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The Role of Lupine, Garlic and Bean Root Exudates in Potato Bacterial Wilt Infection

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Abstract: Root exudates for three plant species [Lupine (*Lupinus albus*), Garlic (*Allium sativum*) and Bean (*Phaseolus vulgaris*)] were c ollected and tested for their effect on growth of *Ralstonia solanacearum* (the causal agent of Potato wilt disease) and on wilt disease severity on potato plants. All tested plant exudates did not inhibit *R. solanacearum* growth, *in vitro*. Screening for production of bacterial pectinases in the presence of root exudates for each plant (using SPAM medium) revealed normal pectinases production in medium. Potato plants that grown in infested soil contained root exudates of different plants (lupine, garlic and bean) indicated reduction of wilt disease index compared with control. Lupine root exudates caused the highest reduction of disease index followed by bean and garlic root exudates, respectively. High activity of dehydrogenase was detected in potato rhizosphere (inoculated by *R. solanacearum*) in the presence of bean exudates (0.40 mM formazan/ 1 g soil) followed by garlic exudates (0.26 mM) and lupine exudates (0.26 mM) compared with infested control (0.02 mM). Inoculated potato plants by *R. solanacearum* showed noticable degradation of salicylic acid compared with healthy control. Low levels of salicylic acid was noticed also in potato plants treated with root exudates of bean, lupine and garlic, compared with control. Seven organic acids were detected by mass spectrometry in root exudates of the three plants tested. Maleic acid, malonic acid, citric acid, succinic acid, lactic acid, tartaric acid and quinic acid were detected in the three plant exudates.

Key words: *Lupinus albus* · *Allium sativum* · *Phaseolus vulgaris* · Root exudates · Potato · *Ralstonia solanacearum*

INTRODUCTION

The most attractive area for different studies is the rhizosphere. Because of its richness of chemical reaction and biological process, plants differ in its secreted compounds from roots that's play main role in symbiotic or defensive reactions depending on the other factors of soil [1]. In addition, it can serve as a source of carbon substrate for microbial growth and also contain chemical molecules that promote chemotaxis of soil microbes to the rhizosphere [2]. Organic acids are the main component in root exudates help in nutrient mobilization strategy. studies on rice, corn and Lupine report the existence of certain mechanisms by which the plant absorbs the mobilized metal–organic acid complex resulting in better nutrition for the plants [3-5]. Therefore, it can affect the soil pH however root exudation of high concentrations of organic acid anions as a result of P deficiency does lower rhizosphere pH, making P and micronutrients such as Mn, Fe and Zn to be more available [6]. The release of organic acids in root exudates is also related to detoxification. Some organic acids such as malic, citric and oxalic are efficient chelators of aluminum, a very toxic metal in some soils [7].

Many plant pathogens are affecte by root exudate. Root exudates of bean can reduce growth of certain plant pathogenic fungi, i.e. *Rhizoctonia*, *Botrytis* and *Macrophomina* [8]. In contrast, Tahat *et al*., [9] reported that tomato and corn root exudates have no inhibition effect on the growth *Ralstonia solanacearum*.

This work aimed to study the role of lupine, garlic and bean root exudates on *R. solanacearum* growth and bacterial wilt disease severity on potato plants.

MATERIALS AND METHODES

Plant Material: Fifty seeds of Lupine (*Lupinus albus*), Garlic (*Allium sativum*) and Bean (*Phaseolus vulgaris*)

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were surface sterilized with Naocl 2% for 5 min and washing with sterile distilled water. Seeds were sown in five pots (25 cm diameter) contained sterile sand clay soil (2:1 w/w) and regularly irrigated with Hoagland's solution twice per week. After 30 days, the plants were removed to collect root exudates. The same pots were sown with potato tubers (Nicola cultivar); one tuber / pot, two days after inoculation of soil with pathogenic bacteria *Ralstonia solanacearum* [10].

Bacterial Inoculum: Virulent isolate of *R. solanacearum* was grown on sucrose peptone agar medium at 28°C for 48 hr. Bacterial cells were suspended in buffer solution (pH 7.2) and adjusted to 3.2 x 10^8CFU/ml, using a spectrophotometer at A_{590} = 0.12. Hundred ml of bacterial suspension were added to each pot. The pots were regularly irrigated and after 2 days planted with potato tubers [11].

Collection of Root Exudates: After 30 days of sowing, lupine, garlic and bean plants were removed gently from soil and their roots were rinsed in 0.01 mol L-1 KOH for 5 min to remove organic anions. Then the roots were carefully washed by tap water and finally completely immersed in flasks containing distilled water for 24 hours. The suspension was collected and filtered through 0.2 µm sterile filters and kept at -2°C for further studies [12, 13].

Effect of Root Exudates on Growth of *Ralstonia solanacearum*: Nutrient agar medium was poured in petri plates then inoculated by 0.1 ml of pure culture of pathogenic bacterial suspension (24hr old). Different root exudates were added to the plates in wells made by corkborer on agar surface. Five plates were used for each treatment. Plates with added water were used as a control. All plates were incubated at 28°C for 48hrs. Presence of clear zone around wells indicated the inhibition of bacterial growth.

Screening for Pectinase Production: Pectinase screening agar medium (PSAM) was used for detecting the release of pectinases of *R. solanacearum* according to Beulah *et al*. [14]. Twenty-five ml of root exudates for each plant was mixed with 50 ml of PSAM medium just before plating. A loopful of bacterial culture was streaked on the middle of solid medium and incubated for 48hr at 28°C. and plates without root exudates served as a control. Positive results for pectinase production were detected by zone of hydrolysis.

Disease Assessment: Bacterial wilt severity on potato plants was assessed after 40 days from sowing. Disease index was calculated according to Kemp and Sequeira, [15] using rating scale from 0 to 5, where 0 = No visible symptoms; 1 = 1-25% of the plant is wilting; 2 = 26-50% wilt; 3 = 51-75% wilt; 4 = more than 75% wilt and 5 = plant died. Percentage of Disease severity (DS) was calculated as follow:

$$DS = (\Sigma R.T / 5 \times N) \times 100$$

where,
R = Disease ratting scale (R = 0, 1, 2, 3, 4 and 5).
T= Number of plants counted within each category.
N = Total number of tested plants.

Dehydrogenase Activity: Soil dehydrogenase activity was determined using the method of Kumar and Tarafdar [16] by the mixture of 0.2 ml of 3% triphenyltetrazoliumchloride (TTC) solution and 0.5 ml of 1% glucose to 1 gm soil sample. Samples were incubated at 28°C for 24 h and then 10 ml of acetone was added and again incubated at 28°C for 1 h. the pull out triphenyl formazan (TPF) was measured by absorbance at 485 nm.

HPLC Analysis of Salicylic Acid: The salicylic acid was analyzed by HPLC, Agilent 1100 equipped with reversed phase column (25cmX4.6mmX4.6µm). The mobile phase composed of phosphate buffer: acetonitrile 60:40. The detection wavelength was 245nm.

The fresh plant sample (2 g) was extracted with 3 ml of methanol (HPLC grade) by grinding in a mortar. The supernatant was separated by centrifugation at 5000 rpm for 10 min. The supernatant was completed to 3 ml with methanol and 5 µl were injected into the HPLC (Agilent 1100) under the following conditions: Mobile phase consists of sodium acetate buffer solution (0.05 M, pH 4.5) (78%) and methanol (28%), flow rate 1ml/min, column Agilent C18, 25 cm X 4.6 mm, the eluted compounds were detected at 270nm using diode array detector [17, 18].

Mass Spectrometry Detection of Organic Acids: The organic acids were detected in the root exudates solution by mass spectrometry using Xivo TQD mass unit, Waters, after electrospray ionization (ESI), using daughter scan mode and positive ion detection [M-H]+. The capillary volts was 2.7 KV, cone volts was 30 V and the desolvation gas flow 600 L/h.

The detected MW and daughter ions are as follows:

Maleic acid MW 134, [M-H]+ 134, daughter ions: 132.9 (114, 73, 71)
Malonic acid MW 104 , [M-H]+ 104, daughter ions: 103 (59.2, 41)
acetic acid MW 60, [M-H]+ 60, daughter ions: 54
Citric acid MW 192, [M-H]+ 192, daughter ions: 191.3(111, 87, 67, 57)
Succinic acid MW 118, [M-H]+ 118, daughter ions: 117(73, 99, 93)
Lactic acid MW 90, [M-H]+ 90, daughter ions: 89(43, 41, 44.9)
Tartaric acid MW 150, [M-H]+ 150, daughter ions:148.9(87, 72.9, 43.2, 59.3)
Quinic acid MW 192, [M-H]+ 192, daughter ions: 191(85, 93, 127, 59, 45)

Statistical Analysis: Analysis of variance (ANOVA) was carried out according to Steel *et al*., [19]. Duncan's multiple range tests were used to compare treatment means at 0.05 level of significant.

RESULTS AND DISCUSSION

Effect of Root Exudates on Growth of *Ralstonia solanacearum* and Pectinase Production: The three tested plant root exudates have no inhibitory effect on *R. solanacearum* growth, *In vitro*. All plates were filled with bacterial growth. In addition, there was no inhibition effect on pectinases production in all plates of SPAM medium in the presence of root exudates of each plant.

Effect of Root Exudates on Disease Severity: Potato plants that grown in infested soil containing root exudates of previously sown plants (lupine, garlic and bean) indicated various percentage of disease index. All treatments showed reduction of disease index compared with control. Lupine root exudates showed the highest reduction of disease index (23.7%) followed by bean root exudates (33.2%) and garlic (50.0%), respectively compared with control (93.1%) (Fig. 1).

It is clear that using garlic in multi-cropping systems serve as antimicrobial mean. [20]. It has previously shown that garlic root exudates can significantly inhibit mycelial growth of *Phytophthora caspsici*, the causal of pepper blight [21]. It affect the plant host itself by increasing the chlorophyll content, photosynthetic rate and activity of antioxidant enzymes, which all produce healthy plants that can resist the pathogens. [22, 23]. Root exudates of lupine and bean may play the same role.

Dehydrogenase Activity: Soil dehydrogenases considered the best indicator for soil microbial activity. They exist intracellular the living microorganisms cells. Dehydrogenases have a main role in oxidation of organic matter in soil, utilization of O2 and other compounds as terminal electron acceptors, nutrients circulation and organic residues decomposition [24].

The results revealed high activity of dehydrogenase in potato rhizosphere (inoculated by *R. solanacearum*) in the presence of bean exudates (0.40 mM formazan/ 1 g soil) followed by garlic exudates (0.26 mM) and lupine exudates (0.26 mM) compared with infected control (0.02 mM). On the other hand, high activity was noticed in non infested soil in the presence of garlic exudates (0.46 mM) more than other treatments (Fig. 2).

Effect on Salicylic Acid Content in Potato Plants: Data in Fig. (3) revealed degradation of salicylic acid in potato plants inoculated by *R. solanacearum* compared with healthy control. Low levels of salicylic acid were noticed also in potato plants treated with root exudates of bean, lupine and garlic, compared with control.

However, salicylic acid not the only way for defense signals of plants. Also, jasmonic acid play a role in plant defense system. *R. solanacearum* can suppress salicylic acid accumulation in leaves in response of its effector proteins secreted through type III secretion system called Rips (*Ralstonia* injected proteins). This proteins increased the jasmonic acid levels in plant [25]. This degradation of salicylic acid allowed *R. solanacearum* growth and enhanced virulence by protecting the bacteria from the toxicity [26].

Mass Spectrometry Detection of Organic Acids: Seven organic acids were detected by mass spectrometry of the root exudates of lupine, garlic and bean plants. Maleic acid, malonic acid, citric acid, succinic acid, lactic acid, tartaric acid and quinic acid were produced in all tested plants. Root exudates (as shown in Figs. 4, 5 and 6) with a difference concentration according to the area under the beak

It is well known that plant root exudates contain mixture of soluble substances, such as sugars, enzymes, organic acids and amino acids. Organic acids (OAs) are considered the main components of exudates which are important for soluble mineral nutrient in soil [27].

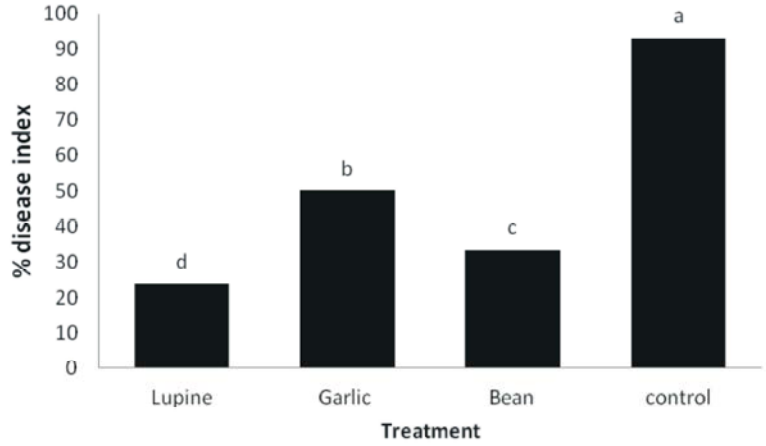

Fig. 1: Disease index (DI) of bacterial wilt disease of potato plants treated with different root exudates

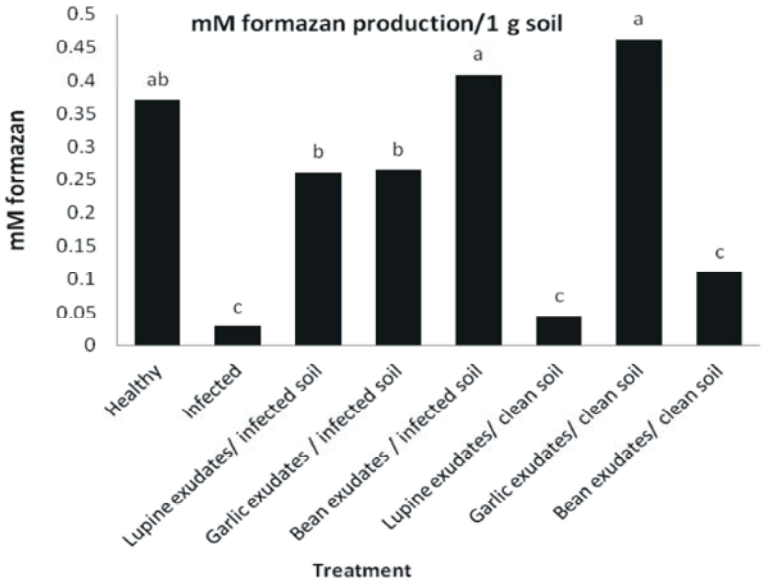

Fig. 2: Dehydrogenase activity in different (infested and non- infested) soil treatment of root exudates

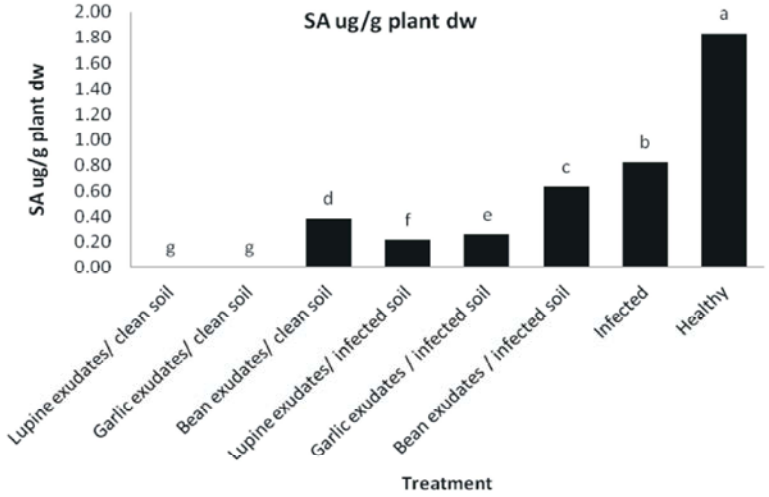

Fig. 3: Induction of salicylic acid in potato plants grown in soil containing different plant root exudates

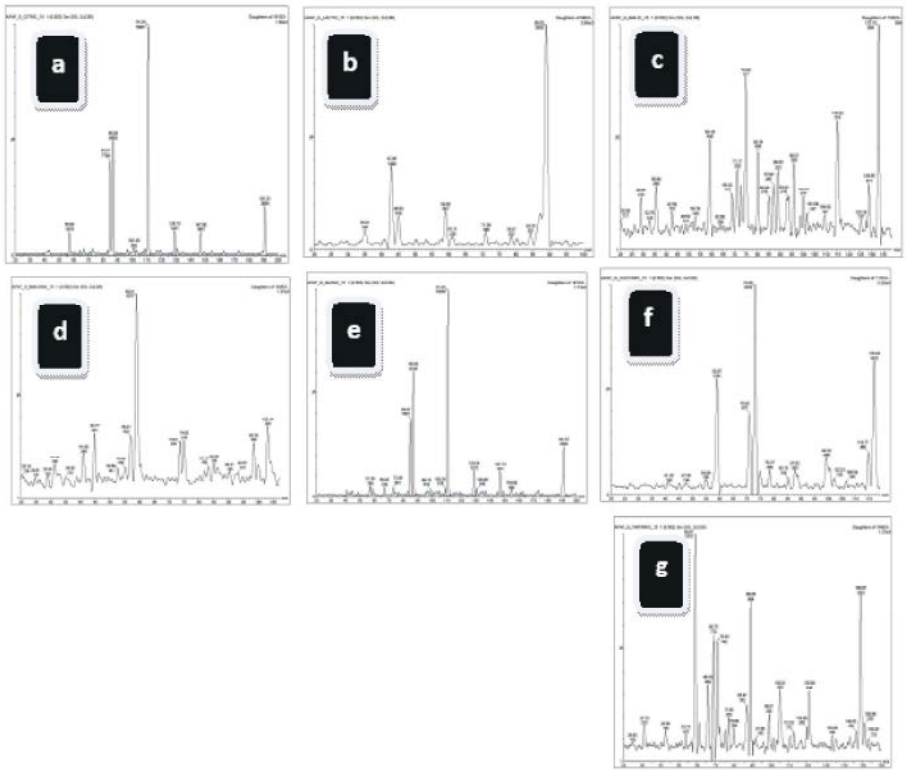

Fig. 4: The mass spectra of organic acids detected in the root exudates of Garlic: a) citric acid b) lactic acid c) maleic acid d) malonic acid e) quinic acid f) succinic acid g) tartaric acid

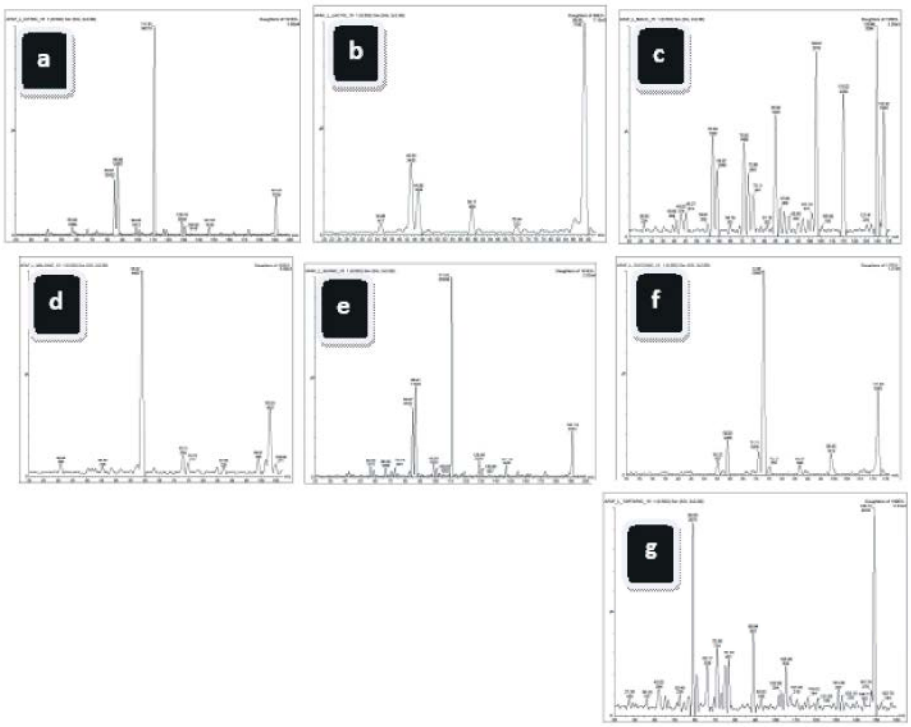

Fig. 5: The mass spectra of organic acids detected in the root exudates of Lupin : a) citric acid b) lactic acid c) maleic acid d) malonic acid e) quinic acid f) succinic acid g) tartaric acid

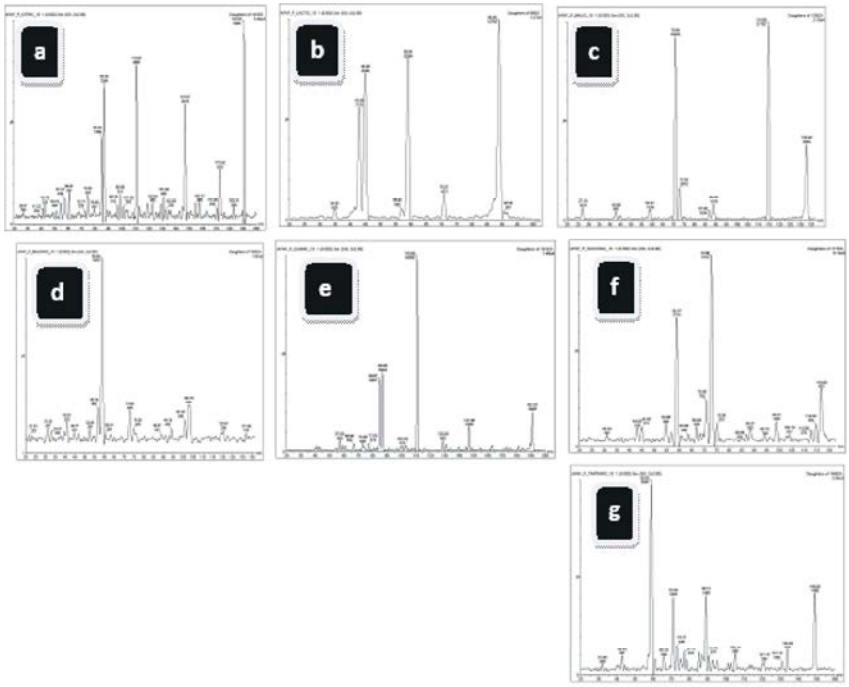

Fig. 6: The mass spectra of organic acids detected in the root exudates of Bean : a) citric acid b) lactic acid c) maleic acid d) malonic acid e) quinic acid f) succinic acid g) tartaric acid

Soil communities are significantly affected by OAs application, either individually or in a mixture. Utilization of OAs by soil microbes leads to changes in soil pH which considered a major driver of change in the microbial community structure in soil [28]. Therefore, OAs, activate a wide range of bacterial taxa, especially that closely related to beneficial groups of bacteria. [29].These bacteria have either 'biofertilizing' or 'biocontrol' effects on plant growth [30]. These bacteria have many roles as nutrients cycling, involve nitrogen, phosphorus and sulfur. Other bacterial species, as *Bacillus* spp. and *Pseudomonas* spp, are considered plant growth-promoting bacteria These bacteria produce different hormones and substances which suppress plant pathogens and pests and can stimulate ectomycorrhizal colonization of roots [31, 32].

REFERENCES

1. Walker, T.S., P.B. Harsh, E. Grotewold and J.M. Vivanco, 2003. Root Exudation and Rhizosphere Biology. Plant Physiology, 132: 44-51.
2. Caetano-Anolles, G., D.K. Crist-Estes and D.W. Bauer, 1988. Chemotaxis of *Rhizobium meliloti* to the plant flavone luteolin requires functional nodulation genes. J. Bacteriol., 170: 3164–3169.
3. Dinkelaker, B., V. Romheld and H. Marschner, 1989 Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell, 12: 285-292.
4. Jones, D.L. and P.R. Darrah, 1994. Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. Plant Soil, 166: 247-257.
5. Jones, D.L. and P.R. Darrah, 1995. Influx and efflux of organic acids across the soil - root interface of *Zea mays* L. and its implications in rhizosphere C flow. Plant Soil, 173: 103-109.
6. Dakora, F.D. and D.A. Phillips, 2002 . Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil, 245: 35-47.
7. Ryan, P.R., E. Delhaize and P.J. Randall, 1995. Characterisation of Al-stimulated efflux of malate from the apices of Al-tolerant wheat roots. Planta, 196: 103-110.
8. El-Gali, Z., 2015. Influence of seeds and roots extract and exudates of Bean plant on growth of some pathogenic fungi. Open Access Library Journal, 2: 1-10.
9. Tahat, M.M., K. Sijam and R. Othman, 2010. The role of tomato and corn root exudates on *Glomus mosseae* spores germination and *Ralstonia solanacearum* growth *In vitro*. International Journal of Plant Pathology, 1(1): 1-12.

10. Steinkellner, S., R. Mammerler and H. Vierheilig, 2008. Germination of *Fusarium oxysporum* in root exudates from tomato plants challenged with different *Fusarium oxysporum* strains. Eur. J. Plant Pathol., 122: 395-401.
11. Michel, V.V. and T.W. Mew, 1998. Effect of soil amendment on the survival of *Ralstonia solanacearum* in different soils. Phytopathology, 88: 300-305.
12. Silva Lima, L.D., F.L. Olivares, R.R. Oliveira, M.R. Garcia Vega, N.O. Aguiar and L.P. Canellas, 2014. Root exudate profiling of maize seedlings inoculated with *Herbaspirillum seropedicae* and humic acids. Chemical and Biological Technologies in Agriculture, 1: 23.
13. Ferna´ndez-Aparicio, M., F. Flores and D. Rubiales, 2008. Recognition of root exudates by seeds of broomrape (Orobanche and Phelipanche) species. Annals of Botany, 10: 1-9.
14. Beulah, D., E.M. Sunitha and T. Srilakshmi, 2015. Production, purification and assay of pectinase enzyme from *Aspergillus niger*. Helix, 2: 673-677.
15. Kemp, J. and L. Sequeira, 1983. Biological control of bacterial wilt of potatoes: Attempts to induce resistance by treating tubers with bacteria. Plant Dis., 67: 499-503.
16. Kumar, P. and J.C. Tarafdar, 2003. 2, 3, 5-Triphenyltetrazolium chloride (TTC) as electron acceptor of culturable soil bacteria, fungi and actinomycetes. Biology and Fertility of Soils, 38(3): 186-189.
17. Sawyer and V. Kumar, 2003. A Rapid High-Performance Liquid Chromatographic Method for the Simultaneous Quantitation of Aspirin, Salicylic Acid and Caffeine in Effervescent Tablets MaryJean. Journal of Chromatographic Science, 41: 393-397.
18. Petreka, J., L. Havela, J. Petrlovab, V. Adamb, C.D. Potesilb, C.P. Babulad and R. Kizek, 2007. Analysis of Salicylic Acid in Willow Barks and Branches by an Electrochemical Method. Russian Journal of Plant Physiology, 54(4): 553-558.
19. Steel, R.G.D., J.H. Torrie and D.A. Dicky, 1997. Principles and procedures of statistics: A biometrical approach, 3rd ed., McGraw Hill, Inc. Book Co., New York, USA, pp: 352-358.
20. Iciek, M., I. Kwiecien and L. Wlodek, 2009. Biological properties of garlic and garlic-derived organosulfur compounds. Environ. Mol. Mutagen., 50: 247-265.
21. Khan, M.A., Z.H. Cheng, X.M. Xiao, A.R. Khanand, S. Ahmed, 2011. Ultrastructural studies of the inhibition effect against *Phytophthora capsici* of root exudates collected from two garlic cultivars along with their qualitative analysis. Crop. Prot., 30: 1149-1155.
22. Xiao, X.M., Z.H. Cheng, H.W. Meng, H.L. Liu, H.Z. Li and Y.X. Dong, 2013. Intercropping of green garlic (*Allium sativuml*) induces nutrient concentration changes in the soil and plants in continuously cropped cucumber (*Cucumis sativus* L.) in a plastic tunnel. PLoS One., 8(4): e62173.
23. Wang, M.Y., C.N. Wu, Z.H. Cheng, H. Meng, M.R. Zhang and H.J. Zhang, 2014. Soil chemical property changes in eggplant/garlic relay intercropping systems under continuous cropping. PLoSOne., 9(10): e111040.
24. Woliñska, A. and Z. Stêpniewska, Dehydrogenase Activity in the Soil Environment, licensee InTech. Lublin, Poland, pp: 183-210.
25. Nakano, N. and T. Mukaihara, 2018. *Ralstonia solanacearum* type III effector RipAl, targets chloroplasts and induces jasmonic acid production to suppress salicylic acid- mediated defense responses in plants. Plant and Cell Physiology, 59(12): 2576-2589.
26. Lowe-Power, T.M., J.M. Jacobs, F. Ailloud, B. Fochs, P. Prior and C. Allen, 2016. Degradation of the plant defense signal salicylic acid protects Ralstonia solanacearum from toxicity and enhances virulence on tobacco. mBio, 7(3): 1-12
27. Koo, B.J., D.C. Adriano, N.S. Bolan and C.D. Barton, 2005. Root exudates and microorganism. Elsever, pp: 421-428
28. Bissett, A., A.E. Richardson, G. Baker, S. Wakelin and P.H. Thrall, 2010. Life history determines biogeographical patterns of soil microbial communities over multiple spatial scales. Mol. Ecol., 19: 4315-4327.
29. Shi, S., A.E. Richardson, M. O'Callaghan, K.M. DeAngelis, E.E. Jones, A. Stewart, M.K. Firestone and L.M. Condron, 2011. Effects of FEMS Microbiol. Ecol., 77: 600-610.
30. Richardson, A.E., J.M. Barea, A.M. McNeill and C. Prigent-Combaret, 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321: 305-339.
31. Emmert, E.A.B. and J. Handelsman, 1999. Biocontrol of plant disease: a (Gram-) positive perspective. FEMS Microbiol. Lett., 171: 1-9.
32. Frey-Klett, P., J. Garbaye and M. Tarkka, 2007. The mycorrhiza helper bacteria revisited. New Phytol., 176: 22-36.