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Efficacy of the active substances of some *Trichoderma* isolates to control early blight in potatoes plants

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ABSTRACT

Alternaria solani causes early blight, which is perhaps of the main disease that influences potatoes at temperatures (24-29°C). Control of early blight with fungicides has become troublesome over the course of the last long time because of its ecological and human wellbeing concerns. As of now, different sorts of *Trichoderma* can be utilized as one of the most encouraging that not just biocontrol specialists influencing improvement and seriousness of *Alternaria solani* disease on potato plants yet in addition as plant development stimulator. Results: In the current review, the applying of different *Trichoderma* segregates on *A. solani* disease also as disconnection of powerful substance from *Trichoderma harzianum* was researched. Biochemical exercises of *T. harzianum* were likewise examined. Results showed that *T. harzianum* followed by *T. hamatum* diminished parasitic development or illness seriousness of *A. solani*, likewise improved the development boundary of potato plants. HPLC examination for assurance of phenol and flavonoids in methanol concentrate of *T. harzianum* recorded the presence of Kampeferol being 840.1 ppm. Conclusions: These outcomes show that acceptance of foundational guard instruments with *T. harzianum* trade could be a competitor among the components that will assume a significant part in the control of potato early blight.

Keywords: *Trichodema*, *Alternaria solani*, HPLC, DNA extraction and biological control, Potato

1. Introduction

Potatoes, including early assortments, are renowned for their high dietary quality. They contain protein of high natural worth, ascorbic corrosive and furthermore the vitamin B complex nutrients, minerals, and dietary fiber. Tubers are additionally a fair wellspring of healthfully significant cell reinforcements like free and bound phenolic compounds, including chlorogenic corrosive and catechuic corrosive (Im *et al.,* 2008). On the contrary hand, overlooking plant microbes can prompt extreme misfortunes in crop yields by 20-40% (Fang and Ramasamy 2015; Savary *et al.,* 2012). Also, the nonstop advancement of pathogenic microbes and growths as far as opposition against manufactured antimicrobial specialists can lead to difficult issues in crop establishes wherever the world. The quality and yield of potato tubers could likewise be diminished by a foul for a harvest, supplement lack, and contaminations brought about by microbes that assault both the over-the-ground portions of potato plants and tubers (Möller *et al.,* 2007; Bouws and Finckh 2008).

Early blight (*Alternaria solani*) is the risky microorganism of vegetables of the potato family Solanaceae, including the early potato cultivars (Haverkort *et al.,* 2009). Since illness, side effects show up at early improvement organizes, the sickness can happen over a large number of climatic circumstances. On potatoes, contamination of the plants might bring about a total loss of the harvest as yields are diminished by harming foliage and natural products.

The utilization of fungicides, other than being costly and implying dangers to the climate connected with the applying of synthetic compounds, isn't viable and may prompt the presence of new safe types of microbes (Bruin and Edgington 1980). Creating elective methods of control is in this

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manner important. Crop security through regular organic options plays an imperative part in maintainable horticulture, subsequently satisfying the requests of the developing populace for quality food sources. Be that as it may, endophytes can safeguard the host plants from certain microbes (Busby *et al.,* 2016; Lareen *et al.,* 2016). Endophytes have the inclination to colonize have plant tissues with practically no damage under the indigenous habitat (Khan *et al.,* 2015). Among the endophytes, parasites have a personal relationship with their host, where it secretes a few optional metabolites that advance plant development (Ikram *et al.,* 2019; Mehmood *et al.,* 2019). Endophytes have the capacities to endeavor through uncommon natural circumstances by delivering intense bioactive that could be useful to different ventures, including medication, designing, and farming (Mehmood *et al.,* 2018). *Trichoderma* spp. is considered a potential organic control specialist, and the methods of activity incorporate mycoparasitism, antibiosis, rivalry, chemical action and prompted plant safeguard (Howell, 2003 and Fatima *et al.,* 2015). The strong metabolites delivered by these species incorporate alkaloids, terpenoids, steroids, quinones, phenols, and flavonoids (Ikram *et al.,* 2019).

The goals of this study were to assess the biocontrol capability of ten *Trichoderma* disengages on the early blight on potato plants and decided the viable substance answerable for fundamental guard components for controlling potato early blight.

2. Materials and Methods

2.1. Collecting of early blight pathogen

Leaves of potato plants with early blight ordinary side effects were gathered from various areas in five governorates, *i.e*. El- Qalyubia (Qaha); Ismailia (Ismailia); Sharqia (Faqus); Beheira (El-Nubaryia) and Kafr El-Sheikh (Sakha, Kafr El-Sheikh and Qellin). The contaminated tissues were cut into little pieces; surface cleaned with sodium hypochlorite (0.5%) for 2-3 minutes and afterward washed a few times with sanitized refined water. These sterilized pieces were dried between two sterilized filter papers and transferred directly onto PDA plates (9 cm) then incubated for 5-7 days under 12h light and 12h dark at 25±1°C in step with (Naik *et al.,* 2010). *Alternaria* spp. isolates were purified and identified according to its morphological features using the descriptions of Singh (1982) and Barnett and Hunter (1987). Pure cultures of the pathogen were maintained on PDA slants and stored at 5-10°C till used.

2.2. Pathogenicity test

Alternaria solani isolates were tested for their pathogenicity on apparently healthy potato plants Spunta as follows:

The inoculum, was ready by refined each of the tried confines on PDA plates at 25°C for 15 days under 12h light and 12h dim, then 10 ml of sterile refined water was added to each plate, and development was painstakingly rejected with a clean fine brush. The subsequent mycelial suspension from each disengage was changed in accordance with 5×10^5 cfu/ml.

Optional the different *A. solani* segregates were done, under nursery states at Plant Pathology Research Institute, Agricultural Research Center (ARC), Giza. Plastic pots (50 cm diam.) were loaded up with disinfected sandy mud soil (1:1 w/w, 5kg/pot). Soil was eliminated by washing all tubers in refined water. The tubers were then surface disinfected by 2% sodium hypochlorite for 3-5 minutes and left for 4h for dried. Three tubers /pot and ten pots for each treatment were used. Plants were sprayed by 5×10^5 cfu/ml inoculum with 30ml/plant when plants had 4–5 leaves. Other sprayed plants with an equivalent amount of distilled water were used as control. To guarantee great spore germination, the sprayed plants were held under polyethylene packs for 48h to maintain high humidity (Chen *et al.,* 2003). Disease assessment was recorded with the presence of normal infection symptoms on control plants (60 days subsequent to planting). Disease severity% was recorded two weeks after artificial inoculation with the tested pathogen, using a scale consisted of five categories ranging from 0 to four assessed as follows; 0 = no leaf injury; 1= sores on < 25% of leaf region; 2 = sore on 26-half of leaf region; 3 = injury on 51-75% of leaf region and 4 = injuries on 76 up to 100 percent of leaf region according to recommended methods by (Cohen *et al.,* 1991), then disease severity was calculated using the formula developed by Townsend & Heuberger, (1943) as follows: $DS\ (\%) = \Sigma\ (nr) / NR \times 100$

Where, n - degree of infection according to the scale; r - number of samples per each category; R - total number of samples examined; N - the highest score of the categories

2.3. Biological control

2.3.1. Isolation and purification of the *Trichoderma* isolates

Trichoderma harzianum (T1), *T. hamatum* (T2), were obtained from previous work (Abou-Zeid *et al.,* 2016). On the other hand, thirteen *Trichoderma* isolates were isolated from healthy potato plants leaves collected from which different governorate fields, *i.e.* El- Qalyubia (Qaha, Tersa); Ismailia (Ismailia); Sharqia (two location of Faqus); Beheira (El-Nubaryia) and Kafr El-Sheikh (Sakha, Kafr El-Sheikh and Qellin) Giza (Elsaf, Giza, Badrashin). Discs (5 mm) were taken from the collected leaves and one gram of each sample was placed in a sterilized flask containing 99 ml of sterilized distilled water and then shacked on a mechanical shaker for 15 min at 100 rpm to wash out the present microorganisms onto the surface of leaf discs into the water to give a suspension. Serial dilutions from 10^{-2} to 10^{-6} were done. Pure cultures of *Trichoderma* isolates were maintained on PDA slants and stored at 5-10°C till used.

2.3.2. Biochemical activity of biocontrol agents

2.3.2.1. Phosphate solubilization

a- Qualitative Assessment

Qualitative Estimation of *Trichoderma* isolates were tried for their capacity to solubilize inorganic phosphate on Modified Pikovskaya's Agar (MPA), one mycelial disc (0.5 cm diam.) of *Trichoderma* isolates were placed on the agar plate and incubated at room temperature for 3 days. The Phosphate Solubility Index (PSI) was estimated and determined according (Alam *et al.,* 2002; Afzal & Bano 2008) as follows:

$$PSI = [\text{colony diameter} + \text{halo zone diameter}] / \text{colony diameter}$$

b. Quantitative Assessment

All types of *Trichoderma* isolates were tried for their capacity to break up inorganic phosphate in Modified Pikovskaya's Broth (MPB). Five mycelial plates (0.5 cm in width) of *Trichoderma* strain were immunized into 250 ml Erlenmeyer flagon containing 100 ml of stock medium and incubated at room temperature in a shaker (GFL 3020) at 120 RPM for 6 days. Then, spores and mycelia of *Trichoderma* isolates were eliminated from stock medium by filtration through 0.45 µm Whatman No.1 and centrifuged by rotator (Z 200 A, HERMLE Labortechnik GmbH) at 5,000 RPM for 10 min. The supernatant of each culture was investigated for pH by utilizing a pH meter (CyberScan pH 510, Singapore). Phosphate fixation in the supernatant was assessed by the spectrophotometric method (Fiske & Subbarow 1925; Saravanakumar *et al.,* 2013). An aliquot of 750 µl culture supernatant was blended in with 750 µl of variety reagent containing ammonium molybdate ($(NH_4)6Mo_7O_{24}•4H_2O$) 1.5% (w/v), sulfuric corrosive (H_2SO_4) arrangement 5.5% (v/v) and ferrous sulfate ($FeSO_4$) arrangement 2.7% (w/v) and afterward estimated by a spectrophotometer (U-1800, Hitachi, Japan) at 600 nm. The degree of phosphate not set in stone by utilizing a standard diagram of potassium dihydrogen phosphate (KH_2PO_4) and expressed as equivalent phosphate in mg-P/L.

2.4. Hormonal production

2.4.1. Estimation of Indole-3-Acetic Acid (IAA) production by *Trichoderma* species

Production of Indole-3-acetic acid (IAA) was estimated for *Trichoderma* species according to (Loper and Schroth 1986). *Trichoderma* isolates were inoculated in 50 ml of broth containing 0.1mg/ml tryptophan and kept in a shaker incubator at 30 ± 2°C for 2days at 180 RPM in obscurity. After incubation, the stock was centrifuged, the supernatant was held and 1 ml of supernatant was blended in with 2 ml of Salkowski's reagent (2% 0.5 $FeCl_3$ in 35% $HClO_4$ arrangement) and stayed in obscurity for no less than 30 min. then, the optical thickness (OD) was estimated at 530 nm.

I. Effect of antagonistic *Trichoderma* isolates on radial growth of *A. solani*

In this study, the antagonistic activity of fifteen *Trichoderma* isolates against *A. solani* was assessed by double culture technique portrayed by (Edington *et al.,* 1971). Plates of PDA medium were inoculated with a 5 mm disc from five-day-old culture of each *Trichoderma* isolate at a distance of 2 cm, from the edge of the plate. a fungal disc (5 mm) of *A. solani* was placed at the same distance from the edge of the plate against of *Trichoderma* Petri dishes inoculated with *A. solani* alone as a comparison

treatment. All dishes were incubated at 25ºC. The percentage of fungal growth reduction in the different treatments was calculated when the pathogen growth was completed in one of the comparison treatments plates.

The reduction in growth diameter was measured and compared to the control. The percentage of *A. solani* growth inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = (C-T) / C \times 100$$

Where "C" is mycelia growth in the control and "T" is mycelia growth of antagonistic isolates.

II. Effect of methanol extraction from *Trichoderma* spp. on radial growth of *A. solani*

a- Methanol extraction from *Trichoderma*

Antifungal compounds were extracted from cell free filtrate of *T. harzianum* (T1), *T. hamatum* (T2) inoculated on (PDA) medium, the stock was centrifuged at 10,000 rpm for 20 min to isolate the *Trichoderma* cells and spores. The supernatant was extracted by methanol solvent used (1:1 ratio) for extraction of antifungal compounds and so separated using separating funnel.

b- Separation of antifungal compounds

The organic solvent extractions were evaporated under vacuum at 40ºC. The crudes were dissolved in DEMSO (1:10 w/v) and allowed to be separated by using pre-coated TLCs (E. Merk, AG, and Darmstadt, Germany) plats. 150 µl of the organic solvent extract was spotted on the TLC plate and using chloroform: methanol (8:2) solvent system as a mobile phase (Kumar *et al.,* 2009). The plats were developed and bands were visualized inspection with UV light at 365 nm. Rf of every fraction were calculated from the obtained crude samples (Sheeba *et al., 2019*).

$$Rf = \frac{\text{Distance travelled by the solute} \times 100}{\text{Distance travelled by the solvent}}$$

c- Evaluation of antifungal activities of TLC- bands on PDA plate (Antibiotic assay)

The antifungal activities of the resulted TLC-bands from solvent extracts were tested against *A. solani* by the agar diffusion method. 50µl of each band was assayed on the well of an agar medium containing a disk agar culture of *A. solani* within the center and 100µl of each band was distributed on the surface medium and containing a disk agar culture of *A. solani* within the center afterward the immunized plates were brooded at 25ºC until the mycelial development cover the medium surface sufficient treatment, control was inoculated by the pathogen alone. Growth inhibition was measured and also the growth reduction is set.

III. Effect of foliar spray treatments on potato early blight diseases under greenhouse conditions.

The experiment was carried out as mentioned before in the pathogenicity test. Ten *Trichoderma* isolates which selected from *in vitro* studies as previously mentioned and applied as foliar spray in this study. Each *Trichoderma* isolate was added as spore suspension (adjusted to $1x10^6$ spores/ml with a haemacytometer slide) (Abd-Alla *et al.,* 2007), On the other side, Bellis fungicide 38% WG (Pyraclorostrobin + Boscalid) was tested as check control in comparison with Antagonistic *Trichoderma* isolates for controlling early blight disease by spraying potato plants at 2 days before inoculation with the causal pathogen *A. solani*. Two infested and un-infested controls were used in this study. Inoculated potato plants were sprayed with water as control. Disease severity (%) was calculated using the equation suggested by (Townsend and Heuberger, 1943) as mentioned before.

V. Efficacy of foliar spray application with *Trichoderma* biocontrol agents and chemical control on early blight disease under field condition.

The field experiment was conducted under the conditions of Abu Rawash district, Giza, Egypt, during two growing seasons of February 2021 and February 2022. All bioagents and chemical treatments were utilized as a foliar spraying 30 days after plants had 4–5 leaves. *Trichoderma* isolates were applied as spore suspension with ($1x10^6$) spore/ml. Potato plants were sprayed with water as control. This experiment was designed as split-plot design with three replicates where potato cultivars

were surveyed in main plot while foliar treatments were evaluated in subplot. Potato seed tubers of each evaluated cultivar established in columns 1m width, 30 cm separated under trickle water system framework and the plot region was 25 m^2. Harvesting was done after 120 days from planting. All trial plots were collected then tubers were counted and weighed to record plant length (cm), potato yield (kg/plot), tuber's number/plot and average tuber weight (g). Disease severity was calculated using the formula developed by Townsend & Heuberger, (1943) as previously mentioned.

2.5. DNA extraction and sequencing for *Trichoderma* isolates:

The virulent isolate of *Trichoderma* (T1) was identified and insertion in the National Center for Biotechnology Information (NCBI).

2.5.1. Extraction of DNA

100 ml of potato dextrose stock in 250-ml Erlenmeyer carafes was inoculated with parasitic circles per culture got from province edges and hatched at 26°C for 7 days in obscurity. Parasitic mats were gathered on channel paper in a Buchner pipe, washed with refined water and freeze-dried for the time being.

DNeasy Plant Mini kit (QIAGEN) extracted DNA. Freeze-dried ground mycelium (50 mg in an Eppendorf tube) was resuspended in 400 µl of extraction cushion AP1 for 10 min at 65°C. The cylinders were modified 2-3 times during brooding, then 130 µl from Buffer AP2 was added, after that it was blended and hatched for 5 min on ice. The arrangement was centrifuged for 5 min at 20,000xg (14,000 rpm). The lysate was taken and pipetted into a QIAGEN Mini twist section in a 2 ml assortment tube, centrifuged for 2 min at 20,000 x g (14,000 rpm), then, at that point, the course through portion was moved into another cylinder without upsetting the pellet. One and half volumes of support AP3/E were added, and blended by pipetting. 650 µl of the blend were moved into a DNeasy Mini twist segment in a 2 ml assortment tube and centrifuged for 1 min at 6000 x g (8000 rpm) and disposed of course through and this step was rehashed with the leftover example. Then the twist segment was put into another 2 ml assortment tube and 500µl from Buffer AW was added, and centrifuged for 1 min at 6000 x g. The course through was disposed of and added another 500 µl from Buffer AW. After that axis for 2 min at 20,000 x g. then moved the twist segment to another 1.5 ml or 2 ml microcentrifuge cylinder, and 100 µl from Buffer AE were added for elution and hatched after that for 5 min at room temperature. At last, centrifuged for 1 min at 6000 xg (Watanabe *et al.,* 2005; sim *et al.,* 2007).

2.6. Primer

The nuclear ribosomal small subunit (nrSSU-18S) region of *Trichoderma* (T1) isolate was amplified, using the Nuclear Primers – (18S rRNA) NS1 (forward) and NS24 (reverse) which were incorporated in the reaction mixture. Primers have the following composition: NS1 (5' - GTA GTCATATGCTTGTCTC-3'), and NS24 (5' AAACCTTGTTACGACTTTTA -3'). The last volume of each PCR response blend (test) was 20 µl containing; 2 µ1 10 × PCR cradle, 1 µl of every groundwork (10 pmol), 2 µl dNTPs (2 mM), 3 µl layout DNA (30 mg/µ1), 1 Taq polymerase unit, then, at that point, finished to 20 µl by expansion of nuclease free disinfected refined water. PCR intensification was performed utilizing the accompanying circumstances: introductory denaturation at 94 °C for 1 min followed by 35 cycles each comprising of conclusive denaturation at 94°C for 30 s, toughening temperature at 55°C for 30 s, beginning expansion for 1 min, and last augmentation at 72°C for 5 min (White *et al.,* 1990). PCR-intensified items were sequenced by using automated software's Phred programed which convert follows into successions that can be saved in a data set inside the space of seconds subsequent to sequencing run according to (Ewing *et al.,* 1998).

2.7. HPLC analysis of phenols and flavonoids in *Trichoderma harzianum* extract

The high-performance liquid chromatography (HPLC) analysis was performed according to Biswas *et al.* (2013). The system thermo (ultimate 3000) consisted of: pump, automatic sample injector, and associated Dell-compatible computer program. Adiode array detector DAD-3000 was used. The Thermo-hypersil switched stage C18 section 2.5×30 cm was worked at 25°C. The versatile stage comprises of refined water (dissolvable A) and methanol (dissolvable B.). The UV-vis retention spectra of the principles as well as the examples were kept in the scope of 230-400 nm. Tests and guidelines arrangements as well as the portable stage were degassed and separated through 0.45um film channel

(Millipore). Recognizable proof of the mixtures was finished by sidekick of their maintenance time and UV ingestion range with those of the principles.

2.8. Microbiological analysis

Nutrient media was used for total microbial counts according to (Nautiyl 1999). microorganisms count utilizing Potato Dextrose Agar medium (PDA) as revealed by (Elad and Freeman 2002). Soil tests were broken down for: Dehydrogenase action as depicted by (Friedel *et al.,* 1994).

2.9. Statistical analysis

Statistical analysis was finished utilizing investigation of change, (ANOVA) (Snedecor and Cochran 1982).

3. Results

3.1. Pathogenicity test

Seven isolates were obtained from infected potato leaves that were gathered from some governorates in Egypt. Data in Table 1 show that all tested *A. solani* had able to infected potato plants causing early blight with various disease severity-percentages after 14 days post inoculation *A. solani*-2 isolate was the most virulent isolate where it recorded highest disease severity 81.13 % followed by *A. solani*-1 isolate which recoded 67.37% of D.S. On the other hand, the least virulent isolate was *A. solani*-4 which recorded 24.45% of D.S.

Table 1: Pathogenicity test of *A. solani* on potato plants under greenhouse condition.

***Alternaria* Isolates**	**Governorates**	**Disease severity (%)**
***A. solani* 1**	El- Qalyubia (Qaha)	67.37
***A. solani* 2**	Ismailia (Ismailia)	81.13
***A. solani* 3**	Sharqia (Faqus)	36.20
***A. solani* 4**	Beheira (El-Nubaryia)	24.45
***A. solani* 5**	Kafr El-Sheikh (Sakha)	48.74
***A. solani* 6**	Kafr El-Sheikh (Kafr El-Sheikh)	27.06
***A. solani* 7**	Kafr El-Sheikh (Qellin)	51.44
Control (without infection)		1.66
L.S.D. at 5%		**5.137**

3.2. Biological control

3.2.1. Effect of antagonistic *Trichoderma* isolates on A. *solani* radial growth

Fifteen *Trichoderma* isolates were collected from healthy potato soil rhizosphere, at different governorates throughout Egypt.

Data in Table 2 showed that all *Trichoderma* isolates significantly suppressed radial growth of *A. solani* on PDA medium compared to the control. *T. harzianum* (T1) was the most suppressive among them (96.7 % growth reduction) and was grown overgrowth the pathogen, followed by *T. hamatum* (T2) (91.1% growth reduction). The lowest percentage of growth reduction was given by (T15) (53.3% growth reduction).

3.3. Biochemical activities of *Trichoderma* isolates

3.3.1. Phosphate solubilization

Phosphorus is a fundamental supplement expelates showed an unmistakable zone on modified Pikovskaya's Agar (MPA) after incubation at 28°C for 2 days which was estimated and determined as Phosphate solubilization percent. For the quantitative assessment of phosphate solubilization, *Trichoderma harzianum* gave P-solubilization in modified Pikovskaya's Broth medium (MPB) as contrasted and a control (stock medium non-inoculated with *Trichoderma* isolates) after incubation at room temperature for 7 days.

Table 2: Effect of different *Trichoderma* isolates on the linear growth of *A. solani* on PDA medium.

Treatments	Governorates	Locations	Linear growth	Reduction %
***T. harzianum* T1**	El-Fayoum	Senoras	0.30	96.7
***T. hamatum* T2**	El-Gharbia	Zefta	0.80	91.1
T3	Kafr El-Sheikh	Kafr El-Sheikh	1.23	86.3
T4	Sharqia	Faqus	1.44	84.0
T5	El- Qalyubia	Tersa	1.50	83.3
T6	Beheira	El-Nubaryia	1.66	81.5
T7	El- Qalyubia	Qaha	1.70	81.1
T8	Kafr El-Sheikh	Sakha	1.73	80.8
T9	Sharqia	Faqus	1.86	79.3
T10	Kafr El-Sheikh	Qellin	1.80	80.0
T11	Kafr El-Sheikh	Kafr El-Sheikh	2.00	77.8
T12	Giza	Elsaf	3.30	63.3
T13	Giza	Giza	3.70	58.9
T14	Giza	Badrashin	3.90	56.6
T15	Ismailia	Ismailia	4.20	53.3
Control			9.00	0.0
L.S.D. at 5%			**0.32**	**--**

Results shown in Table 3 illustrate that *T. harzianum* has capable to produce IAA being 15.93 µg/ml. The production of plant controllers by microorganisms is a significant instrument frequently connected with development excitement since it is undoubtedly a way that *Trichoderma* further develops plant development.

Table 3: Phosphate solubilization and IAA production by *Trichoderma* isolates

Treatments	Qualitative	Quantitative		IAA
	PS%	PH	Phosphorus (mg-P/L)	(µg/ml)
Control	0.0	7.30	5.13	
***T. harzianum* T1**	49.0	5.12	9.87	21.93
***T. hamatum* T2**	36.0	5.39	9.52	18.2
T3	21.6	6.82	7.21	15.34
T4	18.5	6.62	9.13	15.19
T5	29.0	5.52	9.43	17.49
T6	23.0	6.48	6.29	16.12
T7	24.6	5.46	8.71	15.29
T8	28.1	5.84	8.66	14.29
T9	19.0	6.12	9.16	16.28
T10	18.2	6.35	8.41	16.73
T11	20.3	5.42	7.26	15.27
T12	24.2	6.11	8.15	15.82
T13	30.6	6.27	9.06	16.11
T14	26.1	6.10	8.64	16.73
T15	25.7	5.53	8.27	15.20
L.S.D. at 5%	**1.4**	**0.3**	**0.25**	**0.55**

3.3.2. Influence of foliar spray application of *Trichoderma* isolates on early blight diseases severity of potato under greenhouse conditions

Data in Table 4 showed a significant reduction in disease severity. The most effective treatments were in case of fungicide with potato plant, followed by *T. harzianum* (T1) which gave the best biological agent effect with potato plant then *T. hamatum* (T2) which recorded 14.98, 17.55 and 19.68 % D.S respectively compared to control.

Table 4: Effect of *Trichoderma* isolates on potato early blight disease severity under greenhouse conditions.

Treatments	Disease severity (%)
***T. harzianum* T1**	17.55
***T. hamatum* T2**	19.68
T3	65.75
T4	28.30
T5	38.57
T6	53.78
T7	44.90
T8	40.67
T9	21.73
T10	75.70
Bellis (fungicide)	14.98
Infected control	83.40
Un- Infected control	1.3
L.S.D. at 5%	**2.97**

3.3.3. Influence of *Trichoderma* isolates on early blight disease severity under field condition

The promising treatment for decreasing disease seriousness in pot tests were applied under normal field conditions during two growing seasons. Data presented in Table 5 and Table 6 showed disease severity (D.S) %, potato plant length (cm), potato yield (kg/plot), tubers number/plot and tuber weight (g) were significantly affected by tested foliar spraying application in both seasons compared to control treatment. Belies fungicide was the most effective treatment for decreasing D.S% of early blight during the two growing seasons February 2021 and February 2022, respectively (21.8 and 19.64 %) compared to control (44.6 and 40.18%). On the other hand, all applied bioagents had an unrivaled impact in this regard. Obtained results showed that *T. harzianum* (T1) and *T. hamatum* (T2) had higher efficacy for reducing D.S % by (24 and 21.62%); (26.8 and 24.14%) comparison with the other *Trichoderma* isolates at both seasons, respectively.

Table 5: Influence of *Trichoderma* isolates on early blight disease severity under field condition at the first season 2021.

Treatments	Disease severity D.S %	Reduction %	Plant length (cm)	Potato yield (kg/plot)	Tubers number/plot	Average tuber weight (g)
***T. harzianum* T1**	24.0	45.99	99.6	35.00	270.62	129.3
***T. hamatum* T2**	26.8	39.93	102.5	33.25	268.87	123.7
T3	38.4	13.90	85	25.46	259.93	97.95
T4	31.2	29.98	101.2	32.80	279.60	117.3
T5	33.4	25.11	96.16	29.22	264.88	110.3
T6	37.6	15.70	87.5	26.30	265.15	99.2
T7	36.6	17.94	89.4	27.04	270.38	100.0
T8	35.4	20.85	85.6	28.21	270.04	104.6
T9	29.0	35.00	98.1	31.87	259.90	122.6
T10	40.2	9.87	88.2	23.63	268.47	88.0
Bellis (fungicide)	21.8	51.12	97.5	37.54	278.06	135.0
Control	44.6	-	82.1	19.85	249.83	79.45
L.S.D. at 5%	**4.9**	**4.2**	**5.2**	**5.9**	**5.9**	**6.2**

Some of plant morphological characters, *e.g*., plant length (cm), potato yield (kg/plot), tubers number/plot and tuber weight (g) were calculated at the end of the two growing seasons. Data in Table (5 and 6) showed that Bellis (fungicide) was the best effective treatment on increased of all growth parameters compared with other treatments. Results indicated that all *Trichoderma* isolates were significantly improved the quantitative parameters of potatoes yield if compared with untreated plants (control).The most effective treatments among *Trichoderma* isolates observed in T1 followed by application with T2 for all potato yield and average tuber weight, they had high records as (35, 33.25 kg/plot); (34.12, 32.44 kg/plot) and (129.3, 123.7g); (126.58, 121.15g), respectively; whileT4 and T1

were recorded the highest tuber number/plot as (279.6 and 270);(277.16, 269.55), moreover T2 and T4 recorded the highest plant length as (102.5, 101.2 cm);(97.14, 94.71 cm) followed by (99.6 cm);(92.45 cm) at the treatment of T1, in both season, respectively.

Table 6: Influence of *Trichoderma* isolates on early blight disease severity under field condition at the second season 2022.

Treatments	Disease severity D.S %	Reduction %	Plant length (cm)	Potato yield (kg/plot)	Tubers number/plot	Average tuber weight (g)
***T. harzianum* T1**	21.62	41.43	92.45	34.12	269.55	126.58
***T. hamatum* T2**	24.14	35.97	97.14	32.44	267.77	121.15
T3	34.59	12.52	79.98	24.94	256.73	97.14
T4	28.11	27.01	94.71	31.55	277.16	113.83
T5	30.09	22.62	88.63	27.97	262.77	106.44
T6	33.87	14.14	81.83	24.99	263.45	94.86
T7	32.97	16.16	80.54	26.11	269.18	97.00
T8	31.89	18.78	91.44	27.33	268.32	101.86
T9	26.13	31.53	90.73	29.99	257.81	116.33
T10	36.22	8.89	82.14	22.22	266.99	83.22
Bellis (fungicide)	19.64	46.05	101.12	36.76	277.26	132.58
Control	40.18	-	79.14	18.59	248.13	74.92
L.S.D. at 5%	**5.7**	**3.8**	**5.1**	**4.4**	**5.1**	**4.8**

3.3.4. Variations among *Trichoderma* isolates using the nuclear ribosomal small subunit region technique

Data in Table (7) illustrate the similarity % of the two targeted *Trichoderma* isolates under study with the other *Trichoderma* isolates which previously registered worldwide in Gen Bank based on the number of nucleotides sequenced with ribosomal small subunit region technique. In this respect, *T. harzianum* (T1).

Table 7: *Trichoderma* isolates under study registered in Gen Bank based on the number of nucleotides sequenced with ribosomal small subunit region technique

Current Isolate No.	Name	Current Accession No.	Similarity (%)
T1	*Trichoderma harzianum*	OL454813	98%

3.4. Effect of methanol extraction from *Trichoderma sp.* on radial growth of *A. solani*

a- Extraction and separation of antifungal compounds of *Trichoderma* filtrate

Methanol was used for extraction process of antifungal compounds from filtrate of *T.harzianum* (T1),and *T. hamatum* (T2). Fig (1) showed the resulted bands separated from each isolate on TLC plate. The effective band of *Trichoderma harzianum* (T1) which performs this growth reduction had Rf equal 0.61 when the band of *Trichoderma hamatum* (T2) had Rf equal 0.58.

b- Antagonistic activity of the extraction

Fig (1) showed the antagonistic effect of (T1) and (T2) on the mycelial radial growth of *A. solani*. (T1) extract was the foremost significantly effective bioagent which cause growth reduction in well diffusion and distributed on the surface medium by 78-100% followed by *Trichoderma hamatum* (T2) which causes growth reduction by 0-47%. The results indicated that *Trichoderma harzianum* (T1) extract was the foremost significantly effectiveness bioagent for controlling *A. solani*.

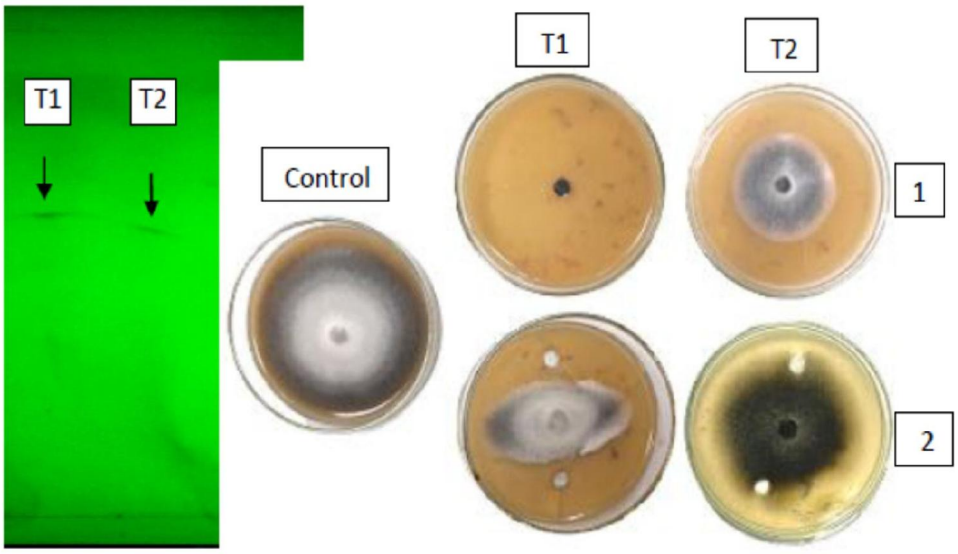

Fig. 1: TLC graph. of methanol extraction of *Trichoderma harzianum* (T1), *Trichoderma hamatum* (T2) on TLC plate and separated bands on growth reduction in
1- Distributed on the surface medium.
2- Well diffusion
All compared with control on PDA medium.

3.5. HPLC analysis for phenolic and flavonoids content of *Trichoderma harzianum*

The HPLC chromatogram for methanol extract of *T. harzianum* showed the presence of quercetin and kaempferol as introduced in (Fig. 2 and Table 8). The present investigation showed the presence of kaempferol content in the methanol extract (233.4 mg/gm) and quercetin (78.15 mg/gm).

Table 8: HPLC analysis for Phenol and flavonoids in *T. harzianum* by methanol extract

No.	Peak name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount ppm
1	**kaempferol**	3.927	67.563	223.693	94.25	85.01	840.6015

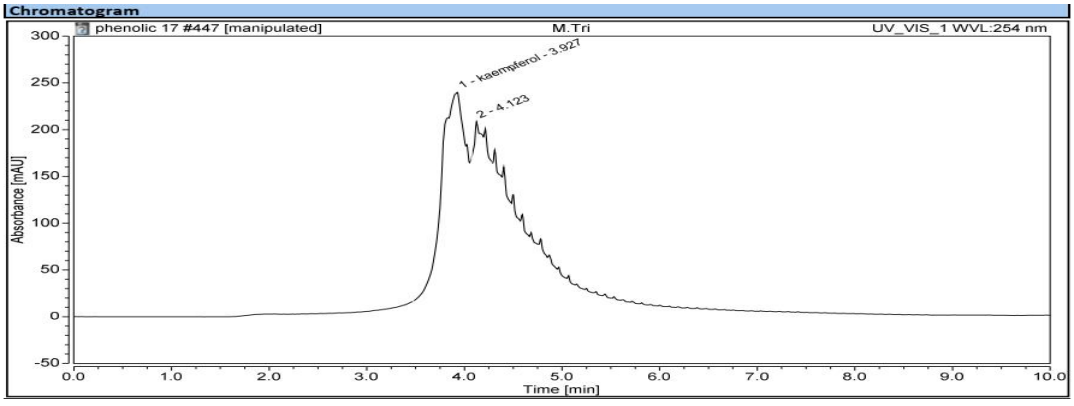

Fig. 2: HPLC analysis for phenolic and flavonoids content of *T. harzianum* by methanol extract Total Bacterial Counts.

An underlying all out microbial count before planting was 56×10^5 cfu/gm dry soil. Information in Table (9) showed that the most elevated counts recorded with *T. harzianum* being 249×10^5 cfu/ gm dry soil this was followed by other bioagent treatments. *Trichoderma* isolates inoculation recorded enhancement in microbial determinations in potato rhizosphere.

3.6. Enzymatic activity

Data in Table (9) showed that the determination of enzymatic activity in rhizosphere area of potato plants. Dehydrogenase enzyme increased with the different *Trichodema* isolates treatments. The highest mean values for Dehydrogenase were recorded with the *T. harzianum* treatment. *Trichoderma* isolates inoculation had a positive effect on the previous parameters and gave the maximum microbial activity including total microbial counts, fungi counts and dehydrogenase activity in the rhizosphere soil.

Table 9: Influence of *Trichoderma* isolates on microbial determination in rhizosphere of potato under field condition (Average of two seasons).

Treatments	Total microbial counts ($\times10^5$cfu/g dry soil)	Fungi counts ($\times10^2$cfu/g dry soil)	Dehydrogenase µg TPF/g dry soil/24 hours
***T. harzianum* T1**	249	31	16.3
***T. hamatum* T2**	227	26	14.2
T3	159	21	11.9
T4	210	24	12
T5	164	18	10.9
T6	138	23	14.1
T7	128	28	12.6
T8	175	12	13.5
T9	143	18	14.2
T10	169	25	11.6
Bellis (Fungicide)	65	5	0.7
Control	105	12	8.2
L.S.D. at 5%	**6.0**	**5.7**	**4.5**

4. Discussion

Our study revealed that, all isolates of *Trichoderma* caused great reduction against the tested pathogen *A. solani* the causal pathogen of early blight of potato. *T. harzianum* was the best bioagent followed by *T. hamatum*. The highest mycoparasitism was seen by *T. harzianum. Trichoderma* species can be a more secure substitute procedure to be incorporated into diseases the executive's projects of early blight of potato. These results agreed with some previous studies on the pathogenicity of *A. alternata* proved that the tested isolates agreed with some previous studies in Egypt on the pathogenicity of *Alternaria* isolates on potatoes (Ahmed, 2017). This mechanism was agreement with by a few different investigators for the strongest effect was given by *Trichoderma* species (*T. harzianum* and *T. viride*) (Gupta *et al.,* 1995); (Santamarina *et al.,* 2002); (Abdul Wahid *et al.,* 2018). While (Chowdappa *et al.,* 2014) found that *T. harzianum* OTPB3 inhibited mycelium growth of *A. solani* under *in vitro* conditions. Also, the antagonistic effectiveness of *Trichoderma* species against six fungal plant pathogens including *A. solani* was demonstrated by (Bell *et al.,* 1982). (Sarfraz *et al.,* 2019) they found that *T. hamatum* had great inhibitory potential in growth inhibition of *A. solani*, all species of *Trichoderma* produced maximum inhibition of the pathogen. The best inhibition was observed by *T. harzianum* followed by *T. hamatum*. However, the strong mycoparasitism was observed by *T. harzianum*. *Trichoderma* species are often a safer alternate strategy to be integrated into disease management programs for early blight of potatoes. These results were matching with many previous findings (Devi *et al.,* 2012; Moosa *et al.,* 2017). *Trichoderma* has varied antagonistic potential and this difference may be due to genetic potential and isolate origin (Moosa *et al.,* 2017). *Trichoderma* inhibits the Linear growth of the pathogen through its random growth potential and competition for food and space (Devi *et al.,* 2012).

Our studies revealed that, *Trichoderma harzianum* capable to produce P-solubilization in modified Pikovskaya's Broth medium (MPB) as contrasted and a control. Saravanakumar *et al.,* 2013 showed that *Trichoderma* species can dissolve insoluble phosphate to soluble phosphate.

At the same time, that *T. harzianum* has capable to produce IAA, which one of a significant component frequently connected with development excitement since it is presumably a manner by which *Trichoderma* works on the improvement of plants. Contreras-Cornejo *et al.,* 2011 found that the production of plant regulators by microorganisms is an important mechanism often associated with growth stimulation because it is mostly way in which *Trichoderma* improves plant growth and may be

important factors in boosting plant immunity. In contrast, (Shtienberg *et al.,* 1994), (Sid *et al.,* 2000) and (Kloepper *et al.,* 2004) found that *T. harzianum* has been reported to stimulate growth and systemic resistance to several soils, seed-borne and foliar diseases of various vegetable crops including tomato and potato. These results are also consistent with those of (Chowdappa *et al.,* 2013), who found that *T. harzianum* OTPB3 induces systemic resistance in tomato seedlings against early and late blight through induction of growth hormones and defense enzymes. Greenhouse and field trials (Yuan-Hang *et al.,* 2014) indicated that *Trichoderma* R-5 and T-15 isolates reduced disease incidence by 72.4% and 70.0%, respectively. Also, (Fontenelle *et al.,* 2011) evaluated the ability of 28 *Trichoderma* isolates to promote the growth of tomato seedlings and to induce systemic resistance (ISR) against *A. solani*, the causative agents of early blight disease.

The outcomes are pretty much like the results found by (Mane *et al.,* 2014) who showed that amongst the bio-agents *T. harzianum* was the best antagonistic one for controlling the early blight of potato. The tested bio-agents tried in the current examination have demonstrated their true capacity and can be utilized in future for the administration of early curse of potato and consequently can lessen the aimless utilization of fungicides by the potato cultivators. Likewise, (Pawar *et al.,* 2013) also worked on safflower, (Meena *et al.,* 2009) on Indian mustard and (Sharma *et al.,* 2002) worked on maize. (Benitez *et al.,* 2004) reported that *Trichoderma* strains exert biological control against fungal phytopathogens either indirectly, by competing for nutrients and space; by modifying the environmental conditions or promoting plant growth, defense and antibiosis mechanisms, or directly, by mechanisms such as mycoparasitism.

Among regular poly-phenolics kaempferol may be a flavonol found in among natural polyphenolics and many edible plants and is reported to possess potent pharmacological and nutraceutical activities. Kaempferol exhibits many pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, antitumor, cardioprotective, neuroprotective, and antidiabetic activities, and is applied in cancer chemotherapy. It is known that the antioxidant properties are responsible for these health benefits (Imran, 2019), (Aboody, and Mickymaray 2020), (Wach *et al.,* 2007).

5. Conclusions

Trichodrma species can be utilized as an incredible option bioagents to control early blight disease on potato brought about by *A. solani* which might diminish the broad of fungicides as integrated pest management. Depending on the results, it was concluded that *T. harzianum* could play an active role against plant pathogens by secreting the bioactive compounds to protect the host plant. HPLC analysis for the determination of phenols and flavonoids in the methanol extract of *T. harzianum* recorded the presence of Kampeferol. Furthermore, the antimicrobial and antioxidant potential of the *T. harzianum* indicates its use in agriculture and pharmaceutical industry.

List of abbreviations

Alternaria solani (*A. solani*) - *Trichoderma* (*T.*) - Potato Dextrose Agar (PDA) - Agricultural Research Center (ARC) - Disease severity (D.S.) - Modified Pikovskaya's Agar (MPA) - Phosphate Solubilization Index (PSI) - Modified Pikovskaya's Broth (MPB) - Indole-3-acetic acid (IAA) - optical density (OD) - National Center for Biotechnology Information (NCBI) - High-performance liquid chromatography (HPLC).

References

Abd EL-Gawad, A.M., and S.A. Omar, 2014. Effect of Biofertilization on the productivity of some forage crops under stress conditions. Egypt. J. of Appl. Sci., 29(2).55-80.

Abd-Alla, M.A., R.S. El-Mohamedy, and N.S. El- Mougy, 2007. Control of sour rot disease of lime fruits using saprophytic isolates of yeast. Egypt J. Phytopathol., 35 (2):39-51.

Abdul Wahid, O., I. Ismail, and A. Abo-Esha 2018. Non-chemical control of potato early blight caused by *Alternaria alternata*. *Catrina.* The International Journal of Environmental Sciences, 1(2): 103-109.

Abou-Zeid, N.M., A.M. Noher and A.S. Rania 2016. Effect of some Biotic and Abiotic applications on control of Fusarium wilt of pepper plants. Egypt J. Phytopathol., 44(2): 103-118.

Afzal, A., and A. Bano, 2008. Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (Triticum aestivum). International Journal of Agriculture and Biology, 10(1), 85-88.

Ahmed, A.I.S., 2017. Biological Control of Potato Brown Leaf Spot Disease Caused by *Alternaria alternata* Using *Brevibacillus formosus* Strain DSM 9885 and *Brevibacillus brevis* Strain NBRC 15304. J. Plant Pathol. Microbiol., 8: (6): 2157-7471. DOI: 10.4172/2157-7471.1000413.

Ainsworth, G.C., P.W. James, and D.L. Hawksworth, 1971. Dictionary of the fungi.

AL-Aboody, M.S., and S. Mickymary 2020 Anti-fungal Efficacy and Mechanism of Flavonoids. Antbiotics.9:45. https://doi.org/10.3390/antibiotics9020045.

Alam, S., S. Khalil, N. Ayub, and M. Rashid, 2002. In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from Maize rhizosphere. International Journal of Agriculture and Biology, 4(4): 454-458. 1560–8530/2002/04–4–454–458.

Alvin, A., K.I. Miller, and B.A. Neilan, 2014. Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Microbiol. Res. 169: 483–495. https://doi.org/10.1016/j.micres.2013.12.009.

Barnett, H.L., and B.B. Hunter 1987. Illustrated Genera of Imperfect Fungi. MacMillan Pub.Co., New York, U.S.A. 218.

Bell, D.K., H.D. Wells, and C.R. Markham, 1982. In vitro antagonism of *Trichoderma* species against six fungal plant pathogens. Phytopathology, 72: 379-382.

Benítez, T., M. Ana, M. Rincón, L.A. Carmen, and C. Codón, 2004. Biocontrol mechanisms of Trichoderma strains. International Microbiology, 7:249-260.

Bhardwaj, A., D. Sharma, N. Jadon, and P.K. Agrawal, 2015. Antimicrobial and Phytochemical Screening of Endophytic Fungi Isolated from Spikes of *Pinus roxburghii* . I. Med. Pub. Journals, 6 (3):1. ISSN 1989-8436.

Biswas, N., B. Pauline, S. Narlakkanti, E. Haque, and M Hassan, 2013. Identification of phenolic Compounds in Processed Cranberries by HPLC Method. J. Nutr. Food Sci., 3:1. http://dx.doi.org/10.4172/2155-9600.100018.

Bouws, H., and M.R. Finckh, 2008. Effects of strip intercropping of potatoes with non-hosts on late blight severity and tuber field in organic production. Plant Pathology, 57 (5): 916–927. https://doi.org/10.1111/j.1365-3059.2008.01860.x.

Bruin, G.C., and L.V. Edgington 1980. Induced resistance to ridomil of some oomycetes. Phytopathology, 70:459–60.

Busby, P.E., R. Mary, and G. Newcombe, 2016. Fungal endophytes: modifiers of plant disease Plant Molecular Biology. DOI 10.1007/s11103-015-0412-0.

Chowdappa, P., N.B.J. Kumar, S. Madhura, M.S.P. Kumar, W.E. Fry, J.N. Squires, D.E.L. Cooke, and K.L. Myers, 2013. Emergence of 13_A2 Blue Lineage of *Phytophthora infestans* was responsible for Severe Outbreaks of Late Blight on Tomato in South-West India. J Phytopathol., 161: 49–58. https://doi.org/10.1111/jph.12031.

Cohen, Y., U. Cisi, and E. Mosinger 1991. Systemic resistance of potato plants against Phytophthora infestans induced by unsaturated fatty acids. Physiol., Molecular, Plant Pathol., 38: 255 – 263. https://doi.org/10.1016/S0885-5765(05)80117-1.

Contreras-Cornejo, H.A., L. Macıas-Rodrıguez, E. Beltrán-Peña, A. Herrera-Estrella, and J. Lopez-Bucio, 2011. Trichoderma-induced plant immunity likely involves both hormonal and camalexin-dependent mechanisms in Arabidopsis thaliana and confers resistance against necrotrophic fungi Botrytis cinerea. Plant Signal. Behav. 6: 1554–1563. https://doi.org/10.4161/psb.6.10.17443.

Devi, S.S., Y. Sreenivasulu, S. Saritha, M.R. Kumar, K.P. Kumar, and P. Sudhakar 2012. Molecular diversity of native *Trichoderma* isolates against *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.). A causal agent of *Fusarium* wilt in tomato (*Lycopersicon esculentum* Mill.). Arch. Phytopathol. *Pl. Prot.* 45(6): 686-698. https://doi.org/10.1080/03235408.2011.591195.

Edington, L.V., K.L. Khew, and G.I. Barron, 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology, 61:42-44.

Elad, Y., and S. Freeman, 2002. Biological control of fungal plant pathogens. In: The Mycota XI: Agricultural Applications. Kempken, ed. Springer-Verlag, Berlin. https://doi.org/10.1007/978-3-662-03059-2_6.

Ewing, B., L. Hillier, M.C. Wendl, P. Green, 1998. Basecalling of automated sequencer races using Phred. I. Accuracy assessment. Genome Res. 8:175–185. doi: 10.1101/gr.8.3.175

Fang, Y., and R.P. Ramasamy 2015. Current and Prospective Methods for Plant Disease Detection 4: 537-561 doi:10.3390/bios5030537. https://doi.org/10.3390/bios5030537.

Fatima, K., K. Noureddine, J.E. Henni, and K. Mabrouk, 2015. Antagonistic effect of *Trichoderma harzianum* against *Phytophthora infestans* in the North-West of Algeria. International Journal of Agronomy and Agricultural Research, 6(4):44-53.

Fiske, C.H., and Y. Subbarow, 1925. The colorimetric determination of phosphorus. J. Biol. Chem, 66(2): 375-400.

Fontenelle, A.D., S.D. Guzzo, C.M. Lucon and R. Harakava, 2011. Growth promotion and induction of resistance in tomato plant against *Xanthomonas euvesicatoria* and *Alternaria solani* by *Trichoderma* spp. Crop Prot., 30: 1492-1500. https://doi.org/10.1016/j.cropro.2011.07.019.

Friedel, J.K., K. Molter, and W.R. Fischer, 1994. Comparison and improvement of methods for determing soil dehydrogenase activity using triphenyl chloride and iodonitrotetrazolium chloride. Biol. Fertil. Soils, 18: 291–296. https://doi.org/10.1007/BF00570631.

Gupta, V.P., A.K.B. Govindaiah, and R.K. Datta, 1995. Antagonistic potential of *Trichoderma* and *Gliocladium* species to *Botryodiplodia thiobromae* infecting mulberry. Indian Journal of Mycology and Plant Pathology, 25: 125.

Haverkort, A.J., P.C. Struik, R.G.F. Visser, and E. Jacobsen, 2009. Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora Infestans*. Potato Research, 52: 249–264. https://doi.org/10.1007/s11540-009-9136-3.

Howell, C.R., 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. Plant Disease, 87: 4–10. https://doi.org/10.1094/PDIS.2003.87.1.4.

Ikram, M., N. Ali, G. Jan, M. Hamayun, F.G. Jan, and A. Iqbal, 2019. Novel antimicrobial and antioxidative activity by endophytic *Penicillium roqueforti* and *Trichoderma reesei* isolated from *Solanum surattense.* Acta Physiologiae Plantarum (2019) 41:164. https://doi.org/10.1007/s11738-019-2957-z.

Im, H.W., B. Suhnh, S.U. Lee, N. Kozukue, M. Ohnisi-Kameyama, C.E. Levin, and M. Friedman, 2008. Analysis of phenolic compounds by high-performance liquid chromatography/mass spectrometry in potato plant flowers, leaves, stems, and tubers and in home-processed potatoes. Journal of Agricultural and Food Chemistry, 56 (9): 3341–3349. https://doi.org/10.1021/jf073476b.

Khan, A.L., J. Hussain, A. Al-Harrasi, A. Al-Rawahi, and I.J. Lee, 2015. Endophytic fungi: resource for gibberellins and crop abiotic stress resistance. Critical reviews in biotechnology, 35(1): 62-74. https://doi.org/10.3109/07388551.2013.800018.

Kloepper, J.W., C.M. Ryu, and S. Zhang, 2004. Phytopathology; 94: 1259-1266. https://doi.org/10.1094/PHYTO.2004.94.11.1259.

Kornerup, A., and J.H. Wanscher, 1989. "Methuen handbook of colour" 3rd edn. London: Eyre Methuen.

Lareen, A., F. Burton, and P. Scha¨fer, 2016. Plant root-microbe communication in shaping root microbiomes. Plant Mol. Biol. 90:575–587. DOI 10.1007/s11103-015-0417-8.

Loper, J.E., and M.N. Schroth 1986. Influence of Bacterial Sources of Indole3-acetic Acid on Root Elongation of Sugar Beet. Article *in* Phytopathology· DOI:10.1094-76-386. https://www.researchgate.net/publication/249298473

Mane, M.M., L. Abhilasha, A. Zghair, Q. Nadhim, and S. Sobita 2014. Efficacy of certain bio agents and fungicides against early blight of potato (*Solanum tuberosum* L.). *Internat. J. Plant Protec.*, 7(2): 433- 436. DOI: 10.15740/HAS/IJPP/7.2/433-436.

Meena, P.D., A. Kumar, C. Chattopadhyay, and P. Sharma, 2009. Eco friendly management of Sclerotiniarot in Indian mustard (*Brassica juncea*). *16th Australian Res. Assembly on Brassicas*, 31:127-128.

Möller, K., J. Habermeyer, V. Zinkernagel, and H.J. Reents 2007. Impact and interaction of nitrogen and *Phytophthora infestans* as yield-limiting and yield-reducing factors in organic potato (*Solanum tuberosum* L.) crops. Potato Research, 49 (4): 281–301. https://doi.org/10.1007/s11540-007-9024-7.

Moosa, A., A. Farzand, and M.Z. Anjum, 2017. *In vitro* evaluation of botanical extracts and fungicides against *Phytophthora* blight of chili pepper (*Capsicum Annuum* L). Pak. J. Phytopathol. 28(2): 201-206.

Naik, M.K., Y. Prasad, K.V. Bhat, and G.S. Rani, 2010. Morphological, Physiological, Pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. Indian phytopthol., 63: 168-173.

Nautiyal, C.S., 1999. An effeicient microbiological growth mediun for screening phosphate solubilizing microorganisms. FEMS Microbiology Letters. 170: 265-270. https://doi.org/10.1111/j.1574-6968.1999.tb13383.x.

Pawar, S.V., U. Dey, V.G. Munde, D.S. Sutar, and D. Pal 2013. Management of seed/soil borne diseases of safflower by chemical and biocontrol agents. African J. Microbiol. Res., 7(18):1834-1837. DOI: 10.5897/AJMR12.2010.

Qu, Y., Q. Wang, Y. Yao, Z. Huang, and Y. Li, 2014. Antagonistic Trichoderma isolates against potato late blight caused by Phytophthora infestans. Mycosystema, 33(6): 1231-1241.

Richardson, A.E., 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Funct. Plant Biol. 28: 897–906. https://doi.org/10.1071/PP01093.

Santamarina, M.P., J. Rosello, R. Liacer, and V. Sanchis, 2002. Antagonistic activity of *Penicillium oxalicum* Corrie and Thom, *Penicillium decumbens* Thom and *Trichoderma harzianum* Rifai isolates fungi, bacteria and insects *in vitro*. Revista Iberoamericana de Micologia, 19: 99-103.

Santos, W.R., W.R. Santos, P.P. Paes, I.A. Ferreira-Silva, A.P. Santos, N. Vercese, and A.P.M. Fernandes, 2015. Impact of strength training on bone mineral density in patients infected with HIV exhibiting lipodystrophy., The Journal of Strength & Conditioning Research, 29(12): 3466-3471. doi: 10.1519/JSC.0000000000001001.

Saravanakumar, S.S., A. Kumaravel, T. Nagarajan, P. Sudhakar, and R. Baskaran, 2013. Characterization of a novel natural cellulosic fiber from Prosopis juliflora bark. Carbohydrate polymers, 92(2): 1928-1933. https://doi.org/10.1016/j.carbpol.2012.11.064.

Sarfraz, M., S.A. Khan, A. Moosa, A. Farzand, U. Ishaq, I. Naeem, and W.A. Khan 2019. Promising antifungal potential of selective botanical extracts, fungicides and *Trichoderma* isolates against *Alternaria solani.* Cercetări Agronomice în Moldova, 1(173):65-74.

Savary, S., A. Ficke, J.N. Aubertot, and C. Hollier, 2012. Crop losses due to diseases and their implications for global food production losses and food security. Food Security, 4(4): 519-537. https://doi.org/10.1007/s12571-012-0200-5.

Sharma, R.C., S.K. Vasal, F. Gonzalez, B.K. Batsa, and N.N. Singh, 2002. Redressal of banded leaf and sheath blight of maize through breeding, chemical and biocontrol agents. Breeding for BLSB Resistance, 5(8): 391-397.

Sheeba, H., M.S. Ali, and V. Anuradha, 2019. Bioactive compounds and antimicrobial activity of fungal crude extract from medicinal plants. Journal of Pharmaceutical Sciences and Research, 11(5): 1826-1833.

Shtienberg, D., D. Blachinski, A. Yaniv, and A. Dinoor, 1994. Control of potato early blight in the northern Negev region of Israel in the fall season. [Hebrew] Hassadeh; 74(4):393-397, 473. 5 ref. AN: 19951000303.

Sid, A.A., C.P. Sanchez, M.E. Candela, and J. Eur, 2000. (*Capsicum annuum*) to Phytophthora capsici using *Trichoderma harzianum* and its relation with capsidiol accumulation. European Journal of Plant Pathology, 106(9):817-824. https://doi.org/10.1023/A:1008780022925.

Sim, M.C., P.E. Lim, S.Y. Gan, and S.M. Phang, 2007. Identification of random amplified polymorphic DNA (RAPD) marker for differentiating male from female and sporophytic thalli of Gracilaria changii (Rhodophyta). Journal of Applied Phycology, 19(6): 763-769. https://doi.org/10.1007/s10811-007-9224-1.

Singh, R.S., 1982. Plant Pathogens "The Fungi" Oxford and IBH publishing Co. New Delhi, Bombay Calcuta, 443.

Snedecore, G.W, and W.G. Cochran, 1982. Statistical methods 7th Ed. Iowa state University, Pres Ames USA.

Townsend, G,R., and J.W. Heuberger, 1943. Method for estimating losses caused by diseases in fungicide experiments. Plant Dis., Reptr., 27: 340.

Wach, A., K. Pyrzyńska, and M. Biesaga 2007. Quercetin content in some food and herbal samples. Food chemistry, 100(2): 699-704. https://doi.org/10.1016/j.foodchem.2005.10.028.

Waqas, M., A.L. Khan, M. Kamran, M. Hamayun, S.M. Kang, Y.H. Kim, and I.J. Lee 2012. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules, 17(9): 10754-10773. https://doi.org/10.3390/molecules170910754.

Watanabe, S., K. Kumakura, H. Kato, H. Iyozumi, M. Togawa, and K. Nagayama 2005. Identification of Trichoderma SKT-1, a biological control agent against seedborne pathogens of rice. Journal of General Plant Pathology, 71(5): 351-356. https://doi.org/10.1007/s10327-005-0217-0.

White, T.J., T. Bruns, S. Lee, and J. Taylor, 1990 Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) In: PCR Protocols: A Guide to Methods and Applications. Academic Press, San Diego, 315–322.