RESEARCH ARTICLE

Statistical Interpretation of Quantitative and Qualitative Changes in Microflora of Rhizosphere and Rhizoplane Induced by Foliar Spray of Gibberellic Acid at Different Growth Stages of Chickpea *(Cicer arietinum)*

Shalini G Pratap, Vinod Kumar, Pokhraj Sahu and Pramod Kumar Singh*

DOI: 10.18811/ijpen.v10i04.09

Abstract

The chickpea (*Cicer arietinum* L.) crop is the third-largest pulse crop in the world, while it was cultivated in a 9 mHa area in India, which is 90% of the world's production. The aim of the present study was to evaluate both the quantitative and qualitative effects of foliar spray of gibberellic acid (GA) on rhizoplane and rhizosphere microflora of chickpea at various growth stages. The foliar spray of GA, *i.e.,* control (without GA), 25 (low), 50 (medium), and 100 ppm (high) concentrations were applied in a complete randomized design (CRD) at the different growth stages of chickpea, *i.e.,* pre-flowering, flowering, and fruiting stages. Results indicated that the number of fungal species in both, *i.e.,* rhizosphere and rhizoplane, were significantly decreased on increasing GA concentration. A notable reduction was observed in the rhizosphere during the pre-flowering stage at high doses, while the flowering stage was visible at medium and high doses. However, the fruiting stage is sustained in all treatments. Regarding the quantity of root nodules and shoot length, a comparable pattern was also observed. As plants became older, the content of sugars and amino acids decreased. At both the rhizosphere and the rhizoplane, the loading of fungal species was moderate to weak, with 88 and 91% variance, respectively, and exhibited a significant correlation.

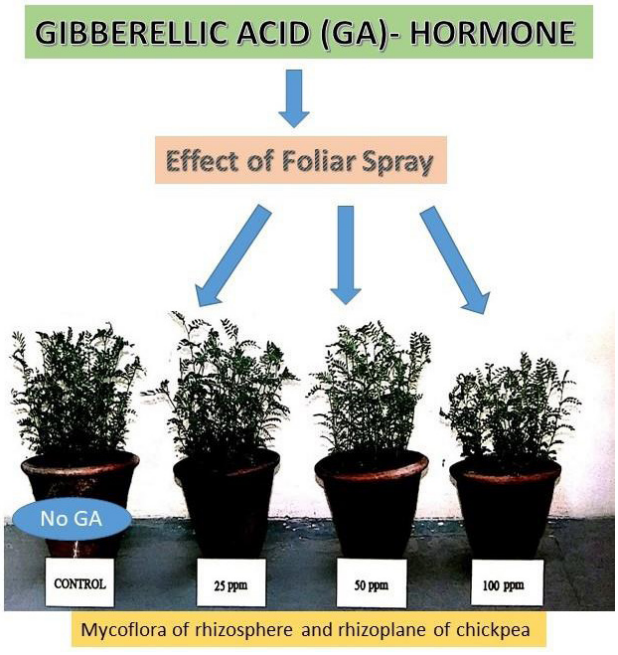
Keywords: Growth promoter, Root exudates, Rhizosphere, Fungi, Amino acid, sugar.

Highlights:

- Foliar spray of Gibberellic acid (GA) influenced the rhizosphere fungal micro-flora of chickpea.
- Application of Gibberellic acid (GA) promotes growth of shoot and root but no. of nodules decreased.
- Fungi/gm dry soil in non-rhizosphere was significantly decreasing with increasing GA.
- The number of amino acids and sugars influenced by the stage of plant growth
- A significant correlation was found between fungal species

International Journal of Plant and Environment (2024); **ISSN:** 2454-1117 (Print), 2455-202X (Online)

Graphical Abstract



Graphical representation of chickpea (Cicer arietinum) plant Study

Division of Environment Science, School of Basic Science, Babu Banarasi Das University, Lucknow-226028

***Corresponding author:** Pramod Kumar Singh, Division of Environment Science, School of Basic Science, Babu Banarasi Das University, Lucknow-226028, Email: singh_p_kumar@rediffmail.com

How to cite this article: Pratap, S.G., Kumar, V., Sahu, P., Singh, P.K. (2024). Statistical Interpretation of Quantitative and Qualitative Changes in Microflora of Rhizosphere and Rhizoplane Induced by Foliar Spray of Gibberellic Acid at Different Growth Stages of Chickpea *(Cicer arietinum)*. International Journal of Plant and Environment. 10(4), 84-96.

Submitted: 03/12/2023 **Accepted:** 09/01/2025 **Published:** 20/01/2025

Introduction

The chickpea is a multifunction crop and has huge nutritional value. In spite of these, chickpea has a diverse microbial population, including both bacterial and fungal species(Sahu *et al*., 2020). It is commonly known that rhizobia attach or adhere close to legume roots and stimulate growth by producing siderophores, phosphate solubilization, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, indole-3-acetic acid

(IAA) production, catalase, oxidase, and other enzymes(Ditta *et al*., 2015; Ditta and Khalid, 2016; Sarfraz *et al*., 2019). The region of soil where the root system of a crop exerts its impact is known as the rhizosphere. The term "rhizosphere effect" refers to the phenomena whereby the exudation of chemicals by the root causes an increase in biomass and microbial activity relative to the bulk of soil (Raaijmakers *et al*., 2009). Plant root tissues have a particular influence on the soil in a specialized ecological niche known as the rhizosphere. According to Manoharachary and Mukerji (2006), it is a poorly defined zone of soil with a microbial gradient where the population of soil microflora varies more when it is close to the root and decreases as it gets away from it.

Both plant species and soil type significantly influence the composition and activity of microbial communities associated with the rhizosphere. The colonization of the rhizosphere is significantly influenced by both the fungal metabolism and the plant metabolism. Compounds are constantly produced and excreted into the rhizosphere by plant roots (Uren, 2000; Rovira, 1956; Clark, 1949). Certain microbial populations in the rhizosphere are enriched in part due to the presence of root exudates. Ions, free oxygen, water, enzymes, mucilage, and a variety of carbon components, including primary and secondary metabolites, are all present in root exudates (Bais *et al*., 2006). At the soil-root interface, the main components are organic acids, sugars, amino acids, coumarins, flavonoids, proteins, enzymes, and aliphatic and aromatic compounds. However, each plant species has a unique combination and makeup of fundamental components. According to Broeckling *et al*., (2008), two plant species models—*Arabidopsis thaliana* and *Medicago trucatula* can support resident fungal populations in the soil, but they are unable to sustain non-resident fungal populations due to the crucial function of root exudates.

Many workers studied the stimulation of fungi in the rhizosphere (Agnihothrudu, 1955; Farzana, 1980; Mohammad, 1985; Kumar *et al*., 2006); Okorski *et al*., 2007; Tamilarasi *et al*., 2008; Berg, (2009). They observed that in general, the fungi in rhizosphere soil were several times greater than that in root-free soil. Cui (2007) reported that the number of fungi per gram in dry soil was greater in rhizosphere soil at the early stage as compared to pre-flowering, flowering and fruiting stages.

The effect of foliar application of gibberellic acid and indol acetic acid (IAA) on nodulation of leguminous plants has been studied by a few workers (Fletcher *et al*., 1959; Galston, 1959; Garg *et al*., 1993). They observed that gibberellic acid had a direct inhibitory effect on the nodulation of leguminous plants. Bano (2002) observed that synthetic ABA inhibited all phases of nodulation (nodule initiation and function). Maity and Bera (2008) reported that foliar application of BR and SA increased in number and fresh weight of nodules.

Therefore, the present investigation was designed to assess the impact of foliar spray of different levels of gibberellic acid on the composition of rhizosphere fungi and their effect on nodulation and plant growth of chickpeas (*Cicer arietinum*). Despite these, the effect of foliar spray of GA on the contents of amino acids and sugars in the root extract was also studied to correlate their impact with the pattern of fungi present in the rhizosphere. Some statistical interpretations were done to correlate them with each other's.

Materials and methods

Treatments

In earthenware pots with evenly blended, unsterilized soil, seeds were sowed. Three pots were maintained for every concentration of gibberellic acid and the control. After six days of sowing, six equal-sized seedlings were selected to stay in each pot while the remaining seedlings were removed. The different levels of gibberellic acid solution were prepared in the glass distilled water (GDW), i.e., 25 ppm (low), 50 ppm (medium), and 100 ppm (high) by vigorously shaking for 15 minutes. The solution was first sprayed on seedlings that were 15 days old. In total, two sprays were applied on two typical days at each of the three stages e.g., pre-flowering, flowering, and fruiting. DW was sprayed in control plants. Sterilized cotton was used to cover the soil surface to avoid falling drops directly on the soil. In each sampling, one complete root system was carefully dug out from each of the triplicate pots with the help of a trowel. The root system gently tapped to remove loosely adhering soil and the roots were cut off with sterilized scissors and were placed in a 250 mL flask containing 100 mL DW. The rhizosphere fungi were analyzed by dilution plate method, after 10 days of each spraying. Isolation of rhizoplane fungi was also assessed in each treatment.

Isolation of mycoflora from the rhizosphere and rhizoplane soil

Three plants (one from each of the three plots that were extremely close to one another) were dug out for each sampling in order to isolate the rhizosphere mycoflora. The adhering soil was then gently tapped away. Using sterile scissors, the roots were removed and placed in a flask holding 100 mL of distilled water that had been sterilized. A vigorous shaking of the flask was used to achieve a homogeneous suspension of soil and water. A total of 15 mL of sterilized and cooled Czapeck's medium containing the following ingredients: KH_2PO_4 (1.0 g), $MgSO_4.7H_2O$ (0.5 g), KCl (1.0 g), $FeSO_4$ (in trace), yeast powder (0.5 g), $NaNO_3$ (0.5 g), dextrose (10.0 g), agar-agar (15.0 g), and 1-mL of aliquot were added to five four-inch petri dishes.

The weight of the oven-dry soil in the remaining suspension was determined after the root system was removed from the soil suspension and it was allowed to dry for 24 hours at 105°C in an electric oven. The weight dry soil was calculated in 1-mL of soil suspension. The number of fungi that appeared in 1-mL of the solution was counted and the average number of colonies was determined. This allowed for the calculation of the number of fungi/g of oven-dry soil.

Rhizoplane fungi were studied using the Harley (1955) technique. After removing the roots from the flask, they were thoroughly cleaned with many changes of distilled water that had been sterilized. First, the roots were dried using Whatman's filter paper No. 44, which had been sterilized. Next, tiny root fragments measuring 10 mm were removed, and five of these fragments were put on a sterile agar medium in Petri dishes. For the rhizoplane study, samples were used in triplicate.

To isolate the non-rhizosphere mycoflora, the soils of the root system were removed. 90 ml of sterilized DW were placed in a 250 mL flask along with 10 g of soil sample. For preparing a

homogeneous soil suspension, the flask was rapidly shaken, and dilution series of 1:100, 1:1000, and 1:10000 were prepared. Each dilution's 1ml suspension was added to plates along with the nutritional medium. Every dilution was made using a different pipette. The average number of fungal colonies across five plates was multiplied by a specific dilution to determine the fungi/gm of dry soil in the non-rhizosphere. The moisture content of the soil was taken into consideration while calculating the fungi/g dry soil. The percentage occurrence of fungi in the rhizosphere and non-rhizosphere was calculated.

Inoculated plates were incubated for 5 to 6 days at 25°C and thereafter, fungi were isolated and identified with the help of 'A manual of soil fungi' by Gian (1956), "The genus *Aspergillus* by Raper (1965), and 'The illustrated genera of imperfect fungi' by Barnett, (1962), Cienera of fungi' by Clements, (1954).

Detection of amino acids and sugars

Qualitative analysis of amino acids and sugar was done by the following method by Block, (1950) using the descending paper chromatography technique. Detection of amino acids and sugars present in the root extract of the plant at pre-flowering, flowering, and fruiting stages was done in which 5 gm of roots were crushed into pestle mortar in 30 ml absolute alcohol. The root debris was filtered and the filtrate was concentrated by mixing it with three parts of chloroform in a separating funnel. Root extract was separated and used for spotting. Separate capillary tubes were used to make the spot on the chromatogram. Following solvents viz., n-butanol, glacial acetic acid, and water in the ratio of 4:1:5 were taken into a separating funnel. Two layers become visible and separated. The lower level of solvent was kept at the bottom of the chromatographic chamber while the upper layer of solvent was used for running the chromatogram which was allowed to run for approximately 8 hours. The chromatogram was dried again at room temperature.0.1% Ninhydrin in n-butanol was used as a spraying agent for the identification of amino acids, whereas 8.3 g phthalic acid in 75 mL distilled water, 425 mL n-butanol and 5 mL aniline were used as spraying agents for the identification of sugars. The chromatogram was dried again at room temperature. Thereafter, it was heated in an oven at 80°C till the clear spots became visible. Amino acids and sugars present in root extract were identified by running the individual known amino acids and sugars.

Statistical Interpretation

SPSS software was used for the interpretation of analyzed data sets and measurement of statistical significance such as mean, standard error mean (SEM), ANOVA, factor analysis, Spearman rho's correlation coefficient, and hierarchical cluster analysis. Factor analysis (FA) was running for the identification of a significant load of fungal species in the percentage occurrence of fungal species isolated from rhizosphere and rhizoplane during the study of foliar application of gibberellic acid at different growth stages of chickpea (*C. arietinum*) plant and spearman rho's correlation coefficient was also applied for identification of the positive, negative or significant correlation among the fungal species. Hierarchical cluster analysis running for measuring the similarity and comparative study of fungal species isolated in the rhizosphere and rhizoplane of the chickpea plant. The least standard deviation (LSD) post hoc test applied through Bivariates analysis of variance (ANOVA) in isolated fungal species isolated in rhizosphere non-rhizospheric soil and rhizoplane and growth of shoot, root, and number of root nodules of chickpea plant during the different growth stage.

Results

Fungal population in the rhizosphere,non-rhizosphere and rhizoplane

During the pre-flowering stage, the percentage of occurrence of the fungal species in the rhizosphere increased as the concentration of GA increased. These species included *Mucor lutense, Cephalosporium coremoides, Aspergillus niger, A. candidus, Stachybotrysatra, Cladosporium herbarum, Fusarium udum,* and white sterile mycelium. In contrast, the percentage of *Rhizopus nigricans*, *A. flavous*, *A. terreus*, *Paecilomyces fusisporus*, *Penicillium citrinum,* and *Myrothecium roridum* decreased. But the *A. luchuensis, Helminthosporium sativum* and *Alternaria humicola* were checked in the treated plant at all concentrations. On the rhizoplane of the GA-treated plant, the percentage occurrence of *Aspergillus flavus*, *A. niger, P. citrinum, Fusarium udum* and white sterile mycelium increased while the growth of *Trichoderma lignorum* was checked (Table 1). The total number of fungi in the rhizosphere during the pre-flowering stage was 21.0 ± 1.16 species in control, which showed a decreasing trend of 18.0 ± 2.08, 16.0 ± 1.00, and 13.0 ± 1.73 species at 25, 50, and 100 ppm, respectively. A significant effect of no. of fungal species was observed at the foliar spray of a high concentration of GA (Table 2).

A. flavus, A. niger, A. luchuensis, Paecilomyces fusisporus, Curvularia lunata, and Fusarium udum were more prevalent in the rhizosphere during the flowering stage, whereas *Rhizopus nigricans*, *A. terreus, P. citrinum, Cladosporium herbarium*, and white sterile mycelium were less prevalent depending on the concentration of GA. At every stage of treatment, the growth of *T. lignorum, Myrothecium roridum, and Cephalosporium coremioides* was monitored in the treated plants. On the rhizoplane of GA-treated plants, the percentage occurrence of Mucor, A. niger, and white sterile mycelium rose while the growth of *A. flavus* and *Alternaria hemicola* was checked (Table 1). The total number of fungi in the rhizosphere during the flowering stage was 16.0 ± 0.33 species in control which was decreasing trend as 14.0 ± 1.00, 11.0 ± 1.16 and 10.0 ± 1.00 species at 25, 50 and 100 ppm, respectively. A significant effect on the no. of fungal species is observed at the foliar spray of medium and high concentrations of GA (Table 2).

When GA hormone concentrations increased during the fruiting stage, the percentage of fungal species in the rhizosphere, namely, *A. flavus, A. niger, Paecilomyces fusisporum,* and *F. udum* increased, whereas the percentage of *R. nigricans, Penicillium citritum*, *Starchybotrysarta*, and white sterile mycelium were decreased. In the treated plants, the growth of yellow sterile mycelium was seen at all GA concentrations. In the rhizoplane of GA hormone-treated plants, the percentage occurrence of *A. niger* and *F. udum* increased when *Alternaria humicola* growth was checked (Table 1). The total number of

Table 1: Effect of foliar spray of different concentrations of gibberellic acid on the frequency (percentage occurrence) of fungi isolated from the rhizosphere and rhizoplane at pre-flowering, flowering and fruiting growth stages of chick pea (*Cicer ariatinum*) plant.

Sl. No.	*Isolated of Fungi Species*	*Rhizosphere*												*Rhizoplane*											
		Pre-Flowering				*Flowering*				*Post-Flowering*				*Pre-Flowering*				*Flowering*				*Post-Flowering*			
		Control	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*
1	Rhizopus nigricans	7	3	4	Abs	5	4	Abs	Abs	6	2	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
2	Mucor luteus	1	3	10	7	Abs	3	16	23	2	2	Abs	Abs	Abs	Abs	Abs	Abs	Abs	25	20	50	Abs	Abs	Abs	Abs
3	Cephalosporium coremiodes	5	7	1	1	7	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
4	Trichoderma lignorum	Abs	5	7	2	2	Abs	Abs	Abs	Abs	Abs	Abs	Abs	4	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
5	Aspergillus flavous	12	4	1	7	7	14	14	1	5	6	6	10	15	11	Abs	19	17	Abs	Abs	Abs	27	17	17	Abs
6	A.niger	4	20	20	31	7	12	16	28	13	23	25	34	13	17	27	44	23	33	40	Abs	25	40	33	33
7	A.luchuensis	7	Abs	Abs	Abs	8	12	Abs	5	5	Abs	1	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	50	Abs	Abs	Abs	Abs
8	A.candidus	3	6	9	5	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
9	A.terreus	5	2	Abs	12	5	4	4	2	5	Abs	1	4	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
10	A.nidulans	5	2	8	Abs	Abs	Abs	Abs	Abs	1	2	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
11	Penicillium citrium	3	2	2	Abs	4	2	Abs	Abs	6	5	1	6	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	10	10	Abs	25
12	Paecilomyces fusisporus	11	10	4	10	4	9	12	27	10	8	18	21	5	Abs	13	Abs	Abs	25	Abs	Abs	Abs	Abs	Abs	Abs
13	Stachybitrys atra	3	Abs	6	6	1	2	1	3	5	2	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
14	Humicola grisea	2	7	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
15	Cladosporium herbarium	5	6	6	Abs	11	9	8	Abs	10	5	2	1	8	Abs	20	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
16	Curvularia lunata	3	4	1	3	6	8	10	3	3	Abs	2	4	Abs	9	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
17	Herliminthosporium sativum	5	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	2	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	20	Abs	Abs	Abs
18	Alternaria humicola	2	Abs	Abs	Abs	5	7	9	4	7	25	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
19	Fusarium udum	3	6	10	6	3	8	7	4	1	1	38	14	32	37	Abs	Abs	25	17	Abs	Abs	15	25	33	Abs
20	Myrothecium roridum	8	6	Abs	9	5	Abs	Abs	Abs	7	7	Abs	2	Abs	Abs	Abs	Abs	25	Abs	Abs	Abs	Abs	Abs	Abs	17
21	Black sterile mycelium	2	4	2	Abs	Abs	Abs	Abs	Abs	3	Abs	Abs	Abs	Abs	Abs	10	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs	Abs
22	White sterile mycelium	2	3	9	1	20	6	3	Abs	9	8	4	4	23	26	27	37	8	Abs	40	Abs	Abs	8	17	25

Table 2: Effect of foliar spray of different concentrations of Gibberellic acid.in number of fungi in dry soil, rhizosphere and rhizoplane during different growth stages of chickpea (*C. arietinum*) plants.

Growth stages of plant	*Number of sprayings*	*Concentration of Gibberellic acid*	*No. of fungal species in rhizosphere*	*No. of fungal species in rhizoplane*	*No. of fungi/g dry soil (x1000)R*
Pre flowering stage	First spray	Control	21.0 ± 1.16	7.0 ± 0.05	113.0 ± 4.41
		25 ppm	18.0 ± 2.08	5.0* ± 0.57	61.0* ± 2.03
		50 ppm	16.0 ± 1.00	5.0* ± 0.07	40.0** ± 2.00
		100 ppm	13.0* ± 1.73	3.0*** ± 0.58	30.0*** ± 0.58
Flowering stage	Second spray	Control	16.0 ± 0.33	5.0 ± 1.00	82.0 ± 2.08
		25 ppm	14.0 ± 1.00	4.0 ± 1.00	58.0* ± 1.53
		50 ppm	11.0* ± 1.16	3.0 ± 0.58	41.0** ± 0.58
		100 ppm	10.0** ± 1.00	2.0* ± 0.05	37.0*** ± 1.33
Fruiting stage	Third spray	Control	17.0 ± 0.57	5.0 ± 1.00	85.0 ± 1.45
		25 ppm	13.0* ± 0.05	5.0 ± 0.05	59.0* ± 0.66
		50 ppm	11.0* ± 1.00	4.0 ± 0.33	47.0** ± 0.58
		100 ppm	10.0** ± 0.58	4.0 ± 0.67	40.0*** ± 1.33

*LSD Post Hoc ANOVA Test * The mean difference is significant at the .05 level multi-comparisons with control sample*
*** The mean difference is significant at the .05 level multi-comparisons with control sample and 25 ppm Gibberellic acid*
**** The mean difference is significant at the .05 level multi-comparisons with control sample, 25 ppm and 50 ppm Gibberellic acid*

fungi in the rhizosphere during the post-flowering stage was 17.0 ± 0.57 species in control which was significantly decreased as 13.0 ± 0.05, 11.0 ± 1.00 and 10.0 ± 1.00 species at 25, 50 and 100 ppm, respectively.

In the non-rhizosphere, the number of fungal population (no. of fungi/g dry soil) was observed significantly decreased on increasing the concentration of GA during pre-flowering, flowering, and fruiting stages (Table 2). The total number of fungi/g dry soil in pre-flowering was 113.0 ± 4.41-species in control which was significantly decreased as 61.0 ± 2.03, 40.0 ± 2.00 and 30.0 ± 0.58 species at 25, 50 and 100 ppm, respectively. A similar trend was also observed in flowering and fruiting stages which were 82 ± 2.08, 58 ± 1.53, 41.0 ± 0.58, and 37.0 ± 1.33species in the flowering stage while 85 ± 1.45, 59 ± 0.66, 47 ± 0.58, and 40 ± 1.33 species respectively (Table: 2). During the pre-flowering, flowering and fruiting, and post-flowering stages of the rhizoplane, fungal species like *Aspergillus niger*, *Cephalosporium coremoides*, *A. candidus*, *A. terreus*, *A. nidulans, Stachybitrys atra, Humicola grisea,* and *Alternaria humicola* were not isolated from the rhizoplane area. At the pre-flowering and fruiting stages, the proportion of occurrence of *A. niger* and white sterile mycelium increased as the concentration of GA increased. The total number of fungi inrhizoplane during the pre-flowering stage was 7.0 ± 0.05 species in control which was significantly decreasing at 5.0 ± 0.57, 5.0 ± 0.07 and 3.0 ± 0.58 species at 25 ppm, 50 ppm and 100 ppm respectively. The percentage occurrence for *Mucor luteus* and *A. niger* were showing increasing trends with the concentration of GA at the flowering stage in rhizoplane. The total number of fungi in rhizoplane during the flowering stage was 5.0 ± 1.00 species in control which was a decreasing trend as 4.0 ± 1.00, 3.0 ± 0.58 and 2.0* ± 0.05 species at 25 ppm, 50 ppm and 100 ppm respectively. The significant effect on no. of fungal species is observed at a foliar spray of 100 ppm gibberellic acid. The percentage occurrence of *Aspergillus flavus* showed decreasing trends with the concentration of GA at the fruiting stage. The total number of fungi in rhizoplane during the post-flowering stage was 5.0 ± 1.00 species in control which was decreased as 5.0 ± 0.05, 4.0 ± 0.33 and 4.0 ± 0.67 species. The effect on the number of fungi in the rhizosphere was not significant at all treatments.

Effect on growth and number of root nodules

Gibberellic acid (GA) acts as a growth promoter hormone. Foliar spray of different concentrations of GA (25, 50 and 100 ppm) increased shoot length (24, 26 and 29 cm) and root length (22, 23 and 25 cm) of chickpea plant (Table: 3). In contrast, the number of nodules (27, 26 and 19) per plant was decreased on increasing the concentration of GA (Table: 3). The growth of shoot length was observed significant at 50 and 100 ppm concentration of

Table 3: Effect of foliar spray of different concentrations of gibberellic acid on the growth of shoot, root and number of root nodules of chickpea (*C. arietinum*)

Concentration of Gibberellic acid (ppm)	*Shoot growth (cm) Mean ± SEM*	*Root growth (cm) Mean ± SEM*	*No. of root nodules per plant Mean ± SEM*
Control	20.0 ± 0.58	21.0 ± 1.73	30.0 ± 1.16
25	24.0 ± 1.53	22.0 ± 1.73	27.0 ± 1.52
50	26.0* ± 1.73	23.0 ± 0.05	26.0 ± 0.33
100	29.0* ± 2.08	25.0 ± 1.53	19.0*** ± 1.53

LSD Post Hoc ANOVA Test
** The mean difference is significant at the .05 level multi-comparisons with the control sample*
*** The mean difference is significant at the .05 level multi-comparisons with control sample and 25 ppm Gibberellic acid*
**** The mean difference is significant at the .05 level multi-comparisons with the control sample, 25 ppm, and 50 ppm Gibberellic acid*

GA. In contrast, root growth was observed but significant. The number of root nodules /plants was decreasing with increasing concentrations of gibberellic acid and a significant decrement was noted on foliar spray at 100 ppm concentration of gibberellic acid on chickpea plants. Thus, it was observed that GA promoted shoot and root length while hindering the nodulation.

Qualitative changes in Amino acids and sugar in root extracts

Chromatographic analysis of the root extract of chickpea treated with different concentrations of GA to assess amino acids and sugars induced changes (Figs 1 and 2), respectively. It was observed that the number of amino acids in root extract was decreased with the age of the plant and a minimum number was observed at the fruiting stage while 8, 7, and 6 amino acids were detected in root extract at pre-flowering, flowering, and fruiting stages respectively (Table 4).

There were no qualitative changes were observed due to GA spray. Major amino acids that were observed in the pre-flowering stage were L Tyrosine, Glycine, DL Iso-Leucine, DLB Phenylalanine, L Ornithine mono HCL, DL thrioline and DL 2 Aminobutyric while there was lacking L Ornithine mono HCL

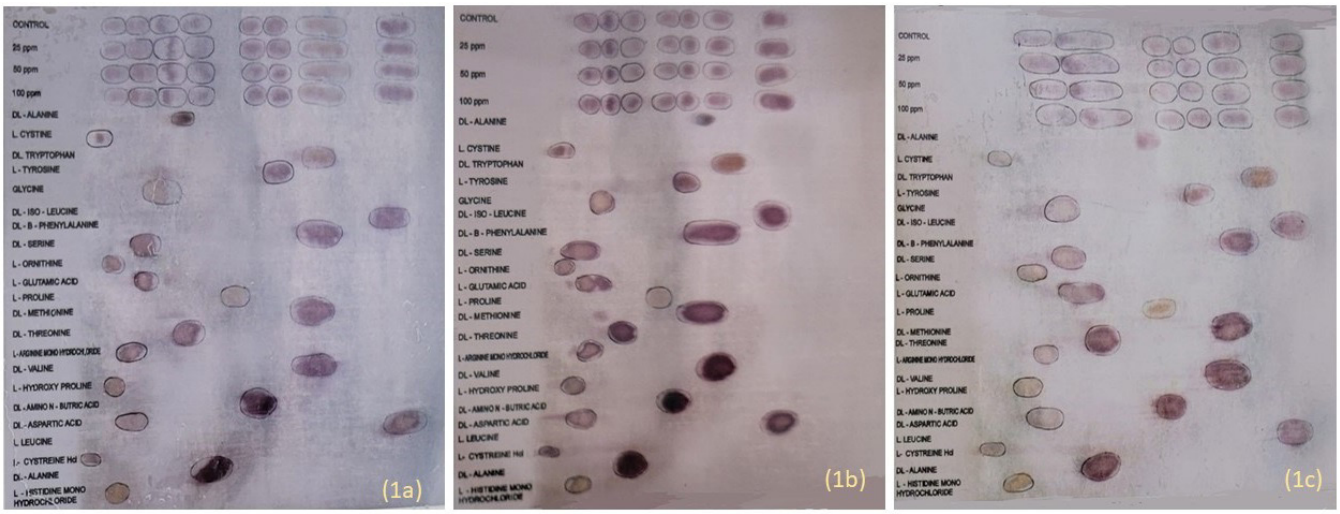


Fig 1: Chromatogram showed the effect of foliar spray of different concentrations of gibberellic acid on the aminoacid (AA) at different stages of plant growth i.e., (1a) pre-flowering, (1b) flowering and (1c) fruiting stages in the root extract of chickpea (*Cicer aietinum*) plant.

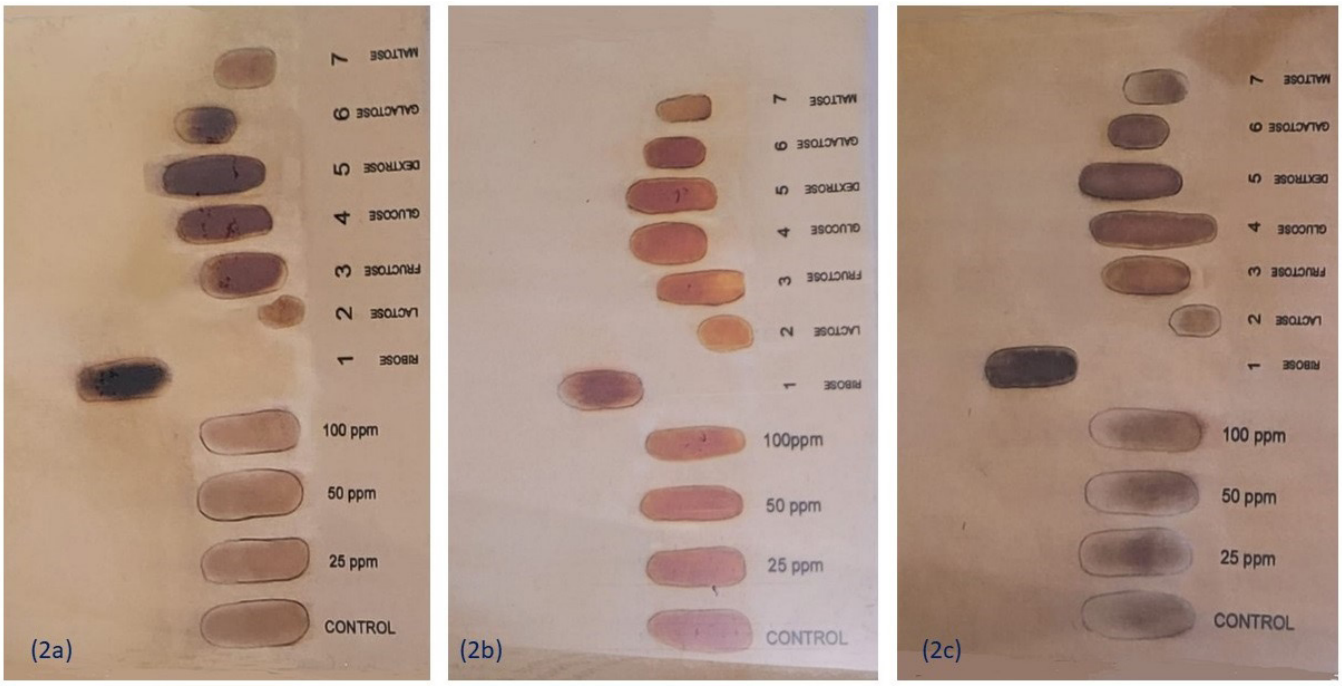


Figure 2: Chromatogram showing the effect of foliar spray of different concentrations of gibberellic acid on the sugars at different stages of plant growth *i.e.* pre-flowering (2a), flowering (2b) and fruiting (2c) stages in the root extract of chickpea (*C. arietinum*) plant.

Table 4: Effect of foliar spray of gibberellic acid on the amino acids present in the root extract in different growth stages of chickpea (*C. arietinum*) plant.

Sl. No.	Known amino-acids	Pre-Flowering stage				Flowering stage				Fruiting stage			
		Control	25 ppm	50 ppm	100 ppm	Control	25 ppm	50 ppm	100 ppm	Control	25 ppm	50 ppm	100 ppm
1.	DL Alanine	-	-	-	-	-	-	-	-	-	-	-	-
2.	L Cystine	-	-	-	-	-	-	-	-	-	-	-	-
3.	DL Tryptophan	-	-	-	-	-	-	-	-	-	-	-	-
4.	L Tyrosine	+	+	+	+	+	+	+	+	+	+	+	+
5.	Glycine	+	+	+	+	+	+	+	+	+	+	+	+
6.	DL Iso Leucine	+	+	+	+	+	+	+	+	+	+	+	+
7.	DLB Phenyl alanine	+	+	+	+	+	+	+	+	+	+	+	+
8.	DL Serine	-	-	-	-	-	-	-	-	-	-	-	-
9.	L Ornithine mono HCL	+	+	+	+	-	-	-	-	-	-	-	-
10.	L Glutamic acid	-	-	-	-	-	-	-	-	-	-	-	-
11.	L Proline	-	-	-	-	-	-	-	-	-	-	-	-
12.	DL. Methionine	-	-	-	-	-	-	-	-	-	-	-	-
13.	DL. Thrioline	+	+	+	+	+	+	+	+	+	+	+	+
14.	L. Arginine mono HCL	+	+	+	+	+	+	+	+	+	+	+	+
15.	DL. Valine	-	-	-	-	-	-	-	-	-	-	-	-
16.	L. Hydroxy proline	-	-	-	-	-	-	-	-	-	-	-	-
17.	DL.2 Amino butyric Acid	+	+	+	+	+	+	+	+	-	-	-	-
18.	DL. Aspartic Acid	-	-	-	-	-	-	-	-	-	-	-	-
19.	L. Leucine	-	-	-	-	-	-	-	-	-	-	-	-
20.	L. Cystne HCL	-	-	-	-	-	-	-	-	-	-	-	-
21.	L. Histidine mono HCL	-	-	-	-	-	-	-	-	-	-	-	-
Total		8	8	8	8	7	7	7	7	6	6	6	6

(+) present and (-) absent

Table 5: Effect of foliar spray of gibberellic acid on the sugars present in the root extract in different growth stages of chickpea (*C. arietinum*) plant.

Sl. No.	*Known amino-acids*	*Pre-Flowering stage*				*Flowering stage*				*Fruiting stage*			
		Control	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*	*Control*	*25 ppm*	*50 ppm*	*100 ppm*
1.	Ribose	-	-	-	-	-	-	-	-	-	-	-	-
2.	Lactose	-	-	-	-	-	-	-	-	-	-	-	-
3.	Fructose	+	+	+	+	+	+	+	+	+	+	+	+
4.	Glucose	-	-	-	-	-	-	-	-	-	-	-	-
5.	Dextrose	-	-	-	-	-	-	-	-	-	-	-	-
6.	Galactose	-	-	-	-	-	-	-	-	-	-	-	-
7.	Maltose	-	-	-	-	-	-	-	-	-	-	-	-
	Total sugars	1	1	1	1	1	1	1	1	1	1	1	1

(+) present and (-) absent

amino acid in flowering stage and L. Arginine mono HCL in fruiting stage. Sugar was also detected in root extract at the pre-flowering, flowering and fruiting stage which was found only one sugar in all stages which was fructose and there were no changes due to the treatment of GA hormones (Table: 5).

Statistical Analysis

Cumulative variance and the Eigenvalues presented in Table 6, and Fig 3, extracted by Factor analysis in the percentage occurrence of fungal species isolated from rhizosphere and rhizoplane during the study of different growth stages of

chickpea plant. Factor analysis (FA) extracted seven factors for both rhizosphere and rhizoplane, which had more than 1 eigenvalue and cumulative variance was 88.911 and 91.808%, respectively.

The fungal species loading in extracted factors showed that the values between 0.01 to 0.49 presented weak loading, 0.50 to 0.74 showed moderate loading, 0.75 to 0.95 represented strong loading and the values ≥0.96 or ~1 denoted significant loading (Table 7). The negative symbol (-) representsthe negative load and the rest of the positive load. Spearman rho's correlation coefficient was also applied for the identification of the positive, negative, or significant correlation among the fungal species. The negative symbol (-) represents the negative correlation and the rest of the positive correlation. Spearman rho's correlation coefficient was analyzed for the percentage occurrence of fungal species isolated in the rhizosphere and rhizoplane during the

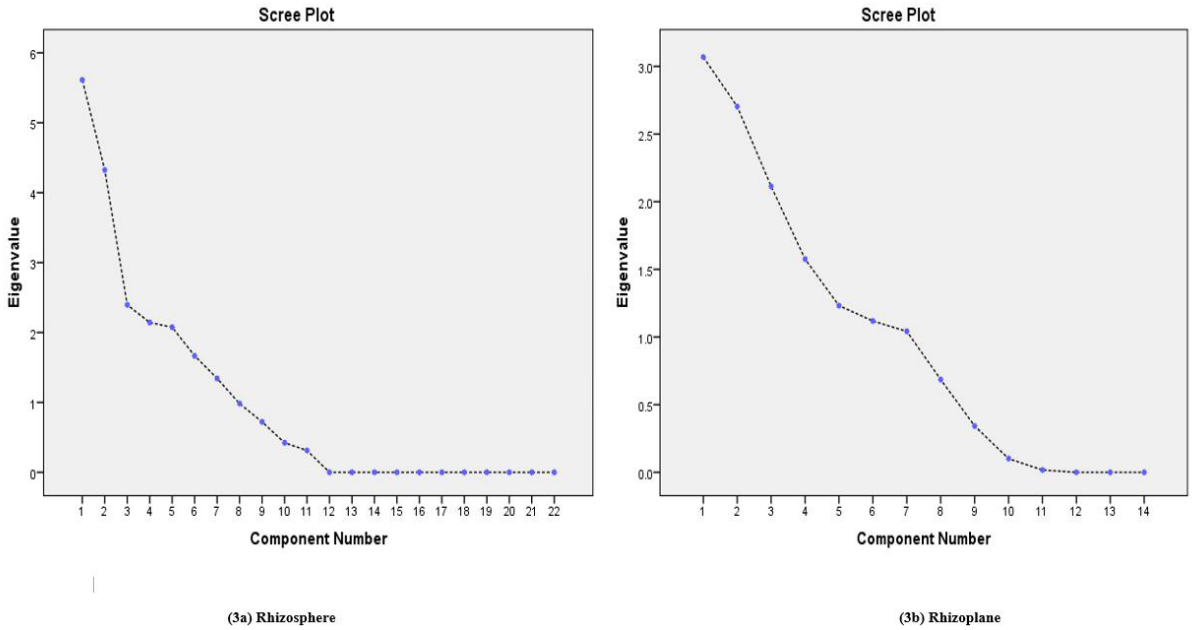


Figure 3: Scree Plot of FA for isolated fungal species in rhizosphere (3a) and rhizoplane (3b) of chick pea (*C. arietinum*) plant.

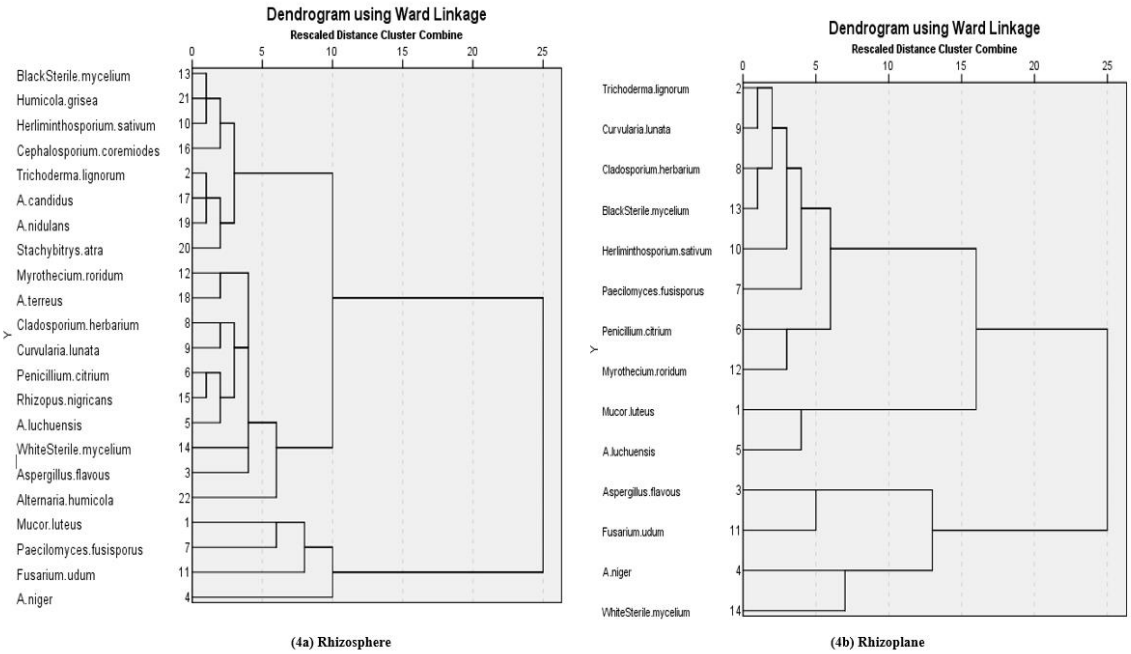


Figure 4: Dendrogram of HCA for measuring the similarity and comparative study of isolated fungal species in rhizosphere (4a) and rhizoplane (4b) of chick pea (*C. arietinum*) plant.

Table 6: Eigenvalues and cumulative variance extracted by factor analysis in percentage occurrence of fungal species isolated from rhizosphere and rhizoplane during the study of different growth stages of chickpea (*C. arietinum*) plant.

	Rhizosphere			*Rhizoplane*		
Component/Factor	*Extraction Sums of Squared Loadings*			*Extraction Sums of Squared Loadings*		
	Eigenvalues	*% of variance*	*Cumulative Variance %*	*Total*	*% of variance*	*Cumulative Variance %*
1	5.613	25.513	25.513	3.069	21.922	21.922
2	4.325	19.659	45.173	2.703	19.308	41.230
3	2.395	10.887	56.060	2.113	15.095	56.325
4	2.143	9.743	65.802	1.576	11.255	67.579
5	2.075	9.431	75.233	1.232	8.800	76.380
6	1.667	7.576	82.809	1.118	7.987	84.367
7	1.342	6.102	88.911	1.042	7.441	91.808

Table 7: Component Loading extracted by factor analysis for number of fungi species isolated from the rhizosphere and rhizoplane

Isolated of Fungi Species	*Factor for Rhizosphere*							*Factor for Rhizoplane*						
	1	*2*	*3*	*4*	*5*	*6*	*7*	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Rhizopus nigricans	.91	-.20	.00	.00	.08	.30	.07	.00	.00	.00	.00	.00	.00	.00
Mucor luteus	-.46	.26	-.12	.63	-.20	.10	.45	-.78	-.56	-.09	-.09	-.17	-.03	-.12
Cephalo sporium coremiodes	.69	.06	.47	-.08	-.09	-.30	.01	.00	.00	.00	.00	.00	.00	.00
Trichoderma lignorum	.46	.73	-.07	.17	-.37	-.16	-.21	.06	.11	.62	.07	.42	-.12	-.57
Aspergillus flavous	-.04	-.70	.40	.14	.11	.05	-.11	.79	-.17	.26	.33	-.09	.19	-.13
A. niger	-.75	.48	-.18	-.17	.13	-.29	-.11	.41	.50	-.54	-.09	-.46	-.14	-.19
A. luchuensis	.31	-.63	.14	.24	-.02	.32	.05	-.67	-.65	.00	-.02	.15	.26	-.04
A. candidus	.41	.83	.08	.24	-.04	.01	-.17	.00	.00	.00	.00	.00	.00	.00
A. terreus	.00	-.15	.24	.49	.67	-.31	-.36	.00	.00	.00	.00	.00	.00	.00
nidulans	.56	.56	-.14	.00	-.08	.49	.04	.00	.00	.00	.00	.00	.00	.00
Penicillium citrium	.39	-.31	-.37	-.59	.19	-.11	-.01	.32	-.05	-.62	.18	.36	-.01	.14
Paecilomyces fusisporus	-.79	.06	.25	-.19	.08	.12	.30	-.47	.34	.15	.28	-.33	-.61	.11
Stachybitrys atra	.23	.38	-.44	.54	.44	.28	-.10	.00	.00	.00	.00	.00	.00	.00
Humicola grisea	.41	.38	.57	-.20	-.13	-.39	.35	.00	.00	.00	.00	.00	.00	.00
Cladosporium herbarium	.69	-.52	-.15	.15	-.38	-.07	.01	-.41	.73	.30	.31	.26	.15	.08
Curvularia lunata	-.08	-.60	.38	.51	-.35	-.23	-.01	.20	-.06	.44	-.52	-.11	.20	.60
Herliminthosporium sativum	.23	-.04	.55	-.24	.34	.63	.04	.35	-.25	-.04	.73	-.17	.35	.11
Alternaria humicola	.02	-.42	-.60	-.16	.06	-.09	.44	.00	.00	.00	.00	.00	.00	.00
Fusarium udum	-.47	.13	.28	-.42	-.29	.27	-.51	.54	-.15	.70	-.09	-.02	-.32	.15
Myrothecium roridum	.50	.04	.01	-.12	.76	-.33	.06	.25	-.08	-.40	-.08	.64	-.38	.24
BlackSterile mycelium	.67	.44	.21	-.12	-.03	-.08	.29	-.45	.72	.07	.30	.10	.21	.31
WhiteSterile *mycelium*	.55	-.37	-.44	-.11	-.31	-.14	-.36	.10	.63	-.10	-.58	.05	.37	-.25

Note: *Weak Loading (0.01 to 0.49), Moderate Loading (0.50 to 0.74), Strong Loading (≥0.75) and Significant Loading (~1) Sahu et al. 2018.*

study of different growth stages of chickpea plants (Table 8). While the correlation coefficient denoted with a significance 0.05 level of α and was significant at 0.01 level of α. The Dendrogram of Hierarchical cluster analysis was given in Fig. 4 for comparative study and measurement of visual dis-similarity in fungal species isolated in the rhizosphere and rhizoplane of chickpea plant. Bivariate analysis of variance (ANOVA) was applied by using LSD post hoc test on the growth of shoot, root, and several root nodules presented in Table 3, and isolated fungal species isolated in the rhizosphere, non-rhizospheric soil and rhizoplane given in Table: 2, of chickpea plant during foliar spray of gibberellic acid on different growth stage. The significant difference in mean value was tested at the .05 level through multi-comparisons between the different concentrations of gibberellic acid (25, 50, 100 ppm) with the control sample, 50 and 100 with 25 ppm of gibberellic acid, 50 ppm with 100 ppm of gibberellic acid their

Table 8: Spearman rho's Correlation coefficient in percentage occurrence Fungal Species rhizosphere and rhizoplane during the study of different growth stages of chickpea (*C. arietinum*) plant.

	(Rn)	*(Ml)*	*(Csc)*	*(Tl)*	*(Af)*	*(An)*	*(Al)*	*(Ac)*	*(At)*	*(Ans)*	*(Pc)*	*(Pf)*	*(Sa)*	*(Hg)*	*(Ch)*	*(Cl)*	*(Hs)*	*(Ah)*	*(Fu)*	*(Mr)*	*(BSm)*	*(WSm)*
Rhizopus nigricans (Rn)	1.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
Mucor luteus (Ml)	-.27	1.00	.00	-.17	**-.60[a]**	-.09	**.63[a]**	.00	.00	.00	-.33	.18	.00	.00	-.25	-.17	-.17	.00	-.37	-.25	-.17	-.29
Cephalo sporium coremiodes (Csc)	.46	-.11	1.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
Trichoderma lignorum (Tl)	.16	.19	**.77[b]**	1.00	.05	-.40	-.09	.00	.00	.00	-.17	.40	.00	.00	**.60[a]**	-.09	-.09	.00	.32	-.13	-.09	.04
Aspergillus flavous (Af)	.01	-.25	-0.12	-.42	1.00	.15	-.32	.00	.00	.00	.19	-.41	.00	.00	-.22	-.05	.50	.00	.37	-.03	-.32	-.16
A. niger (An)	**-.84[b]**	.14	-.36	.00	-.32	1.00	-.48	.00	.00	.00	.23	-.14	.00	.00	-.30	-.31	-.13	.00	-.30	-.09	-.04	.41
A. luchuensis (Al)	**.56[a]**	-.26	0.04	-.31	.24	**-.64[a]**	1.00	.00	.00	.00	-.17	-.17	.00	.00	-.13	-.09	-.09	.00	-.32	-.13	-.09	-.40
A. candidus (Ac)	.21	.30	**.69[b]**	**.77[b]**	-.33	.00	-.38	1.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
A. terreus (At)	.27	-.17	.28	-.05	.50	-.30	.37	-.04	1.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
nidulans (Ans)	**.59[a]**	.05	.39	.33	-.40	-.32	-.20	**.63[a]**	-.32	1.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
Penicillium citrium (Pc)	**.56[a]**	**-.67[b]**	.01	-.14	.03	-.23	.14	-.21	.08	.34	1.00	-.33	.00	.00	-.25	-.17	.46	.00	-.16	.29	-.17	-.23
Paecilomyces fusisporus (Pf)	**-.58[a]**	.05	-.45	**-.57[a]**	.08	.46	-.09	-.31	.04	-.42	-.29	1.00	.00	.00	**.68[a]**	-.17	-.17	.00	.00	-.25	.52	-.07
Stachybitrys atra (Sa)	.31	.49	.07	.20	-.25	-.10	.07	.38	.23	.34	-.13	-.31	1.00	.00	.00	.00	.00	.00	.00	.00	.00	.00
Humicola grisea (Hg)	.37	-.08	**.62[a]**	.23	-.04	-.33	.00	**.55[a]**	.07	**.53[a]**	.05	.05	-.20	1.00	.00	.00	.00	.00	.00	.00	.00	.00
Cladosporium herbarium (Ch)	**.67[b]**	-.14	.19	.14	.19	**-.80[b]**	.40	-.15	.16	0.15	.36	**-.61[a]**	-.07	.01	1.00	-.13	-.13	.00	-.04	-.20	**.74[b]**	.28
Curvularia lunata (Cl)	-.01	.05	.08	-.07	**.62[a]**	-.33	.27	-.26	.48	**-.53[a]**	-.11	.12	-.40	.08	.43	1.00	-.09	.00	.50	-.13	-.09	.22
Herliminthosporium sativum (Hs)	.18	-.45	.11	-.31	.19	-.24	.29	.07	.04	.19	-.04	.31	-.13	.39	-.22	-.28	1.00	.00	-.05	-.13	-.09	-.40
Alternaria humicola (Ah)	.22	.14	-.40	**-.51[a]**	.31	-.45	.30	**-.61[a]**	.04	-.07	.18	-.22	.08	-.28	.49	.21	-.25	1.00	.00	.00	.00	.00
Fusarium udum (Fu)	**-.51[a]**	-.01	-.19	.09	.14	.40	-.26	.11	-.31	-.32	-.35	.30	-.36	-.18	-.26	.14	.12	**-.58[a]**	1.00	-.07	-.32	-.23
Myrothecium roridum (Mr)	.34	-.27	.41	.12	.06	-.08	-.09	.29	**.54[a]**	.33	.36	-.20	.29	.39	-.10	-.22	.11	-.02	**-.64[a]**	1.00	-.13	-.05
BlackSterile mycelium (BSm)	.61	.05	.46	.38	-.42	-.37	-.06	**.60[a]**	.00	**.77[b]**	.29	-.21	.20	**.67[b]**	.31	-.16	.11	-.23	-.25	.31	1.00	.31
WhiteSterile mycelium (WSm)	**.50[a]**	-.42	.02	.18	-.08	-.38	.18	-.20	-.15	.21	**.64[a]**	**-.70[b]**	-.03	-.35	**.72[b]**	.08	.25	.27	.11	-.10	.11	1.00

Note: *Correlation significant at (a) 0.05 level of α and (b) 0.01 level of α*

Fungal	Spearman rho's correlation coefficient for isolated fungal species in rhizosphere
	Spearman rho's correlation coefficient for isolated fungal species in rhizoplane

effect on the growth of shoot, root and number of root nodules and fungal species isolated in the rhizosphere, non-rhizospheric soil and rhizoplane. The mean difference was significant at the .05 level with the control sample marked by "Single Star (*)", the difference is significant with the control sample and 25 ppm of gibberellic acid was marked by "Double Star (**)" and the difference was significant with the

Discussions

The finding of the present study indicated that the foliar spray of different concentrations of gibberellic acid enhanced the growth of shoot and root length but adversely effects on the number of nodules per plant, which was decreased on increasing the concentration of GA. Although gibberellic acid is one of the growth promoters, it enhanced shoot and root length.

but inhibited the root nodulation. Pratap and Singh (2020) was also observed similar findings by using different chemicals in leguminous crops and reported that nodulation in chickpeas was inhibited at higher concentrations (50 and 100 ppm) of fungicide (Bavistin) while at the initial concentration (25 ppm) the number of root nodules was significantly increased but in the case of use of Carbofuran pesticide inhibited the nodulation in chickpea plants on increasing concentration. Pratap and Singh, (2020) observed that the shoot and root length was significantly decreased on increasing the concentration of fungicide and insecticide, while the use of organic manure increases the number of nodules, shoot, and root length of the plant. Thus, use of chemicals in leguminous crops adversely affect root nodulation which is directly correlated with amino acid and protein synthesis which are concerned with the quality of leguminous crops.

The population of fungi was decreased on increasing the concentration of gibberellic acid which was due to the rhizosphere zone being rich in amino acids, carbohydrates, and growth-promoting substances in the form of root exudation, which have already been reported to have a stimulating effect on fungi. The age of the plant profoundly influenced saprophytic and parasitic rhizosphere mycoflora qualitatively as well as quantitatively (Vesely, 1985). The rhizosphere activity increased with the increase in the age of the plant, attaining the highest activity at the peak of vegetative growth of the plant (Khasanov, 1967; Mall, 1973).

The effect of foliar spray of hormones and antibiotic substances on rhizosphere mycoflora has been studied by many workers. Reddy (1968), working with rice seedlings, reported a decrease in rhizosphere effect in the case of a plant treated with hormones and antibiotics. Sethunathan (1970) observed a decrease in rhizosphere microflora in *Cajanus cajan* sprayed with gibberellic acid and maleic hydrazide. Singh (1982) studied the effect of foliar application of hormones on rhizosphere and rhizoplane mycoflora and reported that rhizosphere and rhizoplane mycoflora was enhanced due to spray. Dublish (1986) studied the effect of two hormones on the rhizosphere microflora of *Abelmoschus esculentus* and *Lageneria vulgaris* and reported the effect of both hormones on rhizosphere microflora was statistically significant. Jain and Gupta, 2000 observed a drastic reduction in the rhizosphere mycoflora at a higher concentration of gibberellic acid in *Vigna mungo*. There was an adverse effect of foliar application of indole acetic acid on the rhizosphere microflora of *Vicia faba* (Babu, 2004). Gupta, (1971) also reported adverse effects on rhizosphere microflora due to the foliar application of gibberellic acid. The reason for the decrease in the number of fungi in the rhizosphere soil of gibberellic acid-treated plants may be attributed to the following observed by different workers. Root exudates play an important role in influencing fungi in the rhizosphere (Rovira, 1956). Agnihotri (1964) observed an increase in the exudation of amino acids and glutamine, glucose, and fructose and a decrease in organic acid. Halleck and Cochrane (1950) reported the direct translocation of chemicals to roots and exudates in soil. Starkey (1958), Papavizas and Davey (1961) and Neal *et al*., (1964) reported that the rhizosphere microflora differs both qualitatively and quantitatively from the general soil microflora. Kumar and Gupta (2006) reported a maximum number of fungi/g of dry soil at the early stage. Jia *et al*., (2018) found that restoration time plays the most significant role in the bacterial and fungal composition and bacterial diversity, but it has no effect on fungal diversity in the rhizosphere and non-rhizosphere soil. In addition, the driving factors of microbial composition and diversity varied in the rhizosphere and non-rhizosphere soil among the different restoration time treatments. Gupta and Paliwal (2009) also observed a significant quantitative difference between the rhizosphere and non-rhizosphere microflora which is evident by the R/S ratio. They also observed a maximum number of fungal species at an early stage.

In the present study, a maximum number of amino acids were observed at the pre-flowering stage, which was decreased at the flowering and fruiting stages, which may be one of the reasons for the maximum number of fungi at the pre-flowering stage. On foliar spray of gibberellic acid fungal species decreased on increasing GA concentration wise versa fungal species also decreases which proves that amino acids are a responsible increase in the fungal population in the rhizospheric zone. This finding was in agreement with the findings of Gupta and Paliwal (2009). Bhuvaneshwari and Rao (1957) also reported that root exudates are the main factor that influences the rhizosphere microflora. Its fluctuation with the age of plants has been correlated with the quality and quantity of root exudation, which is supposed to change with the age of plants.

Factor Analysis extracted seven factors with 88 and 91% variance for both rhizosphere and rhizoplane. In the rhizosphere, factor one is strongly positively loaded with *Rhizopus nigricans* and strongly negatively loaded with *Paecilomyces fusisporus* and *A. niger*, while factor 1 in rhizoplane was strongly positively loaded with *Aspergillus flavus* but negatively loaded with *Mucor luteus*. In the rhizosphere, factor two is strongly positively loaded with *Aspergillus candidus*, while factor two has not loaded strongly with any fungus species. Fungal species loading in extracted factors in which values between 0.50 to 0.74 show moderate loading, 0.01 to 0.49 present weak loading given in Table: 7, and negative symbol (-) represents negative load.

Spearman rho's correlation coefficient was conducted for the identification of the significant correlation among the fungal species. A correlation was found significant at 0.05 level of α in the rhizosphere for *A. luchuensis, Aspergillus nidulans, Penicillium citrium, Paecilomyces fusisporus, Fusarium udum,* and white

sterile mycelium with *Rhizopus nigricans. Humicola grisea* with *Cephalosporium coremiodes, Paecilomyces fusisporus Alternaria humicola* with *Trichoderma lignorum, Curvularia lunata* with *Aspergillus flavous, Aspergillus nidulans Humicola grisea, Alternaria humicola* black sterile mycelium with *Aspergillus candidus, Myrothecium roridum* with *Aspergillus terreus*, *Stachybitry satra, Curvularia lunata* with *Aspergillus nidulans,* White Sterile mycelium with *Penicillium citrium, Cladosporium herbarium* with *Paecilomyces fusisporus, Fusarium udum* with *Alternaria humicola, Myrothecium roridum* with *Fusarium udum*. The correlation was found significant at 0.05 level of α in *Aspergillus flavous and A. luchuensis with Mucorluteus, Trichodermalignorum,* and *Paecilomyces fusisporus with Cladosporium herbarium* for rhizoplane.

A correlation was found significant at 0.01 level of α in *Aspergillus niger* and *Cladosporium herbarium* with *Rhizopus nigricans, Penicillium citrium* with *Mucor luteus, Trichoderma lignorum* and *Aspergillus candidus* with *Cephalosporium coremiodes, Aspergillus candidus* with *Trichoderma lignorum, Cladosporium herbarium* with *Aspergillus niger,* Black Sterile mycelium with *Aspergillus nidulans,* White Sterile mycelium with *Paecilomyces fusisporus,* Black Sterile mycelium with *Cladosporium herbarium* for rhizosphere, while correlation was found significant at 0.01 level of α only between *Cladosporium herbarium and* Black Sterile mycelium in rhizoplane.

The hierarchical cluster analysis was executed in the SPSS software to explore the percentage occurrences of fungal species in the sample collected from the rhizosphere and rhizoplane. The distance was calculated through a rescaled distance cluster combined using the ward linkage methods. The hierarchical cluster analysis is an advanced technique for diagrammatic comparison in the data set; it creates a tree plot where the data set contributed within the branches that are similar or close together and deviation or dissimilar data set separated in other branches (Sahu *et al*., 2018). The classified fungal species presented in the dendrogram of hierarchical cluster analysis are given in Fig. 4 (a) for the rhizosphere and Fig. 4 (b) for rhizoplane.

The dendrogram of HCA classifies fungal species in the rhizosphere given in Figure 4. (a) clustered into two major clusters which are further divided into thirteenth sub-clusters step by step, Black sterile mycelium*, Humicolagrisea* and *Herliminthosporiumsativum* represented sub- clusters 1, *Cephalosporiumcoremiodes* separated in sub-clusters 2, *Trichodermalignorum, A.candidus,* and *A.nidulan*re presented sub- clusters 3, *Stachybitrysatra*alienated in sub-clusters 4, *Myrothecium roridum*and*A.terreus*formedsub- clusters 5, *Cladosporium herbarium* and *Curvularialunata* represented sub- clusters 6, *Penicilliumcitrium and Rhizopusnigric an created sub- clusters* 7, *A.luchuensis,* separated in sub-clusters 8, *White sterile mycelium* and *Aspergillus flavous* madesub- clusters 9, *Alternariahumicola* separated in sub-clusters 10, *Mucor luteus* and *Paecilomyces fusisporus* produced sub- clusters 11, *Fusariumudum shaped* sub- clusters 12, and *A.niger*separated in sub-clusters 13 respectively. The dendrogram of HCA classifies fungal species is given in Fig. 4(b) clustered into two major clusters which are further divided into eight sub- clusters step by step, *Trichodermalignorum,* and *Curvularialunat a*re presented sub-clusters 1, *Cladosporium herbarium* and *Black sterile mycelium* represented sub- clusters 2, *Herliminthosporiumsativum* and *Paecilomycesfusisporus* separated in sub-clusters 3 and 4, *Penicilliumcitrium* and *Myrothecium roridum* formed sub- clusters 5, *Mucorluteus* and *A.luchuensis*re presented sub- clusters 6, *Penicillium citrium* and *Aspergillus flavus* and *Fusariumudum shaped* sub- clusters 7, *A.niger*and *White sterile mycelium* built sub- clusters 8 respectively.

Conclusion

Chickpeas (*C. arietinum* L.), is an important crop of dryland farming and are a good source of protein. Numerous factors, such as the amount and quality of root exudates, affect the rhizosphere and root colonization. Additionally, organic acids, which are essential for regulating plant-microbe interactions, were a significant component of root exudates. It is commonly recognized that the physiology of the related mycoflora is influenced by the nutritional state of the plants and vice versa. The rhizosphere population is influenced by root exudates, which are the main source of nutrients that are accessible close to plant roots. Factor Analysis extracted 7 factors with 88 and 91 % variance for both rhizosphere and rhizoplane. Fungal species loading in extracted factors showed moderate loading (between 0.50 to 0.74) and weak loading (0.01 to 0.49). There was a significant fungal species correlation. The dendrogram of HCA classifies fungal species in the rhizosphere and was clustered into two major clusters which were further divided into thirteenth sub-clusters. The foliar spray of GA increases the occurrence of some fungal species while in some, it decreases and in some, it was checked. Thus application of gibberellic acid influenced the rhizosphere fungal microflora in chickpea plants.

Authors Contributions

Shalini G Pratap: Investigation, Conceptualization, and writing- original draft preparation Vinod Kumar: Revised draft preparation and methodology and statistics. Pokhraj Sahu: Assist with draft preparation and guide for experiment design. Pramod Kumar Singh: Supervision, Reviewing, and Editing.

Competing interest

The author declares no competing interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Agnihothrudu, V. (1955). State in which fungi occur in the rhizosphere. Naturwissenschaften, 42(18), 515-516. https://doi.org/10.1007/BF00601214

Agnihotri, V. P. (1964). Effect of the foliar spray of urea on *Aspergilli* of the rhizosphere of *Triticum vulgare* I. *Plant and Soil, 10*(3), 364-370.

Babu, M. (2004). *Study of rhizosphere mycoflora and foliar spray of certain chemicals in relation to nodulation and growth of Vicia faba Linn* (PhD thesis). Dr. B.R. Ambedkar University, Agra.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology, 57*, 234-266.

Bano, A., & Harper, J. E. (2002). Plant growth regulators and phloem exudates modulate root nodulation of soybean. *Functional Biology, 29*(11), 1299-1307.

Gupta, S., & Paliwal, S. P. (2009). Study of rhizosphere, non-rhizosphere, and rhizoplane mycoflora at different stages of growth of *Cicer arietinum*. *Journal of Phytology Research, 22*(2), 287-290.

Barnett, H. L. (1962). *Illustrated genera of imperfect fungi*. Burgess Publishing Co.

Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology and Ecology, 68*(1), 1-13.

Bhuvaneshwari, K., & Subba Rao, N. S. (1957). Root exudates in relation to rhizosphere effect. *Proceedings of the Indian Academy of Sciences, 45*, 299-301.

Block, R. J. (1950). Estimation of amino acids and amides on paper chromatography. *Analytical Chemistry, 22*(10), 1327-1332.

Broeckling, C. D., Broz, A. K., Bergelson, J., Manter, D. K., & Vivanco, J. M. (2008). Root exudates regulate soil fungal community composition and diversity. *Applied and Environmental Microbiology, 74*(3), 738-744.

Clark, F. E. (1949). Soil microorganisms and plant roots. *Advances in Agronomy, 1*, 214-288.

Clements, F. E., & Shear, C. L. (1954). *The genera fungi*. Hofner Publishing Co.

Dublish, P. K. (1986). Foliar application of antibiotics, growth hormones, and urea in relation to rhizosphere mycoflora of *Abelmoschus esculentus* and *Lagenaria vulgaris*. *Indian Phytopathology, 39*(2), 264-268.

Fletcher, W. W., Alcorn, J. W. S., & Raymond, J. C. (1959). Gibberellic acid and nodulation of legumes. *Nature, 184*, 1576.

Galston, A. W. (1959). Gibberellic acid and nodulation. *Nature, 184*, 545.

Garg, N., Garg, O. P., & Dua, T. S. (1993). Effect of gibberellic acid and kinetin on nodulation and nitrogen fixation in chickpea. *Indian Journal of Plant Physiology, 35*(4), 401.

Gian, J. C. (1956). *A manual of soil fungi*. Constable and Co. Ltd.

Gupta, P. C. (1971). Foliar spray of gibberellic acid and its influence on the rhizosphere mycoflora. *Plant and Soil, 34*, 233-236.

Halleck, F. E., & Cochrane, V. W. (1950). The effect of fungistatic agents on the bacterial flora of the rhizosphere. *Phytopathology, 40*, 715-718.

Harley, T. L., & Waid, J. S. (1955). A method of studying active mycelia on living roots and other surfaces in the soil. *Transactions of the British Mycological Society, 38*, 104-118.

Jain, V., & Gupta, V. K. (2000). Effect of foliar spray of gibberellic acid on nodule number, shoot, and root length. Acta Botanica Indica, 28, 91-92.

Jia, T., Cao, M., & Wang, R. (2018). Effects of restoration time on microbial diversity in rhizosphere and non-rhizosphere soil of Bothriochloa ischaemum. International Journal of Environmental Research and Public Health, 15(10), 2155. https://doi.org/10.3390/ijerph15102155

Khasanov, O. K. (1967). Distribution of Penicillium spp. in the rhizosphere of Hibiscus cannabinus in a meadow marsh soil of Chirckik River Valley. Uzbek Biological Journal, 11(1), 12-14.

Kumar, A., & Gupta, V. K. (2006). Effect of foliar spray of gibberellic acid on the rhizosphere and rhizoplane mycoflora of Vigna sinensis L. Journal of Phytology Research, 20(1), 103-109.

Kumar, S., & Kumar, M. (2006). Studies on the rhizosphere mycoflora of Coriandrum sativum L. International Journal of Mendel, 23(1-2), 25-26.

Maity, U., & Bera, A. K. (2008). Effect of foliar application of brassionlide and salicylic acid on growth, yield parameters, and yield of mung bean (Vigna radiata (L.) Wilzek). Indian Journal of Botanical Research, 4(2), 267-274.

Mall, S. (1973). Studies on microbial antagonism in rhizosphere soils of potato varieties differentially susceptible to black scurf and wilt. Plant and Soil, 9(3), 547-554.

Manoharachary, C., & Mukerji, K. G. (2006). Rhizosphere biology – an overview. In K. G. Mukerji, C. Manoharachary, & J. Singh (Eds.), Microbial activity in the rhizosphere (pp. 1-15). Springer.

Mohammad, G. (1985). Influence of alachlor and allechloralin on the rhizosphere and rhizoplane fungi of soybean crop. Journal of Maharashtra Agricultural University, 9(3), 303-305.

Neal, J. I., Bollen, W. B., & Zak, B. (1964). Rhizosphere microflora associated with the mycorrhizae of Douglas fir. Canadian Journal of Microbiology, 10(2), 259-265.

Okorski, A., & Barbara, M. (2007). Fungi isolated from soil before the seedling and after harvest of pea (Pisum sativum L.) after application of biocontrol product EMI. Agrobotanica, 60(1), 113-121.

Papavizas, D. G. C., & Davey, C. B. (1961). Extent and nature of the rhizosphere of Lupinus. Plant and Soil, 14, 215-236.

Pratap, G. S., & Singh, P. K. (2020). Impact of chemicals (pesticide and fungicide) and organic manure on root nodulation, growth of root and shoot of chickpea (Cicer arietinum). Plant Archives, 20, 254-260.

Raaijmakers, J. M., Paulitz, C. T., Steinberg, C., Alabouvette, C., & Moenne-Loccoz, Y. (2009). The rhizosphere: A playground and battlefield for soil-borne pathogens and beneficial microorganisms. Plant and Soil, 321, 341–361. https://doi.org/10.1007/S11104-008-9568-6

Ramachandra-Reddy, T. K. (1968). Plant treatment in relation to rhizosphere effect. I. Pretreatment of root of rice seedlings to certain chemicals and antibiotics on the rhizosphere microflora. Plant and Soil, 28, 242-336.

Raper, K. B., & Fennel, D. I. (1965). The genus Aspergillus. Williams and Wilkins Co.

Rovira, A. D. (1956). Plant root excretions in relation to the rhizosphere effect. Plant and Soil, 7, 178-194.

Sahu, P., Kisku, G. C., Singh, P. K., Shukla, V. K., Kumar, P., & Shukla, N. (2018). Multivariate statistical interpretation on seasonal variations of fluoride-contaminated groundwater quality of Lalganj Tehsil, District Raebareli (UP) India. Environmental Earth Sciences, 77, 484. https://doi.org/10.1007/s12665-018-7658-1

Sethunathan, N. (1970). Foliar sprays of growth regulators and rhizosphere effect in Cajanus cajan Mill. Sp. I. Quantitative change. Plant and Soil, 33, 71-78.

Singh, G. N. (1982). Effect of foliar application of hormone on rhizosphere and rhizoplane mycoflora of oil-yielding plants. Acta Botanica Indica, 17(2), 218-227.

Starkey, R. I. (1958). Interactions between microorganisms and plant roots in the rhizosphere. Bacteriological Reviews, 22, 154-160.

Tamilarasi, S., Nanth, K. K., & Lakshmanaperumalsamy, P. (2008). Diversity of root-associated microorganisms of selected medicinal plants and influence of rhizomicroorganisms on the antimicrobial property of Coriandrum sativum L. Journal of Environmental Biology, 29(1), 127-134.

Uren, N. C. (2000). Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. In Pinton, R., Vrani, Z., & Hanniperi, P. (Eds.), The rhizosphere: Biochemistry and organic substance at soil interface (pp. 19-40). Marcel Dekker Inc.

Vaidehi, B. K., & Farzana, A. (1980). Rhizosphere microflora of pearl millet (Pennisetum typhoides) cultivar BHU. National Academy of Science, 2(8), 255-257.

Vesely, D. (1985). Changes in the distribution of microfungi in the rhizosphere of sugar beet. Sobornik UVTIZ Ochrana Roslin, 21(3), 185-194.

Yu, C., Liu, D. G., Qin, S. J., Guo-Dong, & Liu, G. C. (2007). Microbial flora in Cerasus sachalinensis. Yingyong Shengtai Xuebao, 18(10), 2277-2281.