Efficiency of Rhizobacteria as Elicitors for Controlling Anthracnose in Pepper Plant

Eman E. S. El-Sharkawy[1*], Monga I. Mossa[2] and Mohamed I. Khalil[3]

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt [1], Botany and Microbiology Department Faculty of Science, Arish University, Egypt[2], Botany Department, Faculty of Agriculture Suez Canal University, Egypt[3]

Received :20/4/2024

Abstract: The study aimed to determine the influence of some of plant growth promoting rhizbacteria (PGPRs), i.e., *Serratia marcescens*, *Bacillus pumilus*, *Bacillus siamensis* and *Brevundimonas diminuta* on *Colletotrichum acutatum*, the causal agent of anthracnose in pepper, under both *in vitro* and glasshouse conditions. Under the first condition, *B. pumilus* and *B. diminuta* had the most effective PGPRs in reducing *C. acutatum* mycelial growth with 62.59 and 58.52%, respectively. Under glasshouse experiment, soil drenching and foliar spraying application was performed to evaluate the effects of *B. pumilus* and *B. siamensis* rhizobacteria either as individual or combined treatment against anthracnose in pepper plant. Combined treatment of *B. pumilus* and *B. siamensis* promoted and stimulated the growth parameters of the seedlings of pepper as well as elevated the levels of non- enzymatic compounds (Free phenolic compounds and Total protein) and enzymes activity (Polyphenol-oxidase, Peroxidase and Superoxide dismutase). Also, combined treatment caused a significant decrease in disease severity and showed potential as plant growth-promoting bacteria.

Keywords: Biological control, *Bacillus pumilus*, *B. siamensis*, *Colletotrichum acutatum*.

INTRODUCTION

Colletotrichum species are significant plant pathogens with substantial scientific and commercial impact and ranked among the top ten fungal pathogens (Dean *et al.,* 2012). These fungi are known as greatly destructive, causing numerous diseases such as anthracnose in various agricultural crops, including peppers (Than *et al.,* 2008; Guo *et al.,* 2022). Pepper anthracnose is mainly caused by, *C. gloeosporioides*, *C. coccodes, C. capsici and C. acutatum* (Sim.) which, frequently mentioned globally and linked to anthracnose in bell peppers and chilli peppers (Montri *et al.,* 2009; Damm *et al.,* 2012; El-Sharkawy and ElSharawy, 2023) and caused severe yield and quality losses in crop. Anthracnose in hot peppers appears as sunken spots that range from circular to irregular shapes, featuring dark centers and paler edges on the fruit. These spots can grow larger and may develop a concentric ring pattern. In advanced stages, infected peppers might fall off the plant early, causing a decrease in crop production. The fungus causing this disease flourishes in environments with high humidity and wet foliage (Salotti *et al*., 2022). Different species exhibit varying levels of aggressiveness and susceptibility to fungicides (Chechi *et al.,* 2019). Nevertheless, the extensive usage of chemical fungicides has led to the growth of fungicide-resistant pathogens (Ishii *et al.,* 2022), generating concerns about the long-term consequences for human health and the environment. Consequently, there has been an increasing shift toward using biocontrol agents (BCAs) as a safer alternative to conventional fungicides (Riseh *et al.,* 2022). Recently, great attention has been given for the use of endophytic bacteria in plant protection and plant growth promotion opening newer windows for microbial exploitation (Peia *et al.,* 2023). Conversely, Gram-positive bacteria, including *Bacillus velezensis* LY7 have been investigated primarily for their role in enhancing plant growth parameters and managing plant diseases of anthracnose in pepper plants (Zou *et al.,* 2024). *Bacillus* species are regarded as one of the viable biocontrol agents that can serve as alternatives to chemical pesticides (Etesami *et al.,* 2023). These species have significant potential for governing infections by directly inhabiting pathogen growth through the production of various metabolites, enzymes, low molecular weight compounds, and volatile substances (Rehman and Leiknes, 2018). Their rapid growth in culture, ability to produce highly resilient endospores, and secretion of diverse bioactive compounds make them ideal candidates for commercialization. (Wu *et al.,* 2015). In context of these prospective advantages, the goals of this investigation were to: a) evaluate the potential efficacy of the PGPRs strain (*Serratia marcescens*, *Bacillus pumilus*, *Bacillus siamensis* and *Brevundimonas diminuta*) on the linear growth of *Colletotrichum acutatum* under *in vitro* conditions b) to evaluate the total amount of protein and phenolic compounds and the activity of oxidative enzymes, in order to clarify the potential relationship between their activity and the plant's induced defense mechanisms, and c) to explore the effectiveness of plant growth-promoting rhizobacteria (PGPR) in managing anthracnose disease in pepper plants, both as standalone treatments and in combination therapies.

MATERIALS AND METHODS

Isolation and Identification: *Colletotrichum acutatum* was isolated from pepper plants exhibiting characteristic anthracnose symptoms. Lesions measuring 5 mm in diameter were sliced, and the plant tissues were subjected to surface sterilization. This involved treating them with 1% NaOCl for 1-2 minutes, followed by 70% ethyl alcohol for 30

*Corresponding author e-mail: dremansadek@agr.suez.edu.eg

seconds. The tissues were rinsed twice with sterilized distilled water, dried between two of sterilized filter papers, and then cultured on potato dextrose agar (PDA) medium supplemented with streptomycin. The Petri dish plates were incubated at 25±1 °C for 7 days, as described by Dutta *et al.* (2019). This isolate was identified based on morphological and cultural characteristics, in accordance with (Sutton, 1992). Verification of the identification was performed at the Assiut Mycological Centre, Faculty of Science, Assiut University (AUMC). The culture was stored at 4 °C being used in experiments.

I-*In vitro* experiment:-

Effect of antagonists (PGPR) on linear growth of *C. acutatum:* Strains of *S. marcescens*, *B. pumilus*, *B. siamensis*, and *B. diminuta* were sourced from Dr. Tamer Shawky, Department of Botany, Faculty of Agriculture, Suez Canal University. These biological agents were evaluated against *C. acutatum in vitro* conditions. A 5 mm disc of 7-day-old *C. acutatum* cultured on PDA was positioned near the edge of a Petri dish, while a streak of the test bacterium was applied on the opposite side of the plate. The control treatment consisted of plates inoculated only with *C. acutatum.* Each treatment was carried out in triplicate and the cultures were incubated at 25±1°C (Dutta *et al.,* 2019). The linear growth of *C. acutatum* was measured when it nearly covered the medium surface in the control treatment, and the reduction percentage of mycelial growth was calculated according to Siripornvisal *et al.* (2009). The biocontrol agent demonstrating the most potent antagonistic activity against *C. acutatum in vitro* was chosen for further study. *B. pumilus* and *B. siamensis* strains were identified using amplifying and sequencing the 16S rRNA techniques and deposited in NCBI /GenBank (National Center for Biotechnology Information) database system with accession numbers for strain *B. pumilus* MK501617.1 with 99.84 % similarity and strain *B. siamensis* MK373318.1 with 99.82% similarity.

II-Glasshouse experiments:-

a-Effect of PGPR on growth of pepper plant: Following the methods described by Lamsal *et al.* (2012) with minor modifications, 3-week-old pepper seedlings (Super Ammar F1 cultivar) were grown in pots containing a soil mixture of sand and vermi compost (3:1, v/v). To each pot, a 30 mL dose of the diluted bacterial suspension (1×10^8 CFU/ml) was applied via soil drenching once a week, with treatments spaced a week apart. The seedlings growth was monitored under glasshouse conditions, maintaining over 90% relative humidity, a 16-hour photoperiod, and a temperature of 25 °C. The experiments were arranged in Complete Randomized Design (CRD) with five replications and 3 seedlings /pot. At flowering stage some growth traits like, plant height (cm), leave number/plant, plant fresh weight(g), plant dry weight(g) were measured in the *B. pumilus*, B. *siamensis* and *B. pumilus* + *B. siamensis* treated plants and compared with those of the control plants (sterilized distilled water applied).

b-Effect of PGPR on pepper anthracnose under glasshouse conditions

-Inoculum preparation: Conidial suspensions of *C. acutatum* were obtained from the surface of a 7-day-old of *C. acutatum* at 25±1°C (Dutta et al., 2019) in Petri dishes by adding 30 ml of sterile distilled water to a PDA medium. Conidia were gently removed with a soft-bristled brush and filtered through two layers of sterile cheesecloth. They were then counted using a hemocytometer, and the concentration was adjusted to 1×10^6 spores/ml.

-Rhizobacteria inoculum: According to Starr *et al.* (1981), in 100 ml flasks with 40 ml of sterilized tryptic soy agar (TSA) medium, *B. pumilus* or/ *B. siamensis* strain was cultured. Then these flasks were incubated at 30°C for 3 days, and the viable cell count was adjusted to 1×10^8 CFU/ml.

-Plant treatment: According to methods of Yadav *et al.* (2021) with some modification, Soil drenching and foliar spraying application were carried out to treat the seedling 3- week - old of pepper plants cultivar (super Ammar F1) with suspensions of *B. pumilus* , *B. siamensis* and *B. pumilus* + *B. siamensis* (10^8 CFU/ml) for five times in the entire life cycle of the plant at 20 ml under glasshouse condition (>90% relative humidity, 16 hrs. photoperiod) at 25˚C. After treatment with bioagents at the fruiting stage, the fruits were sprayed with a conidial suspension of *C. acutatum* (10 mL of 1×10^6 spores/ml). They were then covered with autoclaved plastic bags for 96 hours to maintain moisture. Untreated pepper fruits were sprayed with 10 ml of sterile dH_2O as a negative control(control1), while pathogen-treated fruits, serving as the positive control(control 2), were also covered for 96 hours to retain humidity. The development of anthracnose symptoms was observed for 10 days following pathogen inoculation. Disease severity was evaluated using the scale ranging from 0 to 3 proposed by Anaruma *et al.* (2010). However, disease severity index (DSI%) was calculated according to Chakraborty *et al.* (2019). Control efficacy (%) was determined according to Bae *et al.* (2021). Three separate trials were used for the experiment, each with three plants in each treatment. Pepper fruits, which treated with bioagents under the pathogen-challenged condition, positive control and negative control, were harvested at 72 hours post inoculation (hpi) then stored at −80 °C and tagged to determine non-enzymatic compounds and antioxidant enzyme activity.

III-Determination of free phenolic and total protein compounds:-

a-Free phenolic compounds: Fresh fruit was extracted using ethanol following the method of Abdel Rahman *et al.* (1975). In a test tube, 1 mL of the ethanolic extract was combined with 1 mL of 2N Folin-Ciocalteu reagent, 1 ml of 14% Na_2CO_3 solution, and 7 mL of distilled water. A water bath at 70°C was used to heat the mixture. By using the Folin-Ciocalteu method (Horwitz *et al.,* 1970) the phenolic content was

analyzed at 650 nm, applying a correction factor of 0.0042.

b-Total protein measurement: fruit extract was prepared by homogenizing 0.2 g of fresh pepper fruit in 1 ml of 0.1 M phosphate buffer (pH 7). The homogenate was filtered and centrifuged at 10,000 rpm for 15 minutes, following the procedure of Urbanek *et-al.* (1991). The resulting supernatant was used to measure soluble protein concentration using the Bradford method (Bradford, 1976), reported in mg/g fresh weight, with a bovine serum albumin standard curve and a correction factor of 0.00233.

c-Determination of antioxidant enzyme activity: Enzyme extracts were prepared according to (Urbanek *et al.,* 1991).

d-Peroxidase (POD) activity: POD activity was measured spectrophotochemically following the method described by Hammerschmidt *et al.* (1982). The reaction mixture (2.9 mL) consisted of 0.25% (v/v) guaiacol in 10 mM sodium phosphate buffer (pH 6) with 10 mM H_2O_2. To initiate the reaction, 100 µL of the crude enzyme extract was added, and the reaction rate was monitored at 470 nm per minute.

e-Polyphenol-oxidase (PPO) activity: PPO was measured according to (Matta and Dimond,1963), The reaction mixture consisted of 0.1 ml of enzyme extract, 1 ml of 0.2 M potassium phosphate buffer (pH 7), and 1 ml of 10 mM catechol, with the volume adjusted to 6 ml with distilled water. This mixture was incubated for 30 minutes at 30 °C. PPO activity was determined by measuring the change in absorbance every 0.5 minutes at 430 nm, expressed as absorbance per gram of fresh weight per minute using a spectrophotometer.

f-Superoxide dismutase (SOD) activity: The reaction mixture consisted of 0.25 mL of methionine (13 mM), 0.25 mL of nitro blue tetrazolium (NBT, 80 µM), and 0.1 mL of EDTA (0.1 mM), with the total volume brought to 3 mL using a buffer solution. To this mixture, 0.25 mL of riboflavin (50 mM) was added, and the test tube was shaken thoroughly before being positioned 30 cm from a light source. The reaction proceeded for 20 minutes, after which it was halted by turning off the light. Superoxide dismutase (SOD) enzyme activity was determined by measuring its capacity to inhibit the reduction of NBT at 560 nm, based on the method described by Beauchamp and Fridovich (1971).

Statistical Analysis: Data were analyzed by Analnysis of Variance (ANOVA) using CoStat software (version 6.311). Means were separated using Duncan Multiple Range Test (DMRT) at $P < 0.05$ level of significance.

RESULTS

-Effects of different PGPR isolates against *C. acutatum in vitro*: All tested PGPRs significantly differed from the control in their ability to reduce the mycelial growth of *C. acutatum* (Table 1). *B. pumilus* was the most effective, achieving a 62.59% reduction in mycelial growth, while *B. diminuta* was the least effective, causing a 49.48% reduction in *C. acutatum* growth (Table 1).

-Effects of PGPR on the growth parameters of pepper plants under glasshouse Conditions: The tested PGPR treatments significantly improved plant growth parameters compared to the control treatment (Table 2). The combined treatment of *B. pumilus* and *B. siamensis* resulted in the longest plant length (76.13 cm), the highest number of leaves per plant (180.66 leaves/plant), and the greatest total fresh weight (917.7 g) and dry weight (191.75 g). Significant differences were observed among the PGPR treatments in comparison to the control.

-Suppressive effect of strains *B. pumilus* and *B. siamensis* on disease severity index of *C. acutatum*: The data in Table (3) indicate that the fermentation broths of *B. pumilus* and *B. siamensis* can effectively suppress anthracnose in peppers. The combined treatment with *B. pumilus* and *B. siamensis* significantly reduced the rate of disease development in the plants, decreased the disease index, and achieved the highest control efficiency of 69.08%. Statistically significant differences were observed in the Disease Severity Index (DSI) among the tested treatments compared to the control (Table 3).

-Effects of PGPR on free phenolic compounds and protein content in pepper plants: Table (4) presents results indicating a substantial impact of the treatments on non-enzymatic compounds, namely total protein and free phenols, as compared to the control group. The combined treatment of *B. pumilus* and *B. siamensis* resulted in the highest free phenol content at 504.66 mg/g fresh weight (fw), followed by *B. pumilus* alone at 486.72 mg/g fw, compared to the lowest free phenol content in the controls. For total protein, significant differences were observed among the treatments and controls. The *B. pumilus* + *B. siamensis* treatment achieved the highest level of total protein at 24.77 mg/100g fw, while the treated and untreated controls had the lowest levels at 21.32 and 18.99 mg/100g fw, respectively.

-Effects of PGPR on peroxidase, polyphenol-oxidase and superoxide dismutase activity in pepper plants: Data in Table (5) showed that with the presence of *C. acutatum,* application of biocontrol agents (*B. pumilus* and *B. siamensis*) increased the activities of enzymes (POD, PPO and SOD) in pepper plant compared to control application .Combined treatment of *B. pumilus* and *B. siamensis* recorded the highest significant value of enzyme activities of PPO, POD and SOD at (4.29, 2.88 and 2.04 unit/g fresh weight/1min) respectively, compared to controls treatment. Statistical analyses revealed significant differences in POD, PPO, and SOD activity among the tested PGPR treatments and the control treatments.

Table 1. Effect of certain PGPR on mycelial growth of *C. acutatum* and growth reduction %

Bioagent	Linear growth	Reduction %
Control	90.00a	--
B. pumilus	33.66c	62.59a
B. siamensis	37.33c	58.52a
Serratia marcescens	44.33b	50.74b
Brevundimonas diminuta	45.33b	49.48b
LSD 0.05	4.01	5.34

Means in the same column followed by different letters are significantly different (LSD, P< 0.05)

Table 2. Effect of PGPR on the growth parameters of pepper plants

Treatments	Plant height (cm)	Leaves number/plant	Plant fresh weight (g)	Plant dry weight (g)
Control	51.20c	118.67d	635.17c	102.85d
B. pumilus	64.47b	161.33c	803.76b	157.93b
B. siamensis	61.90b	168.33b	770.33b	138.23c
B. pumilus* + *B. siamensis	76.13a	180.66a	917.7a	191.75a
LSD	4.51	6.61	81.98	16.64

Means in the same column followed by different letters are significantly different (LSD, P< 0.05)

Table 3. Effect of PGPR on disease severity index of *C. acutatum* on pepper plants

Bioagent	Disease Severity Index (%)	Efficacy (%)
Control	56.66a	--
B. pumilus	21.53c	62.18b
B. siamensis	25.42b	55.07c
B.pumilus+B. siamensis	17.51d	69.08a
LSD 0.05	2.95	3.69

Means in the same column followed by different letters are significantly different (LSD, P< 0.05)

Table 4. Effect of PGPR on the contents of free phenolic compounds and protein content

Treatments	phenolic compounds mg/g fw	Total protein (mg/100g fw)
Control 1	411.05e	18.99c
Control 2	422.03d	21.32bc
B. pumilus	486.72b	23.49ab
B. siamensis	469.98c	23.03ab
B.pumilus+B. siamensis	504.66a	24.77a
LSD 0.05	10.51	2.66

Means in a column followed by the same letters are not significantly different (LSD, P< 0.05)

Table 5. Effect of PGPR on POD, PPO and SOD activity

Bioagent	Peroxidase (POD) activity	Polyphenol-oxidase (PPO) activity	superoxide dismutase (SOD) activity
Control 1	2.09d	0.82d	0.27c
Control 2	2.85c	1.07d	0.41c
B. pumilus	3.89ab	2.27c	1.55b
B. siamensis	3.56b	2.63b	1.72ab
B.pumilus* + *B. siamensis	4.29a	2.88a	2.04a
LSD 0.05	0.46	0.27	0.33

Means in a column followed by the same letters are not significantly different (LSD, P< 0.05)

DISCUSSION

In an *in vitro* experiment, all tested PGPR isolates demonstrated a reduction in the mycelial growth of *C. acutatum* compared to the control. Notably, *B. pumilus* exhibited the most significant ability to inhibit the mycelium growth of *C. acutatum* with reduction (62.59 %). These results align with the work of Park *et al.* (2013), who documented the antagonistic effects of the *B. vallismortis* strain BS07 against anthracnose in pepper caused by *C. acutatum*, with inhibition zones measuring 28.8 cm in dual culture plates. According to Lamsal *et al*. (2012), all the bacterial isolates demonstrated more than 50% effectiveness in inhibiting the radial growth of *C. acutatum*. *Bacillus* isolates L1-7 and L3-5, derived from pepper leaves, demonstrated inhibition rates of growth at 79% and 80%, respectively, against *C. scovillei* mycelium (Wei *et al*., 2023). Additionally, an *in vitro* assay revealed that *Bacillus* sp. BSp.3/aM exhibited inhibitory effects against *C. capsica* (Jayapalan*et al.,* 2019). Similarly, *B. velezensis* BS1, isolated from pepper, showed a 45.60% inhibition rate on the mycelial growth of *C. scovillei* (Shin *et al.,* 2021). Moreover, the *in vitro* antagonistic activity of *B. subtilis* GYUN-2311 against *C. coccodes*, *C. acutatum*, *C. nymphaea* and *C. fioriniae* resulted in inhibition rates exceeding 70% (Heo *et al*., 2024).

The application of the tested PGPRs, whether used alone or in combination, significantly enhanced various growth parameters in pepper plants. These results are consistent with the findings of Jayapala *et al.* (2019), where *Bacillus* sp. BSp.3/aM was shown to improve the health of chili plants by suppressing anthracnose disease. The study also reported a marked improvement in seedling vigor and a germination rate of 98% in chili seedlings under greenhouse conditions. Also, seven rhizobacterial isolates were studied and AB17 isolate was the greatest enhancement of pepper growth (Lamsal *et al*., 2012) under greenhouse conditions. Shin *et al.* (2021) reported that *B. velezensis* BS1 significantly promoted the growth of chili pepper seedlings, leading to increased plant height, leaf width, leaf length, root length, and root fresh weight compared to untreated plants. Similarly, the ability of *B. velezensis* LY7 to control anthracnose in pepper and enhance growth was demonstrated in pot experiments, where the LY7 strain was found to induce hormone synthesis in pepper plants, boosting disease resistance and promoting growth (Zou *et al.,* 2024). Additionally, Sutariati *et al.* (2014) showed that seed treatments with *P. fluorescens* PG01, either alone or in combination with *B. polymyxa* BG25, led to increased yields and improved the quality of seed under both glasshouse and field conditions.

Under glasshouse conditions, the Disease Severity Index (DSI) of pepper affected by anthracnose was significantly reduced by treatments involving PGPRs. These findings are consistent with those of Lamsal *et al.* (2012), who found that the *P. polymyxa* (AB15) isolate was the most effective in suppressing the severity of pepper anthracnose caused by *C. acutatum* under similar conditions. Similarly, Park *et al.* (2013) reported a significant reduction in anthracnose infection on mature fruits in plants treated with BS07 or BTH compared to untreated controls. Additionally, pot experiments demonstrated excellent control of *C. scovillei* in pepper by *B. amyloliquefaciens* (L1-7) and *B. velezensis* (L3-5), with control rates of 80.64% and 73.39%, respectively (Wei *et al.,* 2023). Application of bioagents (*B. pumilus* and *B. siamensis*), either individually or in combination to pepper plants led to a significant elevation in free phenolic compounds and protein content compared to the controls group. As phenolic compounds accumulated, an infection-induced resistance was produced as reported by Hammerbacher *et al*. (2011). The treatment with Rhizobacteria of *Bacillus* sp. BSp.3/aM induced resistance against anthracnose disease in chili pepper and increased the accumulation of phenolic compounds (Jayapala *et al*., 2019*). B. velezensis* LY7 was found to secrete an antibiotic protein with a cup in domain. RNA-sequence

analysis of the *C. scovillei* transcriptome at multiple time points following exposure to LY7 (24, 48, and 72 hours) revealed the upregulation and downregulation of several genes (Zou *et al.,* 2024).

The results in this study clearly indicate the positive role of PGPRs in upregulating POD, PPO and SOD activities in pepper under biotic stress. Several studies have elucidated that the level enzyme activities of POD, PAL and PPO are an important biochemical indicator of the induction of disease resistance in plants (Zhao *et al*., 2021). Similarly, Jayapala *et al*. (2019) reported that the treatment with Rhizobacteria *Bacillus* spp. induced resistance against anthracnose disease in chili pepper and increased the activities of defense related enzymes (PAL, POX, PPO, LOX, and chitinase). Zou *et al.* (2024) showed that treating pepper plants with LY7 fermentation broth stimulated the production of endogenous hormones, thereby promoting plant growth. This treatment also boosted the activity of antioxidant and defense-related enzymes, such as PAL, PPO, CAT and SOD. Additionally, this treatment increased the activity of defense-related and antioxidant enzymes, including CAT, PPO, SOD, and PAL. Similarly, Sutariati *et al*. (2014) discovered that seed treatment with *P. fluorescens* PG01, either alone or combined with *B. polymyxa* BG25, triggered resistance against *C. capsici*. This induced resistance was linked to increased peroxidase activity and the enhanced biosynthesis of phytoalexins, both of which are key mechanisms in plant disease resistance.

CONCLUSIONS

Based on laboratory and glasshouse experiments, the tested PGPR strains, *B. pumilus* and *B. siamensis,* showed significant potential for controlling anthracnose disease in pepper plants. Inoculating pepper seedlings with these PGPR strains enhanced the plants' tolerance to anthracnose by improving plant vigor and growth parameters, inducing plant defense mechanisms, and exerting an antagonistic effect against the pathogen. Notably, the combined inoculation of these strains led to a marked increase in all measured growth parameters and resulted in a significant reduction in disease severity. These findings suggest that inoculating seedlings with microbial control agents could be a promising strategy for managing anthracnose disease in pepper plants.

REFERENCES

Abdel-Rahman, M., T.H. Thomas, G.J. Doss and L. Howell (1975). Changes in endogenous plant hormones in cherry tomato fruits during development and maturation. Physiol Plant, 34(1): 39-43.

Anaruma, N.D., F.L. Schmidt, M.C.T. Duarte G.M. Figueira, C. Delarmelina, E.A. Benato and A. Sartoratto (2010). Control of *Colletotrichum gloeosporioides* (penz.) Sacc. in yellow passion fruit using Cymbopogon citratus essential oil. Braz. J. Microbiol., 41: 66–73.

Bae, S., J.W. Han, Q.L. Dang, H. Kim and G.J. Choi (2021). Plant disease control efficacy of *Platycladus orientalis* and its antifungal compounds. Plants, 10: 1496. doi:org/10.3390/ plants10081496.

Beauchamp, C. and I. Fridovich (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem., 44 (1): 276 – 287.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem., 72: 248-254.

Chakraborty, N., K. Mukherjee, A. Sarkar and K. Acharya (2019). Interaction between Bean and *Colletotrichum gloeosporioides*: Understanding through a biochemical approach. Plants, 8: 345.

Chechi, A., J. Stahlecker, M. E. Dowlingand and G. Schnabel (2019). Diversity in species composition and fungicide resistance profiles in Colletotrichum isolates from apples. Pesticide Biochem. Physiol. 158 :18–24. doi: 10.1016/j.pestbp.2019. 04.002.

Damm, U., P.F. Cannon, J.H.C. Woudenberg and P.W. Crous (2012). The *Colletotrichum acutatum* species complex. Studies in mycology, 73: 37-113.

Dean, R., J.A.L Van Kan, Z. A. Pretorius, K. E. Hammond-Kosack, A. Di Pietro, P. D. Spanu, J.J. Rudd, M. Dickman, R. Kahmann, , J. Ellis and G.A. Foster (2012). The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol., 13: 414–430.

Dutta, S, E-E. Woo, S-M. Yu, R. Nagendran, B-S. Yun and Y.H. Lee (2019). Control of anthracnose and gray mold in pepper plants using culture extract of white-rot fungus and active compound schizostatin. Mycobiology, 47: 87-96.

El-Sharkawy, E.E.S. and A.A. ElSharawy (2023).The Influence of Resistance Chemical Inducers Against Anthracnose on Cucumber and Pepper Plants. Catrina: The International Journal of Environmental Sciences, 29(1):57-67.

Etesami, H., B. R. Jeong and B. R. Glick (2023). Biocontrol of plant diseases by *Bacillus* spp. Physiol. Mol. Plant Pathol., 126:102048.

Guo, Z., C.-X. Luo, H.-J. Wu, B. Peng, B.-S. Kang, L.-M. Liu, M. Zhang and Q.-S. Gu (2022). Colletotrichum Species Associated with anthracnose disease of watermelon (*Citrullus lanatus*) in China. J. Fungi, 8: 790. doi:org/10.3390/jof8080790

Hammerbacher, A., S.G. Ralph, J. Bohlmann, T.M. Fenning, J. Gershenzon and A. Schmidt (2011). Biosynthesis of the major tetrahydroxystilbenes in spruce, astringin and isorhapontin, proceeds via resveratrol and is enhanced by fungal infection. Plant Physiol., 157:876–890.

Hammerschmidt, R., E.M. Nuckles and J. Kuc (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to

Colletotrichum lagenarium. Physiological Plant Pathology, 20 (1): 73-76.

Heo, Y., Y. Lee, K. Balaraju and Y. Jeon (2024). Characterization and evaluation of *Bacillus subtilis* GYUN-2311 as a biocontrol agent against *Colletotrichum* spp. on apple and hot pepper in Korea. Front. Microbiol., 14:1322641. doi: 10.3389/fmicb.2023.1322641.

Horwitz W, P. Chichilo and H. Reynolds (1970). Official methods of analysis of the association of Official analytical chemists. Washington, DC, USA

Ishii, H., H. Watanabe, Y. Yamaoka and G. Schnabel (2022). Sensitivity to fungicides in isolates of *Colletotrichum gloeosporioides* and *C. acutatum* species complexes and efficacy against anthracnose diseases. Pestic Biochem. Physiol., 182:105049. doi: 10.1016/j.pestbp.2022.105049.

Jayapala, N., N.H. Mallikarjunaiah, H. Puttaswamy, H. Gavirangappa and N.S Ramachandrappa (2019). Rhizobacteria *Bacillus* spp. induce resistance against anthracnose disease in chili (*Capsicum annuum* L.) through activating host defense response . Egyptian Journal of Biological Pest Control, 29:45. doi.org/10.1186/s41938-019-0148-2.

Lamsal, K., S. W. Kim, Y. S. Kim and Y. S. Lee (2012).Application of rhizobacteria for plant growth promotion effect and biocontrol of anthracnose caused by *Colletotrichum acutatum* on Pepper. Mycobiology, 40(4): 244-251.

Matta, A. and A.E. Dimond (1963). Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology, 53: 574-578.

Montri, P., P.W.G. Taylor and O. Mongkolporn (2009). Pathotypes of *Colletotrichum capsici*, the causal agent of chili anthracnose, in Thailand. Plant Disease, 93: 17-20.

Park, J.-W. , K. Balaraju , J.-W. Kim, S.-W. Lee and K. Park (2013). Systemic resistance and growth promotion of chili pepper induced by an antibiotic producing *Bacillus vallismortis* strain BS07. Biological Control, 65: 246–257.

Peia, D., Q. Zhanga, X. Zhua and S. Han (2023). Endophytic *Bacillus subtilis* P10 from Prunus cerasifera as a biocontrol agent against tomato Verticillium wilt. Brazilian Journal of Biology, 83, e244261. doi:org/10.1590/1519-6984.244261.

Rehman, Z. U. and T. Leiknes (2018). Quorum-quenching bacteria isolated from red sea sediments reduce biofilm formation by *Pseudomonas aeruginosa*. Front. Microbiol., 9:1354. doi: 10.3389/fmicb.2018.01354.

Riseh, R. S., M. Hassanisaadi, M. Vatankhah, F. Soroush and R. S. Varma (2022). Nano/microencapsulation of plant biocontrol agents by chitosan, alginate, and other important biopolymers as a novel strategy for alleviating plant biotic stresses. Int. J. Biol. Macromol., 222 :1589–1604. doi: 10.1016/j.ijbiomac.2022.09.278.

Salotti, I., T. Ji and V. Rossi (2022). Temperature requirements of *Colletotrichum* spp. belonging to different clades. Front. Plant Sci., 13:953760. doi: 10.3389/fpls.2022. 953760

Shin, J.-H., B.-S. Park, H.-Y. Kim, K.-H. Lee and K. S. Kim (2021). Antagonistic and plant growth-promoting effects of *Bacillus velezensis* BS1 isolated from rhizosphere soil in a pepper field. Plant Pathol. J., 37(3): 307-314. doi:org/10.5423/PPJ.NT.03.2021.0053.

Siripornvisal, S., W. Rungprom and S. Sawatdikarn (2009). Antifungal activity of essential oils derived from some medicinal plants against grey mould (*Botrytis cinerea*). Asian Journal of Food and Agro-Industry, Special Issue S229-S233.

Starr, M.P., H. Stolp, H.G. Trüper, A. Balows and H.G. Schlegel (1981). The Prokaryotes: A Handbook on Habitats, Isolation and Identification of Bacteria. Springer-verlag, Berlin, Germany

Sutariati, G.A.K., Widodo, Sudarsono and S. Ilyas (2014). Biological seed treatment for controlling anthracnose disease of hot pepper. IJSTAS, (1)1: 29-43.

Sutton, B.C. (1992). The genus Glomerella and its anamorph Colletotrichum. In: Bailey, J.A. and Jeger, M.J., Eds., Colletotrichum: Biology, Pathology and Control, CAB International, Wallingford, UK, 1-26.

Than, P. P., Prihastuti, H., Phoulivong, S., Taylor, P. W. J., and Hyde, K. D. (2008). Chilli anthracnose disease caused by Colletotrichum species. J. Zhejiang Univ. Sci. B. 9: 764–778. doi: 10.1631/jzus.B0860007.

Urbanek, H., E. Kuzniak-Gebarowska and K. Herka (1991). Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. Acta Physiol Plant, 13: 43-50.

Wei, L., C. Yang, L. Cui, M. Jin and R. Osei (2023). Bacillus spp. isolated from pepper leaves and their function and inhibition of the fungal plant pathogen *Colletotrichum scovillei* Egyptian Journal of Biological Pest Control, 33:46.

Wu, L., H. J. Wu, J. Qiao, X. Gao, and R. Borriss (2015). Novel routes for improving biocontrol activity of Bacillus based bioinoculants. Front. Microbiol., 6:1395. doi: 10.3389/fmicb.2015.01395.

Yadav, M., M.K. Dubey and R.S. Upadhyay (2021). Systemic resistance in chilli pepper against anthracnose (Caused by *Colletotrichum truncatum*) Induced by *Trichoderma harzianum ,Trichoderma asperellum* and *Paenibacillus dendritiformis.* J. Fungi, 7: 307. doi:org/10.3390/ jof7040307.

Zhao, J., T. Liu, D. Zhang, H. Wu, T. Zhang and D. Dong (2021). Biocontrol potential of *Trichoderma afroharzianum* TM24 against grey mould on tomato plants. Current Microbiology, 78(12):4115–4126. doi: 10.1007/s00284-021-02671-x.

Zou, X. , J. Ning, X. Zhao, H. Lv, N. Qin, H. Yin and L. Ren (2024). *Bacillus velezensis* LY7 promotes pepper growth and induces resistance to *Colletotrichum scovillei*. Biological Control, 192 :105480.

كفاءة الريزوبكتيريا كمحفزات لمكافحة مرض الأنثراكنوزفى نبات الفلفل

ايمان السيد صادق [1*] ، منجه ابراهيم موسى[2]، محمد ابراهيم خليل[3]

معهد بحوث أمراض النبات، مركز البحوث الزراعية، الجيزة، مصر[1] ,قسم النبات والميكروبيولوجى، كلية العلوم ، جامعة العريش، مصر[2] ,قسم النبات الزراعى كلية الزراعة، جامعة قناة السويس، مصر[3]

تهدف الدراسة لاختبار تأثيرالعزلات البكتيرية لكلا من بكتيريا *Serratia marcescens* و*Bacillus pumilus* و*Bacillus siamensis* و*Brevundimonas diminuta* ضد فطر *Colletotrichum acutatum* المسبب المرضى لمرض الأنثراكنوز علي نباتات الفلفل. أوضحت التجارب تحت الظروف المعملية قدرة العزلات المختبرة *B. pumilus* و*B. siamensis* (PGPRs) أكثر فعالية فى تثبيط النمو الميسليومى لفطر *C. acutatum* بنسبة (59. 62 و 52. 58٪). تم اجراء التجارب تحت ظروف الصوبةعن طريق غمر التربة والرش الورقي لتقييم فعالية العزلات *B. pumilus* و *B. siamensis* سواء بشكل فردى أو مجتمعة كمحفزات لمكافحة مرض الأنثراكنوز على نباتات الفلفل. أوضحت النتائج أيضا المعاملة المشتركة ل (*B. siamensis*) + (*B. pumilus*) ادت الى ذيادة مقاييس النموالخضرى فى نباتات الفلفل. كما أدت الى زيادة مستوى المركبات غير الأنزيمية (المركبات الفينولية الحرة والبروتين الكلي) ونشاط الإنزيمات (بوليفينول أوكسيديز، بيروكسيديز و سوبرأوكسيد ديسموتيز). أيضا أدت المعاملة المشتركة للريزوبكتيريا الى انخفاض كبير فى نسبة شدة الأصابة بالمرض وأظهرت كفاءة مقاومة البكتيريا المشجعة لنمو النبات.