0.917
T. harzianum + Compost tea	1.125	1.214	1.566	1.611	0.618	0.691	1.175	1.461	0.958	0.992	1.424	1.581
B. subtilis + Angel Yeast	1.065	1.100	1.267	1.293	0.542	0.548	0.861	0.881	0.859	0.868	0.878	0.951
B. subtilis + Humic *acid*	1.046	1.067	1.130	1.144	0.522	0.533	0.714	0.746	0.822	0.835	0.777	0.825
B. subtilis + Compost tea	1.119	1.162	1.315	1.448	0.568	0.586	1.036	1.071	0.926	0.942	1.151	1.254
Angel Yeast + Compost tea	0.909	1.013	0.908	0.994	0.484	0.499	0.620	0.664	0.739	0.753	0.692	0.705
Angel Yeast + Humic acd	0.942	0.985	0.844	0.865	0.451	0.475	0.587	0.596	0.701	0.716	0.624	0.652
Compost tea + Humic acid	0.971	0.998	0.870	0.913	0.465	0.479	0.601	0.619	0.721	0.727	0.641	0.689
Control	0.782	0.808	0.491	0.497	0.345	0.361	0.375	0.428	0.527	0.547	0.401	0.439
LSD at 5%	0.150	0.140	0.118	0.140	0.092	0.053	0.092	0.093	0.053	00.053	0.130	0.053

Protein and total phenol:

Obtained data in Table (12) offers insights into the impact of various antagonists and/or natural compound treatments on protein and total phenol levels in sage plants. Remarkably, the combination of *T. harzianum* and compost tea yielded the highest measured values for both protein and total phenol content compared to untreated plants during 2022 and 2023 growing seasons.

Yield characters

Fresh and dry herb yield ton/fed.:

Table (13) shows the impact of the studied treatments on yield fresh and dry herb of sage plants, over two harvests (two cuts) of the first and second seasons. The data indicate that, the superior treatment for obtaining the highest values of yield fresh herb (4.09 and 10.84 ton fed^{-1} for the first and second cuts, respectively, in the first season; and 4.5 and 11.89ton fed^{-1} for the first and second cuts, respectively, in the second season) and yield dry herb (1.2 and 3.12ton fed^{-1} for the first and second cuts, respectively, in the first season; and 1.3 and 3.2 ton fed^{-1} for the first and second cuts, respectively, in the second season) was the combined application of *Trichoderma harzianum* and compost tea. It was followed by the treatment combining *B. subtilis* with compost tea, surpassing other treatments in terms of herb fresh and dry yields.

Conversely, the control group exhibited the lowest values for yield fresh herb ton/fed. (1.84 and 4.30 ton fed^{-1} for the first and second cuts, respectively, in the first season; and 1.87 and 4.79 ton fed^{-1} for the first and second cuts, respectively, in the second season) and herb dry yield ton/fed. (0.47 and 1.45 ton fed^{-1} for the first and second cuts, respectively, in the first season; and 0.55 and 1.10 ton fed^{-1} for the first and second cuts, respectively, in the second season). This consistent trend in growth characteristics persisted throughout both growing seasons in 2022 and 2023. The superior treatment involving the combined application of *Trichoderma harzianum* and compost tea, as well as the treatment combining *B. subtilis* with compost tea, resulting in the highest yield of fresh and dry herb of sage plants, can be attributed to several scientific factors.

Table 12. Effect of some antagonists and/ or in natural compound treatments on protein percentage and total phenol content of sage plants under field conditions during 2022 and 2023growing seasons

Different treatments	2022 growing season				2023 growing season			
	Protein%		Total phenol (mg/GAE)		Protein%		Total phenol (mg/GAE)	
	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut
T. harzianum	14.19	19.22	2.49	1.84	17.48	20.29	2.75	2.51
B. subtilis	14.00	18.35	2.44	1.81	16.58	19.44	2.71	2.49
Angel Yeast	12.06	15.98	2.08	1.64	14.82	17.00	2.39	2.10
Humic *acid*	9.74	11.06	1.84	1.44	10.03	14.04	2.26	1.72
Compost tea	11.27	15.25	1.92	1.52	14.63	16.93	2.34	2.00
T. harzianum + Angel Yeast	16.06	21.01	2.71	2.02	19.32	22.75	3.01	2.70
T. harzianum + Humic *acid*	14.99	20.50	2.60	1.97	18.33	21.32	2.94	2.58
T. harzianum + Compost tea	20.00	22.19	2.86	2.24	20.15	23.91	3.43	2.95
B. subtilis + Angel Yeast	15.56	20.78	2.70	2.00	18.93	22.31	2.98	2.65
B. subtilis + Humic *acid*	14.75	19.69	2.55	1.91	17.9	21.00	2.86	2.53
B. subtilis + Compost tea	19.44	21.20	2.84	2.22	20.00	23.34	3.16	2.85
Angel Yeast + Compost tea	12.90	17.21	2.42	1.78	16.01	18.19	2.68	2.44
Angel Yeast + Humic acid	12.25	16.35	2.13	1.70	15.12	17.19	2.48	2.39
Compost tea + Humic acid	12.50	17.06	2.30	1.71	15.55	17.90	2.59	2.41
Control	11.02	14.38	1.75	1.31	14.45	16.30	2.02	1.45
LSD at 5%	0.56	0.65	0.053	0.053	2.053	1.296	0.092	0.175

Table 13. Effect of some antagonists alone or in combination with natural compound treatments on yield fresh and dry weights of sage plants under field conditions during 2022 and 2023 growing seasons

Different treatments	2022 growing season				2023 growing season			
	Yield fresh weight (ton)/feddan		Yield dry weight (ton)/feddan		Yield fresh weight (ton)/feddan		Yeild dry weight (ton)/feddan	
	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut
T. harzianum	2.66	7.51	0.78	2.06	2.93	8.36	0.97	2.51
B. subtilis	2.43	7.20	0.68	1.95	2.68	7.81	0.85	2.16
Angel Yeast	2.08	5.33	0.60	1.42	2.10	6.00	0.69	1.61
Humic *acid*	1.94	4.28	0.54	1.07	2.01	4.59	0.68	1.09
Compost tea	1.98	5.00	0.62	1.47	2.04	5.59	0.60	1.74
T. harzianum + Angel Yeast	3.78	9.08	1.11	2.44	3.98	9.62	1.39	2.53
T. harzianum + Humic *acid*	3.11	7.61	0.80	2.31	3.53	8.39	1.13	2.66
T. harzianum + Compost tea	4.09	10.84	1.20	3.12	4.50	11.89	1.30	3.20
B. subtilis + Angel Yeast	3.56	8.52	0.97	2.58	3.61	9.16	1.65	2.36
B. subtilis + Humic *acid*	2.82	7.20	0.64	2.01	3.49	8.03	0.91	2.29
B. subtilis + Compost tea	3.93	10.20	0.99	2.61	4.33	10.38	1.16	3.01
Angel Yeast + Compost tea	2.30	6.00	0.69	1.60	2.63	6.87	0.93	1.82
Angel Yeast + Humic acid	2.13	5.33	0.62	1.59	2.25	6.03	0.57	1.85
Compost tea + Humic acid	2.27	5.55	0.51	2.08	2.51	6.42	0.76	1.99
Control	1.84	4.30	0.47	1.54	1.87	4.79	0.55	1.10
LSD at 5%	0.183	0.555	0.190	0.317	0.270	0.928	0.236	0.410

Essential oil percentage and essential oil yield ml/plant:

The greater efficacy of the combined treatment of *Trichoderma harzianum* and compost tea in increasing the essential oil yield percentage in sage plants during 2022 and 2023 growing seasons can be scientifically explained through multiple factors, including potassium, an essential nutrient for plant growth, which is closely linked to the biosynthesis of essential oils, as shown in Table (14).

Essential oil constituents

Data in Table (15) illustrate the effect of some antagonists and/ or in natural compound treatments on essential oil constituents of control and the most effective treatments in sage plants under field conditions during 2023 growing season compared with untreated plants. Fourteen distinct compounds were identified within the sage essential oil, with 1,8-Cineole (ranged from 35.16 to 41.03%) being the dominant component fraction in the sage oil, followed by α-Thujone (ranged from 8.39 to 15.26%) as a predominant component. The other main components were β-Thujone (ranged from 6.35 to 10.43%) , camphor (ranged from 7.97 to 9.53%) and β-Pinene (ranged from 6.55 to 9.14%). The application of *T. harzianum* in combination with compost tea, as well as the sole application of *T. harzianum*, resulted in significantly higher levels of 1,8-Cineole compared to the control treatment in both the first and second cuts, Notably, the combined treatment of *T. harzianum* + compost tea yielded the highest recorded values for 1,8-Cineole content. These outcomes can be attributed to the synergistic effects of these treatments, which likely influenced the biosynthesis of 1,8-Cineole within the sage plants, ultimately leading to enhanced accumulation.

Table 14. Effect of some antagonists and/ or in natural compound treatments on essential oil percentage and yield ml/plant of sage plants under field conditions during 2022 and 2023 growing seasons

Different treatments	2022 growing season				2023 growing season			
	Oil %		Essential oil yield (ml)/plant		Oil %		Essential oil yield (ml)/plant	
	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut	1st Cut	2nd Cut
T. harzianum	3.39	2.54	2.08	4.12	3.53	2.75	2.69	5.44
B. subtilis	3.28	2.11	1.75	3.27	3.43	2.36	2.30	4.02
Angel Yeast	2.90	1.47	1.36	1.64	3.08	1.51	1.66	1.92
Humic *acid*	2.61	1.17	1.10	0.99	2.82	1.46	1.52	1.25
Compost tea	2.78	1.38	1.36	1.61	2.97	1.49	1.40	2.04
T. harzianum + Angel Yeast	3.92	2.89	3.44	5.55	4.01	3.07	4.41	6.13
T. harzianum + Humic *acid*	3.79	2.71	2.37	4.93	3.89	2.91	3.48	6.08
T. harzianum + Compost tea	4.38	3.39	4.11	8.32	4.43	3.53	4.53	8.87
B. subtilis + Angel Yeast	3.82	2.87	2.91	5.85	3.92	3.05	5.08	5.64
B. subtilis + Humic *acid*	3.43	2.63	1.74	4.15	3.56	2.84	2.56	5.12
B. subtilis + Compost tea	4.32	3.12	3.49	6.40	4.37	3.28	3.96	7.77
Angel Yeast + Compost tea	3.27	1.75	1.78	2.20	3.42	2.04	2.51	2.94
Angel Yeast + Humic acid	2.95	1.62	1.44	2.03	3.13	1.57	1.40	2.28
Compost tea + Humic acid	3.05	1.73	1.24	2.82	3.22	2.02	1.94	3.20
Control	2.25	1.24	0.84	1.50	2.49	1.45	1.07	1.25
LSD at 5%	0.560	0.339	0.824	.905	0.677	0.449	1.418	1.172

Table 15. Effect of some antagonists and/ or in natural compound treatments on essential oil constituents of control and most effective treatments in sage plants under field conditions during 2023 growing season

No.	Compound	First Cut			Second Cut		
		Control	*T. harzianum*	*T. harzianum* + Compost tea	Control	*T. harzianum*	*T. harzianum* + Compost tea
1	α-Pinene	3.90	5.22	4.75	6.35	6.18	6.02
2	(+)-Comphene	3.89	0.00	3.16	7.52	5.01	5.62
3	β-Pinene	6.55	8.43	8.54	8.74	9.14	8.07
4	M yrcene	4.02	5.09	4.81	4.59	5.39	3.81
5	1.8-Cineole	35.44	38.77	41.03	35.16	38.66	40.40
6	γ-Terpinene	2.91	2.52	4.17	2.37	3.74	1.46
7	α-Thujone	15.26	15.00	8.39	14.90	11.43	14.29
8	β-Thujone	10.43	8.63	10.10	7.11	6.35	7.53
9	camphor	8.27	8.52	8.98	7.97	9.53	8.34
10	Eugenol	4.15	1.64	0.82	1.40	1.57	0.74
11	*Β*-Caryophyllene	1.13	4.77	1.71	1.86	2.20	1.24
12	Aromadendrene	4.05	-	1.29	1.48	-	0.32
13	α-Humulene	-	1.41	0.71	-	0.19	2.16
14	Viridiflorol	-	-	1.53	0.56	0.60	-

DISCUSSION

The fungi isolated from rotted roots of sage plants revealed that *Rhizoctonia solani* and *Sclerotium rolfsii* exhibited the highest frequency among the isolated fungi from the rotted sage samples collected in Ismailia Governorate. Identification of these fungi was conducted based on their cultural, morphological, and microscopic characteristics, as described by Aziz *et al.* (2013). Notably, *R. solani* emerged as the most virulent soilborne disease, exerting the greatest impact on the incidence of pre- and post-emergence damping-off, as well as root rot. *S. rolfsii* ranked second in terms of virulence. These findings align with those presented by Ahmed *et al.* (2022), who highlighted the synergistic interaction between polygalacturonase and oxalic acid produced by these pathogenic fungi as the causative factors leading to root damage induced by soilborne infections.

Trichoderma harzianum significantly contributed to the most pronounced reduction in mycelial growth, while *Bacillus megaterium* exhibited the least impact. This phenomenon can be attributed to the fact that different pathogens with distinct structures possess varied defense mechanisms against enzymes and toxic compounds produced by different antagonists (Ahmed, 2013 and Ahmed *et al.,* 2016). *Trichoderma* spp., for example, degrades the pathogen's cell wall through the production of lytic enzymes such as chitinases, peroxidase, polyphenoloxidase, and glucan 1-3 B-glucosidases. *B. subtilis,* ranking second after *Trichoderma spp*., may achieve this through the production of enzymes that break down the pathogen's cell wall, antibiotics like bacterocin and subtilisin, as well as the release of volatile compounds and phytotoxic substances (Đurović *et al.,* 2022; Abdel-Latif and Zakarya, 2023). In general, the isolation, purification, and identification of the related microorganisms, mainly fungi, was the first step toward diagnosing the root rot problem in sage plants. This method allowed the particular fungus species responsible for the disease to be identified based on cultural, morphological, and microscopic characteristics.

T. harzianum in combination with Compost tea showed the highest efficacy treatment followed by *B. subtilis* in combination with Compost tea in controlling root rot of sage plants during two seasons 2022 and 2023, respectively. On the other hand, Humic acid in combination with compost tea showed the least efficacy treatment. The results can be elucidated by considering the dual influence of bioagents and the synergistic effects of natural combination treatments. These treatments have been reported to generate growth regulators, as noted in studies by Ahmed (2013) and Marín *et al.* (2013).

Furthermore, the chemical impact of antioxidants has played a discernible role in enhancing plant physiology and metabolism. *Trichoderma* spp. is known to employ various mechanisms, including mycoparasitism and the production of antifungal substances such as endo-chitinase, β-glucosidase, α-1,3-glucanase, and trichodermin (Balode, 2010). On the other hand, some isolates compete for resources and space. The reason behind their varying effectiveness may stem from the survival challenges faced by antagonists, the presence of natural compounds, or the possibility that their antagonistic mechanisms are ineffective in suppressing pathogens within the complex soil environment. It's worth noting that microbial antagonism in soil presents a significant challenge, as many of the mechanisms involving biocontrol by *Trichoderma* spp. are hypothetical, and obtaining concrete proof can be challenging, as mentioned by Ahmed *et al.* (2016).

The superior growth results observed in sage plants under the combined treatment of *T. harzianum* and compost tea, as well as the combined treatment of *B. subtilis* and compost tea, can be attributed to several scientific factors. The sage plants receiving these treatments are consequently better able to tolerate the effects of root rot and direct more energy toward growth. Healthy root systems are essential for plant growth. The biocontrol agents in the treatments may have contributed to improved root health by reducing the incidence of root rot. This, in turn, would enable the sage plants to take up water and nutrients more efficiently, leading to taller plants with more branches. The consistent performance of these treatments in both the 2022 and 2023 growing seasons indicates their reliability and effectiveness. This consistency suggests that the treatments are likely addressing underlying issues related to soil health and pathogen suppression consistently over time. In contrast, the control group, which did not receive these beneficial treatments, exhibited poorer growth characteristics because it lacked the advantages provided by biocontrol agents and nutrient-rich compost tea. This emphasizes the importance of adopting these biologically based treatments in organic agriculture systems to mitigate root rot disease and promote healthier, more productive sage plants. These findings align with the results reported by Babu & Pallavi (2013); Mata *et al.* (2019) and Xi *et al*. (2019).

The observed variations in herb fresh and dry weights among sage plants subjected to different treatments can be explained scientifically as mentioned above. The combined treatment of *T. harzianum* and compost tea is highly effective due to the synergistic action of Trichoderma in suppressing pathogenic fungi, thereby enhancing nutrient uptake and overall plant health, while compost tea enriches the soil with essential nutrients and beneficial microorganisms. Conversely, the control group, devoid of these beneficial treatments, exhibits reduced growth due to the absence of disease suppression and nutrient enrichment, highlighting the significance of biologically based treatments in optimizing sage plant productivity under organic agricultural conditions. This combination promotes vigorous growth and higher herb yields. Similarly, the treatment combining *B. subtilis* with compost tea provides additional biocontrol mechanisms and nutrient enrichment, resulting in improved growth compared to other treatments. These results are in harmony with those of Abd-El-Azim (2003) and Ahmed (2013).

The scientific reasons for the observed increase in NPK levels in sage plants due to the combined treatment of *T. harzianum* + Compost tea can be explained the role of compost tea is known to contain a wide range of beneficial microorganisms, including bacteria and fungi, which can enhance nutrient mobilization in the soil. These microorganisms break down organic matter in compost, releasing essential nutrients like nitrogen (N), phosphorus (P), and potassium (K) in a form that can be readily taken up by plants. This increased nutrient availability in the root zone promotes higher nutrient uptake by sage plants. *T. harzianum,* when applied as a biocontrol agent, can have a positive influence on the overall soil biology. It can promote the growth of beneficial microorganisms in the rhizosphere, which in turn can facilitate nutrient cycling and mineralization. This can result in improved nutrient uptake by the sage plants. *T. harzianum* is known not only for its biocontrol properties against plant pathogens but also for its ability to enhance plant health. Healthy roots are better equipped to absorb and transport nutrients, leading to higher NPK levels in the plant. The combined treatment of *T. harzianum* + Compost tea may have contributed to a balanced nutrient profile in the soil. This balance can optimize the uptake and utilization of NPK elements by sage plants, leading to improved growth and nutrient content. In summary, the observed increase in NPK levels in sage plants with the combined treatment of *T. harzianum* + compost tea can be attributed to enhanced nutrient mobilization, improved soil biology, disease suppression, healthier root systems, and a balanced nutrient environment in the rhizosphere.

These factors work together to promote the overall growth and nutrient content of sage plants. These results are in harmony with those of Scotti *et al.* (2015); Carillo *et al.* (2020) and Gopi *et al.* (2020).

The observed elevation in protein and total phenol levels in sage plants subjected to the combined treatment of *T. harzianum* and compost tea can be elucidated scientifically through several mechanisms. *T. harzianum* is known to trigger a systemic response in plants, inducing the production of defense proteins and secondary metabolites, including phenolic compounds like phenols. This enhanced production of phenols can serve as a plant's defense mechanism against pathogens and environmental stressors. Additionally, compost tea, enriched with beneficial microorganisms, can stimulate plant metabolism, leading to increased protein synthesis. This synergistic effect between *T. harzianum* and compost tea likely resulted in elevated protein content and higher total phenol levels in sage plants, contributing to improved resilience and overall plant health. These findings align with the research outcomes reported by Scotti *et al.* (2015); Carillo *et al.* (2020) and Gopi *et al*. (2020).

The presence of antagonists in the soil protects the root system, increasing the efficiency of potassium absorption which has a direct impact on the plant's ability to create essential oils. *Trichoderma harzianum*, known for its positive effects on plant health and nutrient uptake, likely synergistically contributed to this effect, resulting in increased essential oil production. These findings align with the concept that optimal nutrient management and soil health maintenance can lead to enhanced essential oil content in plants, as supported by the research conducted by Ahmed *et al.* (2016). In addition, Hendi and Metwaly (2020) who worked on the impacts of *T. harzianum, Streptomyces griseus* and two chemical fungicides to control spearmint rust disease and they concluded that, all treatments reduced the incidence and percentage of rust disease severity in compared to untreated plants (control). Additionally, in comparison to the chemical fungicide and control treatments, vegetative growth, essential oil production, mineral content, herb total carbohydrates, and essential oil carvone content were all considerably higher. Moreover, Serag El-Din *et al*. (2020), investigated rosemary root rot and wilt and found that, the treatment of NPK + Potassium silicate + *T. harzianum* was more effective in reducing infection of root rot and wilt diseases as well as superiority increasing of growth characteristics, essential oil content and the total phenols and flavonoids contents.

The effectiveness of the treatment involving the combined application of *Trichoderma harzianum* and compost tea, as well as the treatment combining *B. subtilis* with compost tea, in achieving maximum values for chlorophyll a, chlorophyll b, and B-carotene in sage plants can be explained by several scientific factors. *Trichoderma harzianum and B. subtilis* are well-documented biocontrol agents with the ability to suppress soilborne pathogens and promote plant health. By reducing the impact of pathogens such as *Rhizoctonia solani* and *Sclerotium rolfsii*, these treatments likely contributed to improved plant vigor and reduced stress, which can result in higher chlorophyll and carotenoid pigment production. Additionally, compost tea, being a source of organic matter and beneficial microorganisms, enhances soil structure and nutrient availability. This fosters optimal conditions for photosynthesis and nutrient uptake, which are crucial for chlorophyll and carotenoid biosynthesis. Furthermore, the improved microbial diversity in the soil resulting from compost tea application can positively impact nutrient cycling and availability, supporting the synthesis of these pigments. The combination of biocontrol agents and soil enhancement contributed to enhanced plant photosynthetic capacity, resulting in the observed higher chlorophyll a, chlorophyll b, and Β-carotene levels. In contrast, the control group, lacking these beneficial treatments, experienced the negative influence of root rot disease on plant health and pigment production, resulting in lower chlorophyll and carotenoid levels. This consistent trend in pigment content across both growing seasons underscores the role of these treatments in promoting sage plant health and pigment production. This trend remained consistent throughout both growing seasons in 2021 and 2022. These results are in harmony with those of Babu & Pallavi (2013); Mata *et al.* (2019) and Xi *et al.* (2019).

Trichoderma harzianum and *B. subtilis* are known antagonists against pathogenic fungi, such as *Rhizoctonia solani* and *Sclerotium rolfsii*, which were identified as the predominant causes of sage root rot disease in the isolation trials. These antagonists likely suppressed the growth and activity of these pathogens in the soil, reducing their impact on sage plants and resulting in healthier plants with increased yields. Compost tea, a source of organic matter and beneficial microorganisms, can enhance soil structure, nutrient availability, and microbial diversity. It fosters a more favorable soil environment for plant growth, root development, and nutrient uptake. This combination of biocontrol agents and soil enhancement through compost tea contributed to improved plant health and productivity, resulting in the observed higher yield fresh and dry weights of sage plants in both seasons. Conversely, the control group exhibited lower yields due to the absence of these beneficial treatments, allowing the root rot pathogens to exert their damaging effects on the sage plants. This consistent trend in growth characteristics across both seasons highlights the effectiveness of the combined application of *T. harzianum* and compost tea, as well as *B. subtilis* with compost tea, in mitigating the impact of root rot disease and promoting sage plant growth and productivity (Abd-El-Azim, 2003 and Ahmed, 2013).

The application of *T. harzianum* in combination with compost tea, as well as the sole application of *T. harzianum*, resulted in significantly higher levels of 1,8-Cineole compared to the control treatment in both the first and second cuts. Notably, the combined treatment of *T. harzianum* + compost tea yielded the highest recorded values for 1,8-Cineole content. These outcomes can be attributed to the synergistic effects of these treatments, which likely influenced the biosynthesis of 1,8-Cineole within the sage plants, ultimately leading to enhanced accumulation. These results are in agreement with Radulescu *et al.* (2004) who studied sage oil. The biological properties of sage essential oil were attributed mainly to the constituents 1,8-cineole, α-thujone, β-thujone, and camphor.

CONCLUSION

This thorough study has clarified many facts of sage plant health and performance, especially in relation to managing root rot infections brought on by soilborne pathogens. *Trichoderma harzianum*, *T. album*, *Bacillus subtilis*, and *B. megaterium* all showed potential inhibitory effects during the ensuing investigation into the impact of antagonists on the linear development of these pathogens, with *T. harzianum* showing the best efficiency *in vitro* in comparison with control treatment. In the meantime on to field tests, it was discovered that treating *T. harzianum* with compost tea simultaneously was the most effective method for preventing root rot, increasing in plant survival, yield, herb fresh and dry weights, levels of essential nutrients (NPK), protein, and total phenol. Additionally, higher levels of the predominate compost component also had a positive impact on the constituents and essential oil content in sage plants. Depending on these findings, it can be recommended that, the integrated approach of using *T. harzianum* in combination with Compost tea be considered as a valuable strategy for managing root rot diseases of sage and enhancing the overall its plants growth characters and productivity. This holistic approach addresses not only disease control but also plant nutrition, growth, and secondary metabolite production, making it a promising solution for sage cultivation. In order to improve the benefits of these treatments in commercial sage farming, further study can focus on optimizing the application procedures and timing.

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الملخص العربي

اساليب إدارة مرض عفن الجذورفى المريمية وإنتاجية النبات عن طريق المكافحة البيولوجية في ظل نظام الزراعة العضوية

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تعتبر المريمية واحدة من أكثر النباتات تقديرًا لنشاطها البيولوجي الكبير. لكن مرض تعفن الجذور، وهو مرض فطري منتشر يمكن أن يصيب مجموعة متنوعة من النباتات بما في ذلك المريمية، مما يؤدي إلى موت النباتات وانخفاض كبير للمحصول دفع إلى استكشاف استراتيجيات بديلة لمكافحة المرض. ومن ثم تم إجراء البحث الحالي على مدار موسمي النمو المتتاليين لعامي ٢٠٢٢ و٢٠٢٣ في محطة بحوث القصاصين، مركز البحوث الزراعية (**ARC**)، محافظة الإسماعيلية، مصر، بهدف دراسة تأثير المعاملات التالية ، ***Bacillus subtilis*** ، ***Trichoderma harzianum***، الخميرة ، حمض الهيوميك، شاي الكمبوست وتداخلاتها، على إدارة مرض تعفن جذور المريمية وتأثيره على الإنتاجية داخل نظام زراعي عضوي. وأثبتت نتائج الدراسة في تجربة الصوبة، أن فطر ***Rhizoctonia solani*** يسبب أكثر الأمراض التي تنتقل عن طريق التربة شراسة حيث يتسبب في أعلى معدلات الإصابة بالمرض بنسبة ٤٠,٠% و٣٥,٠% و٢٠,٠% قبل وبعد الظهور لبادرات المريمية وتعفن الجذور، على التوالي. ويليه فى القدرة المرضية فطر ***Sclerotium rolfsii*** حيث بلغت معدلات الإصابة ٣٥,٠%، و٣٠,٠%، و١٢% لهذه المراحل على التوالي. وأظهرت عزلات المضادات الحيوية تباينات كبيرة في قدرتها على تثبيط النمو الميسليومى في المختبر لكلا الفطريات المسببة للأمراض التي تم اختبارها. حيث حققت عزلة ***T. harzianum*** أكبر انخفاض في نمو الفطريات الميسليومى ، مع انخفاض مثير للإعجاب بنسبة ٨٠,٩٥%، يليها المعامة بعزلة ***T. Album*** التى خفضت نمو الفطريات الميسليومى بنسبة ٨٠,٠٥%، ثم ***B. subtilis*** بنسبة ٧٤,٣٠%، و***B. megaterium*** بنسبة ٦٤,٣٧%، في المتوسط في التجربة الحقلية للتطبيق، كما أدت نتائج جميع المعاملات إلى انخفاض كبير في حدوث المرض و تعفن جذور المريمية ، وعززت المعاملات كذلك صفات النمو وإنتاجية الزيت ومحصول العشب لنباتات المريمية في كلا الموسمين. وتميزت المعاملة ب ***T. harzianum*** مع شاي الكومبوست بأعلى فعالية في السيطرة على مرض تعفن الجذورفى المريمية و الحصول على أعلى إنتاجية وجودة للمحصول.

الكلمات المفتاحية: المريمية، عفن الجذور، ***Bacillus subtilis***، ***Trichoderma harzianum***، المحصول.