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Research Paper

Synergy between endophytic *Bacillus amyloliquefaciens* GGA and arbuscular mycorrhizal fungi induces plant defense responses against white rot of garlic and improves host plant growth

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Summary. White rot, caused by *Sclerotium cepivorum*, is a serious and economically important disease of garlic, which leads to losses in the garlic production in most of the tropical, subtropical and temperate areas. Biocontrol potential of an endophytic *Bacillus amyloliquefaciens* GGA and/or arbuscular mycorrhizal fungi (AMF) against this disease was investigated. The *B. amyloliquefaciens* GGA exhibited antagonistic activity against *S. cepivorum in vitro*. Scanning electron microscopy revealed alterations in the morphology of the pathogen in response to the exposure to the bacterial metabolites. Results from a pot experiment demonstrated that application of the dual treatment of the *B. amyloliquefaciens* GGA and AMF reduced disease incidence and severity more than the single treatments, and led to the greatest increases in total phenol content, activities of the defense-related enzymes phenylalanine ammonia-lyase, polyphenoloxidase and peroxidase, and transcriptional expression levels of the defensin and chitinase genes. Growth and yield parameters of garlic plants were enhanced after this treatment. This study showed good efficacy on the tested biocontrol agents for control white rot of garlic plants grown in pots. Future research should evaluate these biocontrol strategies under field conditions.

Keywords. *Allium sativum*, chitinase, defensin, electron microscopy, real-time PCR, *Sclerotium cepivorum*.

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INTRODUCTION

Garlic (*Allium sativum* L.), has been widely used since ancient times for multiple cooking and therapeutic purposes (Bayan *et al.*, 2014). In Egypt, this plant is one of the most important vegetable crops for local consumption and export, and Egypt is the sixth most important garlic-producing country. The

area of garlic cultivation in Egypt in 2018 was 12,782 ha, with total production of 286,213 tons (FAOSTAT, 2019).

White rot, caused by the soil-borne fungus *Sclerotium cepivorum* Berk, is one of the most destructive diseases of garlic, and other members of the *Allium* genus. This pathogen affects crop yields leading to yield losses up to 100% (Siyoum and Yesuf, 2013). The pathogen can produce microconidia and overwintering sclerotia. The sclerotia can remain viable in the soil for many years, and can be transmitted to non-infested fields by poor sanitation practices (Amin *et al*., 2014). Fungicides such as tebuconazole, iprodione, and dicloran are available and widely used against white rot, but these may have adverse effects on environments, human and animal health, and remain in soils for long periods (Yang *et al*., 2011).

Biological control using microbial antagonists may provide an effective, eco-friendly, and safe alternative approach to control of garlic white rot. Among promising biocontrol agents, endophytes have received increasing interest. These organisms are defined as symbionts (bacteria or fungi) that asymptomatically inhabit plant tissues for a period of their life cycles (Clay *et al*., 2016; Strobel, 2018). During these relationships, complex plant-endophyte interactions differentially occur according to the type of endophyte, host plant and environmental conditions (De Silva *et al*., 2019). Biocontrol activity of many endophytic bacteria, including *Bacillus* spp., *Burkholderia* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Serratia* spp., has been studied against different plant pathogens (Hong and Park, 2016; de Almeida Lopes *et al*., 2018). Hazarika *et al*., (2019) reported *B. subtilis* SCB-1 as the most potent antagonist among seven endophytic bacteria which were isolated from sugarcane and screened for the antifungal potential against *Alternaria* sp., *Cochliobolus* sp., *Curvularia* sp., *Fusarium* sp., and *Saccharicola* sp. Their antagonistic activity was attributed to production of the antifungal lipopeptide surfactin. In general, the probable biocontrol mechanisms utilized by endophytic bacteria include direct mechanisms such as antibiosis and competition, and/or indirect mechanisms through triggering plant defense responses against invading pathogens. In addition, they may promote plant growth through phytostimulation and/or biofertilization (Santos *et al*., 2018).

Arbuscular mycorrhizal fungi (AMF), are obligate endophytes which can form mutualistic relationships with most terrestrial plants (Spatafora *et al*., 2016). Biocontrol activity of AMF against various plant diseases has been widely reported (Abdel-Fattah *et al*., 2011; El-Sharkawy *et al*., 2018; Aseel *et al*., 2019). Mustafa *et al*. (2017) reported a 78% reduction in the severity of powdery mildew of wheat plants when the plants were colonized with the *Funneliformis mosseae* under controlled conditions. AMF can also enhance plant tolerance to salinity and drought, and improve plant growth and nutrient uptake (Asrar *et al*., 2014).

The present study aimed to: 1) assess the *in vitro* antifungal activity of an endophytic *B. amyloliquefaciens* strain against *S. cepivorum,* the white rot pathogen of garlic; 2) evaluate the biocontrol potential of application of *B. amyloliquefaciens* and/or AMF on the diseased garlic plants under natural conditions; and 3) investigate probable effects of their application on molecular and biochemical host defense responses, and on growth of garlic plants.

MATERIALS AND METHODS

Microorganisms used in this study

A highly pathogenic isolate of *S. cepivorum* (S6) from a garlic plant showing white rot symptoms, was obtained from the Plant Pathology Research Institute, Egypt. Inoculum was prepared by growing this isolate in 500 mL capacity flasks containing sterilized medium composed of sand: sorghum grains (2:1, v/v) for 15 d at 20°C.

In a preliminary trial, nine isolates of endophytic bacteria were obtained from healthy garlic plants, and these were screened for their antifungal activity. A promising isolate with highly antagonistic activity was selected, identified using the 16S rRNA gene as *B. amyloliquefaciens* GGA (NCBI GenBank accession no. MN592674.1), and used in this study. Inoculum of this strain was prepared by culturing in 500 mL capacity flasks containing sterilized nutrient broth at 37°C for 2 d. The bacterial resulting inoculum was adjusted to 2×10^5 CFU mL^{-1}.

The AMF inoculum used in this study was provided by Prof. Gamal M. Ouf, Botany Department, Mansoura University, Egypt. This was a mixture of AMF species (in equal proportions), of *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *Glomus monosporum* Gerd. & Trappe, *Acaulospora laevis* Gerd. & Trappe, and *Rhizoglomus clarum* (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl. Spores of these AMF were propagated under Sudan grass in sterilized soil for 6 months (83% colonization index).

Assessment of the antagonistic in vitro *activity of the endophytic* B. amyloliquefaciens *GGA*

The antifungal activity of *B. amyloliquefaciens* GGA was assessed against *S. cepivorum* S6, using the dual cul-

ture plate technique. A 5 mm diam. disc, taken from 7 d culture of *S. cepivorum* S6 was placed 1 cm from the edge of each potato dextrose agar (PDA) plate, and a loop of *B. amyloliquefaciens* GGA was streaked 1 cm from the opposite edge of the plate. PDA plates each inoculated only with the fungal disc served as experimental controls. The test was performed in triplicate. The plates were incubated at 20°C and the inward linear growth of the pathogen was measured after 4, 8, and 12 d. The test was ended when fungal growth completely covered the control plates. Fungal growth inhibition was calculated using the following equation:

$$\text{Growth Inhibition (\%)} = \frac{R1 - R2}{R1} \times 100$$

where R1= inward linear growth in the control plate, and R2= inward linear growth in the dual culture plate.

Scanning electron microscopy (SEM)

From a dual culture plate, a PDA block (1 cm^2) of mycelial growth at the edge of the growth inhibition zone was transferred and processed for SEM observation, using the tissue processor (Leica Biosystems, Inc.). Sample fixation using osmium oxide, and dehydration by ethanol and acetone were performed before the sample was dried using a critical point drier (EMS 850), and coated with gold using a sputter coater (EMS 550), as described by Hayat (2000). The sample was then examined using a scanning electron microscope (JEOL JSM-6510LV).

Pot experiment

Pots (25 cm diam.) were each filled with 2.5 kg of autoclaved soil (2:1 clay: sand, v/v). The soil physicochemical properties were: pH, 7.8; electrical conductivity, 170 $\mu S.cm^{-1}$; organic matter, 2.11%; available phosphorus, 6.14 $\mu g\ g^{-1}$; and total nitrogen, 0.58%. Three healthy garlic cloves (cv. Sids 40) were surface sterilized using sodium hypochlorite solution (0.05%) for 3 min, rinsed with sterile water, and then planted into each pot. Ten grams of AMF inoculum (≅50 spores and infected roots pieces g^{-1} soil) was added as a seed bed under each garlic clove at the time of planting. Non-mycorrhizal cloves each received equal amount of autoclaved soil to produce the same nutrients without mycorrhizal propagules. The bacterial inoculum was applied by adding 5 mL (2×10^5 CFU mL^{-1}) onto each garlic clove at the planting time. After 4 weeks from the AMF inoculations, soil infestation was achieved by mixing *S.cepivorum* inoculum with the upper layer of the soil in each pot at the rate of 2% (w/w). The fungicide tebuconazole (50% WP) was used as a positive experimental control, and was applied as a clove dressing at the recommended dose (3mL L^{-1} cloves). Pots treated with tap water were used as negative experimental controls. All pots were arranged in a factorial design (split-split plot ($3 \times 6 \times 2$). Three levels of time (30, 60 or 90 d post-inoculation (dpi) with *S. cepivorum*), six treatments (C, F, P, B, B+P and F+P; see below) and two levels of mycorrhizal status (M or NM, see below) were applied. Twelve pots were used as replicates for each treatment. All the pots were kept under natural outdoor conditions (day temperature 25°C, night temperature 20°C, 16 h light period) and watered when necessary.

The treatments applied are summarized as follows:
CNM = untreated control;
CM = treated with AMF;
FNM = treated with tebuconazole fungicide;
FM = treated with tebuconazole and AMF;
PNM = inoculated with *S. cepivorum*;
PM = inoculated with *S. cepivorum* and treated with AMF;
FPNM = inoculated with *S. cepivorum*, and treated with tebuconazole;
FPM = inoculated with *S. cepivorum,* and treated with tebuconazole and AMF;
BNM = treated with the endophytic bacteria;
BM = treated with the endophytic bacteria and AMF;
PBNM = inoculated with *S. cepivorum*, and treated with the endophytic bacteria, and
PBM = inoculated with *S. cepivorum*, and treated with the endophytic bacteria and AMF.

Disease assessments

Four garlic plants from each treatment were assessed for white rot incidence (DI) and severity (DS) at 30, 60, or 90 dpi. DI was calculated using the following equation:

$$\text{DI (\%)} = \frac{\text{Number of infected plants}}{\text{Total number of inoculated plants}} \times 100$$

The garlic bulbs were visually assessed for white rot severity (DS) using a five point severity scale; where 1 = healthy bulb, 2 = 1–10% bulb rot, 3 = 11–25% bulb rot, 4 = 26–50% bulb rot, and 5 > 50% bulb rot (Entwistle, 1990). DS was then calculated using the following equation:

$$DS\ (\%) = \frac{\Sigma(ab)}{5N} \times 100$$

where a = number of diseased plants having the same disease score, b = the disease score, N = total number of assessed plants and 5 = the highest disease score.

Evaluation of the plant growth and yield parameters

Four plants from each treatment were carefully uprooted at 30, 60, or 90 dpi, and were washed under running water to remove soil particles. The plants were then evaluated for shoot and root lengths and dry weights, and number of leaves per plant. Yield parameters (fresh and dry weights of bulbs, bulb and clove diameters, clove length, and number of cloves per bulb) were also assessed at each harvest time. Dry weights were calculated after oven drying of samples at 80°C for 48 h until constant weight.

Estimation of mycorrhizal colonization

Mycorrhizal colonization was estimated in four garlic plants from each treatment at 30, 60, or 90 dpi. Garlic roots were cut into 1 cm pieces and then stained with trypan blue (Phillips and Hayman, 1970). Forty stained root pieces from each treatment were examined using a light microscope (Carl Zeiss) at ×400 magnification, and the colonization level was estimated according to Trouvelot *et al*. (1986) using the mycocalc program (https://www2.dijon.inra.fr/mychintec/Mycocalc-prg/download.html).

Analyses of biochemical parameters in garlic plants

Estimation of the photosynthetic pigments

The photosynthetic pigment contents (chlorophyll *a*, chlorophyll *b*, carotenoids) were estimated (Harborne, 1984) in four leaves of each garlic plant (0.5 g of fresh leaves) for each treatment at 30, 60, or 90 dpi.

Estimation of nutrients content

For each treatment, nutrient contents (N, P, K, Ca, and Mg) were estimated in leaves (0.5g of dry leaves) from four garlic plants at 30, 60, or 90 dpi. Total nitrogen content was determined by the Kjeldahl method (Sadasivam and Manickam 1992). Total phosphorus content was determined as described by Jackson (1958). Total potassium was estimated using a flame photometer (Corning 400), according to Peterburgski (1968). Total magnesium (Mg) and calcium (Ca) were determined using an atomic absorption spectrometer (ZEEnit 700P, Analytik Jena) and the method of Allen (1989).

Estimation of total phenol content

Total phenol content was estimated in four garlic roots (0.5 g of fresh roots) from each treatmentat 30, 60, or 90 dpi, using Folin-Ciocalteu reagent according to the method of Malik and Singh (1980).

Assay of the defense-related enzymes activities

Activities of three plant defense-related enzymes were assessed in four garlic roots (0.5 g of fresh roots) from each treatment at 30, 60, or 90 dpi. Assessments of enzymes activities were carried out as follows: phenylalanine ammonia-lyase (PAL) according to Beaudoin-Eagan and Thorpe (1985), polyphenoloxidase (PPO) according to Galeazzi *et al*. (1981), and peroxidase (POD) according to Maxwell and Bateman (1967).

Quantitative Real-Time PCR (qRT-PCR)

Garlic roots from each treatment were subjected to qRT-PCR at 30 dpi using a TOPreal™ qPCR 2X PreMIX SYBR Green (Enzynomics). The reaction program was performed using a real-time PCR system (Rotor-Gene Q, Qiagen) as follows: one cycle (15 min, 95°C), and 45 cycles (10 sec at 95°C, 15 sec at 60°C, 30 sec at 72 °C). Primers of the chitinase A gene (F, 5'-GCCCATGGAAGGAATCAGTTATGCGCAAAT-3', R, 5'-GCGGATCCCAACGCACTGCAACCGATTAT-3'), and defensin gene (F, 5'-CCAAATGCCTCGTCATCT-3', and R, 5'-ATTAGAGTCAAGCTCAAAAGG-3') were used. The reference gene used in this reaction was β-actine (F, 5'-GTGGGCCGCTCTAGGCACCAA-3', and R, 5'-CTCTTTGATGTCACGCACGATTTC-3') (Saleha, 2010). The data obtained were analyzed using the comparative method (Ct) (Schmittgen and Livak, 2008).

Statistical analyses

All results were analyzed using analysis of variance (ANOVA) and the statistical analysis software CoStat (version 6.4). Comparisons among means were made using the least significant difference (LSD) or Duncan's multiple range test (Duncan 1955).

RESULTS

Dual culture test

Means of *S. cepivorum* S6 growth inhibition achieved for the *B. amyloliquefaciens* GGA are presented in Table 1. Results obtained indicated that the bacterium exhibited strong antagonistic activity against *S. cepivorum* with a mean of 63.8% inhibition after 12 d compared to the control plates. The dual culture test is illustrated in Figure 1, showing the inhibition zone between the two microorganisms.

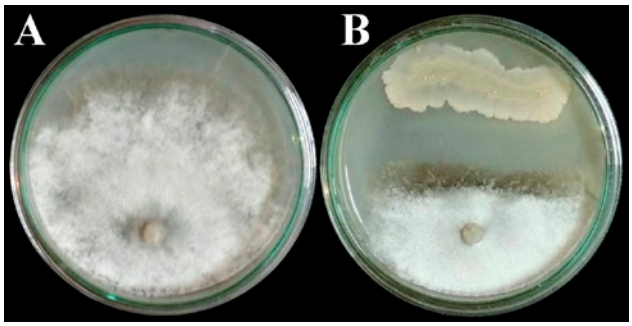

Figure 1. *In vitro* antifungal activity of *Bacillus amyloliquefaciens* GGA against *Sclerotium cepivorum* S6 (8-d old cultures). A, a PDA plate of *S. cepivorum* S6; B, a dual culture plate of *S. cepivorum* S6 and *B. amyloliquefaciens* GGA.

Electron microscopy

The antagonistic effects of *B. amyloliquefaciens* GGA on the morphology of the fungal structures of *S. cepivorum* S6 were examined using SEM to confirm the results of the dual culture test. SEM observations of the fungus from a control plate showed normal spherical sclerotia with intact rough-surfaced external rind layers (Figure 2A), small globose to subglobose rough-walled microconidia on mycelium emerging from the sclerotia (Figure 2B), and typical well-developed branched aerial hyphae (Figure 2C). SEM observations of the fungus from the dual culture plate showed alterations in the morphology of the fungal structures as a response to the exposure to the bacterial metabolites. These included wrinkled sclerotia with depressions in their surfaces and ruptured rinds (Figure 2D), distorted and shrunken microconidia (Figure 2E), and twisted, curled and collapsed hyphae (Figure 2F).

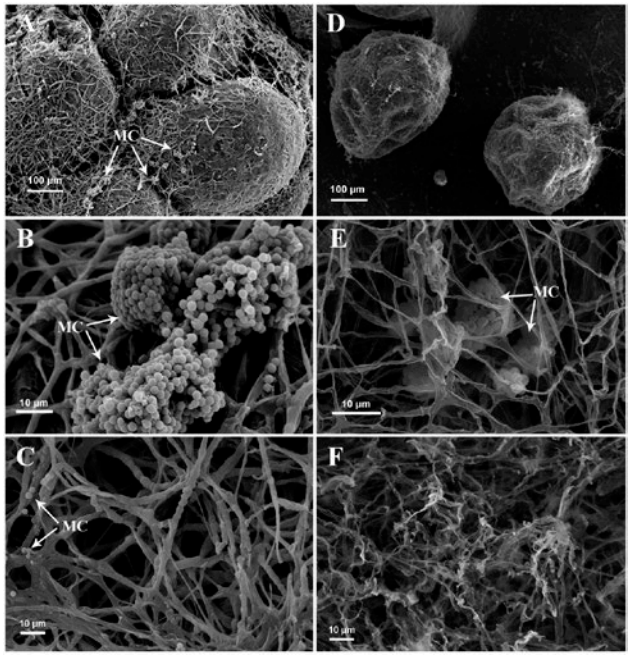

Figure 2. Scanning electron micrographs showing the antifungal effects of *Bacillus amyloliquefaciens* GGA on the morphology of *Sclerotium cepivorum* S6 in the dual culture test. A, normal spherical sclerotia with intact, rough-surfaced external rind layers; B, small globose to subglobose rough-walled microconidia on mycelium emerging from sclerotic; C, typical well-developed branched aerial hyphae; D, wrinkled sclerotia with depressions on their surfaces and ruptured rinds; E, distorted and shrunken microconidia; and F, twisted, curled and collapsed hyphae. MC = microconidia.

Pot experiment

Disease assessments

Mean DS and DI(%) of white rot on garlic plants in response to the tested treatments are illustrated in Figure 3. These results showed that DS and DI increased with increasing the age of the infected garlic plants, compared with the untreated plants. However, the disease severity and incidence in the non-mycorrhizal infected plants were significantly greater than those of the mycorrhizal infected plants at the three harvests. Inoculated plants treated with *B. amyloliquefaciens*

Table 1. Dual culture test between *Sclerotium cepivorum* S6 and *Bacillus amyloliquefaciens* GGA.

Treatment	Mean inward linear growth* (cm) after		
	4 d	8 d	12 d
S. cepivorum S6	3.9	6.8	8.0
S. cepivorum S6 + *B. amyloliquefaciens* GGA	2.2	2.6	2.9

* Each value is the mean of three replicates.

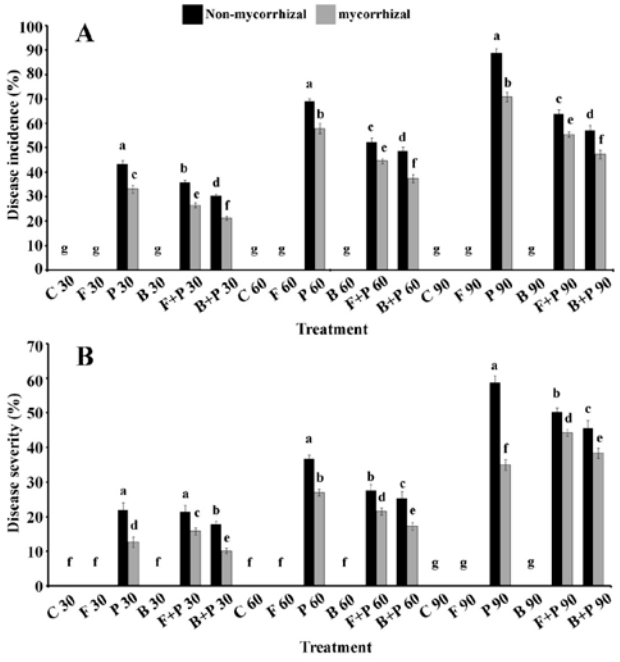

Figure 3. Mean disease incidence (A) and severity (B) of garlic plants in response to the tested treatments. C = untreated control, F = treated with tebuconazole, P = inoculated with *Sclerotium cepivorum*, B = inoculated with *Bacillus amyloliquefaciens* GGA, F+P = treated with tebuconazole and inoculated, and B+P = inoculated with S. cepivorum and treated with the bacteria, at three plant growth stages (30, 60, or 90 days post inoculation). At each growth stage, columns accompanied by the same letter are not significantly different (P=0.05) according to Duncan's multiple range test.

GGA showed disease severity and incidence that were less than the untreated-infected plants. However, garlic plants treated with AMF and *B. amyloliquefaciens* GGA had the greatest reductions in DS and DI, when compared with those for the plants treated with tebuconazole, or from the untreated-infected treatments.

Evaluation of the growth parameters

Effects of application of AMF and/or *B. amyloliquefaciens* GGA treatments on means of the garlic plant growth parameters are presented in Table 2. In general, all evaluated growth parameters increased with the increasing time after inoculations. All the assessed growth parameters were significantly reduced in the plants affected by white rot, compared with the untreated control plants. However, most of these parameters were significantly increased in plants inoculated with AMF compared with the non-mycorrhizal plants at the three harvests, regardless whether the plants were inoculated with *S. cepivorum* or not. In addition, inoculation with *B. amyloliquefaciens* GGA most of the assessed plant growth parameters at the three growth stages, in the healthy and pathogen inoculated plants, when compared with the untreated control plants. However, the combined treatment (AMF plus *B. amyloliquefaciens* GGA) gave the greatest plant growth parameters compared with the other treatments.

Garlic plant yield parameters

Results obtained from the pot experiment for the plant yield parameters in response to application of AMF and/or *B. amyloliquefaciens* GGA are presented in Table 3 and illustrated in Figure 4. The yield parameters were reduced as a result of *S. cepivorum* inoculation compared to the un-inoculated plants. However, these parameters were greater mycorrhizal plants than in the non-mycorrhizal plants. Application of *B. amyloliquefaciens* GGA increased all the assessed yield parameters in the healthy and pathogen inoculated plants compared with the untreated controls. However, the dual treatment of AMF and *B. amyloliquefaciens* GGA gave the greatest mean plant parameters compared with the other treatments.

Estimations of mycorrhizal colonization

Data of mycorrhizal colonization of garlic roots are summarized in Table 4. Amounts of root mycorrhizal colonization increased with increasing plant age for all the treatments, regardless of whether the plants were inoculated with *S. cepivorum* S6 or not. Mycorrhizal colonization was reduced in garlic roots infected with *S. cepivorum* S6, compared with the other treatments. In addition, application of the tebuconazole for plants inoculated with AMF led to reductions in mycorrhizal colonization levels, particularly at 60 and 90 dpi. In contrast, levels of mycorrhizal colonization showed pronounced increases where the AMF-inoculated garlic plants were also treated with *B. amyloliquefaciens* GGA at all the plant harvests. No mycorrhizal colonization was observed in garlic roots not inoculated with AMF.

Biochemical changes in garlic plants in response to the applied treatments

Photosynthetic pigments in garlic plants

Amounts of the photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) in garlic leaves in response to the different treatments are summarized

Table 2. Mean garlic plant growth parameters (± standard error) for plants inoculated with *Sclerotium cepivorum* and treated with AMF and/or *Bacillus amyloliquefaciens* GGA.

Days post pathogen inoculation	Treatment[a]	Mycorrizal status[b]	Shoot dry wt. (g)	Root dry wt. (g)	Shoot height (cm)	Root length (cm)	Number of leaves
30	C	NM	0.81 ± 0.05	0.29 ± 0.003	15.6 ± 0.21	6.80 ± 0.06	5.0 ± 0.0
		M	1.18 ± 0.06	0.56 ± 0.006	22.2 ± 0.23	10.3 ± 0.09	5.7 ± 0.3
	F	NM	0.80 ± 0.07	0.27 ± 0.014	14.6 ± 0.22	6.50 ± 0.06	5.0 ± 0.0
		M	1.15 ± 0.08	0.55 ± 0.003	21.7 ± 0.15	10.1 ± 0.06	5.7 ± 0.3
	P	NM	0.38 ± 0.01	0.11 ± 0.004	10.7 ± 0.12	5.23 ± 0.01	4.0 ± 0
		M	0.67 ± 0.07	0.25 ± 0.002	16.3 ± 0.29	8.94 ± 0.05	5.3 ±0.3
	B	NM	1.15 ± 0.08	0.42 ± 0.002	18.6 ± 0.17	9.18 ± 0.01	6.0 ± 0.0
		M	1.78 ± 0.14	0.65 ± 0.002	26.6 ± 0.12	13.6 ± 0.12	7.0 ± 0.0
	F+P	NM	0.71 ± 0.03	0.19 ± 0.001	12.5 ± 0.06	7.02 ± 0.04	5.0 ± 0.0
		M	0.93 ± 0.02	0.32 ± 0.004	15.7 ± 0.12	9.79 ± 0.05	5.7 ± 0.3
	P+B	NM	1.04 ± 0.03	0.29 ± 0.002	15.1 ± 0.09	8.15 ± 0.01	5.0 ± 0.0
		M	1.38 ± 0.02	0.51 ± 0.001	20.8 ± 0.03	11.2 ± 0.09	7.0 ± 0.0
60	C	NM	1.94 ± 0.03	0.59 ± 0.002	22.8 ± 0.06	10.4 ± 0.15	6.3 ± 0.3
		M	2.91 ± 0.04	0.80 ± 0.004	30.7 ± 0.09	16.6 ± 0.12	7.3 ± 0.3
	F	NM	1.85 ± 0.07	0.58 ± 0.001	21.4 ± 0.21	10.1 ± 0.06	6.0 ± 0.0
		M	2.64 ± 0.23	0.79 ± 0.002	29.7 ± 0.12	16.3 ± 0.25	6.7 ± 0.3
	P	NM	1.10 ± 0.07	0.30 ± 0.001	16.8 ± 0.09	8.17 ± 0.09	5.0 ± 0.0
		M	1.99 ± 0.09	0.50 ± 0.002	22.4 ± 0.21	11.6 ± 0.17	6.3 ± 0.3
	B	NM	3.13 ± 0.04	0.73 ± 0.002	29.4 ± 0.06	17.4 ± 0.24	8.0 ± 0.0
		M	3.95 ± 0.02	0.95 ± 0.003	40.3 ± 0.12	24.6 ± 0.09	8.3 ± 0.3
	F+P	NM	1.81 ± 0.01	0.38 ± 0.002	18.7 ± 0.12	9.70 ± 0.15	6.7 ± 0.7
		M	2.07 ± 0.02	0.61 ± 0.002	25.5 ± 0.19	12.6 ± 0.15	8.3 ± 0.3
	P+B	NM	2.37 ± 0.09	0.61 ± 0.002	28.6 ± 0.18	15.4 ± 0.27	6.6 ± 0.3
		M	3.09 ± 0.01	0.75 ± 0.002	38.8 ± 0.09	23.3 ± 0.25	7.0 ± 0.0
90	C	NM	3.02 ± 0.09	0.91 ± 0.001	33.4 ± 0.22	20.6 ± 0.15	9.0 ± 0.0
		M	4.44 ± 0.03	1.14 ± 0.017	42.7 ± 0.09	29.7 ± 0.09	9.7 ± 0.3
	F	NM	3.05 ± 0.08	0.89 ± 0.002	32.6 ± 0.09	20.1 ± 0.06	8.3 ± 0.3
		M	4.22 ± 0.07	1.10 ± 0.009	40.9 ± 0.14	28.3 ± 0.24	9.3 ± 0.3
	P	NM	1.83 ± 0.04	0.59 ± 0.002	24.8 ± 0.09	17.4 ± 0.03	7.7 ± 0.3
		M	3.45 ± 0.23	0.88 ± 0.004	32.2 ± 0.15	26.7 ± 0.12	8.7 ± 0.3
	B	NM	4.15 ± 0.04	1.07 ± 0.012	40.7 ± 0.09	35.5 ± 0.17	11.0 ± 0.0
		M	4.95 ± 0.09	1.52 ± 0.038	56.7 ± 0.19	48.4 ± 0.47	13.0 ± 0.0
	F+P	NM	2.73 ± 0.09	0.71 ± 0.003	30.3 ± 0.18	18.2 ± 0.06	8.3 ± 0.3
		M	3.89 ± 0.12	0.99 ± 0.001	36.6 ± 0.15	27.6 ± 0.18	9.0 ± 0.0
	P+B	NM	3.67 ± 0.14	0.95 ± 0.001	35.5 ± 0.18	33.7 ± 0.41	10.0 ± 0.0
		M	4.13 ± 0.04	1.21 ± 0.020	50.4 ± 0.19	51.3 ± 0.68	12.0 ± 0.0
LSD (P<0.05)			0.379	0.025	0.431	1.464	0.717
Treatment			**	**	**	**	*
Harvest			**	***	**	***	*
Harvest × Treatment			**	**	**	**	*
Mycorrhiza			***	***	***	***	**
Mycorrhiza × Treatment			**	**	**	**	*
Mycorrhiza × Harvest			***	***	**	***	*
Mycorrhiza × Harvest × Treatment			**	**	**	**	*

[a] C, untreated control; F, fungicide; P, inoculated with *S. cepivorum*; B, treated with bacteria; F+P, inoculated with *S. cepivorum* and treated with fungicide; P+B, inoculated with *S. cepivorm* and treated with bacteria.
[b] NM, non-mycorrhizal; M, mycorrhizal.
* Significant at P<0.05, ** significant at P<0.01, and *** significant at P<0.001.

Table 3. Mean garlic plant yield parameters (± standard error) for plants treated with AMF and/or *Bacillus amyloliquefaciens* GGA as bio-control agents against *Sclerotium cepivorum*.

Treatment[a]	Mycorrhizal status[b]	Fresh wt. of bulb (g)	Dry wt. of bulb (g)	Bulb diameter (cm)	No. of cloves per bulb	Clove length (cm)	Clove diameter (cm)
C	NM	19.8 ± 0.09	5.74 ± 0.29	3.30 ± 0.19	13.7 ± 0.33	2.60 ± 0.03	1.80 ± 0.04
	M	23.1 ± 0.10	8.59 ± 0.14	4.18 ± 0.04	16.7 ± 0.33	3.55 ± 0.04	2.08 ± 0.04
F	NM	19.1 ± 0.20	5.62 ± 0.26	3.07 ± 0.02	12.3 ± 0.33	2.47 ± 0.02	1.68 ± 0.05
	M	23.2 ± 0.3	8.21 ± 0.13	4.05 ± 0.03	16.3 ± 0.33	3.22 ± 0.02	2.04 ± 0.03
P	NM	12.7 ± 0.45	4.13 ± 0.05	2.05 ± 0.03	10.3 ± 0.33	1.37 ± 0.07	1.08 ± 0.06
	M	17.7 ± 0.92	6.49 ± 0.22	3.07 ± 0.02	13.3 ± 0.33	2.40 ± 0.01	1.85 ± 0.12
B	NM	24.8 ± 0.26	10.1 ± 0.11	5.20 ± 0.03	21.3 ± 0.33	4.12 ± 0.01	2.36 ± 0.04
	M	35.6 ± 0.46	14.1 ± 0.07	6.19 ± 0.04	26.3 ± 0.88	5.02 ± 0.01	2.98 ± 0.07
F+P	NM	14.5 ± 0.53	4.26 ± 0.06	3.06 ± 0.02	11.3 ± 0.33	2.14 ± 0.02	1.37 ± 0.08
	M	19.4 ± 0.44	5.37 ± 0.14	3.88 ± 0.06	14.3 ± 0.33	2.76 ± 0.04	1.87 ± 0.10
P+B	NM	21.9 ± 0.37	7.69 ± 0.17	4.44 ± 0.07	15.3 ± 0.33	3.79 ± 0.04	2.09 ± 0.17
	M	28.0 ± 0.55	10.3 ± 0.15	5.95 ± 0.05	19.3 ± 0.33	4.42 ± 0.04	2.59 ± 0.06
LSD (P<0.05)		4.06	1.78	0.69	2.53	0.54	0.35
Treatment		***	***	***	**	***	***
Mycorrhiza		***	***	***	**	***	***
Mycorrhiza × Treatment		***	***	**	**	**	**

[a] C, untreated control; F, fungicide; P, inoculated with *S. cepivorum*; B, treated with bacteria; F+P, inoculated with *S. cepivorum* and treated with fungicide; P+B, inoculated with *S. cepivorm* and treated with bacteria.
[b] NM, non-mycorrhizal; M, mycorrhizal.
** Significant at P<0.01, and *** significant at P<0.001.

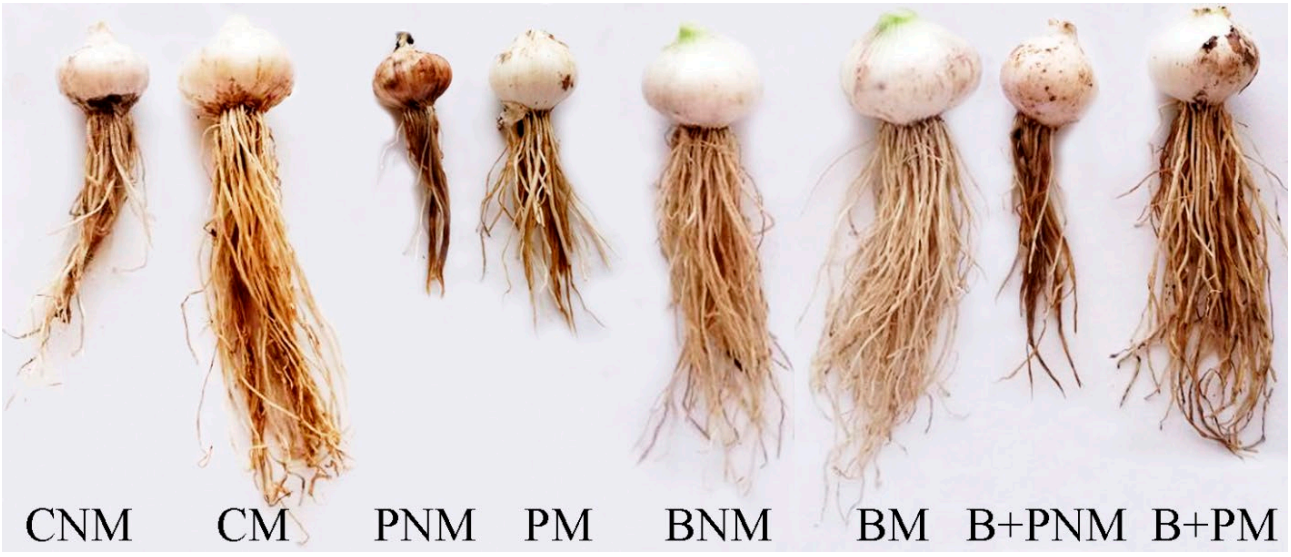

Figure 4. Photograph showing effects of different treatments on resulting bulbs of garlic plants. The treatments were: CNM = untreated control, CM = treated with AMF, PNM = inoculated with *Sclerotium cepivorum*, PM = inoculated with *S. cepivorum* and treated with AMF, BNM = treated with *Bacillus amyloliquefaciens* GGA, BM = treated with *B. amyloliquefaciens* GGA and AMF, PBNM = inoculated with *S. cepivorum* and treated with *B. amyloliquefaciens* GGA, and PBM = inoculated with *S. cepivorum* and treated with *B. amyloliquefaciens* GGA and AMF.

in Table 5. For all the treatments, amounts of these pigments increased from 30 to 60 dpi at which they reached the maximum values then decreased at 90 dpi. Infection of garlic plants with *S. cepivorum* led to decreases in the amounts of pigments compared with the untreated garlic plants. The pigment quantities in the mycorrhizal

Table 4. Mean proportions (± standard error) of mycorrhizal colonization in garlic plant roots at intervals after different treatments were applied.

Days after pathogen inoculation	Treatment [a]	Mycorrhizal status [b]	F (%)[c]	I (%)[c]	A (%)[c]
30	C	NM	0.0	0.0	0.0
		M	81.6 ± 0.76	37.5 ± 0.91	13.8 ± 0.61
	F	NM	0.0	0.0	0.0
		M	81.0 ± 0.35	36.8 ± 0.63	13.3 ± 0.23
	P	NM	0.0	0.0	0.0
		M	59.4 ± 0.8	19.4 ± 0.3	7.2 ± 0.37
	B	NM	0.0	0.0	0.0
		M	83.8 ± 1.65	46.1 ± 0.92	23.0 ± 0.83
	F+P	NM	0.0	0.0	0.0
		M	74.6 ± 0.43	25.77 ± 0.61	10.1 ± 0.28
	P+B	NM	0.0	0.0	0.0
		M	81.7 ± 0.55	41.46 ± 0.28	21.1 ± 0.52
60	C	NM	0.0	0.0	0.0
		M	94.1 ± 0.89	65.1 ± 0.84	37.8 ± 0.71
	F	NM	0.0	0.0	0.0
		M	91.6 ± 0.61	62.1 ± 0.67	35.3 ± 1.13
	P	NM	0.0	0	0
		M	76.6 ± 0.53	33.6 ± 0.51	25.6 ± 0.61
	B	NM	0.0	0.0	0.0
		M	100	71.3 ± 0.54	58.5 ± 0.73
	F+P	NM	0.0	0.0	0.0
		M	84.8 ± 0.09	48.6 ± 0.73	31.0 ± 0.35
	P+B	NM	0.0	0.0	0.0
		M	97.8 ± 0.32	75.8 ± 1.18	48.4 ± 0.64
90	C	NM	0.0	0.0	0.0
		M	100	73.0 ± 1.53	57.1 ± 0.91
	F	NM	0.0	0.0	0.0
		M	95.6 ± 0.74	54.2 ± 0.78	55.1 ± 0.22
	P	NM	0.0	0.0	0.0
		M	83.1 ± 0.94	86.7 ± 0.63	39.6 ± 0.66
	B	NM	0.0	0.0	0.0
		M	100	83 ± 2.08	64.0 ± 1.24
	F+P	NM	0.0	0.0	0.0
		M	90.66 ± 0.55	65.4 ± 0.87	49.5 ± 2.68
	P+B	NM	0.0	0.0	0.0
		M	100	79.9 ± 0.42	61.5 ± 0.69
LSD (P<0.05)			1.27	1.56	1.85
Treatment			***	***	***
Harvest			***	***	***
Harvest × Treatment			***	***	***
Mycorrhiza			***	***	***
Mycorrhiza × Treatment			***	***	***
Mycorrhiza × Harvest			***	***	***
Mycorrhiza × Harvest × Treatment			***	***	***

[a] C, untreated control; F, fungicide; P, inoculated with *Sclerotium cepivorum*; B, treated with bacteria; F+P, inoculated with *S. cepivorum* and treated with fungicide; P+B, inoculated with *S. cepivorm* and treated with bacteria.
[b] NM, non-mycorrhizal; M, mycorrhizal.
[c] F, frequency of root colonization; I, intensity of cortical colonization; A, frequency of arbuscules.
*** Significant at P<0.001.

Table 5. Mean concentrations (μg g^{-1} fresh weight, ± standard error) of photosynthetic pigments in garlic plants at intervals after different *Sclerotium cepivorum* inoculation and microbial treatments were applied.

Days after pathogen inoculation	Treatment[a]	Mycorrhizal status[b]	Chlorophyll *a*	Chlorophyll *b*	Carotenoids
30	C	NM	590 ± 2.89	392 ± 1.45	301 ± 1.86
		M	614 ± 2.33	413 ± 3.18	387 ± 1.13
	F	NM	540 ± 2.91	394 ± 2.61	315 ± 2.82
		M	664 ± 2.08	443 ± 1.63	402 ± 3.75
	P	NM	441 ± 4.41	315 ± 2.84	241 ± 4.42
		M	541 ± 2.07	385 ± 2.72	318 ± 4.29
	B	NM	690 ± 4.62	422 ± 1.53	397 ± 2.17
		M	796 ± 1.86	483 ± 2.08	431 ± 3.35
	F+P	NM	517 ± 6.51	354 ± 2.33	285 ± 2.56
		M	594 ± 2.32	412 ± 1.45	326 ± 2.92
	P+B	NM	630 ± 2.83	398 ± 1.52	341 ± 2.09
		M	715 ± 2.89	426 ± 1.67	411 ± 2.64
60	C	NM	609 ± 1.58	441 ± 1.86	320 ± 2.86
		M	870 ± 2.64	615 ± 2.33	421 ± 2.09
	F	NM	598 ± 4.16	439 ± 3.48	420 ± 2.81
		M	824 ± 3.63	601 ± 1.86	491 ± 2.12
	P	NM	515 ± 1.47	389 ± 0.77	311 ± 2.09
		M	621 ± 2.09	430 ± 3.21	401 ± 1.85
	B	NM	715 ± 2.33	588 ± 0.88	425 ± 2.86
		M	820 ± 2.89	718 ± 1.15	590 ± 2.62
	F+P	NM	547 ± 1.81	399 ± 0.75	335 ± 2.88
		M	667 ± 1.45	518 ± 1.67	430 ± 2.90
	P+B	NM	687 ± 2.09	440 ± 2.89	403 ± 3.51
		M	737 ± 4.31	509 ± 3.78	464 ± 2.33
90	C	NM	479 ± 3.19	399 ± 3.17	365 ± 3.84
		M	595 ± 2.83	435 ± 2.88	434 ± 2.09
	F	NM	465 ± 2.88	389 ± 3.18	415 ± 2.88
		M	590 ± 2.37	404 ± 1.45	442 ± 3.71
	P	NM	375 ± 2.84	295 ± 0.88	211 ± 2.09
		M	441 ± 1.86	333 ± 1.86	292 ± 3.15
	B	NM	502 ± 3.18	422 ± 2.12	310 ± 2.89
		M	617 ± 2.84	482 ± 2.52	371 ± 3.53
	F+P	NM	365 ± 1.29	306 ± 1.67	296 ± 3.51
		M	500 ± 1.82	371 ± 3.84	381 ± 3.28
	P+B	NM	471 ± 2.14	315 ± 2.89	289 ± 1.88
		M	541 ± 2.08	429 ± 3.78	339 ± 3.19
LSD (P<0.05)			9.94	7.68	8.93
Treatment			*	**	**
Harvest			**	*	**
Harvest × Treatment			*	*	**
Mycorrhiza			**	**	**
Mycorrhiza × Treatment			*	**	*
Mycorrhiza × Harvest			**	*	**
Mycorrhiza × Harvest × Treatment			**	**	*

[a] C, untreated control; F, fungicide; P, inoculated with *S. cepivorum*; B, treated with bacteria; F+P, inoculated with *S. cepivorum* and treated with fungicide; P+B, inoculated with *S. cepivorm* and treated with bacteria.
[b] NM, non-mycorrhizal; M, mycorrhizal.
* Significant at P<0.05, and ** significant at P<0.01.

Table 6. Mean amounts (mg g^{-1} dry weight, ± standard error) of mineral nutrients in garlic plant leaves contents at intervals after different *Sclerotium cepivorum* inoculation and microbial treatments were applied.

Days after pathogen inoculation	Treatment[a]	Mycorrhizal status[b]	N	P	K	Ca	Mg
30	C	NM	12.4 ± 0.21	0.95 ± 0.021	10.9 ± 0.32	5.93 ± 0.10	0.55 ± 0.01
		M	15.5 ± 0.42	1.18 ± 0.038	13.2 ± 0.41	6.86 ± 0.12	0.81 ± 0.02
	F	NM	12.1 ± 0.23	0.94 ± 0.014	10.8 ± 0.42	5.73 ± 0.12	0.51 ± 0.01
		M	14.7 ± 0.23	1.24 ± 0.012	12.9 ± 0.64	6.54 ± 0.20	0.80 ± 0.02
	P	NM	8.80 ± 0.27	0.78 ± 0.023	8.74 ± 0.52	3.98 ± 0.10	0.22 ± 0.01
		M	10.7 ± 0.51	0.82 ± 0.002	9.37 ± 0.74	4.61 ± 0.11	0.38 ± 0.01
	B	NM	17.8 ± 0.33	1.03 ± 0.002	14.1 ± 0.67	8.53 ± 0.17	0.82 ± 0.01
		M	23.2 ± 0.26	2.40 ± 0.032	17.5 ± 0.52	10.3 ± 0.10	0.95 ± 0.02
	F+P	NM	10.3 ± 0.13	0.80 ± 0.009	9.14 ± 0.36	5.22 ± 0.14	0.42 ± 0.01
		M	12.6 ± 0.12	0.89 ± 0.004	10.9 ± 0.35	5.94 ± 0.17	0.54 ± 0.01
	P+B	NM	15.7 ± 0.55	0.97 ± 0.012	12.2 ± 0.29	7.64 ± 0.14	0.76 ± 0.01
		M	20.7 ± 0.65	1.89 ± 0.003	16.1 ± 0.51	9.06 ± 0.11	0.84 ± 0.01
60	C	NM	15.6 ± 0.13	1.53 ± 0.007	15.4 ± 0.72	8.63 ± 0.05	0.96 ± 0.01
		M	19.7 ± 0.37	1.84 ± 0.004	19.2 ± 0.63	10.2 ± 0.17	1.14 ± 0.02
	F	NM	14.8 ± 0.25	1.51 ± 0.011	15.2 ± 0.64	8.36 ± 0.16	0.92 ± 0.01
		M	18.7 ± 0.46	1.83 ± 0.004	18.9 ± 0.42	10.0 ± 0.15	1.07 ± 0.01
	P	NM	8.70 ± 0.71	0.99± 0.008	10.5 ± 0.45	5.12 ± 0.19	0.45 ± 0.01
		M	11.4 ± 0.53	1.34 ± 0.006	12.8 ± 0.73	5.92 ± 0.07	0.74 ± 0.01
	B	NM	21.5 ± 0.55	1.89 ± 0.002	18.9 ± 0.56	11.9 ± 0.14	1.16 ± 0.05
		M	30.3 ± 0.34	2.66 ± 0.003	23.4 ± 0.37	13.3 ± 0.01	1.69 ± 0.11
	F+P	NM	12.5 ± 0.48	1.43 ± 0.002	13.2 ± 0.46	7.81 ± 0.11	0.77 ± 0.01
		M	15.8 ± 0.33	1.64 ± 0.015	16.3 ± 0.69	8.25 ± 0.41	0.87 ± 0.02
	P+B	NM	20.7 ± 0.65	1.79 ± 0.006	17.1 ± 0.38	10.1 ± 0.11	1.11 ± 0.05
		M	22.3 ± 0.43	2.23 ± 0.008	21.1 ± 0.31	12.2 ± 0.09	1.57 ± 0.01
90	C	NM	14.5 ± 0.43	1.41 ± 0.007	14.3 ± 0.29	7.12 ± 0.12	0.84 ± 0.01
		M	16.8 ± 0.52	1.58 ± 0.017	15.4 ± 0.47	8.01 ± 0.26	0.98 ± 0.01
	F	NM	14.1 ± 0.36	1.40 ± 0.002	14.1 ± 0.32	7.02 ± 0.06	0.81 ± 0.01
		M	16.3 ± 0.53	1.53 ± 0.009	14.9 ± 0.44	7.88 ± 0.07	0.92 ± 0.02
	P	NM	8.36 ± 0.47	0.82 ± 0.002	9.19 ± 0.59	4.78 ± 0.03	0.37 ± 0.03
		M	10.2 ± 0.34	0.96 ± 0.006	10.3 ± 0.32	5.22 ± 0.06	0.53 ± 0.01
	B	NM	20.4 ± 0.52	1.53 ± 0.010	17.6 ± 0.69	10.0 ± 0.08	1.10 ± 0.03
		M	24.9 ± 0.69	1.99 ± 0.008	20.1 ± 0.22	11.9 ± 0.07	1.51 ± 0.02
	F+P	NM	11.1 ± 0.52	0.97 ± 0.003	11.6 ± 0.41	6.7 ± 0.06	0.67 ± 0.02
		M	12.9 ± 0.55	1.11 ± 0.009	13.9 ± 0.55	7.06 ± 0.10	0.76 ± 0.02
	P+B	NM	17.6 ± 0.49	1.43 ± 0.003	15.8 ± 0.67	9.4 ± 0.07	1.01 ± 0.02
		M	20.9 ± 0.34	1.77 ± 0.007	18.9 ± 0.39	10.5 ± 0.11	1.33 ± 0.06
LSD (P<0.05)			1.327	0.221	2.36	1.135	0.179
Treatment			**	**	*	**	**
Harvest			**	***	**	**	**
Harvest × Treatment			**	**	*	**	**
Mycorrhiza			***	**	**	***	**
Mycorrhiza × Treatment			***	***	**	**	**
Mycorrhiza × Harvest			**	***	**	***	**
Mycorrhiza × Harvest × Treatment			**	**	**	**	**

[a] C, untreated control; F, fungicide; P, inoculated with *S. cepivorum*; B, treated with bacteria; F+P, inoculated with *S. cepivorum* and treated with fungicide; P+B, inoculated with *S. cepivorm* and treated with bacteria.
[b] NM, non-mycorrhizal; M, mycorrhizal.
* Significant at P<0.05, ** significant at P<0.01, and *** significant at P<0.001.

plants were greater than those of the non-mycorrhizal plants, at all three stages of garlic growth, whether the plants were pathogen-inoculated or not, so mycorrhizal colonization of the inoculated plants reduced the negative effects of the pathogen, compared with the non-mycorrhizal plants. In contrast, treating the garlic plants with *B. amyloliquefaciens* GGA led to increases of all photosynthetic pigments at the three plant growth stages in the inoculated non-inoculated plants. Application of AMF and *B. amyloliquefaciens* GGA in combination increased photosynthetic pigment contents at the three plant growth stages, compared with the untreated control plants.

Mineral nutrients in garlic plants

Amounts of mineral nutrients in leaves of garlic plants receiving the different treatments are summarized in Table 6. Inoculation with *S. cepivorum* led to reductions in all amounts of assessed elements, compared with the untreated control plants. Nutrient contents in the mycorrhizal-infected plants were greater than those for non-mycorrhizal *S. cepivorum* inoculated plants at the three harvests, when compared to control plants. Application of *B. amyloliquefaciens* GGA increased all leaf nutrient contents, whether inoculated or not, when compared with the untreated control treatment. The highest nutrient contents were measured for the uninfected garlic plants treated with AMF and *B. amyloliquefaciens* GGA, when compared with the untreated plants.

Total phenol contents and activities of defense-related enzymes in garlic plants

Effects of applications of AMF and/or *B. amyloliquefaciens* GGA on the total phenol content and activities of defense-related enzymes of garlic plants infected with white rot disease are summarized in Table 7. Inoculation with *S. cepivorum* led to significant increases in the total phenol content and activities of defense-related enzymes of mycorrhizal and non-mycorrhizal plants, compared with untreated control plants. The increases in the mycorrhizal plants were greater than in the non-mycorrhizal plants at all harvests. The maximum values of total phenol contents and enzyme activities occurred after 60 days and decreased after 90 days from inoculation with the pathogen.

In addition, garlic plants (whether *S. cepivorum*-inoculated or not) which were treated with *B. amyloliquefaciens* GGA had greater amounts of phenol and greater enzyme activities compared with the corresponding untreated plants. The greatest amounts total phenol and enzyme activity were for the pathogen inoculated plants treated with the combined treatment of AMF and *B. amyloliquefaciens* GGA, when compared with the control plants, particularly at 60 dpi.

Transcript levels of the defense-related enzymes (qRT-PCR)

The transcript levels of the defensin gene in garlic plants was quantified using qRT-PCR in response to the different applied treatments (Figure 5A). There were significant inductions of defensin expression from all the treatments, at both harvests, compared to the control treatment. The induction effect was greatest at 30 dpi than at 60 dpi. For the two harvests, *S. cepivorum* inoculated plants treated with AMF and *B. amyloliquefaciens* GGA had the greatest gene expression (13.2-fold increase at 30 dpi, and 9.5-fold at 60 dpi).

Expression of the chitinase gene (Figure 5B) was also affected by varying degrees. Expression at 30 dpi was greater than at 60 dpi. Plants inoculated with *S. cepivorum* and treated with AMF and *B. amyloliquefaciens* GGA gave the greatest gene expression levels (8.9-fold increase at 30 dpi), compared with the untreated control treatment. At 60 dpi, greatest chitinase gene expression was recorded for the non-pathogen inoculated plants treated with AMF and *B. amyloliquefaciens* GGA, compared with the untreated control plants.

DISCUSSION

White rot, caused by *S. cepivorum*, is a serious disease of garlic leading to considerable yield losses. This study investigated the synergistic interactions between endophytic microorganisms (AMF and *B. amyloliquefaciens* GGA) and their effects on biochemical and molecular plant defense-responses against white rot, as well as effects on garlic plant growth.

Results obtained from the dual culture tests showed potent *in vitro* antagonistic activity of *B. amyloliquefaciens* GGA against *S. cepivorum*, which was confirmed by SEM observations of *S. cepivorum* mycelium, sclerotia, and microconidia. Fungitoxic activity of *B. amyloliquefaciens* has been studied by many researchers against a wide range of soil-borne fungi (Li *et al*., 2016; Lee *et al*., 2017). Several antifungal metabolites have been reported to be produced by *B. amyloliquefaciens*, including lipopeptides (e.g. bacillomycin, fengycin, and surfactin), volatile compounds, hydrolytic enzymes, and siderophores such as bacillibactin (Yuan *et al*., 2012; Hanif *et al*., 2019). However, their production may be

Table 7. Mean amounts (± standard error) of the defense-related enzymes phenylalanine ammonia-lyase (PAL), polyphenoloxidase (PPO) and peroxidase (POD), and total phenol in garlic plants receiving different treatments of *Sclerotium cepivorum* inoculation or AMF and/or *B. amyloliquefaciens* GGA.

Days after pathogen inoculation	Treatment[a]	Mycorrizal status[b]	PAL (μmol *t*-cinnamic acid h^{-1} g^{-1} f wt)	PPO (Δ A_{420} min^{-1} g^{-1} f wt)	POD (Δ A_{470} min^{-1} g^{-1} f wt)	Total phenol (μg g^{-1} f wt)
30	C	NM	200.7 ± 2.18	4.50 ± 0.05	25.3 ± 0.58	185.0 ± 2.88
		M	236.7 ± 9.28	5.36 ± 0.03	39.9 ± 0.07	394.3 ± 5.46
	F	NM	197.7 ± 5.04	4.43 ± 0.05	25.1 ± 0.46	184.3 ± 1.86
		M	235.3 ± 9.94	5.31 ± 0.04	39.9 ± 0.10	385.3 ± 6.49
	P	NM	394.0 ± 3.05	5.88 ± 0.03	59.3 ± 0.62	410.0 ± 2.88
		M	422.0 ± 3.07	6.36 ± 0.02	66.3 ± 0.25	590.7 ± 3.84
	B	NM	415.3 ± 1.45	6.01 ± 0.02	66.2 ± 0.61	339.7 ± 3.17
		M	541.7 ± 7.26	6.81 ± 0.08	73.1 ± 0.98	544.6 ± 5.82
	F+P	NM	382.0 ± 3.06	4.41 ± 0.05	24.1 ± 0.08	393.0 ± 2.52
		M	421.3 ± 8.99	5.24 ± 0.02	37.9 ± 1.08	540.3 ± 4.84
	P+B	NM	436.0 ± 5.19	6.10 ± 0.03	61.7 ± 0.98	355.0 ± 2.88
		M	551.7 ± 9.89	6.98 ± 0.17	74.3 ± 0.88	630.0 ± 9.58
60	C	NM	361.7 ± 9.28	6.88 ± 0.06	30.7 ± 0.31	200.7 ± 6.69
		M	504.3 ± 7.82	7.19 ± 0.06	41.1 ± 0.48	413.0 ± 6.11
	F	NM	363.3 ± 9.16	6.89 ± 0.04	30.3 ± 0.23	199.6 ± 2.73
		M	500.7 ± 2.13	7.30 ± 0.08	40.4 ± 0.26	407.3 ± 4.18
	P	NM	509.3 ± 2.97	7.10 ± 0.05	72.1 ± 1.02	463.3 ± 9.93
		M	652.7 ± 8.29	8.95 ± 0.03	80.9 ± 0.38	661.7 ± 8.98
	B	NM	657.7 ± 7.33	8.12 ± 0.06	70.3 ± 0.14	397.3 ± 8.12
		M	794.7 ± 8.86	9.08 ± 0.03	77.8 ± 1.40	596.0 ± 6.24
	F+P	NM	412.7 ± 3.81	5.14 ± 0.02	30.2 ± 0.15	448.3 ± 9.08
		M	560.3 ± 3.85	6.11 ± 0.02	47.5 ± 1.11	565.7 ± 8.39
	P+B	NM	689.0 ± 7.09	8.72 ± 0.05	72.5 ± 0.44	410.3 ± 5.48
		M	908.0 ± 8.51	9.78 ± 0.24	83.1 ± 0.78	717.7 ± 8.76
90	C	NM	173.7 ± 4.19	3.50 ± 0.05	21.6 ± 0.54	146.0 ± 7.21
		M	225.0 ± 5.73	4.23 ± 0.09	31.5 ± 0.77	305.6 ± 4.08
	F	NM	172.7 ± 3.92	3.45 ± 0.04	21.1 ± 0.69	149.6 ± 7.96
		M	209.7 ± 2.40	4.11 ± 0.06	30.9 ± 0.57	304.7 ± 2.60
	P	NM	355.7 ± 5.81	4.85 ± 0.04	30.7 ± 0.66	296.0 ± 4.93
		M	401.7 ± 8.81	5.11 ± 0.02	55.7 ± 0.18	520.0 ± 5.77
	B	NM	374.0 ± 9.18	5.05 ± 0.03	59.5 ± 0.31	323.3 ± 9.89
		M	447.3 ± 6.88	5.83 ± 0.12	65.8 ± 0.12	524.3 ± 7.89
	F+P	NM	371.7 ± 3.89	4.09 ± 0.06	22.2 ± 0.42	311.7 ± 4.41
		M	367.0 ± 6.81	4.67 ± 0.07	32.8 ± 0.23	508.7 ± 3.18
	P+B	NM	406.3 ± 5.24	5.74 ± 0.09	58.8 ± 0.92	333.0 ± 7.23
		M	519.3 ± 7.38	6.02 ± 0.07	66.7 ± 0.46	545.7 ± 5.21
LSD (P<0.05)			34.33	0.212	12.24	21.62
Treatment			**	**	*	**
Harvest			**	*	**	*
Harvest × Treatment			*	*	**	*
Mycorrhiza			***	***	***	***
Mycorrhiza × Treatment			**	**	*	**
Mycorrhiza × Harvest			**	*	**	*
Mycorrhiza × Harvest × Treatment			**	**	**	**

[a] C, untreated control; F, fungicide; P, inoculated with *S. cepivorum*; B, treated with bacteria; F+P, inoculated with *S. cepivorum* and treated with fungicide; P+B, inoculated with *S. cepivorm* and treated with bacteria.
[b]NM, non-mycorrhizal; M, mycorrhizal.
* Significant at P<0.05, ** significant at P<0.01, and *** significant at P<0.001.

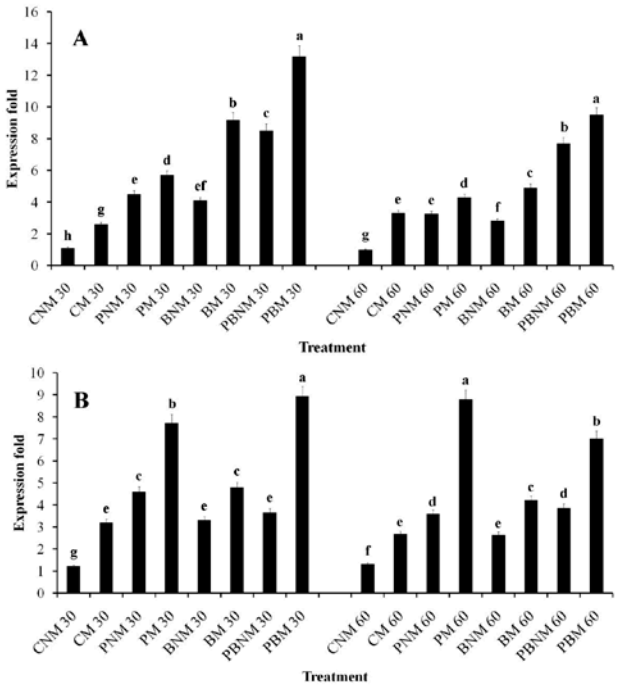

Figure 5. Mean relative transcription expression levels of the defensin gene (A) and chitinase gene (B) in garlic plants after different treatments of *Sclerotium cepivorum* inoculation or AMF and or *B. amyloliquefaciens* GGA at 30 or 60 dpi. CNM = untreated control, CM = treated with AMF, PNM = inoculated with *S. cepivorum*, PM = inoculated with *S. cepivorum* and treated with AMF, BNM = treated with *B. amyloliquefaciens* GGA, BM = treated with *B. amyloliquefaciens* GGA and AMF, PBNM = inoculated with *S. cepivorum* and treated with *B. amyloliquefaciens* GGA, and PBM = inoculated with *S. cepivorum* and treated with *B. amyloliquefaciens* GGA and AMF. Each value represents the mean of three biological replicates, each analyzed in triplicate. Error bars are standard errors. At each growth stage 30 or 60 dpi), means accompanied by the same letter(s) are not significantly different ($P<0.05$), Duncan's multiple range test.

induced in a species-specific manner, so that production may vary in type or quantity depending on the particular fungal pathogen (Li *et al*., 2014). The probable mechanisms of action of these metabolites include interference with cell membrane components, particularly sterol and phospholipid molecules, altering their structure and affecting membrane permeability (Sur *et al*., 2018). In addition, inhibition of fungal DNA biosynthesis and cell lysis were also reported to cause cell death (Tao *et al*., 2011; Liu *et al*., 2011).

Results from the pot experiment demonstrated that single inoculation of *B. amyloliquefaciens* GGA or AMF reduced severity and incidence of white rot on garlic plants. Dual inoculation with both of these organisms gave the greatest disease reductions. This result is similar to that of Haggag and Abdel-latif (2001), who reported a synergistic effect of the combined treatment of *G. mosseae* and *B. subtilis* against the root rot pathogens *F. solani* and *Macrophomina phaseolina* on geranium plants.

Biocontrol activity of AMF, alone or in combination with other biocontrol agents, has been extensively studied against different fungal diseases of many plant species (Abdel-Fattah *et al*., 2011; El-Sharkawy *et al*., 2018; Rashad *et al*., 2020). Berdeni *et al*. (2018) found that resistance of apple trees (*Malus pumila*) was induced against canker caused by *Neonectria ditissima*, when plants were inoculated with AMF. Various defense-related mechanisms have been reported to be induced in host plants in response to the colonization with AMF. These include physical, biochemical and molecular changes. Lignification of host cell walls is one of the main induced defense-related responses against phytopathogenic fungi. Cell wall lignification acts as a physical barrier which restricts pathogen spread within host tissues, and diffusion of pathogen-produced toxins into plant cells. Lignification also obstructs passage of water and nutrients from cells to invading pathogens (Miedes *et al*., 2014). Rashad *et al*. (2020) reported the triggering effect of sunflower colonization by *Rhizophagus irregularis* on transcriptional expression of lignification-related genes. Cell wall thickening of bean roots against the Rhizoctonia root rot pathogen as a result to AMF colonization was also observed by Abdel-Fattah *et al*. (2011). Triggering of host cells for production of some fungitoxic phenolic compounds as a result of AMF colonization has also been reported. This mechanism was confirmed by the results obtained in the present study, where high total phenol contents were recorded in the infected garlic plants inoculated with AMF. El-Sharkawy *et al*. (2018) found that mycorrhizal stem rust-infected wheat plants inoculated with AMF had greater amounts of phenolic compounds than the non-mycorrhizal plants. AMF colonization also leads to elicitation of flavonoids and chlorogenic acid-related genes in tomato and sunflower against invading pathogens (Aseel *et al*., 2019; Rashad *et al*., 2020). Phenolic compounds are antimicrobial substances which are defensively produced by infected plants from adjacent cells encircling pathogen infections, in order to restrict pathogen growth into healthy cells. This is known as localized acquired resistance (Ewané *et al*., 2012). Induction of some defense-related enzymes and accumulation of phytoalexins was also reported for AMF colonization (Song *et al*., 2011). Biochemical data from the present study revealed induction of the defense-related enzymes PAL, PPO, and POD. In addition, triggering of the transcriptional expression levels of defensin and chitinase genes was also observed in the

mycorrhizal garlic plants compared to the non-mycorrhizal plants, suggesting that induction of host systemic resistance is likely to be another defense mechanism induced by AMF.

Results from the present work demonstrated the biocontrol activity of *B. amyloliquefaciens* GGA against white rot of garlic. This result is similar to that of Zouari *et al*. (2016), who reported the biocontrol potential of endophytic *B. amyloliquefaciens* CEIZ-11 against damping-off of tomato, caused by *Pythium aphanidermatum*. Different biocontrol mechanisms were reported to be involved for *B. amyloliquefaciens* against many fungal pathogens, including production of fungitoxic secondary metabolites such as lipopeptides, volatile compounds, hydrolytic enzymes, and siderophores (Cawoy *et al*., 2015). Induction of plant systemic resistance against invading pathogens has also been reported as a biocontrol mechanism of *B. amyloliquefaciens*. Li *et al*. (2015) reported that cucurbit seedlings treated with *B. amyloliquefaciens* LJ02 or culture filtrates of the bacterium reduced the infection by *Sphaerotheca fuliginea*, and triggered biosynthesis of the defense-related enzymes superoxide dismutase, peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase. Salicylic acid production and the transcriptional expression of the pathogenesis-related gene *PR-1* were also elevated, indicating that salicylic acid-mediated defense responses were induced. Similar to these results, the present study revealed considerable increases in the activities of defense-related enzymes (PAL, PPO, and POD), as well as up-regulation of the transcriptional expression of the defense-related genes defensin and chitinase, as responses to treating of garlic plants with *B. amyloliquefaciens* GGA and/or AMF. This indicates that induction of the host systemic resistance contributed the biocontrol behavior of both the tested biocontrol agents against the garlic white rot pathogen. In addition, competition with the pathogen for nutrients may play a part in the biocontrol activity (Ntushelo *et al*., 2019).

On the other hand, data from the pot experiment in this study showed that treating of garlic plants with endophytic *B. amyloliquefaciens* GGA and/or AMF improved the plant growth and enhanced the yield. One of the most beneficial effects of colonization by mycorrhizal fungi on the host plant is the elevation in the uptake of macro- and micronutrients from the soil via the extraradical mycelium network of AMF, specifically of phosphate (Ingraffia *et al*., 2019) which lead to increased biomass accumulation. Enhancing of the photosynthetic pigments in the host leaves and improving the plant water supply from the soil (Zhang *et al*., 2018) were also reported in the mycorrhizal plants, which promote the plant growth and yield (Begum *et al*., 2019). Promoting effect of *B. amyloliquefaciens* on different crop plants was reported in previous studies. In this regard, Kim *et al*. (2017) found that treating of Chinese cabbage, radish, tomato, and mustard plants with *B. amyloliquefaciens* H-2-5 led to enhancement of their growth. Production of the phytohormones gibberellins (GA4, GA8, GA9, GA19, and GA20) and phosphate solubilization ability were the used mechanisms. Production of indole-3-acetic acid has been also reported to contribute to their plant-growth-promoting effect (Shao *et al*., 2015). These mechanisms seem to contributed to the plant growth promoting potential of *B. amyloliquefaciens* GGA on garlic plants.

In conclusion, the present study demonstrated the biocontrol activity of AMF and/or the endophytic *B. amyloliquefaciens* GGA against the white rot of garlic. However, the synergistic effect of application of AMF and the endophytic *B. amyloliquefaciens* GGA as a dual biocontrol treatment was also confirmed. Both of them played important roles in triggering the garlic resistance to the infection with *S. cepivorum* through improving plant nutrition, growth, stimulating photosynthetic pigments, accumulation of some antimicrobial substances (phenolic compounds and defense-related enzymes), and activation of some defense-related genes. For upcoming work, we suggest studying application of these biocontrol agents under open field conditions to evaluate their efficacy, survival, and microbial interactions with the soil microbiome.

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