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INVESTIGATING THE EFFECT OF SOME ELICITORS ON BROWN ROT DISEASE AND TUBER YIELD OF POTATO (*Solanum tuberosum* L.)

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ABSTRACT

Brown rot of potato is a worldwide disease that causes huge agricultural and economical losses. Using synthetic chemicals like antimicrobial pesticide may have adverse effects on consumers and on the environment. The recent trend of control plant disease is the application of eco-friendly tools to control disease. In the present investigation, and this study streptomycin, Pleurotus columbinus spent wheat straw (PCSWS), wheat straw (WS), three bacterial species (Bacillus subtillus, B. polymyxa and Pseudomonas fluorescens), plant essential oils of anethole, mustard, caraway and neem were tested to control brown rot disease of potato cv. Spunta in Baramoon Research Station, Dakahlia Governorate, Egypt under field conditions, during the two winter seasons of 2013/14 and 2014/15.

The vegetative growth parameters, tuber yield, tuber grading, tuber dry matter, specific gravity and infected tubers were investigated. PCSWS led to significant increase in all studied criteria, compared to check or other treatments. Furthermore, PCSWS or caraway oil gave the best results in reduction of infected tubers in vitro. The direct antibacterial effect of studied treatments against the causal pathogen of brown rot of potato; Ralstonia solanacerum. Only streptomycin, anethole and caraway oil affected the growth of R. solanacearum. Pots experiment was carried out to study the ability of the tested treatments to induce systemic resistance (ISR).

***Conclusively,** Bacillus subtillus, caraway and neem oils significantly increased the production of total phenols, polyphenol oxidase and peroxidase, respectively, which enhanced potato plant health, increased chlorophylls and plant height as well as, decreased disease rating that increased tuber weight after 70 days of planting.*

Key words: Potato, production, brown rot, essential oils, organic residues, streptomycin.

INTRODUCTION

Harmful bacteria or pathogens can affect agricultural crops and produce economic losses. *Ralstonia solanacearum*, a plant pathogen, causes bacterial brown rot in vegetables such as potato, tomato, eggplant and many other plants (Hayward, 1994). This bacterium causes wilt by infecting plants through roots and colonizing stem vascular tissue. *Ralstonia solanacearum* can overwinter in plant debris or diseased plants, wild hosts, seeds or vegetative propagative organs. As well as the bacteria can survive a long time in water (up to 40 years at 20–25 °C in pure water) and the bacterial population is reduced in extreme conditions *e.g.*, temperature, pH, salts. Sometimes infected land cannot be used again for susceptible crops for several years (Denny, 2006). Moreover, *R. solanacearum* causes a vascular wilt disease and has been ranked as the second most important bacterial pathogen in potatoes (Yuliar and Toyota, 2015). It is one of the most destructive pathogens identified for induces rapid and fatal wilting symptoms in host plants. The host range is extensively wide, over 200 species; the pathogen is distributed worldwide and induces a destructive economic impact (Kelman, 1998). Direct yield losses by *R. solanacearum* vary widely according to the host, cultivar, climate, soil type, cropping pattern, and strain. For example, yield losses vary from 0 to 91% in tomato, 33 to 90 % in potato, 10 to 30% in tobacco, 80 to 100% in banana, and up to 20% in the groundnut (Elphinstone, 2005).

Essential oils has an known are antibacterial properties and may provide a solution to tackle brown rot such as caraway oil that contains S-(+)-carvone. Based on previous studies, it has proven to inhibit bacteria such as *E. coli*, *S. aureus* and *K. pneumoniae* (Seidler-Łożykowska *et al*., 2013). Neem oil contains different effective phytoconstituents such as alkaloids, glycosides, trepenoids, steroids and tannins act against a wide array of bacteria. It has also been proven to inhibit *E. faecalis*, *S. mutans* and *S. aureus* (Prabhat and Navneet, 2010). Thyme and eucalyptus oils also suppress on *R. solanacearum* (Hosseinzadeh *et al*., 2013) and thymol on *Xanthomonas axonopodis* (Kotan *et al*., 2007). El-Zemity *et al*. (2008) showed that growth of *Pectobacterium carotovorum* on potato was inhibited by thyme oil. Black mustard (*Brassica nigra*) has an antimicrobial activity, when applied by direct contact into the liquid medium or by exposure in the vapor phase,

against the growth of *Aspergillus niger*, *Aspergillus ochraceus*, or *Penicillium citrinum* (Beatriz *et al*., 2015).

Incorporating organic amendments and managing crop residues (type and quality) have a direct impact on plant health and crop productivity. The organic amendments, manures and composts are rich in nitrogen may reduce soil borne diseases by releasing allelochemicals generated during product storage or by subsequent microbial decomposition (Bailey and Lazarovits, 2003).

Plant growth promoting rhizobacteria (PGPR), *i.e*., *Pseudomonas* spp., *Bacillus* spp. enhance plant health and control many plant diseases by many different ways. They can antagonize plant pathogen by inhibiting its growth. PGPR produce phytohormones and encourage induced systemic resistance (ISR). As well as help plant to tolerate abiotic stress as water deficiency (Compant, *et al*. 2005; Bhattacharyya and Jha, 2012; Yuliar *and Toyota* 2015 and Noumavo *et al*., 2016).

Elicitors are biotic or abiotic factors that enhance the resistance of plant against plant pests and increase induced resistance of plants. Use of elicitors to control plant disease is one of the recent trend to control plant disease (Bhattacharyya *et al.*, 2012).

Therefore, this is study aimed to investigate the ability of different bio-origin products to reduce bacterial wilt disease of potato and improve the productivity, explain their abilities as antibacterial *in vitro* experiment, their ability to act as elicitors encouraging ISR and enhance plant growth in pot experiment.

MATERIALS AND METHODS

Plant and chemicals

Potato tubers cultivar (Spunta) was obtained from Nubaria district, Bahaira Government, Egypt. Raw synthetic streptomycin ($(C_{21}H_{39}N_7O_{12})_2.3H_2SO_4$) was used as antibacterial solution (Sigma Chemicals Co., USA), which contains 800 International Units per mg.

Bacteria strains:

Bacterial pathogen: Brown rot local pathogen was isolated and identified as *R. solanacearum* (race 3 biovar 2) virulent strain and provided by the method described with Moussa (2006).

Bacterial bio-agent.

Three isolates of *Bacillus subtilis*, *Bacillus polymyxa* and *Pseudomonas fluorescens* had PGPR characters and were provided kindly from

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Preparation of bacterial pathogen and bacterial bio-agents:

Bacterial pathogen (*R.* solanacearum) and PGPR bio-agents of *B. subtilis*, *B. polymyxa* and *P. fluorescens* were inoculated in nutrient growth Glucose Broth (NGB) and incubated in shaker incubator (28 °C and 120 RPM) for 48 hrs. Bacteria were harvested by centrifugation (10000 rpm/ min for 20 min.), each bacterial species was re-suspend in sterilized tap water and each bacterial suspension was adjusted to 10^8 cfu / ml concentration.

R. solanacearum bacterial suspension was used for moist soil in pot experiment before 48 hrs of planting. Bacterial bio-agents' suspensions were used to soaking potato seeds for 30 min. before planting in the field and pot experiments.

For preparation the bacterial suspension to *in vitro* experiments, studied bacteria were seeded on nutrient glucose agar (NGA) slants for 48 hrs, harvested, re-suspended in sterilized tap water and adjusted to 10^8 cfu / ml concentration.

Organic wastes

Pleurotus columbinus spent wheat straw (PCSWS) was provided from Mas Mushroom Co., Talkha, Dakahlia governorate, Egypt. Only, wheat straw (WS) powder was prepard from the same Mushroom Co. that used for *P. columbinus* cultivation. PCSWS and WS were grinded well to fine powder. PCSWS and WS were used for coating the tubers before planting.

Essential oils extraction

Seeds of caraway (*Carum carvi* L.; Umbelliferae), anise (*Pimpinella anisum* L.; Apiaceae) and mustard (*Brassica nigra* L.; Brassicaceae) (200 g seeds from every one) used for oil extraction by hydro-distillation (2-3 hr.) according to Chialva *et al*. (1982) and Charles and Simon (1990). The monoterpene "anethole" fraction used in this study was obtained from anise oil. Essential oils were subjected to GC-MS analysis using a Gas Chromatography (Singh *et al*., 2005).

Neem (*Azadirachta indica* A. Juss; Meliaceae) oil was used as a commercial product (Nimbecidine®, T. Stanes & Co. Ltd.) extracted from the seeds of the neem plant. The product component contains azadirachtin (neem bitter), 3.5%, neem oil 75%, emulsifier 10%, diluent 9% and other limonoids including meliantriol, salanin, nimbin and a host of other terpinoids in the ratio as it occurs naturally in neem.

Application technique

The different solutions concentration were prepared as follow; Streptomycin solution (2.5 g/L); anethole (5 ml/L); mustard oil (5 ml/L) and caraway oil (2.5 ml/L). Few drops of tween 80 were added to emulsify solution. Neem oil concentration was 30 ml/L. All bacterial suspensions were adjusted to 10^8 cfu / ml. *Pleurotus columbinus* spent wheat straw (PCSWS) and wheat straw powder (WS) (nearly 50 g) mixed with sticker materials (super-film®) and added to seed tuber.

Full potato seed tubers were soaked in streptomycin solution, essential and neem oils, anethole emulsions and bacterial suspension for 30 minutes for each individual treatment or completely coated by PCSWS and WS fine powder before planting.

Test of sprout inhibition

Each studied treatment was used in experiment to know the possibility of inhibition. Streptomycin, anethole, neem oil, mustard and caraway oils were used in 5 concentrations (0.25, 0.50, 1, 2 and 3 g / l for streptomycin and % v/v for others). While, the tested bacteria (*Bacillus subtilis*, *Bacillus polymyxa* and *Pseudomonas fluorescens*) were used in one concentration 10^8 cfu / ml bacterial suspension. Three potato tubers were immersed in each solution or suspension for 30 minutes. In addition, PCSWS and WS powders were moistened by little water and used to cover potato seed tubers (three replicates for each treatment). Immersing potato seeds in tap water for 30 minutes used as a control treatment. Potato tubers were examined after two weeks to see the effect of sprout in potato pieces.

Detection of presence of R. solanacearum in invitro:

The field of this study had history of infection of brown rot disease of potato. To confirm the presence of *R. solanacearum* in this field, soil samples were taken from different locations. Tenfold serial dilutions were applied for the composite sample of this soil suspension of the soil mixture of different samples and 0.1 ml of dilutions 10^{-3} and 10^{-4} dilution was spotted and spread on the surface of solidified plate SMSA medium (Elphinstone *et al*., 1996). Three replicates were prepared. Approximately after 2–5 days of incubation, number of colonies of *R. solanacearum* was recorded for each replicate and the mean value of cfu /gm was calculated.

Field experiment:

The experiments were conducted at Baramoon Research Station, Dakahlia, Egypt (+7m altitude, 30° 11- latitude and 28° 26- longitude), during winter seasons of 2013/2014 and 2014/2015. The soil is a clay loam in texture, with 1.2% organic matter and pH 7.9. Extractable soil P and K levels in the plots used in this 2-yr trial were in the range of 12.2 to 13.6 mg / kg for P and 290 to 302 mg / kg for K. Local climate is Mediterranean type, warm and rainy during the winter season. Temperature range was between 5.5 and 30.2 °C. The weight of tubers were within range of 40 to 60 g, sown was on October 15th in both growing seasons.

The plot area was 11.25 m^2. It contains three lines with 5 m in long and 0.75 m distance among lines. Calcium superphosphate was mixed within the upper soil layer (0-25 cm) before planting. All treatments were planted in hills 25 cm apart. After planting, the soil can be ridged up around the plants, either along rows or around individual plants as hills. This can also be done when plants are around 20 cm tall, as part manual wedding operations. Earthing up the soil around the base of stems favors tuber formation. Nitrogen (ammonium nitrate 33.5% N), phosphorus (mono-superphosphate 15.5 % P_2O_5) and potassium (potassium sulphate 48% K_2O) were applied in the rate of 180, 75 and 96 kg / fed., respectively. Nitrogen fertilizer was added at three equal doses, *i.e.*, after emergence, and with 2nd and 3rd irrigation, respectively. Potassium was added at two times with two equal portions with the second and third additions of N-fertilizer. The other agricultural practices were carried out according to the recommendation of Ministry of Agriculture. A complete randomized blocks design with three replicates was used and the treatments were allocated in their replicates randomly.

Vegetative growth, yield and yield components:

A random sample of three plants from each experimental plot was taken at tuberization stage (60 days after planting) to determine plant height (cm), leaf area(m^2) and dry weight (g). At harvest time, 110 days after planting, the total tuber yield (ton/fed.) and grading the tubers into three size categories based on tubers diameters (<30, 30: 60 and >60 mm) were recorded.

Increase % in total potato tuber yield was calculated according to the following equations:

$$\text{Increase \% in total potato tuber yield} = \frac{\text{(Total tuber yield of treatment – Total tuber yield of control)}}{\text{Total tuber yield of control}} \times 100$$

Tuber quality

The quality of the harvested tuber for each treatment was determined. The percentage of tuber dry matter was calculated by drying 100 grams of fresh tubers in oven at 70 °C tills a constant weight. Starch was determined according to the methods described by AOAC, 1990. A sample of 20 tubers randomly selected from each plot was determined for specific gravity using the weight-in-air/weight-in-water method.

Storage of potato tubers after harvest time

In order to show the latent infection of brown rot disease of potato, after harvest time, the tubers (5 kg) for each experimental unit were stored at room temperature (24-28 °C) and kept under thick layers of rice straw (50 cm thickness) for 30 days (curing). The tubers were examined for the presence of the studied disease at the end of the storage period (4th of March). The percentage of infected tubers was calculated for each treatment after storage period and mean value of percentage of total infected tubers was calculated. Decrease % in infected tuber was calculated according to the following equation:

$$\text{Decrease \% of infected tuber} = \frac{\text{(Infected tuber \% of control – Infected tuber \% of treatment)}}{\text{Infected tuber \% of control}} \times 100$$

Antibacterial activity against R. solanacearum:

Disk diffusion method: Molten semi-solid nutrient glucose agar (NGA) medium (3 ml) was inoculated with 0.1 ml of *R. solanacearum* 48 hr. old (10^8 cfu /ml) suspension, shacked well and pour onto previously solidified NGA medium petri dishes. Paper disks of 5 mm diameter (Watman No. 1 filter paper) were immersed in the three tested oils, anethole emulsions and 0.025 % (w/v) streptomycin solution, then left to dry. Paper discs were immersed in sterile distilled water and dipped in powders of PCSWS and WS. Immersing paper discs in sterile distilled water only was applied as control treatment. One disc was placed onto the middle of each previously prepared inoculated plates. Three replicates were used for each treatment. The plates were incubated at 28 °C for 48 hrs. (Kalemba and Kunicka, 2003). Each plate was examined. The inhibitory effect of each treatment against *R. solanacearum* was measured by recording the inhibition zone diameter (mm) around paper disk.

Determination of minimal inhibitory concentrations (MICs):

The minimum inhibitory concentrations (MICs) for the studied essential oils and streptomycin were determined by using agar dilution method

(Hammer *et al*., 1999) with some modifications. A final concentration of 0.5% (v/v) Tween-80 was incorporated into the agar after autoclaving to enhance oil solubility. A series of dilutions of each treatment, (4, 3, 2, 1, 0.5 and 0.25 % (v/v), was prepared in molten NGA medium with 0.5% (v/v) Tween-80. Plates were dried prior to inoculation with *R. solanacearum*. Inoculation was carried out with streaking *R. solanacearum* by sterilized loop from liquid culture onto the prepared plates. Plates of NGA medium, with 0.5% (v/v) Tween-80 and without any treatment, was used as a growth control. Inoculated plates were incubated at 28 °C for 48 hrs. The MICs were determined as the lowest concentration of the treatment that completely inhibits the growth of bacteria on the agar plate.

Investigation of antagonistic activity of studied bacteria against R. solanacearum:

The methods described by Waksman (1967) with some modifications were used for determination of antagonistic activity of studied bacteria against brown rot disease.

Double layer method:

Only one loop of each tested antagonistic bacteria species cultures (10^8 cfu/ml) was seeded in the middle of solidified NGA plates as straight line. These plates were incubated at 28 ºC for 2 days. A second layer of semi-solid agar containing 48 hrs. old of virulent culture of *R. solanacearum* (0.1 ml of 108 cfu / ml bacterial suspension added into 3 ml molten semi-solid NGA vortexed well, poured for each plate) rotated gently by palm to homogenous distribution onto the plate. Three replicates were used for each studied bacterial species. The plates were incubated at 28 ºC for 48 hrs. The presence of clear zone around the straight line of tested antagonistic bacterial species indicates the positive antagonistic activity against *R. solanacearum*. The diameters of clear zones (if present) were measured.

Disk agar method

Each tested bacterial species was cultured by adding 1 ml of 108 cfu/ ml bacterial culture on each 9 ml petri dish, pour 15 ml molten NGA medium and incubated at 28 ºC for 2 days. Disks of each tested bacterial species of diameter 9 mm were prepared by sterilized cork borers. The bacterial disks were appropriately placed onto the center of solidified NGA plates freshly seeded with *R. solanacearum*. Three replicates were prepared for each treatment. The plates were incubated at 28 ºC for 48 hrs. The inhibition zones were measured (in millimeters).

Pots experiment

This experiment was carried out at the Farm of Fac. Agric., Mansoura Univ., Egypt during the period of 19th November 2015 to 30th January 2016 at open air. The plastic sacks of 35 cm diameter were filled with 20 kg non-sterilized soil. This soil was mixture of clay and sand 1:1 (w: w). Soils of each pot were soaked with water and left to dry for 72 hours. The sacks were infested by *R. solanacearum* (about 106 CFF O.D. 0.1 at 600 nm); 100 ml for each sack. The sacks were left for about 48 hours before sowing.

The studied treatments were prepared as mentioned before in field experiment. Two potato seed tubers were planted in each sack (pot). After emergency, one plant was harvested after 40 days for estimation plant growth hormones and other plant was harvested after 70 days for estimation of chlorophylls, plant length, foliage fresh and dry weights and tuber number and its weight. Three replicates were prepared for each treatment. This experiment was designed according to complete randomized design.

Estimation of growth regulator hormones of the three bacterial bio-agent:

Bacterial suspensions of the three biocontrol agent (*B. subtilis*, *B. polymyxa* and *P. fluorescens*) grown in nutrient glucose broth (NGB) of 48 hrs., old were adjusted to 10^8 concentrations. These suspensions were centrifuged (10000 rpm/ min for 20 mints.). Supernatants were sterilized by millipore filters (0.450 µm).

Sterilized filtrates of bioagent bacterial suspensions were used in the biochemical analysis. All biochemical analysis was performed in Mansoura Unit of Soils, Water and Environment, Agricultural Research Center, Egypt.

Determination of indole acetic acid (IAA) was carried out according to the procedure of Glickmann and Dessaux (1995), gibberellic acid (GA_3) by the procedure of Holbrook *et al*., (1961) and cytokinin by the procedure reported by Hoyerováa *et al*. (2006).

Disease assessment and biochemical testes under greenhouse conditions:

After 40 days from sowing, the total phenol content was determined using folin ciocalteau reagent according to the method described by Maliak and Singh (1980), whereas, extraction and assay of polyphenoloxidase (PPO) and peroxidase (POD) were carried out according to the methods described by Maria *et al*. (1981) and Maxwell and Bateman (1967), respectively. Estimation of chlorophylls was done according to Nagata and Yamashita (1992) at 70 days from planting.

Disease rating: Wilt symptoms severity were recorded daily according to the scale of Kempe and Sequeira (1983) where, (0 = no symptoms, 1= up to 25 % wilt, 2 = 26-50 % wilt, 3 = 51-75 % wilt, 4 = 76-99% of the foliage wilted and 5 = dead plants. Each replicate was examined and disease ratting (severity) was recorded. Mean value for each treatment was calculated.

Statistical analysis:

Data were analyzed with the statistical analysis software CoStat (version 6.4), by the one-way randomized completely blocks design(RCBD). Duncan's multiple range test (Duncan, 1955) was used to compare the means at probability (*P*) level 0.05.

RESULTS AND DISCUSSION

Sprouting

Immersing potato seed tubers in tap water (control), or 10^8 suspension of *B. subtilis, B. polymyxa and P. fluorescens* did not inhibit sprout of potato pieces. Moreover, covering potato pieces with *Pleurotus columbinus* spent wheat straw powder and wheat straw powder did not any inhibition on potato sprout. In addition, all five tested concentrations of anethole and neem oil did not affect potato sprout (Table 1). On the contrast, streptomycin, mustard oil and caraway oil had effects on potato sprouting. Streptomycin concentrations 0.25, 0.5 and 1g did not refection any inhibition. While, streptomycin at 2g inhibited 33 % of potato pieces, at 3g concentration, 100 % inhibit of potato infection were observed. Furthermore, only the concentration 0.25 at % of mustard and caraway oil did not inhibit potato sprout, while other tested concentrations of the two oil affected the potato sprout. The two concentrations, 0.25 and 0.5 % of mustard oil inhibited 33 % of potato pieces, while, the two concentrations, 2 and 3 % inhibited 66.67 % of potato pieces. As well as, the concentration of 0.5 % of caraway oil inhibited 33 % of potato pieces. The concentration of 1% of caraway oil inhabited 66.67 % of potato pieces. While, the two concentrations, 2 and 3 % of caraway oil completely inhabited the potato seeds (100%) as shown in Table 1.

Table 1: Sprouting of potato seed tubers (*cv.* Spunta) as affected by treatments**.**

Treatments	Studied concentration	Sprouting inhibition (%)
1. Control	-	0
2. Streptomycin	0.25 g / l	0
	0.50 g / l	0
	1.00 g / l	0
	2.00 g / l	33.33
	3.00 g / l	100
3. *Pleurotus columbinus* spent wheat straw	Powdering	0
4. Wheat straw	Powdering	0
5. *Bacillus subtilis*	10^8 CFU / ml	0
6. *Bacillus polymyxa*	10^8 CFU / ml	0
7. *Pseudomonas fluorescens*	10^8 CFU / ml	0
8. Anethole	0.25 %	0
	0.50 %	0
	1.00 %	0
	2.00 %	0
	3.00 %	0
9. Neem oil	0.25 %	0
	0.50 %	0
	1.00 %	0
	2.00 %	0
	3.00 %	0
10. Mustard oil	0.25 %	0
	0.50 %	33.33
	1.00 %	33.33
	2.00 %	66.67
	3.00 %	66.67
11. Caraway oil	0.25 %	0
	0.50 %	33.33
	1.00 %	66.67
	2.00 %	100
	3.00 %	100

Studying the effect of studied treatments on potato sprout before the field experiment to be sure that the used concentrations of these treatments did not inhibit potato sprout and did not inhibit potato seedling growth. The results indicated that 10^8 CFU/ml bacterial suspension of *B. subtilis*, *B. polymyxa* and *P. fluorescens* did not suppress potato sprout. In addition, *Pleurotus columbinus* spent wheat straw and wheat straw did not inhibit

potato sprout. Anethole and Neem oil of concentration 3 % or under this concentration did not inhibit potato sprout. Potato sprout inhabited completely by streptomycin concentration 3% and caraway oil (2 and 3%). These results had the agreement of Sonli *et al*. (2010), they reported that potato sprout suppressed by essential oils.

Field experiment

Although, the field of this experiment had history of bacterial wilt disease of potato, the presence of *R. solanacearum* was confirmed by cultivation soil extract on SMSA medium. The mean number of colonies of *R. solanacearum* in the soil was 2 x 10^5 cfu / gm soil. So, this field was surely naturally infested by *R. solanacearum*.

All tested treatments gave a significant effects on vegetative growth parameters, in comparison with the control. Coating potato seeds with *Pleurotus columbinus* spent wheat straw (PCSWS) led to a significant effect on plant height, leaf area and dry weights per plant in comparison with other treatments (Table 2). This is true in both growing seasons. Caraway oil and streptomycin came the second and third treatments in all studied criteria.

Table 2: Vegetative growth characters of potato as affected by tested treatments to control brown rot disease in 2013/14 and 2014/15 seasons.

Characters / Treatments	Plant height (cm)		Leaf area / plant (m^2)		Dry weight of foliage / plant (g)	
	2013/14	2014/15	2013/14	2014/15	2013/14	2014/15
1. Control	47.00 d	46.00 d	0.41 e	0.43 d	27.88 e	26.00 f
2. Streptomycin	58.67 ab	56.33ab	0.50 b	0.51 b	34.12 ab	33.76 ab
3. *Pleurotus columbinus* spent wheat straw	60.67 a	58.00a	0.54 a	0.54 a	36.76 a	34.84 a
4. Wheat straw	52.00 c	52.33bc	0.42 e	0.39 e	28.55 de	27.16 ef
5. *Bacillus subtilis*	52.33 c	54.67abc	0.42 e	0.43 d	28.42 de	26.76 ef
6. *Bacillus polymyxa*	54.67 bc	54.00abc	0.43 e	0.42 d	29.18 de	28.70 def
7. *Pseudomonas fluorescens*	56.00 bc	57.00a	0.46 d	0.48 c	31.27 bcd	32.73 abc
8. Anethole	54.67 bc	55.00abc	0.43 e	0.42 d	28.95 de	29.15 de
9. Neem oil	52.33 c	51.33c	0.49 bc	0.49 c	33.12 bc	32.00 bc
10. Mustard oil	57.33 ab	56.00ab	0.45 d	0.47 c	30.52 cde	27.30 ef
11. Caraway oil	58.00 ab	56.33ab	0.48 c	0.48 c	31.00 b-e	30.80 cd

Means followed by the same letter (s) within each column do not significantly differ using Duncan's Multiple Range Test at the level of 5%.

Organic amendments to soil have direct impacts on plant health and crop productivity. They are advantageous because they improve the physical, chemical, and biological properties of soil, which can have positive effects on plant growth (Bailey and Lazarovits, 2003). The degradation of organic matter in soil can directly affect the viability and survival of a pathogen by restricting available nutrients and releasing natural chemical substances with varying inhibitory properties (Bailey and Lazarovits, 2003). Moreover concluded that carbon released during the degradation of organic matter contributes in increasing soil microbial activity and thereby enhances the likelihood of competition effects in the soil (Bailey and Lazarovits, 2003). Organic amendments to soil have been shown to stimulate the activities of microorganisms that are antagonistic to pathogens (Akhtar and Malik, 2000). In addition, organic amendments often contain biologically-active molecules such as vitamins, growth regulators, and toxins, which can affect soil microorganisms and enhance plant growth. Recently, Youssef and Tartoura (2013) reported that plant resistance against the bacterial wilt pathogen was enhanced through the augmented activities of ascorbate peroxidase, monodehydro ascorbate reductase, dehydroascorbate reductase, and glutathione reductase following the application of organic residues.

The three tested PGPR (*B. subtilis, B. polymyxa* and *P. fluorescence*) increased significantly vegetable growth characters more than control treatment. This may be due to they act as bio-fertilizer by phosphate solubilization, siderophores production, exopolysaccharides production and/or biofixation of atmosphere nitrogen (Noumavo *et al*., 2016).

The three tested essential oils (mustard, caraway and anethole) and neem enhanced vegetative growth characters of potato plant characters in the field experiment. These results had conformity of Momol *et al*. (2005) who used essential oil in field experiment to control bacterial wilt of tomato.

All tested treatments exhibited significant differences ($P \leq 0.05$) in total tuber yield (ton/ fed.) in the first season (Table 3). PCSWS gave the highest yields (14.5 and 13.6 ton/fed.) in both seasons, respectively, followed by caraway oil treatment, *Pseudomonas fluorescens* and streptomycin while the control had the least. The percentage of increase of PCSWS over the control reached 49.51 and 50.88 % and 46.81 and 48.23 % for caraway oil, in both seasons.

The proportion of grade-sized tubers 30: 60 mm and over 60 mm were significant in both seasons and it took the manner of total tuber yield as previously mentioned. Science, total yield increases were due to primarily the

increase in tuber size in larger and medium grades and decrease of the small grades. In this respect, the treatment of PCSWS increased significantly weights of large (> 60 mm) and medium tubers (30: 60 mm) and significantly decreased small tuber weight (< 30 mm), in both seasons, respectively. There were no significant differences ($P \leq 0.05$) among tested elicitors for tuber weight <30 mm, in both seasons. There are no differences between the tested essential oils on medium tubers weight in both growing seasons.

These results may be due to that PCSWS contains antibacterial compound(s) which was soluble in water. These results were confirmed by the results of Tan and Wahab (1997). They reported that *Pleurotus sajor-caju* grown on cotton waste produces relatively low levels of three components namely cellobiohydrolase, CMCase and β-glucosidase. Also, Velázquez-Cedeño *et al*. (2002) indicated that the two basidomycetes *Pleurotus ostreatus* and *P*. *pulmonarius* had the ability to produce some extracellular lignocellulytic enzymes.

At around tuberization stage, the plants' water demand is very high and they wilt rapidly due to the blockage of the xylem tissue by the bacterial mass. In addition, due to high transpiration rates, the plants take up a lot of water (together with bacteria in the soil water) and hence wilt rapidly. Total tuber yield was higher in the first season than the second. This is most likely due to lower temperatures experienced during the first season compared to the second season (data not shown). Disease expression in the field is favored by high temperatures (EPPO, 2004).

There were a significant differences in potato tuber quality parameters. Application of PCSWS significantly increased tuber dry matter in both seasons, starch and specific gravity of tubers (1^{st} season, only) and significantly decreased infected tubers (both seasons), in comparison with other treatments (Table 4).

These increases in dry matter, starch and specific gravity may be attributed to PCSWS had antimicrobial agents against pathogenic bacteria and fungi and this of course is reflected in the quality of tubers (Rai and Tidke, 2005). These results are confirmed with those of Okamoto *et al*. (2002) and Velázquez-Cedeño *et al*., (2002). Furthermore, PCSWS may directly or indirectly suppress disease with enhancing production of decomposed products by antagonistic microbial population or stimulation the production of lytic enzymes involved in the degradation of plant pathogens (Gamliel *et al*., 2000).

PGPR significantly increased tuber yield and quality. This may be due to phytostimulation by production of auxins, cytokinins, gibberellins, which regulate the hormones system in plant and increasing plant growth (Table 2), tuber yield (Table 3) and then increasing tuber quality (Noumavo *et al*., 2016). Tested essential oils and anethole increase tuber yield and quality. The authors suggest that may be due to their elicitor effect on potato plant enhancing production of some growth hormones.

The aggressiveness of the pathogen is affected mainly by temperature and moisture; high temperature and high soil moisture during the first half stage of plant age promote survival, reproduction, infectivity, and spread of the bacterium, and hence disease development (Martin and French, 1985). This high soil bacterial population combined with the vigorous vegetative plant growth probably led to the rapid increase in the disease incidence (infected tubers) in the field (Table 4).

Data in (Table, 4) illustrated that, PCSWS, *Pseudomonas fluorescens* and caraway oil had a significant reduction in percentage of tubers infected (Table 4). The possible mechanisms of action of the plant residues are mainly considered to be antimicrobial activities, followed by the indirect suppression of the pathogen through improved physical, chemical, and biological soil properties that reflected on growth and yield (Cardoso *et al*., 2006). Respecting the role of antimicrobial compounds, the antimicrobial compounds from *Tagetes patula* that suppressed *R. solanacearum* in an *in vitro* experiment were identified as 5-(3-buten-1-ynyl)-2,2'-bithienyl (BBT) and 5-(4-acetoxy-1-butynyl)-2,2'-bithienyl (BBTOAc) (Terblanche and Villiers, 1998). Lansiumamide B isolated from the seeds of *Clausena lansium* suppressed tobacco bacterial wilt more than an antibiotic streptomycin when applied at a density of 100 mg / kg (Li *et al*., 2014).

The three-tested PGPR decreased tubers infected % may be due to their antibacterial activities and/or increasing ISR activity of plant (Battacharyya and Jha, 2012). Suppression mechanisms are typically attributed to the antibacterial metabolites produced by PGPR or those present in natural products; however, the number of studies related to host resistance to the pathogen is increasing. Enhanced/modified soil microbial communities are also indirectly involved in disease suppression (Yuliar and Toyota, 2015). In this respect, *Pseudomonas fluorescens* had a significant reduction of infected tubers percent.

The three essential oils and neem decreased infected tubers. Caraway oils gave the best results (0.00%). In this respect, Alamshahi and Nezhad (2015) reported that thyme essential oil caused significant reduction in soft rot and bacterial wilt incidence on potato by 41 and 44%, respectively. The same treatment on tomato caused 50% reduction of wilt compared with the control sample. Under field conditions, suppression of bacterial wilt (*R. solanacearum*) was observed by treating with thymol oil (Ji *et al*., 2007). Also, this finding had confidence of Momol *et al*. (2005), who showed that essential oils were effective tools to fight bacterial wilt disease in field experiment. This may be due to their antagonistic effect against *R. solanacerum* and/ or their role as an elicitor enhancing ISR. In addition, this may be due to their antagonistic effect against other soil microflora, so, they change soil microbial community that indirect inhibited *R. solanacearum* and reduced the disease severity, hence decrease infected tubers. Using of antibiotics as streptomycin was a good inhibitor for the bacteria growth compared with pencilin and ampicillin (Khalil, 2008).

Inhibition effects and MICs

Results in Table 5 show that streptomycin, anethole and caraway oil inhibited the growth of *R. solanacearum*. On contrary, neem and mustard oil did not suppress *R. solanacearum* growth. The minimal inhibition concentration (MIC) of anethole is largest value (3%). While, MIC of streptomycin is lowest one (≥ 0.25 %). The MIC of caraway oil is 0.25 %. These results were in the same harmony of Moussa (2006) who found that some essential oil inhibited growth of *R. solanacearum* and others did not inhibit the growth of this bacterium. He also found that anise oil had inhibitory effect against the studied bacterium, then, that confirmed the inhibitory effect of anethole (one component of anise oil) against the same bacterium. Also, Wang *et al*. (2015) reported that streptomycin suppresses the growth of *R. solanacearum*. In addition, Abo-Elyousr *et al*. (2014) revealed that caraway oil inhibits the growth of *R. solanacearum*. On the other hand, these results were in contrary with those of Sood and Kumar (2015) who mentioned that neem oil inhibited growth of *R. solanacearum*. Many investigators suggested that difference in the effect of the same essential oil against the same bacterium may be due to the variation of plant part which essential oil was extracted, diversity of plant varieties, variance in soil, in which plant was cultivated and/ or other reasons. This variation may be due difference in studied pathogenic bacterial strains.

Table 5: Inhibition effects and minimal inhibition concentrations of (MICs) of some tested treatments.

Treatments	Inhibition zone (mm)	MIC (%)
1. Control	0	N.D.
2. Streptomycin	1.7	≥ 0.25
8. Anethole	1.5	3
9. Neem oil	0	N.D.
10. Mustard oil	0	N.D.
11. Caraway oil	1.7	0.25

*N.D.: Not detected.

Table (6) was confirmed by Iacobellis *et al*. (2005) who showed that the caraway essential oil which active against *R. solanacearum* contain carvone, limonene, and trans-dihydrocarvone. Furthermore, Kotan *et al*. (2013) used carvacrol (pure compound of caraway oil) to control of *R. solanacearum.*

Table 6: The main compounds identified in the methanol extract of tested essential oils by using GC-MS.

No.	Caraway oil		Anise oil		Mustard oil	
	Compounds	Concentration %	Compound	Concentration %	Compounds	Concentration %
1	Myrcene	0.48	Anethole	80	Ally isothiocyanate	94
2	Limonene	15.50	Estragole	10	4-hydroxbenzyl isothiocyanate	6
3	α-Terpinolene	0.10	Fenchone	7.5		
4	Trans limonene oxide	2.01				
5	Trans dihydro carvone	0.33				
6	Trans carvol	0.31				
7	Estragole	20.23				
8	Carvone	50.80				
9	Perilla alcohol	1.48				
10	Carvacrol	0.34				
11	β-Caryophyllene	0.41				
12	β-Pinene	6.50				

Furthermore, no inhibition zone was recorded with PCSWS and WS when disk diffusion method was applied. These finding mean that PCSWS and WS had no direct antibacterial effect against *R. solanacerum*. El-Fallal and Moussa (2008) showed that water extract of PCSWS and WS had inhibitory effect against *R. solanacerum.* They illustrated that water extract of PCSWS had higher inhibitory effect than that of WS. This difference may be

due to extracellular enzymes *P. columbinus*, which may play a role in increasing the inhibitory effect against *R. solanacerum*.

In addition, the three tested (*B. subtilis, B. polymyxa and P. fluorescens*) bacteria did not inhibit the growth of *R. solanacearum* where there is no inhibition zone was recorded when double layer and disk agar method were applied. These results are contrariwise those of Kheirandish and Harighi (2015) who found that different strains of *P. fluorescens* antagonized the growth of *R. solanacerum*. Also, these results are on the contrary of those of Kwon and Kim (2014) who demonstrated that *B. subtilis* suppressed the growth of *R. solanacerum*. In addition, Nath *et al*. (2016) had counteractive results of this investigation where they showed that *B. subtilis* and *P. fluorescens* inhibited the growth of *R. solanacearum*. Some investigators suggest that these counteractive findings of the inhibitory effect of the same bacterial bio-agent against the same bacterial pathogen may be due to the genetic variation of the different strains of bio-agents from different locations and environment and/ or differences in different isolates of the same bacterial pathogen.

Phytohormones secreted by bio-agents

The three tested of bio-agent bacteria produced indole acetic acid (IAA), gibberellic acid (GA_3) and cytokinin (Table 7). *B. subtilis* produced the highest value of IAA (22.16 µg / ml) and cytokine (13.11 µg / ml) and the lowest value of GA_3 (359.22 µg / ml). *P. fluorescens* secreted the largest quantity of GA_3 (588.11 µg / ml), lowest value of cytokinin (8.016 µg / ml) and middle value of IAA (16.98 µg / ml). While, *B. polymyxa* have the lowest value of IAA (18.545 µg / ml) and cytokinin (4.486 µg / ml) and middle one of GA_3 (4.93.6 µg / ml). These results have confirmation of Noumavo *et al*. (2016) and Wu *et al*. (2016) they revealed that these bacteria produced these phytohormones and they are plant growth promoting rizobacteria (PGPR).

Table 7: Phytohormones of secreted by the three tested bacteria.

Treatments	Indole acetic acid (IAA) (µg / ml)	GA_3 (µg / ml)	Cytokinin (µg / ml)
1. *Bacillus subtilis*	22.16	359.22	13.11
2. *Bacillus polymyxa*	8.545	4936	4.486
3. *Pseudomonas fluorescens*	16.98	588.11	8.016

Physiological aspects in pot experiment

Data in Table (8) illustrate that all tested treatments were effective elicitor where approximately all treatments significantly increased the ability of potato plants in pot experiment to produce total phenols, polyphenol oxidase (PPO) and peroxidase (POD). The best elicitor encouraging production of total phenols was *B. subtilis*, while neem oil and *B. polymyxa* did not increase total phenols. Caraway oil was the first elicitor in increasing PPO, while *B. polymyxa* was the lowest elicitor significantly increasing it. Neem oil was the number one in increment the production of POD, while PCSWS caused non significantly increase in its production. These findings confirm that the tested elicitors increase induced systemic resistance (ISR). These results had the same harmony of Compant *et al*. (2005), Bhattacharyya and Jha (2012) and Noumavo *et al*. (2016), they illustrated that PGPR used as elicitor increasing ISR of plant against pathogens. In addition, PCSWS and WS encouraged the ISR of potato plant. These results confirmed by Gamliel *et al*., (2000) who showed that organic amendment enhanced the ISR of plants.

Table 8: Physiological characteristics of potato plants at 40 days after planting affected by tested treatments in pots experiment.

Treatments	Total phenol (mg catechol / g F.W.)	Polyphenol oxidase (Unit / min. / g F.W.)	Peroxidase (Unit / min. / g F.W.)
1. Control	13.48 e	13.12 e	0.00 d
2. Streptomycin	25.51 c	13.05 e	0.00 d
3. *Pleurotus columbinus* spent wheat straw	14.15 e	25.22 b	0.00 d
4. Wheat straw	14.48 e	14.67 de	20.13 b
5. *Bacillus subtilis*	34.86 a	12.33 e	0.00 d
6. *Bacillus polymyxa*	12.82 e	24.33 b	7.67 c
7. *Pseudomonas fluorescens*	20.83 d	23.33 b	19.67 b
8. Anethole	32.51 b	29.00 a	0.00 d
9. Neem oil	12.82 e	18.13 c	44.12 a
10. Mustard oil	25.50 c	17.06 cd	0.00 d
11. Caraway oil	24.50 c	17.45 cd	0.00 d

Means followed by the same letter (s) within each column do not significantly differ using Duncan's Multiple Range Test at the level of 5%.

Results in Table (9) reveal that all tested elicitors enhanced the plant health by increasing the amounts of chlorophylls (a, b and total). Application of PCSWS and WS had significant effects on these pigments. PCSWS gave the highest value of chlorophyll a (708 mg /g) and total chlorophyll (0.891 mg/g)

Table 9: Chlorophylls of potato plants at 70 days after planting as affected by tested treatments to control brown rot disease in pots experiment.

Treatments	Chlorophyll *a* (mg / g F.W.)	Chlorophyll *b* (mg / g F.W.)	Total chlorophylls (mg / g F.W.)
1. Control	0.316 k	0.070 j	0.386 h
2. Streptomycin	0.433 h	0.132 e	0.565 e
3. *Pleurotus columbinus* spent wheat straw	0.708 a	0.183 b	0.891 a
4. Wheat straw	0.623 b	0.215 a	0.838 b
5. *Bacillus subtilis*	0.521 f	0.133 e	0.654 d
6. *Bacillus polymyxa*	0.584 c	0.126 f	0.710 c
7. *Pseudomonas fluorescens*	0.561 d	0.150 d	0.711 c
8. Anethole	0.426 i	0.175 c	0.601 f
9. Neem oil	0.559 e	0.108 g	0.667 d
10. Mustard oil	0.443 g	0.095 h	0.538 f
11. Caraway oil	0.372 j	0.088 i	0.460 g

Means followed by the same letter (s) within each column do not significantly differ using Duncan's Multiple Range Test at the level of 5%.

while, the highest value of chlorophyll b (0.215 mg / g) recorded with WS. This had confirmation of Wu *et al*. (2012) and Suzuki *et al*. (2014) who demonstrated that the application of PGPR led to increment of chlorophyll content of plants. Furthermore, increasing in chlorophyll content due application of WS and PCSWS had the computability of Roy *et al*. (2015) who illustrated that mushroom substrate enhanced plant health and increased plant chlorophyll content.

Data in Table (10) at 70 days of planting show that, all tested elicitors enhanced plant health and significantly increased the plant height. The longest plant recorded PCSWS and WS. While the shortest plant recorded with *B. subtilis* and *B. Polymyxa*. In addition, all studied treatments significantly decreased bacterial wilt disease rating. No wilting symptoms were recorded with streptomycin, *P. fluorescence*, neem oil, mustard oil and caraway oil. While, WS, *B. subtilis* and, *B. polymyxa* reduced disease rating. Furthermore, all tested factors significantly increased tubers weight/ plant. The best treatment in this factor was PCSWS which increased tuber yield by 53.20 %, while the lowest one was *B. subtilis* that caused increment by 16.04 %.

The pot experiment had the same harmony of the field experiment of this investigation. There were differences in results due to differences of environment of pot and field.

Conclusively, the results of this investigation showed that all tested treatments acted as elicitors encouraging ISR of potato plants. Only three elicitors had a direct antibacterial activity against *R. solanacearum*; streptomycin, anethole and caraway oil, while other treatments had direct antibacterial effect. Therefore, these elicitors were promising tools for eco-friendly management of brown rot disease of potato. The most effective elicitors are PCSWS, caraway oil, *P. fluorescens* and streptomycin. They can be applied before planting for improving yield and its quality, as well as, control brown rot disease of potato.

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دراسة تأثير بعض المستحثات على مرض العفن البني ومحصول الدرنات في البطاطس

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يعتبر مرض العفن البني في البطاطس من الأمراض المنتشرة في جميع أنحاء العالم، ويسبب خسائر زراعية واقتصادية ضخمة. استخدام بعض المركبات الكيميائية الاصطناعية مثل المبيدات المضادة للميكروبات قد يكون لها آثار سلبية على المستهلكين وعلى البيئة. يعتبر تطبيق استخدام مواد صديقة للبيئة للسيطرة على المرض من الاتجاهات الحديثة في هذا الإطار. في هذه الدراسة تم اختبار عشرة معاملات للسيطرة على هذا المرض، بالإضافة إلى معاملة الكنترول، وهذه المعاملات كالآتي: استخدام الاستربتوميسين، المخلفات العضوية (التبن) الناتجة من زراعة المشروم من النوع المحاري PCSWS، تبن القمح الناعم، وثلاثة أنواع بكتيرية باسلس ساتيلس *Bacillus subtillus*، باسلس بوليمكسا *B. polymyxa*، وسيدوموناس فلوريسنس *Pseudomonas fluorescens*، وثلاثة أنواع من الزيوت العطرية الطيارة (أنيثول: مشتق من زيت الينسون، الخردل والكراوية) بالإضافة إلى زيت النيم. أجريت تجارب حقلية على محصول البطاطس (صنف اسبونتا) في محطة بحوث البرامون، محافظة الدقهلية، مصر خلال العروة الشتوية لموسمي ٢٠١٣/٢٠١٤ و٢٠١٤/٢٠١٥م. تم أخذ مقايس النمو الخضري المختلفة، المحصول الكلي، تدريج الدرنات، المحتوي من المادة الجافة، الكثافة النوعية للدرنات ونسبة الدرنات المصابة.

وكانت النتائج المتحصل عليها فى انه قد أدى استخدام تبن القمح المستهلك بواسطة فطريات المشروم إلى زيادات كبيرة في جميع المقاييس التي تمت دراستها، مقارنة مع غيرها من المعاملات. أعطت معاملات PCSWS أو زيت الكراوية أفضل النتائج في خفض نسبة الدرنات المصابة. تم دراسة التأثير المباشر المضاد للبكتريا من العامل الممرض ر. الستونيا سولانيسيرم *R. solanacearum* المسبب للعفن البني في المختبر، حيث أدت إضافات الاستربتوميسين، الأنيثول وزيت الكراوية إلى تثبيط نمو البكتيريا المسببة للعفن البني. في تجربة أصص موازية أجريت لدراسة اختبار قدرة المعاملات المختلفة علي حث المقاومة الذاتية الجهازية للنبات (ISR)، وجد أن بكتيريا *B. polymyxa* وزيت الكراوية وزيت النيم أدت إلى حدوث زيادة كبيرة في إنتاج النبات للفينولات، وإنزيمات البوليفينول أوكسيديز والبيروكسيديز، على الترتيب، مما يعزز من الحالة الصحية لنباتات البطاطس في وعاء التجربة فانعكس ذلك على محتوي الأوراق من الكلوروفيل وطول النبات مع انخفاض تصنيف المرض وزيادة وزن الدرنات بعد ٧٠ يوما من الزراعة.