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Suppression of *Sclerotium cepivorum* Using Biofumigation By Glucosinolate-Containing Plant and Cyanogenic Bacteria

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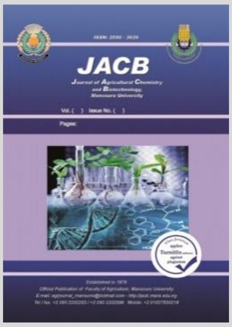

ABSTRACT

In dual culture antagonism test, six of eight cyanogenic bacteria were able to reduce the growth of *Sclerotium cepivorum*. The strains *Glocoacetobacter diazotrophicus* and *Paenibacillus. polymyxa* GQ375783.1 were the most effective bacteria that recorded the highest inhibition of *S. cepivorum* mycelial growth by 75% and 70% respectively. Based on HCN production, two strains recorded complete inhibition of fungal growth. Moreover, the ground tissues of the nasturtium plant were able to inhibit the mycelium growth of *S. cepivorum* by 100%. Results of the greenhouse experiment emphasized that white-rot incidence percentage recorded the lowest values in onion cultivated in soil amended with nasturtium plant and inoculated with *G. diazotrophicus* plus *P. polymyxa* GQ375783.1. This treatment recorded the highest values of onion vegetative growth and their yield. Under field conditions, the soil treated with investigated two methods of biofumigation led to suppress white rot disease by the rate of 100% while using each one individually, it reduced disease by the rate of 94.4 and 94.9 % respectively. All onion treatments that inoculated with cyanogenic bacteria showed higher records of DH and N_2-ase activity than uninoculated ones. The highest values DH and N_2-ase were recorded with soil amended with nasturtium and inoculated with cyanogenic bacterial strains. Additionally, the same treatment gave the highest values of peroxidase and polyphenoloxidase activity, onion NPK content, and the plant's vegetative growth as well as their yield. Therefore, the biofumigation using nasturtium and investigated bacterial strains can be recommended in controlling White-rot disease in onions and increasing their productivity.

Keywords: Biofumigation, Isothiocyanate-containing plant, *Glocoacetobacter diazotrophicus, Paenibacillus polymyxa*, White- rot disease, Onion

INTRODUCTION

In general, the management of soil-borne diseases is a complex process and is considered a challenge in agricultural production (Dutta *et al*, 2019) In particular, *Sclerotium cepivorum*, due to its sclerotia remains viable in the soil for many years and is stimulated to germinate through the presence of a susceptible crop. Therefore, treating the infested soil with this fungus occupies the thinking of many people interested in the field of agriculture.

Under intensive conventional cultivation systems, chemicals are the method currently available to farmers, among them, the volatile compounds that are used frequently in a practice known as soil fumigation (Leite and Lopes, 2018). Fumigation aims to sterilize the soil, which is incompatible with the philosophy and principles of production systems that value the biological activity of the soil, such as organic or agroecological systems (Santos *et al*, 2021).

But the prohibition of these chemicals due to the detrimental effect on human health and the environment led to limitations in some production sectors, such as vegetables, flowers, and seedlings. So, scientists have tended to consider alternative strategies to combat them that are safer and more in line with natural farming systems (Morris *et al*, 2020).

One such option is biofumigation, which offers a creative solution to an old problem, employing the use of naturally produced compounds to control plant pathogens as the application of residues from plant species or microorganisms capable of releasing gases with bioactive or biofumigant action which is effective at controlling phytopathogens (Dutta *et al*, 2019; Brennan *et al*, 2020).

The glucosinolate-containing plants as biofumigant agents have been successfully applied for the management of some soil-borne pathogens as *Rhizoctonia solani* (Abdallah *et al*, 2020), but no studies have been achieved against *Sclerotium cepivorum* the causative agent of onion white rot disease. When the ground tissues of ITCPs are incorporated into the soil, the glucosinolates and myrosinase enzyme come into contact and are hydrolyzed to release various forms of volatile isothiocyanates which have broad fungicidal activity (Vig *et al*, 2009).

Plants such as broccoli, cauliflower, mustard, rapeseed and nasturtium contain organic compounds called glucosinolates. When the tissues of these plants are damaged, biologically active chemicals are produced. One of the most important compounds released is isothiocyanate (ITC). The pungency of horseradish and the spicy taste of hot mustard are caused by ITCs released when the tissues are macerated. At low concentrations, ITCs are considered beneficial to human health. At high concentrations, ITCs are general biocides that behave much like commercial pesticides. Several commercial pesticides including Dazomet, Vapam, and Vorlex depend on an ITC as the

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active ingredient for pest control (Srivastava and Ghatak, 2017).

The cyanogenic bacterial species release several volatile and non-volatile antifungal compounds that influence the surrounding environment and are supposed to contribute to the biological suppression of soil-borne plant pathogens, among these compounds the hydrogen cyanide (HCN) (Jayaprakashvel *et al*, 2010). The production of the respiratory poison hydrogen cyanide (HCN) is called cyanogenesis. However, in the last decade, increasing evidence has been brought about that non-cyanogenic bacteria can also inhibit fungi by emitting volatiles. Until recently, HCN was the only volatile molecule known to be active against fungi (Weisskopf, 2013).

Therefore, the present work focused on the suppression of the soil-borne disease *sclerotium cepivorum* using biofumigation by glucosinolate-containing plants (nasturtium) and cyanogenic bacteria (*Gluconacetobacter diazotrophicus* and *Paenibacillus polymyxa* GQ375783.1).

To achieve this goal, *in vitro* antagonism assays served to assess the efficiency of selected strains against onion white rot causative agent, then they were further tested alone or with biofumigation treatment using nasturtium *in vivo* greenhouse assay, and in field trials in terms of biocontrol efficiency and crop yield.

MATERIALS AND METHODS

Microorganisms and plant materials

Cyanogenic bacterial strains viz, *Gluconacetobacter diazotrophicus*, *Paenibacillus polymyxa* GQ375783.1, and six isolates belong to *Gluconacetobacter* sp. (RS 1-6) that were used as the antagonistic strains were obtained from Agric. Microbiology Dept., Fac. of Agric., Benha Univ., Egypt. The two strains and six bacterial isolates were chosen based on plant-growth-promoting features especially their ability to produce large amount of HCN.

Sclerotium cepivorum strain that is characterized as a highly virulent pathogen to onion was obtained from Plant Pathology Dept., Fac. of Agric., Benha Univ., Egypt. The original bacterial cultures were maintained on nutrient agar while fungal strain was maintained on potato dextrose agar (PDA). The cultures were kept at 4 °C.

Onion seedlings *(Allium cepa* "Giza 20 cv.") were surface-sterilized by soaking in ethanol 70% and 2%sodium hypochlorite solution for 10 min of each one, then thoroughly washed four times in sterile distilled water and air-dried on a sterile plastic sheet.

The garden nasturtium (*Tropaeolum majus* L.) belongs to the family Tropaeolaceae. The plant is one of the glucosinolate-containing plants. The plants were grown in field plots at Fac. of Agric., Benha Univ., Egypt. Aerial parts were harvested, chopped up, and used in the biofumigation treatment of soil.

Fungal and bacterial inocula preparation

The fungal inoculum was prepared in 250 ml glass jars containing the following substrates per jar (75 g grain barley, 25 g coarse sand, and 25 ml tap water). The jars were autoclaved at 121°C for 30 minutes and left to cool, then inoculated with 5 mm^2-disk of the 7-days-old culture of *S. cepivorum* and incubated at 25°C for 14 days to obtain sufficient growth of fungus. However, bacterial inocula were prepared by growing the bacterial strains in the nutrient broth medium with continuous shaking (140 rpm) at 30 °C up to optimum growth. Then the cells were harvested by centrifugation (4000 rpm) at 4°C for 10 minutes. The cells were suspended using water peptone to make suspension containing 10^8 CFU/ml by optical density method.

Fungal growth inhibition assays

The antagonistic nature of *Gluconacetobacter diazotrophicus*, *Paenibacillus polymyxa* GQ375783.1, and six isolates belong to *Gluconacetobacter* sp. (RS 1-6) against *S. cepivorum* was determined using dual culture technique as described by Hariprasad and Niranjana (2009).

The inhibition zone (mm) and growth inhibition percentage were calculated using the formula: $\%inhibition=(R-r)/R\times100$, where r is the radius of the fungal colony opposite the bacterial colony and, R is the maximum radius of the fungal colony away from the bacterial colony.

Inhibition of fungal growth by HCN compounds produced by *Gluconacetobacter diazotrophicus*, *Paenibacillus polymyxa* GQ375783.1, and nasturtium plant was determined according to Montealegre *et al*, (2003) with some modification, 0.1 ml of overnight bacterial culture (10^8 CFU ml^{-1}) was placed and spread on one-half Petri dish containing nutrient agar medium. Regarding the nasturtium plant, the fresh plant was ground and placed in half Petri dish then sterile distilled water at a rate1:2 (w/v) was added to the meal to induce releasing the isothiocyanates (ITCs). 10-mm disk four-days-old pure culture of pathogenic fungi was placed at the center of another half Petri dish containing PDA. Both half plates were placed face to face after placing tap of Whatman filter paper No. 1 that was soaked in 2% sodium carbonate in 0.5% picric acid solution, between two halves plate, then plates were sealed and wrapped with parafilm to isolate the inside atmosphere and to prevent loss of volatiles formed.

Plates were incubated at 22°C for 3-7 days, the growth of the fungal pathogen was measured and compared to control developed in the absence of antagonist, results are expressed as means % inhibition of the growth for pathogenic fungi in the presence of tested bacterial isolates.

Biocontrol assays under greenhouse conditions

A greenhouse trial was conducted to evaluate the efficiency of biofumigation using cyanogenic bacterial strains and nasturtium plants, individually or in combinations, on the incidence of onion white rot disease. The experiment was carried out in pots using onion seedlings (Giza- 20 c.v.) during the 2019 season at the Faculty of Agriculture Experiment Station, Fac. of Agric., Benha Univ., Egypt. Sterilized plastic pots (30 cm in diameter) were filled with sterilized soil (clay soil, pH 8.02, organic matter 30.1 g. Kg^{-1}, bulk density 1.38 g cm-3, field capacity 52.3%, wilting point 16.95%). Pots and soil were sterilized by using 5% formaldehyde solution then left two weeks until dry to remove formaldehyde before use. Except for control, pots were infested with *S. cepivorum* inoculum.

The fungal inoculum was mixed with the soil of each pot at the rate of 20g/kg soil. The infested soil was watered for two weeks. Before transplanting, the infested pots were divided into two groups one of them treated with chopped nasturtium plants at the rate of 100 g fresh weight pot^{-1} and left two weeks before transplanting after covering with a plastic sheet. The seedlings in all bacterial treatments soaked in cells suspension of bacterial inocula. Two seedlings were transplanted in each pot and five replicates for each treatment were used. Pots were organized in a completely randomized plot design (Table 1). Onion plants were weakly irrigated and the boost doses of bacterial inocula were added at 30 and 60 DAT. Chemical fertilizers were supplemented to all treatments with the recommended dose (4 g N pot^{-1} as ammonium sulfate (20.5% N), 3 g P_2O_5 pot^{-1} as superphosphate (15.5% P_2O_5) and 1.5 g K_2O pot^{-1} as potassium sulfate (48% K_2O).

Table1. Pot experiment treatments.

Cod	Treatments
T1	Non-infested soil (control)
T2	Soil infested with *S. cepivorum*
T3	Infested soil + *G. diazotrophicus*
T4	Infested soil + *P. polymyxa*
T5	Infested soil + *P. polymyxa* + *G. diazotrophicus*
T6	Infested soil +nasturtium
T7	Infested soil + nasturtium + *G. diazotrophicus*
T8	Infested soil + nasturtium + *P. polymyxa*
T9	Infested soil + nasturtium+ *P. polymyxa* + *G. diazotrophicus*

Biocontrol assays under field conditions

In 2019-2020 experimental plots were carried out in clay soil naturally infested with *S. cepivorum* located at an especial farm in Qaluiobia Governorate (30°12′18.7″N31°12′49.2″E). The soil was chosen to estimate the efficiency of biofumigation using nasturtium plants and cyanogenic bacteria in reducing onion white rot under field conditions. The more effective treatments that were obtained in the greenhouse were used. The experimental design was a randomized complete plot with three replicates. The experimental unit area was 10.5 m^2 (3.5 × 3 m) and included four rows. Before starting an experiment, the plots that would be treated with biofumigation planted with nasturtium seeds in mid-August. In mid-December (flowering stage), nasturtium plants have been chopped up and incorporated in the soil and covering with plastic sheets. After two weeks, the onion was transplanted at a rate of 70 seedlings per row on two sides. Before the seedling's transplantation, half number of onion seedlings were soaked by dipping the root system and blubs in a mixture of cell suspensions of each *G. diazotrophicus* and *P. polymyxa* GQ375783.1 (equal volumes of each) and 10% Arabic gum as an adhesive agent for 30 min. The onion seedlings were transplanted into the field according to the following treatments: Untreated soil (control), soil treated with nasturtium plants, soil inoculated with *P. polymyxa* + *G. diazotrophicus*, and soil treated with nasturtium plants and inoculated with *P. polymyxa* + *G. diazotrophicus.* NPK mineral fertilizers were applied at the recommended dose by the Ministry of Agriculture and Land Reclamation.

Disease assessment

Disease incidence was calculated as the percentage of dead onion plants due to white rot, and the percentage of healthy plants were recorded 50 DAT. The percentage of disease incidence (DI) was calculated using the following formula:

DI = (No. of dead plants /Total sowing seedlings) ×100.

The percentage of efficiency was calculated as follows:

% Efficiency = DI in control treatment – DI in different treatments / DI in control × 100

Measurements of microbial enzymatic activities

The microbial enzymatic activities were determined in the rhizosphere at 30, 60 and 120 DAT. The activity of dehydrogenase (DH) was measured using a spectrophotometer (SCO-Tech, SPUV-19, Germany) at 464 nm as described by Schinner et al. (1996). However, nitrogenase (N2-ase) activity was measured by the technique given by Diloworth (1970). The acetylene reduction activity was measured using Gas-liquid chromatography GLC.

Determination of defense enzymes activities

The effect of the biofumigation using nasturtium plants and cyanogenic bacteria on the activities of the defense enzymes peroxidase and polyphenoloxidase in onion plants grown under field conditions were estimated at 45 DAT according to the method described by Lee (1973), and Bashan et al. (1985), respectively.

Effect of treatments on growth traits and onion yield

Growth characters of onion including the number of leaves and plant height dry weight were recorded after 70 days of transplanting. At harvesting time, total onion yield, bulb diameter and total soluble solids (TSS) were calculated.

Statistical analysis

Analysis of variance (ANOVA) was performed using CoStat version 6.4 (CoHort software, Monterey, CA, 93940, USA). Mean values among treatments were compared by the Duncan test at 5% level (p = 0.05) of significance.

RESULTS AND DISCUSSION

Inhibitory effect of *Sclerotium cepivorum* by cyanogenic bacteria *in vitro*

In the dual culture test, six of eight cyanogenic bacteria were able to reduce the growth of *S. cepivorum*. Significant differences in inhibition percentage of *S. cepivorum* were observed for the investigated cyanogenic bacteria (Table 2). The strain *G. diazotrophicus* showed the highest inhibition percentage followed by *P. polymyxa* strain. However, *Gluconoacetobater* sp RS6 isolate gave the lowest value.

Data presented in Table 2 and illustrated in Fig 1 showed that *G. diazotrophicus* and *P. polymyxa* were the most effective bacteria that recorded the highest inhibition of mycelia growth by 75% and 70%, respectively. So, two strains were chosen and tested for inhibition of fungal growth by HCN production (Fig 2). Data in Fig 2 showed that the two strains were able to produce HCN compound which inhibited the phytopathogenic fungi growth by 100%.

Moreover, the ground tissues of the nasturtium plant were able to inhibit the mycelium growth of *S. cepivorum* by 100%. Many reports emphasized the activity of aqueous extracts of the glucosinolate-containing plant against pathogens or others. Aqueous extracts of mustard (*B. campestris*) inhibited *Verticillium chlamydosporium* (Owino *et al,* 1993). Studies of pathogen inhibition by glucosinolate-derived allelochemicals have also been described for soils. Cabbage vapors passed through soil columns reduced *Rhizoctonia solani* contained within that soil (Lewis and Papavizas 1974). Volatiles emanating from cabbage-amended soil that were passed through tubes containing *Pythium ultimum* and *Sclerotium rolfsii* cultures inhibited growth of the cultures, especially when warmed to 38°C (Gamliel and Stapleton 1993).

Table 2. Growth inhibition percentage of *S. cepivorum* by cyanogenic bacteria by dual culture technique.

Strains	Inhibition zone (mm)	Inhibition %
Gluconoacetobater sp RS1	0.0g	Nilf
Gluconoacetobater sp RS2	0.0g	Nilf
Paenibacillus polymyxa	10.1b	70b
Gluconoacetobater diazotrophicus	10.2a	75a
Gluconoacetobater sp RS3	7.0c	62.5c
Gluconoacetobater sp RS4	5.0e	62.5c
Gluconoacetobater sp RS5	6.0d	55.0d
Gluconoacetobater sp RS6	4.0f	42.5e

The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

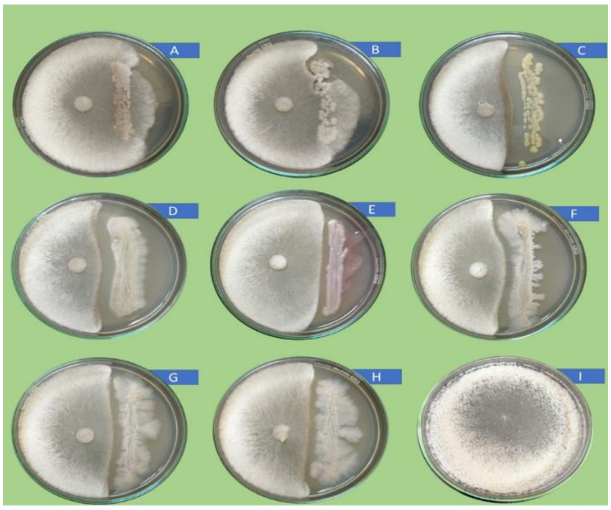

Fig .1. Growth inhibition percentage of *S. cepivorum* by cyanogenic bacteria by dual culture technique.

A: *Gluconoacetobater* sp RS1, B: *Gluconoacetobater* sp RS2, C: *P. polymyxa* GQ375783.1, D: *Gluconoacetobater diazotrophicus*, E: *Gluconoacetobater* sp RS3, F: *Gluconoacetobater* sp RS4, G: *Gluconoacetobater* sp RS5, H: *Gluconoacetobater* sp RS6 and I: *S. cepivorum* (control)

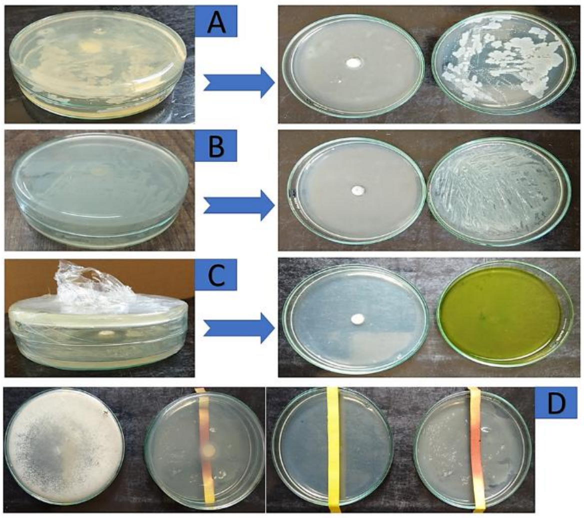

Fig .2. Effect of volatiles HCN compounds from two cyanogenic strains and ground tissues of nasturtium on mycelial growth of *S. cepivorum* (left column).

Efficacy of biofumigation in greenhouse

Greenhouse trial was carried out to evaluate the efficiency of biofumigation using cyanogenic bacterial strains and nasturtium plant, individually or in combinations on the incidence of onion white rot disease, vegetative growth, yield and yield quality.

Effect of biofumigation treatments on disease incidence percentage

Results in Table 3 indicated that disease incidence was significantly reduced in all treatments in comparison with the control. No significant differences were found between the efficiency values exhibited by individual treatment of bacterial strains (*P. polymyxa* GQ375783.1 and *G. diazotrophicus*). However, the soil inoculation with either *P. polymyxa* GQ375783.1 or *G. diazotrophicus* gave the lower percentage of disease incidence than soil amended with nasturtium plant.

The combination of two bacterial strains *P. polymyxa* GQ375783.1 and *G. diazotrophicus* resulted in increasing the disease reduction efficiency comparing with the individual application of bacterial strains. The decrement of incidence percentage in the treatments that inoculated with cyanogenic bacteria may be due to volatile and non-volatile antifungal compounds released by several species of cyanobacterial that influence the surrounding environment and are supposed to contribute to the biological suppression of soil-borne plant pathogens, (Jayaprakashvel *et al*, 2010). among these compounds the hydrogen cyanide (HCN). Until recently, HCN was the only volatile molecule known to be active against pathogenic fungi (Weisskopf, 2013).

Furthermore, data showed that using two types of biofumigation (nasturtium plants and cyanogenic bacteria) have a great effect on disease incidence reduction. This may be due to the activation relationship for each of them to hydrogen cyanide production, which inhibits the fungus. Biofumigation refers to the suppression of soil-borne pathogens by volatile biocidal compounds, mainly isothiocyanates, this happened when the glucosinolates in glucosinolate-containing plants are hydrolyzed during the breakdown in the soil (Noble *et al*, 2002; Harvey *et al*, 2002 and Kirkegaard *et al*, 2006). Moreover, in the present investigation, nasturtium tissues extract and cyanogenic bacterial strains proved to be effective for controlling the causal pathogens of white-rot under laboratory conditions. They had resulted in the suppression of fungal growth of the tested fungi as compared with the control.

White rot incidence percentage in onion that cultivated in soil amended with nasturtium plant and inoculated with *G. diazotrophicus* was lower than that amended with nasturtium plant plus *P. polymyxa* GQ375783.1. The lowest value of disease incidence was found when the soil was amended with nasturtium plant and inoculated with both investigated bacterial strains.

Effect of biofumigation treatments on onion vegetative growth and yield

Obtained results recorded in Table 4 emphasized the adverse relationship between the white-rot incidence and the vegetative growth & final yield. So, all biofumigation treatments increased onion vegetative characteristics and yield in comparison with infested control.

Table 3. Effect of biofumigation using nasturtium plants and cyanogenic bacteria on *Sclerotium cepivorum* infection under greenhouse conditions.

Treatments	Disease incidence %	Efficiency
Non-infested soil (healthy control)	0.0g	100
Soil infested with *S. cepivorum*	85.3a	-
Infested soil + *G. diazotrophicus*	26.9c	68.5
Infested soil + *P. polymyxa*	28.5bc	66.6
Infested soil + *P. polymyxa*+ *G. diazotrophicus*	22.2d	74.0
Infested soil +nasturtium	31.4b	63.2
Infested soil + nasturtium + *G. diazotrophicus*	15.3e	82.1
Infested soil + nasturtium + *P. polymyxa*	17.8e	79.1
Infested soil + nasturtium+ *P. polymyxa*+ *G. diazotrophicus*	11.2f	86.9

The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

The highest values of onion vegetative characters, yield, and yield quality were obtained when infested soil was biofumigated by nasturtium plant combined with both bacterial strains. Concerning, the TSS % and bulb diameter, data showed increasing the TSS % and bulb diameter by inoculation with cyanogenic bacteria than uninoculated ones. Moreover, soil biofumigation by nasturtium plant combined with either *G. diazotrophicus* or *P. polymyxa* GQ375783.1 exhibited higher values of vegetative growth characters and yield than using each one individually with superiority for inoculation with *G. diazotrophicus* than *P. polymyxa* GQ375783.1.

Many reports emphasized the positive effect of biofumigant crops in enhancing crops growth and productivity and it can summarize as follow, use of biofumigant crops can have benefits in addition to disease suppression such as a potential increase in organic matter, better soil structure and nutrient release, all of which may increase plant vigor and growth, hence indirectly reducing the impact of soilborne plant pathogens. (Jaffee *et al.,* 1998 and Fayzalla *et al*, 2009).

Table 4. Effect of biofumigation using nasturtium plants and cyanogenic bacteria on onion vegetative growth and yield under greenhouse conditions.

Treatments	Vegetative characters			Yield and quality		
	Leaves no./plant	Plant height (cm)	Plant dry weight (g)	Bulb weight/pot (g)	Bulb diameter (cm)	TSS (%)
Non-infested soil (healthy control)	8.2c	42de	21.5c	187.6d	7.3b	11.2b
Soil infested with *S. cepivorum*	4.5f	29f	9.5e	61.6h	5.1d	9.0d
Infested soil + *G. diazotrophicus*	6.7de	43cde	19.0d	162.4f	7.3b	10.9b
Infested soil + *P. polymyxa*	7.1d	45bcd	20.0d	169.4e	7.0bc	10.8b
Infested soil + *P. polymyxa*+ *G. diazotrophicus*	8.7bc	47ab	24.0b	194.6c	7.6ab	11.8a
Infested soil +nasturtium	6.2e	40e	19.5d	155.4g	6.4c	10.2c
Infested soil + nasturtium + *G. diazotrophicus*	9.0b	46ab	22. 0c	190.4d	7.3b	11.2b
Infested soil + nasturtium + *P. polymyxa*	9.3b	47ab	24.5b	203b	7.6ab	11.7a
Infested soil + nasturtium+ *P. polymyxa*+ *G. diazotrophicus*	10.0a	50a	27.5a	238a	8.3a	12.0a

The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

Moreover, the indirect effect of cyanogenic bacteria in promoting plant growth. *G. diazotrophicus* and *P. polymyxa* GQ375783.1 are a nitrogen-fixing bacteria that were found to occur in the roots, stems, leaves (Li and MacRae 1991; Reis *et al*, 1994 and Abdel-Rahman *et al*, 2021a), rhizosphere soil, and is considered as an obligate endophyte (Tejera *et al,* 2003) It can excrete about half of its fixed nitrogen in a form that plants can use. It has also been reported that besides N_2 fixation, *G. diazotrophicus* and *P. polymyxa* GQ375783.1 produced indole acetic acid. Furthermore, they have been reported its ability to solubilize inorganic phosphates in the soil and make available P for the inoculated crops and enhancing their growth and productivity (Hemlata *et al,* 2010 and Abdel-Rahman *et al*, 2021a).

Efficacy of biofumigation treatments against onion white rot pathogen in field

The ability of biofumigation using nasturtium or cyanogenic bacteria alone or in combinations, to control *S. cepivorum* was evaluated under field conditions. Results in Table 5 indicated that disease incidence was significantly reduced in all treatments in comparison with the control. No significant differences were found between the efficiency values exhibited by biofumigation using nasturtium and biofumigation using two cyanogenic strains. However, the soil treated with cyanogenic bacteria gave lower values of disease incidence percentage as compared with the soil amended with nasturtium plant. The highest value of disease incidence percentage showed in untreated soil (control), while the lowest value was in the soil treated with both cyanogenic bacterial strains and nasturtium plant.

The decrement of disease incidence in the soil treated with nasturtium plant and cyanogenic bacteria observe the integrated effect of two types of biofumigation in controlling onion white-rot disease. These results are in agreement with those obtained by the current study regarding the ability of cyanogenic strains to inhibit *S. cepivorum* growth in dual culture technique (Table 2 and Fig 2). There have been positive reports on the suppressive effect of biofumigated crops on soil-borne phytopathogens, such as *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii*, *Fusarium* spp., and *Phytophthora* spp. The growth of these agents has been reported to decrease in vitro, and the incidence of diseases that cause in the investigated host species has also been reported to decrease (Rubayet *et al*, 2018; Campanella *et al*, 2020).

Also, Fayzalla *et al*, (2009) indicated that glucosinolate-containing plants such as mustard seed meal reduced the disease incidence over the control by 69.7% four months after planting. Moreover, biofumigant crops act as break crops, disrupting the life cycle of pests and diseases. Suppression may result from direct biocidal toxicity as well as indirectly through changes in the soil fauna and microbial community (Murumkar *et al*, 2017). For example, *Pseudomonas* strains that are known to have beneficial properties in controlling disease were observed to be favored due to biofumigation. They act as antagonists against soil-borne pathogenic fungi (De Corato, 2020).

Therefore, a biofumigation-induced increase in plant-growth-promoting and disease-suppressing bacteria seems to be an important mechanism in biofumigation efficiency (Palaniyandi *et al*, 2013).

It worth mentioning that soil treated with two methods of biofumigation (nasturtium plant and cyanogenic bacteria) led to suppress white rot disease by the rate of 100% while using each one individually reduced disease by the rate of 93.7 and 94.2 % respectively.

Table 5. Effect of biofumigation treatments on onion disease incidence under field conditions.

Treatments	Disease incidence (%)	Efficiency
Untreated soil (control)	38.0a	-
Soil treated with nasturtium	2.4b	93.7
Soil inoculated with cyano-B	2.2b	94.2
Soil treated with nasturtium and cyano-B	0.0c	100

cyano-B: cyanogenic bacteria (*G. diazotrophicus* and *P. polymyxa* GQ375783.1), The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

Effects on microbial enzymes activity

Soil enzymes activity has been suggested to be an index of soil fertility as well as it has been used as an accurate fertility index. Accordingly, the dehydrogenase and nitrogenase were estimated to evaluate the effect of biofumigation on soil microbial community and activity. Periodically changes of dehydrogenase and nitrogenase during the experiment were tabulated in Table 6. Data revealed that all treatments have affected the two enzymes activity compared to control. All onion treatments that inoculated with cyanogenic bacteria showed higher records of DH and N_2-ase activity as compared with uninoculated ones. Also, the soil amended with chopped nasturtium plant showed a positive effect on the two enzymes activity especially after 60 and 120 DAT compared with untreated soil (control). A high activity of DH and N_2-ase may be due to the glucosinolate-containing crops enhanced the population of beneficial microorganisms in the soil of both celery and onion fields. At the same time, the population of plant-pathogen decrease (Wang *et al*, 2009).

The highest values of DH and N_2-ase were recorded in soil amended with nasturtium and inoculated with cyanogenic bacterial strains. Nevertheless, the lowest values of two microbial enzymes activity were recorded with control. The decrement of microbial enzymes activity in case of control treatment and increasing their activity in case of biofumigation treatment reflect the role of inoculation with cyanogenic bacterial strains and biofumigation crops on enhancing microbial population and their activities in the plant rhizosphere. At the same time, their role in increasing the growth of the crops (Murumkar *et al*, 2017). Addionlly, Srivastava & Ghatak (2017) stated that populations of beneficial microorganisms, including mycorrhizal fungi, have been found to increase after biofumigant crops.

It is noted through the data in Table 6, the activity of DH was gradually increased from initial up to 120 DAT while the activity of N_2-ase gradually increased from initial up to 60 DAT and decreased again at 120 DAT. Accordingly, the highest value of DH activity was recorded at 120 DAT with the soil amended with nasturtium and inoculated with the two bacterial strains. On the other hand, the highest value of N_2-ase was recorded with the same treatment at 60 DAT.

Table 6. Effect of biofumigation treatments on microbial enzymes activity in onion soil rhizosphere under field conditions.

Treatments	Dehydrogenase (µg TPF.g^{-1} dry soil. $24h^{-1}$)				Nitrogenase (µL $C_2H_4.g^{-1}$ dry soil.h^{-1})			
	Initial	30	60	120 DAT	Initial	30	60	120 DAT
Untreated soil (control)	32.5a	38.2d	40.2c	41.5d	18.2b	30.2d	41.5d	39.5d
Soil treated with nasturtium	22.1b	40.2c	44.5b	48.2c	12.3d	35.8c	45.7c	40.1c
Soil inoculated with cyano-B	34.1a	50.6b	57.8a	60.3b	20.1a	44.5b	57.2b	48.7b
Soil treated with nasturtium and cyano-B	23.5b	52.3a	59.3a	65.1a	13.4c	46.7a	59.4a	50.6a

cyano-B: cyanogenic bacteria (*G. diazotrophicus* and *P. polymyxa* GQ375783.1), The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

It is important to mention that the activity of the microbial enzyme in all soil treatments amended with nasturtium recorded lower values from the initial experiment than untreated ones. This result may be due to biofumigation can alter the soil microbial community.

Moreover, Yim *et al*, (2016) reported that four weeks after biofumigation in field experiments, there was a shift in soil bacterial community and even more so in fungal community composition: some strains vanished while other strains were promoted due to biofumigation. In general, GLS hydrolysis rapidly occurs, and ITCs and other hydrolysis products generally have a short lifespan in the soil, with a rapid decrease in their concentration within a few days and a mean soil persistence of up to 14 days (Kirkegaard, 2009). However, residues with high GSL levels can inhibit the growth of microorganisms for two weeks after use (Marschner & Rengel, 2010).

Effects on defense enzymes

Data in Table 7 showed that a significant increase in the induction of defense enzymes (peroxidase, and polyphenoloxidase) was recorded with all biofumigation treatments compared to the control. The inoculation with *G. diazotrophicus* and *P. polymyxa* GQ375783.1 induced higher activities of peroxidase, and polyphenoloxidase by increasing 31.4 and 54.9 %, respectively, over the control. Moreover, soil amendment with nasturtium plants and onion inoculation with the cyanogenic bacterial strains led to more increasing of peroxidase, and polyphenoloxidase by 39.4 and 61.5 %, respectively, over the control. This result clears the role of microbial inoculation in induced-resistance mechanism that explains the indirect control of biocontrol agents to suppress phytopathogens in plants. The obtained result is congruent with Abdel-Rahman *et al*, (2021b) who reported that the highest value of peroxidase and polyphenoloxidase activity of green onion plant were recorded with plants inoculated with the combination of plant growth-promoting bacteria, *Bacillus subtilis,* and *Pseudomonas fluorescens*. Moreover, Govindasamy *et al*, (2010) stated that multiple species of *Bacillus* and *Paenibacillus* affect the crop growth and its health by three different ecological mechanisms viz, promotion of host plant nutrition and growth, antagonism against fungal, bacterial, nematode pathogens and insect pests, and stimulation of host defense mechanisms. Specific strains of both *Bacillus* spp. and *Paenibacillus* spp. are known to elicit induced systemic resistance similar to that of *Pseudomonas* spp. which leads to the stimulation of host defense mechanisms against multiple pathogens on diverse crop plants.

Table 7. Effect of biofumigation treatments on onion defense enzymes under field conditions.

Treatments	Peroxidase	Polyphenoloxidase
Untreated soil (control)	0.923d	0.345d
Soil treated with nasturtium	1.012c	0.425c
Soil inoculated with cyano-B	1.345b	0.765b
Soil treated with nasturtium and cyano-B	1.524a	0.897a

cyano-B: cyanogenic bacteria (*G. diazotrophicus* and *P. polymyxa* GQ375783.1), The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

In the current study, the reduction of onion white rot disease incidence due to inoculation with *G. diazotrophicus* and *P. polymyxa* GQ375783.1 may be due to an increase in the defense-related enzymes such as peroxidase and polyphenoloxidase. The defense enzymes play an important role in induced resistance by incorporating toxic products into plant cell walls, which reduce fungal activity. For example, the oxidation of phenols to oxidized toxic products, incorporation of phenolic compounds, and lignification of plant cell walls (Małolepsza *et al*, 2017). Meanwhile, the ability of those strains to produce CN compounds destroyed the cell of the pathogen. CN compounds play an important role in the biofumigation process. Moreover, they able to produce many inhibitory substances such as siderophores

Peroxidase and polyphenoloxidase activity significantly increased in onions cultivated in soil inoculated with *G. diazotrophicus* and *P. polymyxa* GQ375783.1 and amended with nasturtium plants than that inoculated with *G. diazotrophicus* and *P. polymyxa* GQ375783.1 solely. The highest values of peroxidase and polyphenoloxidase activity showed in onions treated with two types of biofumigation treatments.

Effects on onion NPK content

Nitrogen, phosphorus, and potassium play a key role in plant nutrition and productivity. Data in Table (8) showed that a significant increase in N, P and K percentage was recorded with all biofumigation treatments compared to the control. Moreover, data indicated that significant increases were found in nitrogen, phosphorus, and potassium percentage of onion in soil inoculated with investigated cyanogenic bacterial strains compared with uninoculated ones. The increment of NPK percentage in inoculated plants may be due to the positive role of inoculation with PGPR strains in improving the availability and absorption of these nutrients. Many reports emphasize that *G. diazotrophicus* and *Paenibacillus* spp. promote plant growth directly by providing nitrogen to the host plant. They also solubilize insoluble phosphates and other minerals in soil by various mechanisms (Murumkar *et al*, 2017 and Govindasamy *et al*, 2010).

Table 8. Effect of biofumigation treatments on onion NPK content under field conditions.

Treatments	Mineral content (%)		
	N	K	P
Untreated soil (control)	3.3c	2.3c	0.44b
Soil treated with nasturtium	3.5bc	2.6b	0.48a
Soil inoculated with cyano-B	3.7ab	2.7b	0.50a
Soil treated with nasturtium and cyano-B	3.9a	3.1a	0.54a

cyano-B: cyanogenic bacteria (*G. diazotrophicus* and *P. polymyxa* GQ375783.1), The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

Additionally, the higher values of NPK percentage were shown in onions that were treated with nasturtium plants and cyanogenic bacterial strains than each one individually. The highest value of NPK percentage of onion was recorded with plants inoculated with cyanogenic bacterial strains in soil amended with nasturtium plants. These results observe other benefits of the biofumigation process that may be obtained by using glucosinolate-containing plants in pest control strategies. Organic matter is replenished in the soil after incorporation of the biofumigant crop. As microorganisms break down organic matter, they produce sticky substances that bind soil particles together into soil aggregates. This in turn, improves nutrient holding capacity, overall biological activity and organic matter also buffer against changes in pH. Better biological activity can lead to improve nutrient cycling and crop nutrient uptake. (Srivastava and Ghatak, 2017). Moreover, *Brassica napus* is efficient at absorbing phosphorus from deficient soils (Grinsted *et al*. 1982).

The increment of NPK percentage in inoculated onion may be due to the enhancement of various bacterial inoculants in increase activity of some soil enzymes (as shown in Table 6) as well as improve soil nutrition. Similar results were obtained by Abdel-Rahman *et al*, (2021b).

Effects on onion vegetative growth, yield and yield quality

From the above-mentioned results, the effect of biofumigation treatments on reducing the incidence of white rot disease and increasing the activity of microbial enzymes in onion rhizosphere is evident as well as increasing the percentage of NPK for plants, which was reflected on onion vegetative growth and yield. Data in Table 9 showed significant increases in leaves number, plant height, and dry weight of onion plants in all biofumigation treatments compared with control. Moreover, all biofumigation treatments have a positive effect on plants vegetative growth as well as their yield. The highest value of all estimated vegetative growth characters and yield were recorded in plants inoculated with *G.* diazotrophicus and *P. polymyxa* GQ375783.1 in soil amended with chopped nasturtium plants. While, the lowest values of leaves number, plant height, dry weight, and yield of onion plants were found at control treatment. It is not a surprising result that inoculation of onion plants with cyanogenic bacterial strain gave higher values of all estimated vegetative growth characters than uninoculated ones (Fig 3). This result is in agreement with Samayoa *et al*, (2020) who reported that PGPRs are beneficial microbes that increase the growth and yield of onion (*Allium cepa* Linn.).

Table 9. Effect of biofumigation treatments on onion vegetative growth, yield and yield quality under field conditions.

Treatment	Vegetative growth			Yield and quality		
	Leaves no. /plant	Plant height (cm)	Plant dry weight (g)	Average bulb weight (g)	Bulb yield (Ton/fed.)	TSS (%)
Untreated soil (control)	7.4c	45.6c	17.8d	143d	7.8b	11.3b
Soil treated with nasturtium	9.0b	47.5bc	19.2c	149c	10.4ab	11.4b
Soil inoculated with cyano-B	9.7ab	50.8b	22.4b	156b	11.2ab	12.0b
Soil treated with nasturtium and cyano-B	10.7a	55.4a	26.8a	190a	12.3a	13.0a

cyano-B: cyanogenic bacteria (*G. diazotrophicus* and *P. polymyxa* GQ375783.1), The same letter within a column is not significantly different at P= 0.05 when compared by Duncan test

Moreover, Mahaffee and Kloepper (1997) and Seldin *et al*, (1998) stated that species of *Bacillus* and *Paenibacillus* can promote plant growth and health in a variety of ways. Some species can promote plant growth directly by synthesizing plant hormones or increasing mineral nutrient uptake by fixing atmospheric nitrogen, solubilization of soil phosphorus, and other methods. Some populations suppress plant pathogens by producing antibiotic metabolites, while others stimulate plant host defenses before pathogen infection (Govindasamy *et al*, 2008), which indirectly contributes to increased crop productivity. Concerning, the role of biofumigated plants in enhancing onion growth and productivity, Jaffee *et al*, (1998) reported that the use of biofumigant crops can have additional benefits in addition to isothiocyanate-based disease suppression by increasing organic matter, better soil structure, and nutrient release, all of which may increase plant growth and productivity.

Regarding the TSS % of onion bulbs data in Table 9 indicated that there was no significant differences between all treatments except bulbs of plants that inoculated with cyanogenic strains in soil amended with biofumigated plant. This treatment gave the highest value of TSS% with a significant difference compared to all treatments.

Fig .3. Effect of biofumigation treatments on onion growth,

A, B: onion in soil treated with nasturtium plants and cyanogenic bacteria, C: onion in soil treated with cyanogenic bacteria, D: onion in soil treated with nasturtium, E: onion in untreated soil (control), F: disease area from control treatment, G: onion plant with a typical symptom of white rot, H: the focus of G to show sclerotia of the pathogen on blub.

CONCLUSION AND RECOMMENDATION

According to our knowledge, there are no studies with onion white rot disease that deliberate the combination between biofumigation using glucosinolate-containing plants and cyanogenic bacteria what makes this research study a significant contribution in this field. Laboratory, greenhouse, and field experiments emphasized that the strains of *G. diazotrophicus* and *P. polymyxa* recorded the highest inhibition of *S. cepivorum* mycelial growth. The ground tissues of the nasturtium plant were able to inhibit the mycelium growth of *S. cepivorum* by 100%. The highest values of onion vegetative characters, yield, and yield quality were obtained when soil was biofumigated by nasturtium plant combined with both bacterial strains.

It can be recommended that biofumigation treatments with cyanogenic bacteria *G. diazotrophicus* and *P. polymyxa* GQ375783.1 in soil amended with chopped nasturtium plants can be effective tool for suppression onion white rot disease caused by *S. cepivorum* .

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اخماد فطر الاسكيرولشيم سيبوفورم باستخدام التبخير الحيوي بالنباتات المحتوية على الجلوكوسينولات والبكتيريا المنتجة لسيانيد الهيدروجين

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تم اجراء مجموعة من الاختبارات في المعمل والصوبة والحقل لدراسة تأثير التبخير الحيوي باستخدام البكتيريا المنتجة لسيانيد الهيدروجين ونبات ابوخنجر. ففي اختبار التضاد، تمكنت ستة من أصل ثمان بكتيريات منتجة لسيانيد الهيدروجين من تقليل نمو الفطر المسبب لمرض العفن الأبيض في البصل *S. cepivorum.* . كما أظهرت النتائج أن السلالات *Glocoacetobacter diazotrophicus* و GQ375783.1 *Paenibacillus. polymyxa* كانت الأكثر فاعلية في تثبيط النمو الفطري بنسبة تثبيط 75٪ و 70٪ على التوالي . وعند اختبار السلالتين بناءً على إنتاج سيانيد الهيدروجين، فقد سجلت تثبيط كامل لنمو فطر *S. cepivorum*. علاوة على ذلك ، كان لمستخلص انسجة نبات أبو خنجر القدرة على تثبيط النمو الفطري بنسبة 100 ٪. أكدت نتائج تجربة الصوبة أن نسبة الإصابة بالعفن الأبيض سجلت أدنى مستوياتها في البصل المزروع في التربة المعاملة بنبات ابوخنجر والملقحة بسلالتي البكتيريا *G. diazotrophicus* و *P. polymyxa* GQ375783.1. كما سجلت هذه المعاملة أعلى قيم للنمو الخضري لنبات البصل وإنتاجيته. وتحت ظروف الحقل ، تجدر الإشارة إلى أن معالجة التربة بطريقتين من طرق التبخير الحيوي (نبات ابوخنجر والبكتيريا المنتجة لسيانيد الهيدروجين) أدت إلى خفض نسبة الاصابة بمرض العفن الأبيض بنسبة 100٪ كما تجدر الأشارة الي انه أثناء استخدام كل طريقة على حدة ، فإن معدل الاصابة بالمرض قد انخفض بنسبة 93.7 و 94.2 ٪ على التوالى. أظهرت جميع معاملات البصل التي تم تلقيحها بالبكتريا المنتجة لسيانيد الهيدروجين أعلى نشاط لإنزيم الديهيدروجينيز والنيتروجينيز مقارنةً بتلك غير الملقحة. كما تم تسجيل أعلى قيم لنشاط الانزيمين مع التربة المعاملة بنبات ابوخنجر والتي تم تلقيحها بسلالات البكتريا المنتجة لسيانيد الهيدروجين. بالإضافة إلى ذلك ، أظهرت هذه المعاملة الأخيرة أعلى قيم نشاط البيروكسيديز والبولي فينول أوكسيديز ، ومحتوى البصل من النيتروجين والفوسفوروالبوتاسيوم ، والنمو الخضري للبصل بالإضافة إلى زيادة المحصول. لذلك ، يمكن التوصية بهذه المعاملة في مكافحة مرض العفن الأبيض في البصل وزيادة إنتاجيته.