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Identification of amino acids and sugars in root exudate of mungbean (*Vigna radiata* L.)

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Abstract
Root exudates represent an important source of nutrient for microorganisms in the rhizosphere and seem to participate in early colonization inducing chemotactic responses of rhizospheric bacteria. Plant root exudates consist of a complex mixture of organic acid anions, phytosiderophores, sugars,vitamins, amino acids, purines, nucleosides, inorganic ions (e.g. HCO_3^-, OH^-, H^+), gaseous molecules (CO_2,H_2), enzymes and root border cells which have major direct or indirect effects on the acquisition of mineral nutrients required for plant growth. Root exudates have traditionally been grouped into low- and high-molecular weight compounds. For the search of primary metabolites (amino acids and sugar) in root exudate, mung bean (*Vigna radiata* L. var. HUM 1) was grown in Thronton's media for 30 days in culture tube. *Bradyrhizobium* (strain MO_5) was used for nodulation in mung bean. The root exudates in media (500mL) were collected and concentrated for the identification of primary metabolites (amino acids and sugars) through paper chromatography. The amino acids identified in root exudates of mung bean (*Vigna radiata* L.) were glutamic *acid, proline, hydroxyl proline and threonine* and the sugars identified were rhamnose, *arabinose and D-ribose*.

Keywords: root exudate, mung bean, amino acids and sugars

Introduction
In addition to accumulating biologically active chemicals, plant roots continuously produce and secrete compounds into the rhizosphere (Bais *et al.*, 2001; Gleba *et al*., 1999) [4, 9]. Root exudation includes the secretion of ions, free oxygen and water, enzymes, mucilage, and a diverse array of carbon-containing primary and secondary metabolites (Bertin *et al.*, 2003; Uren, 2000) [5, 25]. Root exudation can be broadly divided into two active processes. The first, root excretion, involves gradient dependent output of waste materials with unknown functions, whereas the second, secretion, involves exudation of compounds with known functions, such as lubrication and defense (Bais *et al*., 2003; Uren, 2000) [2, 25]. Roots release compounds via at least two potential mechanisms. Root exudates are transported across the cellular membrane and secreted into the surrounding rhizosphere. Root exudates are often divided into two classes of compounds. Low-molecular weight compounds such as amino acids, organic acids, sugars, phenolics, and other secondary metabolites account for much of the diversity of root exudates, whereas high molecular weight exudates, such as mucilage (polysaccharides) and proteins, are less diverse but often compose a larger proportion of the root exudates by mass. Root exudation clearly represents a significant carbon cost to the plant (Marschner, 1995) [20], and the magnitude of photosynthates secreted as root exudates varies with the type of soil, age, and physiological state of the plant, and nutrient availability (Brady *et al*, 1999; Brimecombe, *et al*., 2001) [6, 7]. Although the functions of most root exudates have not been determined, several compounds present in root exudates play important roles in biological processes (Bais *et al*., 2001; 2002, 2003, Kneer *et al*., 1999) [4, 3, 2, 16].
Stimulation of microorganisms present in the rhizosphere seems to be due to the presence of organic compounds released by the roots and representing up to 20% of the plant dry weight. This material includes flaked cells of the root cap, mucilage, and soluble and non-soluble exudates, which may contain free amino acids, proteins, carbohydrates, alcohols, vitamins, or hormones (Hawes *et al.*, 1998, 2000) [10, 11].
The main functions of the 'hidden' part of the plant, its root system, have traditionally been thought to be anchorage and uptake of nutrients and water. However, roots secrete an enormous range of compounds into the surrounding soil. This area, called the rhizosphere, can be divided into three zones: endorhizosphere (root tissue, including the endodermis and cortical layers), rhizoplane (the root surface with the epidermis and mucilage) and ectorhizosphere (the soil nearby the root) (Lynch, 1987) [19]. The first observation that microbes are more abundant in the rhizosphere than in distant soil was made by Hiltner (1904) [12],

and the first indication concerning root exudation and microbe abundance was provided by Knudson (1920) [20] and Lyon and Wilson (1921) [19]. In recent years, the field of rhizosphere biology has explored the relative importance of root exudates in mediating interactions with neighbouring plants and microbes (Bais *et al*., 2004, 2006; Weir *et al*., 2004, Broeckling *et al*., 2008) [1, 27, 8]. Root exudation is part of the rhizodeposition process, which is a major source of soil organic carbon released by plant roots (Hutsch,Augustin and Merbach, 2000, Nguyen, 2003). The quantity and quality of root exudates are determined by plant species, the age of an individual plant and external factors like biotic and abiotic stresses.

Although there was a quite good advancement in root biology, but roots are still considered an unexplored biology frontier. In contrast, knowledge of rhizosheric processes mediated by root exudates has not developed at the same pace. As highlighted in this update, several line of evidence indicates that root exudates in their various forms may regulate plant and microbial communities in the rhizosphere. The understanding of the biology and biochemistry of root exudation products and processes may contribute to devising novel strategies for improving plant health and the identification and isolation of novel value added compounds found in the exudates.

Mung bean (*Vigna radiata* L. Wilezek) is very common annual k*harif* pulse crop in India. In the name greengram is more commonly used the mung bean. There is quite lacking information of chemical composition of root exudates of Indian cultivars. Considering this technical knowledge gap, the compounds specifically for carbon and energy sources for soil micro-organisms viz. amino acids and sugars from the root exudates of mung bean were identified under Brady-rhizobium infected condition for better understanding of the biochemistry of root exudates of Indian cultivars.

Materials and Methods

Plant Materials

HUM-1 variety of mung bean was used in this entire experiment obtained form the Department of Genetics and Plant Breeding, Banaras Hindu University. Seed were small and nearly globular, green in colour with white hilum. The colour of cotyledon was yellow. Germination of seed was epigeal type.

Seed sterilization

Seeds used for the experiment was healthy, bold, free from diseases. Seed sterilization was done under laminar flow. The seed was first surface sterilized with 0.1% $HgCl_2$ solution for 2 minutes, then subsequently washed thoroughly with alcohol (95% ethanol) for 2-3 minute and then finally washed three times with distilled water.

Germination of seed

Sterilized seeds were kept in 90 mm petri dishes lined with a single layer of filter paper and on an average 10mL sterilized distilled water was used to moist the filter paper. In petri plate approximately 20 seeds were taken. Then these petri plate kept under controlled condition (28±1ºC) for germination in incubator for 48 hours. After germination of seed, through sterilization forceps seed coat had been removed carefully under the sterilized condition under laminar flow.

Plant growth medium and plant growth and development

Thronton media (1930) [25] was used for the growth of germination seeds. Composition of medium (1000mL) presented in Table 1.

Sixteen culture tubes containing Thronton media @ 60 mL/tube were taken. The filter paper in form of alphabet M was taken in such a way that it enter into test tube. The M shaped filter paper was placed in such a way that half of its part was above the medium and remaining half was in the medium. Three Holes had been on this filter paper for placing the germination seeds. Sterilized germination seeds were placed in 'M' shaped filter paper within culture tube in such a manner that the radical just touch the medium. In this whole experiment 16 culture tubes were taken, 19.5 x 3.5 cm, 3 seeds per tubes were placed. The tubes were firmly plugged with rolled (not tight), non-absorbent cotton plug. Before placing seeds in tube whole set-up was autoclaved for 20 minutes at 121°C.

Table 1: Thronton nutrient solution used to culture nodulated legumes

Mineral	Amount
$Ca(PO_4)_2$	2.0 gL^{-1}
K_2HPO_4	0.5 gL^{-1}
$MgSO_4$	0.2 gL^{-1}
NaCl	0.1gL^{-1}
$FePO_4$	1.0gL^{-1}
$FeCl_3$	0.01gL^{-1}
*Micronutrients	1ml L^{-1}
***Micronutrients/ Trace elements composition**	
H_3BO_3	0.05%
$MuCl_2$	0.05%
$ZnSO_4$, $7H_2O$	0.05%
Na_2MoO_4	0.005%
$CuSO_4$, $5H_2O$	0.002%

Rhizobium Strain

Bradyrhizobium strain, MO_5 (Table 2.) was used for nodulation in entire study. The strain was selected on the basis of its better performance in symbiotic nitrogen fixation among the other strain available either locally or from the USDA, on *Vigna radiata* L. plants. MO_5 strain was maintained in the Department of Genetics and Plant Breeding, Banaras Hindu University. After a 24 hours of planting seeds in broth media (Thronton's media). MO_5 strain was inoculated at root zone. Uninoculated tubes that were served as control. The pH of the medium was adjusted 6.8 by using 0.1 N NaOH before adding agar.

Table 2: The composition of yeast extract mannitol agar

Mineral	Amount (all in 1000 m L)
KH_2PO_4	0.5g
$MgSO_4.7H_2O$	0.2 g
NaCl	0.1 g
$CaCO_3$	3.0 g
Mannitol	10.0 g
Yeast extract	0.4 g
Agar	15.0 g

The Rhizobium strain (MO_5) was maintain in yeast extract mannitol agar (YEMA)

Extraction of root exudate

Thirty days after inoculation with *Bradyrhizobium* culture strain MO_5 in *Vigna radiata* L., exudates of roots were collected. For this purpose, first roots were washed in their respective tubes containing media. The root exudate in media was collected in sterilized 500 mL conical flask. 4-5 Drops of toluene was added in the solution to check microbial

activities, centrifuged the solution at 8000-9000 rpm for 8 minutes. Supernatent solution was collected in 500 mL conical flask. Ten drop of toluene was added to preserve the solution in refrigerator.

Identification of primary metabolites in root exudate

Out of 300 m L of total media supernatent solution, 150 mL was used for the identification of primary metabolites (amino acids and sugars). The 150 mL root exudates solution was concentrated to 5 mL and it was ready for identification of free amino acids and sugars in root exudates. Decending paper chromatography was used for the identification of primary metabolites. In paper chromatography, cellulose filter paper (Whatman No. 1) was used as a stationary phase and solvent mixture was used as mobile phase. Different chromogenic reagents were used for the detection of different metabolites.

Identification of amino acids in root exudate

For the identification of amino acids a pencil line was drawn at one end of paper chromatography length wise as the line was covered the total length of paper. Then standard amino acids solutions were prepared. For the preparation of various standard amino acids, 10% isopropanol was used for dissolving neutral amino acids, acetic acid was used for dissolving acidic amino acids and ammonium hydroxide was used for dissolving basic amino acids

The amino acids used as a standard for this experiment were as follow: *nor-leucine, iso dl-leucine, iso L-leucine, proline, tryptophane, histidine, isoleucine, cystine, methionine, aspartic acid*, *threonine, serine* and *valine*. The amino acids standard and the concentrated root exudate solution of *Vigna radiata* L. was applied at one point on the line. The spots were dried using a hot air driyer and the exudate sample and standard were repeated applied followed by drying. Chromatogram was developed in a chamber using developing solvent, n-butanol-acetic acid-water (4:1:5, v/v) by decending chromatographic method. Solvent front, was marked by using pencil. After 16 hours, paper was taken out from the chamber and allowed to air dry thoroughly (for 14-16 hr) until the paper was free from the solvent vapour.

After drying paper was sprayed with chromogenic reagent (Ninhydrin: 200 mg in 100mL. 95% warmed alcohol). Paper was allowed to dry at room temperature for 10-15 minutes and warmed the paper was at 100°C for 5-8 minutes in a well ventilated oven. Then various shades of purple or yellow spots were marked by pencil and their Rf values were then calculated by using formulae and the compared with the standard spots of amino acids for identification.

$$R_f = \frac{\text{Distance moved by substance from origin (cm)}}{\text{Distance moved by the solvent from origin (cm)}}$$

Identification of sugars in root exudates

Same procedure was used for the identification of sugars as used for amino acids. The standard sugars used were *D-glucose, D(-) fructose, L(+) arabinose, α-L(-) rhamnose. D(+)-mannose* and starch. Developing solvent used was n-butanol: acetic acid: water (4:1:5 v/v). Chromogenic reagent used for sugar identification was $AgNO_3$. It was prepared by adding 2.5 mL of saturated solution of $AgNO_3$ in 500 mL of acetone. The black spots on white background was marked on chromatography paper by pencil and their R_f values were calculated and compared with the standard spots of sugar for identification.

Results and Discussion

For the purpose of identification of organic compounds, which are used as energy and carbon sources, by the microbial population of the rhizosphere, the sugars and amino acids were identified in the root exudate of mung bean (*Vigna radiata* L.) through paper chromatography.

Amino acids in the root exudate of mung bean (*Vigna radiata* L.)

The R_f values (expected) of different amino acids recorded in standard text (Jayaraman,1981) by paper chromatography are presented in Table 3 in two different developing solvent systems, n-butanol: acetic acid: water (4:1:5, v/v) and phenol: water (4:1, v/v) respectively. The R_f values (observed) of different standard amino acids investigated in this study in laboratory condition are expressed in Table 4 in single developing solvent system (n-butanol: acetic acid: water, 4:1:5, v/v). The colour of the spots of the standard amino acids were violet except proline (yellow) in presence of chromogenic reagent, ninhydrin. It was found from the Table 4, that the R_f values ranged from 0.13 to 0.77, indicating the potential difference among the polarity of the amino acids. It was observed that the R_f values were significantly different even within the same amino acid, i.e. *Leucine,* but difference in stereoposition, viz. *iso-l-leucine, iso-dl-leucine and nor leucine.* The R_f values of visible chromatographic spots of amino acids in paper chromatogram of root exudate of mung bean *(Vigna radiata* L.) are presented in Table 5.

Table 3: R_f values (expected) of amino acids by paper chromatography

Amino Acid	Solvent	
	n-Butanol: acetic acid: water (4:1:5, v/v)	**Phenol: water (4:1, v/v)**
Histidine	0.07	0.69
Serine	0.10	0.36
Lysine	0.10	0.48
Arginine	0.11	0.59
Aspartic acid	0.13	0.15
Glutamic acid	0.16	0.25
Glycine	0.17	0.40
Alanine	0.22	0.54
Threonine	0.22	0.50
Proline	0.30	0.91
Tyrosine	0.32	0.64
Methionine	0.40	0.80
Valine	0.47	0.77
Trytophan	0.47	0.83
Isoleucine	0.55	0.86
Phenylalanine	0.58	0.89
Leucine	0.6	0.86

Table 4: R_f values (observed) of standard amino acids by paper chromatography

Amino acin	Solvent
	n-Butanol: acetic acid: water (4:1:5, v/v)
Lysine	0.13
Histidine	0.19
Aspartic acid	0.24
Threonine (L)	0.31
Threonine (dL)	0.31
Proline	0.32
Methionine	0.58
Valine	0.61
Tryptophan	0.62
Isoleucine	0.67
Leucine (iso L)	0.71
Leucine (iso dL)	0.72
Leucine (nor)	0.77

Table 5: Amino acids identified in root exudate of mung bean (*Vigna radiata* L.)

R_f value	Amino acid	Intensity of spot colour in chromatograph
0.16	Glutamic acid	+
0.25	Hydroxy poline	+
0.30	Proline	++
0.31	Threonine	+

In comparison to the R_f values (expected/observed) of standard amino acids, it was revealed that, four amino acids were identified in root exudates of four weeks growth mung bean plant viz. *glutamic acid, hydroxy proline, proline* and *threonine*. A close observation of colour of the spots of exudate developed in chromatogram also revealed that intensity of the spots of the amino acids present in root exudate were comparatively very low with the standard amino acids. Scanning the colour intensity of the amino acids in root exudate, it was found that proline was present comparatively higher than the other amino acids. The amino acids compositions in root exudate of mung bean were comparable with the observation of Ray *et al*., (1988) [24]. These amino acids are the possible source of C, N and energy of the microbes in rhizosphere.

Sugar in the root exudate of mung bean (*Vigna radiata* L.)

The R_f values (expected) of different sugars recorded in the standard literature (Partridge, 1948) [23] by paper chromatography are presented in Table 6 in two different developing solvent systems, n-butanol: acetic acid: water (4:1:5, v/v) and phenol-NH_3 (with HCN) respectively. The R_f values (observed) of six standard sugars studied under laboratory condition are presented in Table 7 in single developing solvent system (n-butanol: acetic acid: water, 4:1:5, v/v). The colour of the spots of all the standard sugar molecules were black to dark brown in presence of chromogenic reagent, silver nitrate. Browsing of the R_f values of the standard sugars, revealed that the polarity of the studied sugars were not greatly varied. Thus, for resolution of R_f values of different sugars, third decimal of R_f values were taken for comparison. The R_f values of visible chromatographic spots of sugar in paper chromatogram of root exudate of mung bean are presented in Table 8. In comparison to the R_f values (expected/observed) of standard sugars, it was concluded that three sugars were identified in root exudates of four weeks growth mung bean plant, viz. *rhamnose, arabinose and D-ribose.* The sugars compositions in root exudate of mung bean were well comparable with the observation of Odunfa and Werner (1981) [22].

Table 6: R_f values (expected) of sugars by paper chromatography

Amino Acid	Solvent	
	n-Butanol: acetic acid: water (4:1:5, v/v)	Phenol: water (4:1, v/v)
D-Arabinose	0.21	0.54
D-Deoxyribose	0.33	0.73
L-Fucose	0.27	0.63
D-Fructose	0.23	0.51
D-Galactose	0.16	0.34
D-Glucose	0.18	0.39
Lactose	0.09	0.38
Maltose	0.11	0.36
D-Mannose	0.20	0.45
L-Rhamnose	0.37	0.59
D-Ribose	0.31	0.59
L-Sorbose	0.20	0.42
Sucrose	0.14	0.39
D-Xylose	0.28	0.44
D-Galaturonic Acid	0.14	0.13
D-Glucuronic Acid	0.14	0.89
Leucine	0.12(0.32)*	0.12

*due to lacton

Table 7: R_f values (Observed) of standard sugar by paper chromatograpy

Sugar	Solvent
	n-Butanol: acetic acid: water (4:1:5, v/v)
D-Glucose	0.261
Starch	0.266
Rhamnose	0.271
Mannose	0.273
Fructose	0.278
Arabinose	0.291

Table 8: Sugars identified in root exudates of mung bean (*Vigna radiate* L.)

Rf value	Sugar	Intensity of spot colour in chromatography
0.271	Rhamnose(Fucose)	+++
0.291	Arabinose	++
0.311	D-Ribose	+

The R_f values of rhamnose was mostly similar with fucose. Thus, the sugars present in exudates might be rhamnose/fusose, arabinose and D-ribose. The colour intensity of sugars in paper chromatogram similarly revealed that the amount of sugar present in exudate were very low. A close observation of the colour intensity of sugars of root exudate was also concluded that rhamnose/fucose concentration was comparatively higher, followed by arabinose and D-ribose. The sugars content in root exudate of pulse plant was also recorded by Moody *et al*., (2001) [21]. These sugars are chief source of carbon and energy of rhizospheric microbes.

Conclusion

The chemicals secreted into the soil by roots of the plant are broadly referred to as root exudates. Through the exudation of a wide variety of compounds, root may regulate the soil microbial community in their immediate vicinity, cope with herbivores, encourage beneficial symbiosis, change the chemical and physical properties of soil and inhibit the growth of competing plant species. Nearly 5% to 21% of all

photosynthetically fixed carbon being transferred to the rhizosphere through root exudates (Marschner, 1995) [20]. Root exudates have traditionally been grouped into low- and high-molecular weight compounds. Low molecular weight compound such as amino acids, sugars, organic acids, phenolics (including flavonoid compounds) and various other secondary metabolites are believed to comprise the majority of root exudates, whereas high molecular weight exudates primarily include mucilage (high molecular weight polysaccharides) and proteins.

Mung bean (*Vigna radiata* L.) is one of the important kharif pulse crop in India, particularly Uttar Pradesh. For searching the chemical composition of root exudate of Indian cultivar, mung bean (HUM-1) was growing in culture tubes through inoculating Brady rhizobium (strain MO_5). The root exudates with culture media was collected. The amino acids and sugars were identified in the extracted, purified and concentrated root exudates through paper chromatography using ninhydrin and silver nitrate respectively as chromogenic reagents and n-butanol: acetic acid: water (4:1:5, v/v) as a developing solvent.

The amino acids identified in root exudate of mung bean were glutamic acid, proline, hydroxyproline and threonine. The sugars identified in exudate were rhamnose/fucose, arabinose and D- ribose. The microbial communities in rhizosphere generally utilize these sugars and amino acids as a source of carbon and energy and these microbes indirectly helpful for plant nutrition and pest suppression.

References

1. Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Science, 2004; 301:1377-80.
2. Bais HP, Park SW, Stermitz FR, Halligan KM, Vivanco JM. Exudation of fluorescent beta-carbolines from *Oxalis tuberosa* L. roots. Phytochemistry, 2003; 61:539-43.
3. Bais HP, Walker TS, Schweizer HP, Vivanco JM. Root specific elicitation and antimicrobial activity of rosmarinic acid in hairy root cultures of sweet basil (*Ocimum basilicum* L.). Plant Physiol. Biochem, 2002; 40:9837.
4. Bais HP, Loyola-Vargas VM, Flores HE, Vivanco JM. Root-specific metabolism: the biology and biochemistry of underground organs. *In Vitro* Plant, 2001; 37:730-41.
5. Bertin C, Yang XH, Weston LA. The role of root exudates and allelochemicals in the rhizosphere. Plant Soil, 2003; 256:67-83.
6. Brady NC, Weil RR. The Nature and Property of Soils. Upper Saddle Hall, NJ: Prentice Hall, 1999.
7. Brimecombe MJ, De Leij Frans AAM, Lynch JM. Nematode community structure as a sensitive indicator of microbial perturbations induced by a genetically modified *Pseudomonas fluorescens* strain. Biol. Fertil. Soils, 2001; 34:270-75.
8. Broeckling CD, Broz AK, Bergelson J, Manter DK, Vivanco J.M. Root exudates regulate soil fungal community composition and diversity. Applied Environmental Microbiology, 2008; 74:738-744.
9. Gleba D, Borisjuk NV, Borisjuk LG, Kneer R, Poulev A, Skarzhinskaya M, *et al*. Use of plant roots for phytoremediation and molecular farming. Proc. Natl. Acad. Sci. USA, 1999; 25:5973-77.
10. Hawes MC, Brigham LA, Wen F, Woo HH, Zhu Z. Function of root border cells in plant health: pioneers in the rhizosphere. Annual Reviews of Phytopathology, 1998; 36:311-327.
11. Hawes MC, Gunawardena U, Miyasaka S, Zhao X. The role of root border cells in plant defense. Trends in Plant Science, 2000; 5:128-133.
12. Hiltner L. Uber neure erfahrungen und probleme auf dem Gebiet der Boden-bakteriologie und unter besondere Berucksichtigung der grundungung und Bracke. Arbeiten der Deutschen Landwirtschaft Gesellschaft. 1994; 98:59-78.
13. Hawes MC, Brigham LA, Wen F, Woo HH, Zhu Y. Function of root border cells in plant health: Pioneers in the rhizosphere. Annu. Rev. Phytopathol. 1998; 36:311-327.
14. Hawes MC, Gunawardena U, Miyasaka S, Zhao X. The role of root border cells in plant defense. Trends Plant Science, 2000; 5:128-133.
15. Jayaraman J. Laboratory manual in biochemistry, Wiley Eastern Limited, new Delhi, 1981; 65-67.
16. Kneer R, Poulev AA, Olesinski A, Raskin I. Characterization of the elicitor-induced biosysnthesis and secretion of genistin from roots of *Lupinus luteus*. Journal of experimental botany, 1999; 50:1553-59.
17. Knudson L. The secretion of invertase by plant roots. American Journal of Botany. 1920; 7:371-379.
18. Lynch JM. *The Rhizosphere*. Wiley Interscience, Chichester, UK, 1987.
19. Lyon TL, Wilson JK. Liberation of organic matter by roots of growing plants. New York Agricultural Experiment, 1921.
20. Marschner H. Mineral Nutrition of Higher Plants. 2nd Edn. Academic Press, London, 1995; 889 pp.
21. Moody SF, Clarke AE, Bacic B. Structural analysis of secreted slime from wheat and cowpea roots. Phytochemistry, 1988; 27(9):2857-2861.
22. Odunfa SA, Werner D. Root exudates in relation to growth and nitrogenase activity of Rhizobium japonicum. Zeitsehriff fur allegeneine Mikrobiologie, 1981; 21(8):601-606.
23. Partridge SM. Filter Paper partition chromatography of sugar. 1. General description and application to the qualitative analysis of sugars in apple juice, egg white and foetle blood of sheep. Biochemistry Journal. 1948; 42:238-240.
24. Ray TC, Callow JA, Kennedy JF. Composition of root mucilage polysaccharide from *Lepidium sativum*. Journal of Experimental Botany. 1988; 39:1249-1261.
25. Thronton HG. The early development of root nodules of Lucerne (*Medicago sativa* L.) Ann. Bot., Loud, 1930; 44:385-392.
26. Uren NC. Types, amounts and possible functions of compounds released into the rhizosphere by soil-grown plants. The Rhizosphere, Biochemistry and Organic Substances at the Soil–Plant Interface (eds R. Pinton, Z. Varanini & P. Nannipieri), 2000; 19–40. Marcel Dekker, New York, NY, USA.
27. Weir TL, Park SW, Vivanco JM. Biochemical and physiological mechanisms mediated by allelochemicals. Current Opinion in Plant Biology, 2004; 7:472-479.