ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 09, pp.38376-38382, September, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

EFFICIENCY OF SOME BIOAGENTS FOR TREATMENT OF TOMATO PLANTS INFECTED WITH *BOTRYTIS CINEREA*

***Lubna S. Nawar**

Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

ARTICLE INFO

Article History:
Received 22nd June, 2016
Received in revised form
27th July, 2016
Accepted 16th August, 2016
Published online 30th September, 2016

Key words:

Botrytis cinerea,
Streptomyces, Bioagent,
Biocontrol, biocides,
Bacillus, *Trichoderma harzianum*.

ABSTRACT

Botrytis cinerea is quite destructive and damaging plant disease, which infect tomato and many other plants. Effective means of protection using bioicides is of interest in recent years. The used bioagents were Streptomyces anulatus SM21, S. coelicolor SM1, *Bacillus pumilus* and *Trichoderma harzianum.* They were selected for the high production of lytic enzymes including chitinase and protease on solid and broth media. Effect of these bioicides on spore germination and radial growth of *Botrytis cinerea in vitro* were recorded. Streptomyces anulatus SM21 was the most active bioagent and inhibited *Botrytis cinerea* spore germination by 83% and inhibited fungal growth (inhibition zone diameter, 26.5 mm). Tomato plants were grown in sterile soil and infected with *Botrytis cinerea.* The infected plants recoded significantly higher level of pectinase, peroxidase and catalase enzymes compared to control plants (non infected plants) while treatment with biocides decreased levels of both peroxidase and catalase to levels near to that of healthy plants. Moreover, inoculation of the infected plants with the bioagent decreased the fungal infection and enhanced plant growth criteria. Shoot height (cm /plant) root depth (cm/plant), number of leaves, dry weights of shoot and leaves were enhanced compared to infected plants. Infection of the plants with *Botrytis cinerea* decreased Chlorophyll a+b content of fresh leaves, Nitrogen (N), Phosphorous (P) and Mg^{+2} contents (mg/g) of the shoot system compared to healthy plants while increased total soluble protein and total soluble sugars. Treatment of plants by biocides removes almost the bad changes associated with the plant fungal infection. The most effective treatment was using Streptomyces anulatus SM21. In conclusion, *Botrytis cinerea* can be controlled using Streptomyces anulatus SM21, **S.** coelicolor SM1, *Bacillus pumilus* or *Trichoderma harzianum* due to the presence lytic enzyme and their effects were similar to that obtained by the antifungal Mancozeb.

Citation: Lubna S. Nawar, 2016. "Efficiency of some bioagents for treatment of tomato plants infected with *botrytis cinerea*", *International Journal of Current Research*, 8, (04), 38376-38382.

INTRODUCTION

The well-known fungus, *Botrytis cinerea* cause gray mold infection to a wide host ranges including tomato, potato, onion, grapes and strawberry and cause yield losses (Janisiewicz, 1994, De Curtis *et al*., 1996). It can live and survive over a widely distributed area, under wide range of conditions and for long period as saprophytic fungus. *Botrytis cinerea* isolates reduced crop productions and resist many chemical fungicides which are unsafe and have potential to be carcinogens. Biocontrol of this harmful fungus using certain microbes including bacteria, fungi or yeasts has clinical, industrial and agronomic interest and is an alternative treatment to chemical fungicides (Mahmoud *et al*., 2004, Mouekouba *et al*., 2013).

****Corresponding author: Lubna S. Nawar,***
Department of Biology, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

Biocontrol of pathogenic fungi have been studied for potential applications in the field and preservation of foods (Lowes *et al*., 2000, Sabaratnam and Traquair, 2002). The possibility of using the bioagent *Streptomyces* as novel and effective biocontrol agents against fungi of environmental and agronomical significance is of growing interest. Sabaratnam and Traquair (2002) used Streptomyces as biological control agent for damping-off disease in tomato seedlings, caused by *Rhizoctonia*. Van Minh *et al*. (2015) reported a potent antifungal activity for Streptomyces sp. A3265 against some fungal plant pathogens including Botrytis cinerea**,** Colletotrichum gloeosporioides, and Rhizoctonia solani. They added that Streptomyces sp. A3265 can be used to biocontrol ginseng root rot and damping-off disease due to the presence of guanidylfungin A and methyl guanidylfungin A which showed antifungal activity against various fungal and bacterial plant pathogens. Biological processes using *Trichoderma*

harzianum are known antagonists to other fungi and have been shown to be very potent biocontrol agents for plant harmful fungi (Ait-Lahsen *et al*., 2001). Some yeast secreted killer toxins with pretentious nature which is lethal to other yeasts and some fungal isolates (Santos *et al*., 2004) which lived under environmental stress (Marquina *et al*., 2002). Walker *et al*. (1995) found that *Pichia anomala*, *P. membranifaciens* and *S. cerevisiae* suppress some wood decaying and plant pathogenic fungi including *B. cinerea* and *Rhizoctonia solani*. For example, *P. membranifaciens* protect grape vine plants against *B. cinerea* (Masih *et al*., 2001) and Candida sake protects apples and pears against *Penicillium expansum* and *B. cinerea* (Nunes *et al*., 2002).

The interactions between yeasts, fungi and bacteria may play a key role in the natural process of biocontrol, although the molecular mechanisms involved are still largely unknown. Secretion of cell wall degrading enzymes (Masih *et al*., 2001), competition for nutrients (Filonow, 1998), predation and production of toxins (Woo *et al*., 2002) are possible mechanisms of biocontrol. Moreover, the rhizosphere Bacillus subtilis, obtained from Chilli, had high antagonistic activity for Colletotrichum gloeosporioides, with inhibition zone diameter of 0.5–1.0 cm (Ashwini and Srividya, 2014). Furthermore, many species of the genus Trichoderma were used as biocontrol agent of a broad range of phytopathogenic fungi (Benítez *et al*. 2004). Schilly *et al*. (2014) reported that T. asperellum significantly inhibited the fungus *Fusarium* oxysporum f. sp. cubense which is devastating casual agent of *Fusarium* wilts of banana, worldwide dangerous problem in banana industry. The antagonistic properties of the genus Trichoderma are due to multiple mechanisms which are in synergistic interaction. Trichoderma inhibited fungal growth through mycoparasitism, nutrients and space competion for, enhancing plant growth and development through production of material like biofertilisation (Benítez *et al*. 2004; Harman *et al*. 2006) and Trichoderma are mainly resistant to fungicides, thus they represent a biocontrol agent of banana leaf pathogens under commercial conditions. Samuelian *et al*. (2016) identify many Trichoderma strains from Musa acuminata leaves and develop a strategy to support banana leaves colonization by Trichoderma spores under natural conditions and support the evidence of using Trichoderma spp. as a biological agent in integrated management program. In this study, the potential biocontrol of the grey mould *B. cinerea* is studied and the antagonistic potential of some local bioagents against the tomato pathogen *Botrytis cinerea in vivo* and *in vitro* is investigated.

MATERIALS AND METHODS

Isolation and screening of chitinolytic bacteria

Bacillus pumils, Streptomyces anulatus SM21 and Streptomyces coelicolor SM1 and *Trichoderma harzianum* NRRC-143 were obtained from the culture collection of the Biology Department, Faculty of Science, KAU. *Bacillus pumils*, S. anulatus SM21 and S. coelicolor SM1were isolated from shrimp, characterized and genetically identified by Aly *et al*., (2011a,b) while *Trichoderma harzianum* NRRC-143 was from the Microbial Properties Research Unit, USA. The bacteria were grown and preserved on Nutrient agar medium with 2% yeast extract (ATCC Medium 9) containing (g/l) Nutrient Agar (BD 213000) 23.0, yeast extract 20.0 while the fungus was grown on Potato dextrose agar (PDA) medium.

Chitinase detection

For chitinase production on agar plates, the tested microbes were grown on chitin medium (g/l): colloidal chitin 1.0 (Trachuk *et al*., 1996), KH_2PO_4 0.7, K_2HPO_4 0.3, NaCl 4 and $MgSO_4.7H_2O$ 0.5 (Kim *et al*., 2003). For one liter, 20 g agar was added when solid medium was needed. The plates were incubated at 37°C for 2 days for bacteria and at 25°C for days for *Streptomyces* and fungi. All plated were examined for the presence of clear zone and the mean diameters were recorded. Second screening were carried out in 250 ml Erlenmeyer flasks contained 48 ml of chitin broth medium and inoculated with 2 ml of cell suspension containing $2x10^6$ cfu/ml for bacteria and $4x10^4$ for fungi and all flasks were incubated at 37°C and 80 rpm for 2 days for bacteria and at 25°C for 5 days for *Streptomyces* and fungi. The activity of chitinase in the culture filtrates was determined using dinitrosalicylic acid method as described by Miller, (1959). One chitinase unit is defined as the enzyme quantity which is needed for one µmol production at 50°C of reducing sugar per min. Protein content of the culture filtrate was fetermined (Bradford,1976).

Proteolytic activity

For proteinase production on agar plates, the tested microbes were grown Casein agar medium and the clear zone was examined. In liquid medium, the proteolytic activity of the tested microbes was determined according to Ramakrishna and Pandit (1988). The Casein substrate, 0.5 ml of 1% (w/v) in 50 mM phosphate buffer was incubated with 0. 25 ml microbial filtrate. After incubation at room temperature for 2 hours, 0.5 ml of trichloroacetic acid was added for stopping reaction. The mixture was centrifuged as before under cooling, and proteolytic activity (absorbance 280 nm) was determined against blank and enzyme unit (U) is the amount of enzyme that causes 0.1 increases in the absorbance/hr. (Tremacoldi and Carmona, 2005).

Inoculum preparation, extraction and the inhibitory effects on *Botrytis cinerea*

Streptomyces anulatus SM21 and S. coelicolor SM1 cells were grown in starch nitrate broth medium (Shirling and Gottlieb, 1966) while *Trichoderma harzianum* NRRC-143 was grown Czapeks dox broth medium and growth was carried out for 5 days at 25°C and 80 rpm. *Bacillus pumils* was grown in Nutrient broth for 2 days at 37°C and 80 rpm. Finally, the microbial cells were collected, washed and suspended in sterile saline solution at concentrations, 6 x 10^6 cfu/ ml) and then used for plant treatments (foliar application). Each culture filtrate was dried by lypholiztion and the obtained residues were dissolved in sterile dist. water (1g/10 ml, wt/v) and filter sterilized and used to prepare water agar. Water agar (20 g/l) was prepared, filter sterilized and water agar plates containing the tested extract (20:1 v/v) were prepared. Then, 1 ml of *B. cinerea* spore suspension ($4x10^4$ cfu/ml) was spread on the surface of water agar plates and % of germination of *Botrytis*

cinerea spores was determined after 12 hrs. of incubation at 25°C in the dark using a light microscope. Using agar well diffusion assay method described by Holder and Boyce (1994), the effect of the tested microbial extracts on fungal growth was determined in Petri dish plates containing potato dextrose agar and inoculated with 1 ml of fungal spore suspension of *B. cinerea* (4 x 10^4 CFU/ml). All inoculated plates were incubated at 25°C for 7 days and the antifungal activity was determined by measuring the inhibition zone mean diameter (mm).

Soil preparation and plant growth and infection

Tomato seeds (Solanum lycopersicum) were obtained from local market, Jeddah, Saudi Arabia. Seeds were disinfected by soaking in 2% sodium hypochlorite for 15 min. and were rinsed several times using sterile water. The sterile seeds were put under the soil surface (5 seeds/ pot) and irrigated for 2 weeks with dist. water. Tomato plant shoots of 20 day old were infected by spraying using 20 ml of *Botrytis cinerea* spore suspension containing 4x10^4 spores/ml in 1% Tween 80. About 1 and 2 weeks after infection, infected plants of each pot were sprayed with 20 ml of each bioagent or Mancozeb (SHANGHAI) at 1000 ppm. Plants in each pot were left to be air-dried, sprayed with 15 ml of distilled water and covered with plastic bags for 2 h to maintain the high humidity atmosphere around the leaves. The plant was irrigated with tap water for 2 months and fertilization of soil with NPK was applied using 0.3 g of urea/kg soil, 0.37 g of $Ca_3(PO4)_2$/kg soil, and 0.125 g of K_2SO_4/kg soil. The effect of the tested microbes on the growth of tomato plants infected with *Botrytis cinerea* was determined.

Physiological measurements

After 60 days, plants were harvested and peroxidase, catalase and pectinase enzymes were determined in leaves (units/ml). One unit is the amount of enzyme reacts with 1 mmole of substrate/min. Fresh tomato leaves (about 0.5 g) were grinding in 50 mM phosphate buffer (pH 7), under cooling and the supernatant was collected after centrifugation (6000 rpm, 15 min.) and used for enzyme assay. Peroxidase and catalase assays were carried out according to Kato and Shimizu, (1987) while pectinase was assayed according to Somogyi (1952). The acetonic extract of green leaves was used for chlorophyll measurement (Arnon (1949, Moran and Porath, 1980). Finally, shoot and leave dry weights were recorded after drying at 60°C for 3 days. Total nitrogen and phosphorus concentrations of the plant were determined by micro-Kjeldahl method (Jacobs, 1958) and molybdenum blue method (Page, 1982), respectively while Mg^{+2} was quantified as described by Allen *et al*. (1974) using atomic absorption. Total soluble sugars (TSS) and total soluble proteins (TSP) in the plants were estimated according to Naguib (1963, 1964, and Lowry *et al*. (1951), respectively.

Statistical analysis

The obtained data were the mean of tree readings and statistically analysis using T- test one way analysis of variance or least significant difference (LSD). The differences at $p \leq 0.5$ were considered significant.

RESULTS AND DISCUSSION

Detection of chitinase and protease in Streptomyces anulatus SM21, S. coelicolor SM1, *Bacillus pumils*, and *Trichoderma harzianum* NRRC-143 were confirmed in solid and liquid media (Table 1 and 2). Maximum production of chitinase was obtained by Streptomyces anulatus SM21 (1.8 U/ml) while moderate production was recorded for the others tested microbes and no production was found in uninoculated medium. Streptomyces anulatus SM21, S. coelicolor SM1 and *Trichoderma harzianum* produce higher quantities of protease enzyme compared to control or *Bacillus*. The previous enzymes have interesting roles in fungal growth inhibition and fungal death. Streptomyces anulatus SM2, S. coelicolor SM1, *B. pumilus Trichoderma harzianum* were grown in suitable broth media and the culture filtrates were filter sterilized, concentrated using lyophilization and the antifungal activity on *Botrytis cinerea* spore germination and growth were determined (Table 3).

Table 1. Chitinase detection and activity for some bacterial isolates and one fungal isolate on and in chitin media

Tested microbe	Chitinase enzyme detection			
	Solid medium		Liquid medium	
	Growth on chitin agar medium	Clear zone formation (mm)	Absorbance	U/ml
Streptomyces anulatus SM21	++++	11.1±0.53	0.70 ±0.25	1.4±0.53
Streptomyces coelicolor SM1	+++	12.2±0.49	0.93±0.25	1.8±0.49
Bacillus pumilus	++	6.8±0.33	0.51±0.17	1.0±0.33
Trichoderma harzianum	+	10.1±0.02	0.22±0.08	0.4±0.15
Control (un- inoculated medium)	ND	ND	ND	ND
LSD	Significant difference at $p \leq 0.05$			

++: moderate growth, +++: High growth, ++++: very high growth, ND: Not found

Table 2. Protease detection and activity for some bacterial isolates and one fungal isolate on and in Casein media

	Protease enzyme detection			
	Solid medium		Liquid medium	
Tested microbe	Growth on casein agar medium	Clear zone formation (mm)	Absorbance	U/ml
Streptomyces anulatus SM21	+++	17.1±2.0	2.8 ±0.25	28.0±3.33
Streptomyces coelicolor SM1	+++	19.0±4.44	2.4±0. 52	24.0±5. 9
Bacillus pumilus	+	8.0±1.30	1.1±0.11	11.0±1.03
Trichoderma harzianum	+++	20.0±3.22	2.9±0.54	29.0±5.17
Control (un- inoculated medium)	ND	ND	ND	ND
LSD	Significant difference at $p \leq 0.05$			

++: moderate growth, +++: High growth, ++++: very high growth, ND: Not found

Culture filtrate of S. anulatus SM21 showed the highest inhibition in spore germination (83%), followed by S. coelicolor SM1 (70%), *B. pumilus* (66%) and finally *Trichoderma harzianum* (39%). Mancozebs showed inhibition in germination percentage (43%) of fungal spores. The antifungal effects of the culture filtrate of the tested microbes in addition to Mancozeb on *Botrytis cinerea* growth were determined using agar well diffusion method (Table 3). Maximum inhibition was recorded for Mancozeb (33.1 mm), followed by *T. harzianum* (29.5 mm) and S. anulatus SM21 (26.5mm). The two isolates Streptomyces anulatus and S. coelicolor were isolated from shrimp shells and showed the best growth and chitinase production in liquid medium containing soluble chitin (Aly *et al*., 2011a). Several species of the genus *Bacillus*, Streptomyces and *Trichoderma* were antagonistic for the fungal pathogens of different crops. *Bacillus* subtilis suspensions were used to control Alternaria citri, P. digitatum and Geotrichum candidum, isolated from citrus fruits (Singh and Deverall, 1984). In recent years, the demand for safer and 'sustainable food is increased and factories must be in areas close to agricultural plantations to limit using chemical spraying which cause fungal resistance and loss of effective control or increased the price of application. Reduction of chemical residues in food is of interest, thus the use of biological products particularly antagonistic bacteria or fungi which are believed to have the highest potential as a biofungicides around the world (Lorito *et al*. 2010; Mukherjee *et al*. 2014).

Most of the research conducted has investigated the potential of biological agents as alternatives for systemic chemicals for the control of different fungal pathogens. The biocontrol agent is clean strategy has many environmental benefits, decreased not only the fungal disease and replace the chemical fungicides but also reduced the appearance of resistant fungal isolates (Samuelian *et al*., 2016). Most studies were carried to find a biological agents which can be used as an alternatives to synthetic fungicides (Jacobsen *et al*. 2004) and the use of combination of biological agents with fungicides decreased infections and lead to disease suppression like fungicide treatment (Elad *et al*. 1993a, b; Monte 2001) and prevent fungicide resistance. Moreover, local isolates showed effective biocontrol activity than that obtained from different environments due to adaptation of these isolates to their environment. The effect of Streptomyces anulatus SM21, S. coelicolor SM1, *Bacillus pumils*, and *Trichoderma harzianum* NRRC-143 on *Botrytis cinerea* growth and development was determined *in vivo* using tomato plant as a host. The plants were grown in sterile soil and infected using spore suspension of *Botrytis cinerea*. Infected plants were treated with either Streptomyces anulatus SM21, S. coelicolor SM1, *Bacillus pumils*, or *Trichoderma harzianum*. Fungal infection increased some plant enzymes in fresh plants (pectinase, catalase and peroxidase) and treatment of the infected plant with the bioagents decreased the previous plant enzymes in fresh plants compared to infected plants (Table 4).

Table 3. % of Germination of *Botrytis* spores and the inhibition zone (mm) using culture filtrates of the tested biocontrol agents involving two species of *Streptomyces, Bacillus* and *Trichoderma harzianum* and compared to Mancozeb (1000 ppm)

	Streptomyces anulatus SM21	Streptomyces coelicolor SM1	*Bacillus pumilus*	*Trichoderma harzianum*	Mancozeb (1000 ppm)	Control
% of germination of spore	*83%*	*70%*	66%	39%	43%	0.0 %
Inhibition zone (mm)	26.5±4.58	16.0±1.33	19.0±1.18	29.5±3.00	33.1±5.12	0.0

Table 4. The means of the enzyme activities of fungal infected tomato plants and treated with three bacterial isolates, *Trichoderma harzianum,* Mancozeb and compared to control (non infected plants)

Plant enzyme tested	Control (Healthy plants)	Infected and non-treated	Infected plants and treated with				
			Streptomyces anulatus SM21	Streptomyces coelicolor SM1	*Bacillus pumilus*	*Trichoderma harzianum*	Mancozeb (1000 ppm)
Peroxidase	3.32	5.76*	*3.52*	*4.95*	4.44	3.88	3.7
Catalase	3.17	6.92*	*3.66*	*4.97*	3.92	4.12	4.6
Pectinase	3.88	7.55*	3.99	4.5	4.9*	5.3*	5.9*

*: in the same column followed by the same letter are not significantly different at the 0.05 level according to LSD

Table 5. Some growth criteria of 2 months old tomato plant infected with *Botrytis cinerea* and either treated with some biocontrol agents or Mancozeb or untreated and compared to control (healthy plant)

Tested parameter	Infected plants with *B. cinerea* spores						Control (Healthy plants)
	Treated plants with bioagents					Infected and non-treated	
	Streptomyces anulatus SM21	Streptomyces coelicolor SM1	*Bacillus pumilus*	*Trichoderma harzianum*	Mancozeb (1000 ppm)		
Shoot height (cm /plant)	60.1±3.3	49.1±1.5	53.1±3.2	55.11±3.4	51.11±5.5	31.1±3.0	69.1±3.5
Root depth (cm/plant)	6.1±0.5	6.0±0.5	5.1±1.3	4.91±3.5	6.1±0.5	4.22±0.5	6.1±3.5
Leaf number/plant	10.1±0.4	11±2.0	11±2.5	10.61±2.4	10.33±2.1	7.1±1.5	10.9±2.4
Shoot DW (g /plant)	9.5±2.4	7.15±2.4	7.44±2.4	9.25±2.4	8.55±2.0	2.04±0.05	10.8±2.4
Leaf DW (g /plant)	2.4±0.2	2.0±0.9	2.99±0.5	1.90±0.12	2.4.0±0.35	0.99±1.5	3.0±0.22
Chl a + b (mg/g f.wt)	10.4±0.2	10.31±0.1	8.78±.5	11.80±1.5	11.44±0.5	9.1±0.35	11.44±.50
TSS (mg/g)	279.4±9.2	213.5±7.2	190.4±10.2	222.4±13.0	189.9±22.2	200.3±9.1	252.4±6.9
TSP (mg/g)	165.55	155.77	143.55	148.77±4.9	155.7	151.44±7.9	146.66±2.1
N (mg/g)	23.40±2.31	21.40±0.76	22.40±0.741	20.40±0.43	20.10±0.11	24.6±2.15	24.40±0.71
P (mg/g)	14.80±0.21	15.20±0.31	15.40±0.71	13.30±0.71	14.10±0.71	11.5±1.3	17.90±3.33
Mg^{+2} (mg/g)	5.50±1.7	5.90±0.39	6.40±1. 1	5.40±0.61	6.4 ±0.66	5.6±0.9	6.91±3.5

DW (g /plant): Dry weight (g/plant), mg/g f.wt: mg/g fresh weight, Chl a + b: Chlorophyll a+b, TSS: Total soluble sugars, TSP: Total soluble protein, N: Nitrogen, P: phosphorous, Mg: magnesium

High indication of the antioxidant enzymes, peroxidase and catalase in the fungal infected plants is a plants protecting mechanism against the effect of pectinase on the plant cell walls and the degree of infection depend on the ability of the pathogens to secrete pectinase (Mahmoud *et al.,* 2004). Oxidative enzymes influence the oxidation of phenolic compounds such as quinones which is highly toxic to plants. The changes in pectinase activity under the different biocontrol agents may due the degree of pathogen inhibition. Table 5 showed that shoot height, root depth, leaf numbers, shoot and Leaf dry weighs chlorophyll a+b content of the tomato fresh leaves decreased with fungal infection due to cell wall hydrolysis and cell death while the biocontrol agents increased the previous criteria. Moreover, the quantities of TSS, TSP, nitrogen, phosphorous and Mg^{+2} contents were differed in dried plant materials of infected and control (healthy) plants. The variation in nitrogen, phosphorous and Mg^{+2} contents of the treated plants may due to the influence of these treatments on the uptake and metabolism in plant. The decrease in mineral content of the infected plants might due to the fungal consumption and removal of this effect by plant spraying with some bioagents which have antifungal effects. Fungal infection reduced photosynthetic pigments (Chl a + b) which may due to variation in nitrogen and Mg^{+2} content of the tomato leaves. Nitrogen and magnesium are major components of chlorophyll molecules (AbuGrab and Ebrahim, 2000). As it is well known, changes in carbohydrate concentrations with the plant treatments could be attributed to their effects on the Chl content of leaves (Aly *et al*. 2003). Therefore, we recommend the use of *T. harzianum, Streptomyces, Bacillus* to control the growth and development of *B. cinerea*). The endophyte *Clonostachys rosea,* a fungal species of the family Bionectriaceae, colonizes living plants and induce resistance against a number of plant pathogens especially *Botrytis cinerea* which cause gray mold disease in tomato leaves (Mouekouba et *al*., 2013). They also indicated that *C. rosea* treatment stimulated the activity of the superoxide dismutase (SOD), the nitric oxide (NO) and hydrogen peroxide (H_2O_2), thus reduced the incidence and severity of gray mold on tomato plant. They added that infection of tomato leaves with *B. cinerea* decreased by the presence of *C. rosea* which is excellent biocontrol agent for gray mold of tomato plants due to induction activities of SOD, NO and H_2O_2. Peighami-Ashnaei (2009) *in vitro* tested some bacteria for biological control of *Botrytis cinerea* and found that *Pseudomonas fluorescens* P-35 and *Bacillus subtilis* B-16 showed the most inhibitory zone against *B. cinerea* and *in vivo* study they showed considerable inhibition of *B. cinerea* on apple fruits and reduced the grey mould from 100% to less than 35 % while application of thiabendazol at 1500 mg/litre reduce the disease from 100% to 30% and 60%, after 10 and 20 days respectively. They also indicated that there is no significant difference among the treatments (thiabendazol and bacterial strains), so, bacterial strains could not only control the disease but also be a reliable replacement instead of Thiabendazol. Treatment of injured fruit by covering it with bacterial suspensions or powders of B. subtilis markedly controlled the decaying process of Pencillium digitatum and other decaying fungi (Singh and Deverall, 1984). Similarly, about 19 *Bacillus* isolates were used to control the pathogenic fungus *Macrophomina phaseolina* and the author reported that the antifungal activities may due to lytic enzymes as chitinase, siderophores and volatile and non-volatile compounds and they added that the most promising isolate RP5, obtained from wild castor bean rhizoplane, effectively controlled *M. phaseolina* growth (Villela Marroni, 2015). The genus *Streptomyces* from the rhizosphere have antagonistic activity against fungi and the isolate *Streptomyces sioyaensis* TM32 exhibited distinctive antagonistic activity to control root disease, caused by Rigidoporus sp. (Nakaew *et al*., 2015). They added that the isolate TM32 produced indole-3-acetic acid, chitinase and siderophore which suppressed the disease and this isolate can be used as an alternative treatment for soil-borne fungal control of rubber tree. Trichoderma isolates as T. harzianum and T. virens showed antagonistic effect to banana leaf pathogens Mycosphaerella musicola**,** Cordana musae, and Deightoniella torulosa *in vitro* and *in vivo* under glasshouse conditions. In conclusion, the bioagents **S.** anulatus SM21, S. coelicolor SM1, *B. pumilus* or *T. harzianum* can be used to controlled *Botrytis cinerea* on tomato and their antifungal effects were similar to the antifungal Mancozeb.

REFERENCES

Abu-Grab, O.S. and Ebrahim, M.K.H. 2000. Physiological response of field grown onion to some growth regulators. *Egypt J. Hort*., 27 (1): 117-130.

Ait-Lahsen, H., Soler, A.´S., Rey, M., La Cruz, J., Monte, E. and Llobell, A. 2001. An antifungal Exo-1,3-Glucanase (AGN13.1) from the biocontrol fungus *Trichoderma harzianum*. *Applied and Environmental Microbiology*, Vol. 67, No. 12: p. 5833–5839.

Allen, S.G., Grimshaz, H.M., Parkinson, J.A. and Quarmby, C. 1974. Chemical analysis of ecological materials. Oxford: Blackwell Scientific Publishing.

Aly, M. M., Tork, S., Al-Garni, S. M. and Kabli, S. A. 2011a. Chitinolytic enzyme production and genetic improvement of a new isolate belonging to *Streptomyces anulatus*. *Annal. Microbiol.,* Volume 61(3), 453-461.

Aly, M.M., Tork, S, Alakilli, S. Y. 2011b. Molecular characterization of chitiniolytic *Bacillus pumilus*, isolated from marine habitats and enhancement of chitinase production by mutation. *Advanced Scholar in Biotechnology,* Volume 1(2):14-21.

Arnon, D.I. 1949. Copper enzymes in isolated choloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiol*., 24:1-15

Ashwini, N. and Srividya, S. 2014. Potentiality of Bacillus subtilis as biocontrol agent for management of anthracnose disease of chilli caused by Colletotrichum gloeosporioides OGC1. 3 *Biotech.,* 4(2): 127–136.

Benítez, T., Rincón, A.M., Limón, C.M., Codón, A.C. 2004. Biocontrol mechanisms of Trichoderma strains. *Int Microbiol.,* 7:249–260.

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: 248–54

De Curtis, F, Torriani S, Rossi E, De Cicco, V. 1996. Selection and use of Metschnikowia *pulcherrima* as a biological control agent for postharvest rots of peaches and table grapes. *Ann. Microbiol. Enzimol*. 46, 45–55.

Elad, Y., Kirshner, B., Gokkes, M., Peer, R. 1993a Disease symptoms caused by *Botrytis cinerea* in *Ruscus hypoglossum* plants and their control. *Phytoparasitica,* 21(1):53–61.

Elad, Y., Zimand, G., Zaqs, Y., Zuriel, S., Chet, I. 1993b. Use of Trichoderma harzianum in combination or alternation with fungicides to control cucumber grey mould (Botrytis cinerea) under commercial greenhouse conditions. *Plant Pathol.*, 42:324–332

Filonow, A.B. 1998. Role of competition for sugars by yeasts in biocontrol of gray mold of apple. *Biocontrol Sci. Technol.,* 8, 243–256.

Harman, G.E. 2006. Overview of mechanisms and uses of Trichoderma spp. Phytopathology, 96:190–194.

Holder, I.A. and Boyce, S.T. 1994. Agar well diffusion assay testing of bacterial susceptibility to various antimicrobials in concentrations non-toxic for human cells in culture. Burns, 20(5):426-9.

Jacobs M.B. 1958. The chemical analysis of food and food products. New York: D. Van Nostrand Co. Inc.

Jacobsen, B.J., Zidack, N.K., Larson, B.J. 2004. The role of bacillus-based biological control agents in integrated pest management systems: plant diseases. *Phytopathology;* 94(11):1272–1275.

Janisiewicz, W.J. 1994. Enhancement of biocontrol of blue mold with the nutrient analog 2-deoxy-D-glucose on apples and pears. *Appl. Environ. Microbiol*. 60, 2671–2676.

Kato, M. and Shimizu, S. 1987. Chlorophyll metabolism in higher plants. VII. Cholorophyll degradation in senescing Tobacco leaves; phenolic-dependent peroxidative degradation. *Can J. Bot*, 65: 729-735.

Kim, K. Ja, Yong Joon, Kim, J.G. 2003. Purification and characterization of chitinase from *Streptomyces* sp. M-20. *Journal of Biochemistry and Molecular Biology*, 2003, vol. 36, no. 2, p. 185-189.

Lorito, M., Woo, S.L., Harman, G.E., Monte, E. 2010. Translational research on Trichoderma: from omics to the field. *Annu Rev Phytopathol* 48:395–417

Lowes, K.F., Shearmen, C.A., Payne, J., MacKenzie, D., Archer, D.B., Merry, R.J., Gasson, M.J. 2000. Prevention of yeast spoilage in feed and food by the yeast mycocine HMK. *Appl. Environ. Microbiol*., 66, 1066–1076.

Lowry, O.H., Rosebrough, N.J., Farr, L.A. and Randall, R.J. 1951. Protein measurements with the folin-phenol reagent. *J. Biol. Chem.,* 193: 265-275.

Mahmoud, Y. A., Ebrahim, M. K. H. Aly Magda, M. 2004. Influence of Some Plant Extracts and Microbioagents on Some Physiological Traits of Faba Bean Infected with *Botrytis fabae*. *Turk. J. Bot.,* 28 519-528

Marquina, D., Toufani, S., Llorente, P., Santos, A., Peinado, J.M 2002. Killer activity in yeast isolates from olive brines. *Adv. Food Sci*., 19, 41–46.

Masih, E.I., Slezack-Deschaumes, S., Marmaras, I., Ait Barka, E., Vernet, G., Charpentier, C., Adholeya A., Paul, B. 2001. Characterisation of the yeast *Pichia membranifaciens* and its possible use in the biological control of *Botrytis cinerea*, causing the grey mould disease of grapevines. FEMS Microbiol. Lett., 202: 227–232

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal Chemistry.,* 31: 426–429

Monte, E. 2001. Understanding Trichoderma: between biotechnology and microbial ecology. *Int Microbiol*. ;4:1–4

Moran, R. and Porath, D. 1980. Cholorophyll determination in intact tissues using N N-dimethyl formamide. *Plant Physiol* 65: 478- 479.

Mouekouba, L., Zhang, Z. , Olajide, E. K. 2013. Wang Ai and Wang A. Biological control of *Botrytis cinerea* in tomato leaves.

Mukherjee, A.K., Sampath, Kumar, A, Kranthi, S., Mukherjee, P.K. 2014. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. 3 Biotech 4:275–281.

Naguib, M.I. 1963. Colorimetric estimation of plant polysaccharides. Zucker 16: 15-18.

Naguib, M.I. 1964. Effect of sevin on carbohydrate and nitrogen metabolism during germination of cotton seeds. *Ind. J. Expt. Bid*., 2: 149-152.

Nakaew, N., Rangjaroen, C. and Sungthong, R. 2015. Utilization of rhizospheric *Streptomyces* for biological control of *Rigidoporus* sp. causing white root disease in rubber tree. *European Journal of Plant Pathology*, Volume 142, Issue 1, pp 93-105.

Nunes, C., Usall, J., Teixido´, N., Torres, R., Vin˜as, I., 2002. Control of *Penicillium expansum* and Botrytis *cinerea* on apples and pears with the combination of *Candida sake* and *Pantoea agglomerans*. J. Food Protect. 65, 178–184.

Page, A.L. 1982. Chemical and microbiological properties (Part 2) In: Page AL, Baker DE, Roscoe-Ellis J, Keeney DR, Miller RI &

Peighami-Ashnaei, S., Sharifi-Tehrani, A., Ahmadzadeh, M., Behboudi, K. 2009. Selection of bacterial antagonists for the biological control of *Botrytis cinerea* in apple (*Malus domestica*) and in comparison with application of thiabendazole. *Commun Agric. Appl. Biol. Sci*., 74(3):739-43.

Ramakrishna, T. and Pandit, M. W. 1988. Self-association of α-chymotrypsin: effect of amino acids. *Journal of Biosciences*, vol. 13, n. 3, p. 215-222.

Rhoades, J.D. (eds). Methods of Soil Analysis. 2nd ed. Madison, Wisco, USA.

Sabaratnam, S. and Traquair, J.A. 2002. Formulation of a *Streptomyces* biocontrol agent for the suppression of *Rhizoctonia* damping-off in tomato transplants. *Biological Control,* 23(3): 245-253.

Samuelian, S. 2016. Potential of *Trichoderma harzianum* for control of banana leaf fungal pathogens when applied with a food source and an organic adjuvant. Biotech., 6(1): 8-13.

Santos, A., Marquina, D. 2004. Ion channel activity by Pichia membranifaciens killer toxin. Yeast, 21, 151–162.

Schilly, A, Chaves, N., Guzmán, M., Sandoval, J., Staver, C., Dita, M. 2014. Exploring root-associated entophyte microorganisms from *Musa* spp. for enhancing plant health, 29th International horticultural congress, 17–22, Brisbane, Australia.

Shirling, E.B. and Gottlieb, D. 1966. Methods for characterization of *Streptomyces* species. *Int J Sys Bacteriol,* 16: 313-340.

Singh, V. and Deverall, B.J. 1984. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. Transactions of the British Mycological Society, Volume 83, Issue 3: 487-490

Somogyi, M. 1952. Notes in sugar determination. *J. Biol. Chem.* 195:19–23

Trachuk LA, Revina LP, Shemyakina TM, Chestukhina GG, Stepanov VM (1996) Chitinases of *Bacillus lichenitinolytic* B-6839: isolation and properties. Canadian Journal of Microbiology 42, 307–315.

Tremacoldi, C. R. and Carmona, E. C. 2005. Production of extracellular alkaline proteases by *Aspergillus clavatus*. *World Journal of Microbiology and Biotechnology,* 21(2):169-172.

Van Minh, N., Woo, E., Kim, Jl, Kim, D., Hwang, B., Lee, Y., and Yun, B. 2015. Antifungal Substances from *Streptomyces* sp. A3265 Antagonistic to Plant Pathogenic Fungi. Mycobiology, 43(3): 333–338.

Villela, M. I. 2015. Screening of bacteria of the genus *Bacillus* for the control of the plant-pathogenic fungus *Macrophomina phaseolina*. *Journal: Biocontrol Science and Technology,* 25(3): 302-315

Walker, G.M., McLeod, A.H., Hodgson, V.J. 1995. Interactions between killer yeasts and pathogenic fungi. *FEMS Microbiol. Lett.,* 127, 213–222.

Woo, S., Fogliano, V., Scala, F., Lorito, M. 2002. Synergism between fungal enzymes and bacterial antibiotics may enhance biocontrol. *Antonie Van Leeuwenhoek* 81, 353–356.

****