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COMPOST INDUCED CHANGES IN SECONDARY METABOLITES DURING THE INTERACTION BETWEEN POTATO PLANTS AND THE BACTERIAL WILT PATHOGEN *Ralstonia solanacearum*

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ABSTRACT

The aim of this investigation was to study the interaction between the bacterial wilt pathogen *Ralstonia solanacearum* and potato *Solanum tuberosum* plants in relation to plant growth, secondary metabolism and antioxidant system in response to compost application. Single potato eyepieces were germinated and grown in pots containing sandy soil with or without compost at a rate of 7.5 g kg^{-1} soil. Non-compost- and compost-treated plants (CTP) were inoculated with *R. solanacearum* 21 days after planting and then were subjected to biochemical analysis and growth parameters determinations after 14 days of inoculation. The obtained results revealed that pathogen infection caused a remarkable decrease in plant growth related parameters and an increase in disease incidence. However, compost substantially improved plant growth and decreased disease incidence. Data also indicated that there were significant increases in salicylate (SA), phenolics, flavonoids, lignin, and DPPH (2,2-diphenyl-w1-picrylhydrazyl) activity in infected CTP compared with infected non-CTP. In addition, other biochemical indicators of potato enzymatic activities of glucose-6-phosphate dehydrogenase (G6PDH), shikimate dehydrogenase (SKDH), phenylalanine ammonia-lyase (PAL), cinnamic alcohol dehydrogenase (CADH), polyphenol oxidase (PPO), and guiacole peroxidase (GPX) in infected CTP were significantly higher than those in the infected non-compost-treated ones, indicating induction of critical metabolites playing major roles in plant defense to pathogen. Taken together, the results suggested that compost provides effective protection against the Ralstonia bacterial pathogen via stimulating growth and inducing production of secondary metabolites.

INTRODUCTION

Bacterial wilt disease, one of the most destructive plant diseases worldwide, is caused by *Ralstonia solanacearum* and leads to substantial yield losses in several hundred important crops such as tomato, tobacco, potato, eggplant and banana (Hayward 1991). The soil-borne bacterium invades plant roots and multiplies in the vascular systems, resulting in wilting of the host plant (Vasse *et al.* 1995). It is generally difficult to control this disease due to the genetic diversity of *R. solanacearum* and its ability to survive easily in adverse environments (Schell 2000). Various techniques, such as soil amendments, biological control and cultural practices, have been developed to control bacterial wilt disease. For sustainable agriculture, there is an increasing demand to reduce agriculture dependence on chemical bactericides and to find innovative control methods that are effective, economic and environmental friendly. The exploitation of induced resistance in plants has been studied previously by treatments with a variety of inducers (Walters *et al.* 2005) which could lead to new and alternative management strategies. Soil amendments are public acceptable and interesting practice. It

is well known, compost addition to soil favours plant development and improves soil quality and structure as well as expressing a suppressive effect on many soilborne plant pathogens (Aldahmani *et al.* 2005; Temorshuizen *et al.* 2006; Bonanomi *et al.* 2007; Ntougias *et al.* 2008; Fujiwara *et al.* 2012). Organic amendments not only act by improving soil structure and providing a source of nutrients, but also strongly influence the soil microflora (Crecchio *et al.* 2001) and contribute to sustainable land use. Furthermore, compost has received much attention after documenting its ability to induce multiple forms of resistance, i.e. systemic acquired resistance and/ or induced systemic resistance, against plant pathogens (Zhang *et al.* 1996; Kavroulakis *et al.* 2005; Yogev *et al.* 2010; Sang and Kim 2011).

Plants defend themselves against pathogen infection by producing a large variety of secondary metabolites (Dong 1998; Glazebrook 2005; Metlen *et al.* 2009). The phenolic compound such as salicylic acid (SA) and antimicrobials, including flavonoids, phenols and terpenes, are compounds that destroy or inhibit the growth of bacteria and fungi (Cowan 1999). Presently, no such reports indicating the role of secondary metabolites induced by compost in alleviating bacterial pathogen infection are available. It has been hypothesized that compost application affect plant secondary metabolic pathways during the interaction between potato plants and the bacterial wilt pathogen *Ralstonia solanacearum*. To verify such assumption, investigation of the effect of compost application on the patho-physiological features of potato plants exposed to bacterial pathogen with a focus on the composition of secondary metabolites, and secondary metabolite-related enzymes activities were conducted.

MATERIALS AND METHODS

Compost production and soil properties:

Compost was prepared from a mixture of cattle manure, chicken manure, brassica and rice husk at a ratio of 3:1:1:1, respectively, by volume. The raw materials were thoroughly mixed and allowed to decompose in a pile under aerobic conditions. Moisture content was maintained at 50–60% throughout the active composting period. The thermophilic stage (T^{o} >55 ^{o}C) lasted for three months, during which three turnings over were conducted in order to ensure complete eradication of pathogens. After approximately five months of the initial composting process, the temperatures reached the ambient level. The resultant matured compost was left additional two months for curing to promote organic matter humification, stability and maturity (Jindo *et al.* 2012; Larchevêque *et al.* 2010). The composting processes were conducted in triplicate and monitored for up to 7 months. The stabilized final product had the following data based on dry matter: organic matter (518 g kg^{-1}), organic C (298 g kg^{-1}), and total N (20.5 g kg^{-1}). The pH and EC with 1: 5 (w/v) compost: H_2O extracts were 6.5 and 3.5 dSm^{-1}. Germination index of compost was 87%, according to the method described by Zucconi *et al.* (1981).

Soil was prepared by sieving sand through a 6-mm sieve before placing into the pots. The soil texture is predominantly sandy (73.1 % coarse

sand, 19.6 % fine sand, 5.0 % silt and 2.3 % clay). The experimental soil had the following analytical data based on dry matter basis: total OM (4.95 g kg^{-1}), organic carbon (C) (2.87 g kg^{-1}), total nitrogen (N) (0.28 g kg^{-1}), total phosphorus (P) (0.12 g kg^{-1}) and available potassium (K^+) (28.2 mg kg^{-1}). The pH and electrical conductivity (EC) with 1: 1 (w/v) soil: H_2O extracts were 7.91 and 1.49 dSm^{-1}.

Isolation and inoculum preparation of *R. solanacearum:*

Ralstonia solanacearum, a highly pathogenic isolate (RS4), was isolated from a diseased potato plant with bacterial wilt symptoms collected from naturally infested soil Ismailia district, Egypt, using a semi-selective SMSA medium (French *et al.* 1995). *R. solanacearum* RS4 belongs to race-3 biovar-2. The caudex from an infected plant was washed three times with sterile distilled water, cut into small pieces, homogenized in sterile mortar and filtered. The filtered liquid was diluted with sterile water at an appropriate dilution, spread onto a plate containing SMSA medium and incubated at 28°C for 3 days.

An aqueous suspension of *R. solanacearum* (RS4) was prepared by inoculation of 1L flask containing 200 ml King's B medium (KB), and the culture was shaken 150 rpm at 28°C for 3 days. Cultures were centrifuged at 10,000 rpm at 10°C for 8 min. Cells were suspended in sterile water and adjusted to 10^8 CFU mL^{-1} using spectrophotometer at 680 nm. The suspensions were used to inoculate potato plants under the experimental conditions.

Plant materials, growth conditions and treatments:

Experiment 1: Promotion of plant growth and suppression of bacterial wilt

A single potato eyepiece was germinated and grown in each plastic pots (35 cm diameter x 35 cm height with holes 2 cm in diameter drilled in the bottom of the pots) contained a homogeneous mixture of 10 kg sandy soil supplemented or not with compost at rate of 7.5% (w/w). The rate of compost was selected based on the result of a preliminary experiment (data not shown). Three weeks before planting, all pots were weekly watered to stimulate compost microbial activity (Minz *et al.* 2010). Each planted pot was regularly watered each morning to avoid water stress and the water volume was increased as the plants grew. The experiment was carried out under natural day/ night conditions [photosynthetic active radiation (PAR) >900 µmol m^{-2} s^{-1}, day/ night temperature of 25/18^oC, relative humidity of 65/70%] at the Faculty of Agriculture Farm, Suez Canal University, Ismailia, Egypt, and was conducted in a completely randomized design with three replicates and 5 pots per replicate, giving a total of 60 pots. Twenty one days after planting, half of the non-compost and compost-treated plants (CTP) were drenched with 60 ml of *R. solanacearum* RS4 suspension at concentration 2.8×10^8 CFU mL^{-1} and labeled non-compost-, and compost-treated plants. The remaining plants were irrigated with 60 ml of sterilized water as control treatments: non-compost-treated plants and compost-treated ones. After 14 days of inoculation, data of disease incidence, and plant growth-related parameters were recorded, respectively, as described below. The disease

index was recorded, based on a scale of 0– 4, as described by Kempe and Sequeira (1983). The disease incidence was calculated as follows:

Disease incidence = [∑(The number of diseased plants in this index x Disease index)/(Total number of plants investigated x The highest disease index)] x 100%.

To determine plant growth related parameters, five plants were harvested in each replicate to measure the shoot and root fresh weights (FW), shoot and root dry weights (DW), root/ shoot ratios and total fresh and dry weights.

Experiment 2: Effect of compost on secondary metabolites and their related enzymes activities:

Non-CTP and compost treated ones were germinated and grown and then inoculated with *R. solanacearum*, as previously described to determine the level of secondary metabolites and their related enzymes activities. On the 14 days after inoculation, samples were collected for biochemical analysis as described below.

a- Determination of salicylate, phenolics, flavonoids, lignin and DPPH activity:

Total SA was extracted and quantified according to the method described by Halim *et al.* (2004). Phenolics were extracted following the method of Kováčik *et al.* (2011). After centrifugation, the supernatant (200 µL) was mixed with 150 µL of Folin–Ciocalteu's reagent (Singleton and Rossi, 1965). The mixture was added with 2 mL of 2% (w/v) Na_2CO_3, incubated at 25 ^{o}C for 20 min and then centrifuged at 4000 rpm for 10 min. The absorbance of the supernatant was measured at 735 nm, and the standard curve was prepared with gallic acid. For the determination of flavonoids, roots (0.2 g) were homogenized in 2 mL of methanol/HCl (99:1, v/v). The extract was shaken at room temperature for 12 h and then centrifuged at 4000 rpm for 20 min. The mixture containing the supernatant (300 µL), 300 µL of 5% (w/v) $NaNO_2$ and 300 µL of 10% (w/v) $AlCl_3$ was prepared, and 2 mL of 1 N NaOH was added 6 min later (Zhuang et al., 1992). Lignin was determined by the method of Suzuki *et al.* (2009). For the DPPH free radical scavenging assay, the method of Yen and Chen (1995).

b-Determination of G6PDH, SKDH, CADH, PAL, PPO, and GPXactivities:

For the measurement of G6PDH, SKDH and CADH activities, leaves (0.3 g) were homogenized in 2 mL of 100 mM K-phosphate buffer (pH 7.4), containing 0.5 mM DTT, 2 mM l-cysteine, 2 mM EDTA, 8 mM β-mercaptoethanol and 2% (w/v) polyvinylpyrrolidone (PVPP). The extract was centrifuged at 14,000 rpm for 20 min. G6PDH activity (EC 1.1.1.49) was measured according to the method of Debnam and Emes (1999). The reaction was initiated by the addition of the enzyme and an increase in absorbance was monitored at 340 nm. SKDH (EC 1.1.1.25) assay was based on the method of Díaz and Merino (1997). The absorbance was recorded at 340 nm following the reduction of NADP. CADH activity (EC 1.1.1.195) was measured on the basis of the rate of increase in absorbance at 400 nm following the oxidation of coniferyl alcohol (Mitchell et al., 1994). To determine the changes of PAL activity, 0.3 g of plant tissue was extracted with 2 mL of 50 mM boracic acid buffer (pH 8.8), containing 8 mM β-mercaptoethanol and 2% (w/v) PVPP. The homogenate was centrifuged for

20 min at 14,000 rpm. PAL (EC 4.3.1.5) assay was performed with l-phenylalanine as the substrate at 290 nm (Zucker, 1965). PPO (EC 1.10.3.1) and GPX (EC 1.11.1.7) activities were measured by the increase in absorbance at 398 and 470 nm as described by Lamikanra and Watson (2001) and Hammerschmidt *et al.* (1982), respectively. All operations were carried out at 4 ^{o}C. All spectrophotometric analyses were conducted on a spectrophotometer.The soluble protein content was determined according to the method of Bradford (1976), using bovine serum albumin as the standard.

Statistical analysis:

All data are expressed as the mean of three independent determinations. The data were analyzed by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test for comparisons of all treatments using COSTAT software program (CoHort Computer Software, Berkeley, CA, USA). All data presented are the mean values and the means were compared by the LSD test at 5 % probability level.

RESULTS

Experiment 1: Promotion of plant growth and suppression of bacterial wilt :

As indicated in Table (1), After 5-10 days of inoculation, typical symptoms of *R. solanacearum* wilt were observed on plants grown on *Ralstonia* infested soil. All wilted plants were died shortly after the wilting symptoms had appeared. When a cross section of stem was examined, a milky-white, sticky exudate, which indicates the presence of bacterial cells, oozed from stem cross sections of infected plants supplemented or not with compost with different density. The disease symptoms of CTP were decreased compared with non-compost treated ones. Disease incidence was 19.7% of CTP compared with 62.2% of non-compost-treated ones at 14 days after inoculation. Thus compost application resulted in a 68.3% disease reduction. As expected, no signs of disease were observed on non-inoculated plants whether or not supplied with compost. Fourteen days after inoculation, the bacterial population was significantly higher in caudex of non-CTP compared to CTP.

As also shown in Table (1), the total fresh- and dry weight of the infected compost-treated plants were significantly increased by 23.0% and 26.2% compared with the infected non-compost-treated ones. Compost increased the fresh and dry mass of shoot and root, and shoot/root ratios of fresh- and dry mass of the compost-infected plants compared with the non-compost-infected ones.

Experiment 2: Effect of compost on secondary metabolites and their related enzymes activities:

a- Determination of salicylate, phenolics, flavonoids, lignin and DPPH activity:

As illustrated in Table (2), the contents of salicylic acid, phenolics, flavonoids, and lignin in potato roots of infected compost-treated plants were significantly higher than the infected non-compost-treated ones (Table 2). Moreover, the compost application significantly increased all the previous

parametes relative to the control plants. DPPH activity, a key parameter associated with the antioxidant potential, had a strong parallelism with phenolics of infected and non-infected plants.

Table (1):Effect of compost application on soil infested with *R. solanacearum* isolate (RS4) in potato plants. Disease incidence, and plant growth-related parameters were determined 14 days after inoculation with *R. solanacearum*

	Non-compost-treated soil	Compost-treated soil	Soil infected with pathogen without compost	Soil infected with pathogen with compost	*LSD* (*P ≤ 0.05*)
Disease incidence (%)	0.0	0.0	62.2a	19.7b	3.54
Shoot FW (g plant^{-1})	35.8b	43.3a	27.4c	33.2b	4.12
Shoot DW (g plant^{-1})	3.03b	3.75a	2.35c	2.81b	0.34
Root FW (g plant^{-1})	2.95c	4.44a	2.60c	3.65b	0.49
Root DW (g plant^{-1})	0.25c	0.45a	0.22c	0.36b	0.03
Shoot/root FW ratio	12.1a	9.74c	10.5b	9.09d	0.61
Shoot/root DW ratio	11.6a	8.17c	10.4b	7.77c	0.70
Total FW (g plant^{-1})	38.7b	47.7a	29.9c	36.8b	4.61
Total DW (g plant^{-1})	3.29b	4.21a	2.67c	3.37b	0.43

Values are the mean of 3 replicates. Treatment means having different letters are statistically different at p≤0.05

Table(2):Effect of compost application on soil infested with *R. solanacearum* isolate (RS4) on activities of salicylate, phenolics, flavonoids, lignin, and DPPH in the roots of potato plants after 14 days of inoculation.

	Non-compost-treated soil	Compost-treated soil	Soil infected with pathogen without compost	Soil infected with pathogen with compost	*LSD (P ≤ 0.05*
Phenolics (mg g^{-1} FW)	2.02d	3.29b	2.65c	4.62a	0.38
Flavonoids (mg g^{-1} FW	18.43d	29.5b	23.0c	35.1a	3.20
Lignin (mg g^{-1} FW)	1.76c	2.82a	1.98ab	2.62ab	1.01
Salicylate (ng g^{-1} FW)	106d	171b	133c	267a	22.1
DPPH	38.2d	59.6b	50.5c	71.6a	6.43

Values are the mean of 3 replicates. Treatment means having different letters are statistically different at p≤0.05

b-Determination of G6PDH, SKDH, CADH, PAL, PPO, and GPX activities:

To verify whether compost application affect the regulation of enzymes related to secondary metabolism, activities of five related enzymes were determined. Potato roots enzymatic activities of G6PDH, SKDH, PAL, CAD, PPO, and GPX in infected compost-treated plants were significantly higher than the infected non-compost-treated ones. Moreover, the compost application significantly increased all the previous enzymatic activities in CTP, relative to non-compost-treated ones, as shown in Table (3).

Table (3): Effect of compost application on soil infested with *R. solanacearum* isolate (RS4) on G6PDH, SKDH, PAL, CADH, PPO, and GPX in the roots of potato plants 14 days after inoculation.

	Non-compost-treated soil	Compost-treated soil	Soil infected with pathogen without compost	Soil infected with pathogen with compost	*LSD (P ≤ 0.05*
G6PDH activity (nmol min^{-1} mg^{-1} protein)	32.7d	62.2b	43.3c	78.4a	4.81
SKDH activity (nmol min^{-1} mg^{-1} protein)	44.4c	65.2b	58.6b	89.7a	7.76
PAL activity (unit mg^{-1} protein)	2.78d	5.07b	4.47c	6.50a	0.55
CADH activity ((nmol min^{-1} mg^{-1} protein)	9.71d	22.5b	15.8c	27.1a	2.31
PPO(unit mg^{-1} protein)	9.70d	16.4b	13.2c	20.7a	1.76
GPX (µmol min^{-1} mg^{-1} protein)	3.79d	9.62b	6.12c	12.8a	1.00

Values are the mean of 3 replicates. Treatment means having different letters are statistically different at p≤0.05

DISCUSSION

It is well known that application of compost leads to improve soil properties due to increases soil organic matter, humic substances, cation exchange capacity, water holding capacity and microbial activity. Furthermore, compost application improves soil fertility due to increase of mineral nutrients levels, which in turn improve plant growth and productivity (Walker and Bernal 2008). Application of mature and stable compost has become common practices of pathogen remediation in infested soils and has constituted an important way in maintaining productivity and disease suppression in the modern sustainable agricultural ecosystems (Bastida *et al.* 2008; Mylavarapu and Zinati 2009; Yogev *et al.* 2010; Duong *et al.* 2013). Data in table 1 indicated that *R. solanacearum* caused reduction in plant growth related parameters and increment of disease incidence of bacterial wilt which were significantly alleviated by compost application. Improvement of plant growth and disease suppression by compost addition to infested soil were reported by other researchers (Aldahmani *et al.* 2005; Hiddink *et al.* 2005; Bonanomi *et al.* 2007; Joshi *et al.* 2009; Ntougias *et al.* 2008; Fujiwara *et al.* 2012, and Youssef and Tartoura 2013). The plant growth improvement could be attributed to compost components which positively affect photosynthesis, resulting in higher biomass production, as reported by Amaya- Carpio *et al.* (2009) and Antolín *et al.* (2010). Moreover, compost has been reported as a protective factor against pathogens induced oxidative damage via induced systemic resistance (Zhang *et al.* 1996; Kavroulakis et al. 2005; Sang and Kim 2011).

In the present work, compost application effectively protected plants from the oxidative stress induced by plant pathogen via induction and accumulation of salicylic acid, phenolics, flavonoids and lignin in the roots of infected plants. In fact, relatively few studies have examined the direct effects of compost on secondary metabolism. It is believed that phenolic compounds

and flavonoids are powerful antioxidants that act as free radical scavengers and reducing agents (Rice-Evans et al., 1996; Seo *et al.* 2012). In addition, flavonoids and lignin were known to be directly involved in plant defense systems. Furthermore, plant antimicrobials compounds, including flavonoids, phenols and terpenes, are compounds that destroy or inhibit the growth of bacterial and fungal pathogens Cowan (1999). Hence, the improved status of *R. solanacearum* infected plants in response to compost application might attribute, at least in part, to the increased level of salicylic acid, phenolics, flavonoids and lignin.

There are over-whelming evidences that plant enzymatic activities involved in secondary metabolism have been associated with abiotic and biotic stresses including wounding, pathogen attack, and environmental stresses (Oh et al., 2009; Nascimento and Fett-Neto, 2010). In the present study, compost caused a significant increase in the activities of enzymes associated with secondary metabolism. Among such enzymes, G6PDH which is the key enzyme of the pentose-phosphate pathway (Filosa et al., 2003) and SKDH which catalyses the conversion of dehydroshikimate to shikimic acid (Díaz and Merino, 1997) provide precursors for the synthesis of secondary metabolites. Phenylpropanoids are natural products derived from the amino acid l-phenylalanine by deamination by PAL which is considered the rate-limiting enzyme in secondary metabolism (Dixon and Paiva, 1995). Furthermore, PAL is a well characterized and a key limiting enzyme in the phenylpropanoid pathway. PAL is critical for the biosynthesis of salicylate (Lee et al., 1995; Huang et al., 2010). In this study, GPX activity was higher in infected plants, but infected CTP recorded much higher activity. This high level of GPX activity in infected plants might be involved in reduction of peroxides and thus protecting membranes and proteins from oxidation. Moreover, lignification is mediated by CAD and GPX, as suggested by Whetten *et al.* (1998). Consequently, the enhancement of G6PDH, SKDH, PAL, CAD, and GPX activities promotes the accumulation of salicylate, phenolic acids, flavonoids, and lignin. On the other hand, it has been reported that PPO catalyses the oxidation of phenols to quinines (Mayer, 2006). This study suggests that PPO activity may regulate the level of phenolic compounds and become involved in the phenylpropanoid pathway. Total phenolic content and lignin deposition were significantly higher in infected CTP compared to infected non-CTP. This higher level of phenolics and lignin, formed through phenylpropanoid pathway, may contribute in mounting an aggressive resistance mechanism by CTP against the bacterial pathogen. Based on the foregoing results, it may be suggested that compost played a crucial role in the disease resistance of potato plants by stimulating growth and inducing the production of secondary phytomedicinal metabolites, thereby eliminating ROS damage and sustaining membrane stability. The focus of future research should emphasize on the mechanism by which compost confers tolerance to *R. solanacearu*m at cellular and molecular levels.

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استخدام الكمبوست فى تحفيز مواد الأيض الثانوية لنباتات البطاطس المصابة ببكتيريا *Ralstonia solanacearum*

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الهدف من هذا البحث هو دراسة تأثيرالكمبوست على نباتات البطاطس المصابة ببكتريا فيما يتعلق بنواتج الأيض الثانوية و مضادات الأكسدة النباتية. *Ralstonia solanacearum*

تم انبات درنات البطاطس اما فى تربة رملية فقط أو تربة رملية مضاف إليها الكمبوست بمعدل ٧.٥ جرام لكل كيلو جرام تربة فى أصص بلاستكية تحت ظرف البيئية الطبيعية. تم عدوى نباتات البطاطس المعاملة وغير المعاملة بالكمبوست عمر ٢١ يوما بالمسبب المرضى للذبول البكتيرى . استخدم فى هذا البحث عدد أربعة معاملات هى كالآتي: (١) كنترول دون أى معاملات، (٢) نباتات معاملة بالكمبوست فقط، (٣) نباتات معاملة بالمسبب المرضى فقط، (٤) نباتات معاملة بالكمبوست والمسبب المرضى معا.

أظهرت النتائج أن الإصابة بالمسبب المرضى أدت الى حدوث انخفاض فى معدل نمو النباتات وأدت المعاملة بالكمبوست الى زيادة نمو النباتات وتقليل حدوث الإصابة بالمسبب المرضى. أوضحت النتائج اجماليا أن استخدام الكمبوست أدى الى تخفيف الآثار السلبية الناجمة من المسبب المرضى للذبول البكتيرى وقد أدى استخدام الكمبوست فى تربة خالية من المسبب المرضى الى حدوث تأثيرات ايجابية ذات تأثير معنوي على النمو الطازج والجاف مقارنة بالنباتات غير المعاملة بالكمبوست. أدت المعاملة بالكمبوست الى وجود ارتفاع ملحوظ لمستويات حمض الساليسيلك، الفينولات، ، الفلافونيدات ، واللجنين، ونشاط مضادات الأكسدة فى النباتات المعاملة بالكمبوست و المصابه بالبكتريا مقارنة بالنباتات الغير معاملة بالكمبوست والمصابة بالبكتيريا. بالإضافة الى ذلك فان تقدير الأنشطة الانزيمية لإنزيمات

glucose-6-phosphate dehydrogenase (G6PDH), shikimate dehydrogenase (SKDH), phenylalanine ammonia-lyase (PAL), cinnamic alcohol dehydrogenase (CADH), polyphenol oxidase (PPO), and guiacole peroxidase (GPX)

فى النباتات المعاملة بالكمبوست والمصابة بالبكتيريا أوضحت ارتفاعا معنويا للأنشطة الانزيمية فى تلك النباتات مقارنة بمثيلاتها للنباتات المصابة والغير معاملة بالكمبوست والتي تشير الى تأثير الكمبوست فى تحفيز مواد الايض الثانوية وانزيماتها التى تلعب دور رئيسى فى مقاومة نبات البطاطس ضد المسبب المرضى تحت الدراسة.

يستنتج مما سبق أن استخدام الكمبوست الناتج من المخلفات الزراعية يخفف من تأثير الإصابة لنباتات البطاطس النامية فى وجود المسبب المرضى للذبول البكتيرى وقد انعكس ذلك واضحا فى تحسين النمو وتنشيط المركبات الدفاعية المتمثلة فى حمض الساليسليك والفينولات والفلافونيدات واللجنين وأنشطة الانزيمات ذات العلاقة بتحولاتها ويدل ذلك على أن مكونات الكمبوست المتمثلة فى الكربون والنيتروجين العضوي والمغذيات الكبرى والصغرى بالإضافة الى المواد الدبالية قد شاركت بأدوار فعالة فى التغيرات الفسيولوجية والبيوكيماوية ذات العلاقة بتخفيف حدوث الإصابة لنباتات البطاطس المعاملة بالكمبوست والنامية تحت ظروف الإجهادات الحيوية.

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