Potentials of Co-inoculation of Microbial Organisms and Sewage Sludge on Growth of a Pulse Crop and Microbial Population

Mudassara Hasan and Fauzia Naushin

Research Journal of Agricultural Sciences
An International Journal

P- ISSN: 0976-1675
E- ISSN: 2249-4538

Volume: 13
Issue: 04

Res. Jr. of Agril. Sci. (2022) 13: 1046–1050

CARAS

www.rjas.org

Res. Jr. of Agril. Sci. (Jul-Aug 2022)
13(4): 1046–1050

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

Full Length Research Article

Potentials of Co-inoculation of Microbial Organisms and Sewage Sludge on Growth of a Pulse Crop and Microbial Population

Mudassara Hasan*[1] and Fauzia Naushin[2]

Received: 27 Apr 2022 | Revised accepted: 14 Jul 2022 | Published online: 16 July 2022

ABSTRACT

A field study was carried out to determine the interactive effects of two species of arbuscular mycorrhizal fungi [*Funneliformis caledonius* (Fc) and *Glomus bagyarajii* (Gb)], *Rhizobium* (R), and sewage sludge (SS) on the growth, physiology, microbial population and N, P, K content in chickpea grown in autoclaved garden soil (S) and soil mixed with 20% sewage sludge. The growth parameters of chickpea were recorded 60 days after sowing. The plant height, fresh and dry weight, biochemical content (total chlorophyll, carotenoid, protein content and nitrate reductase activity), microbial population (Rhizobial nodule count and percent root colonization by AM fungi) as well as N, P, K content increased significantly on application of 20% sewage sludge in the soil. Microbial inoculation in the sewage sludge amended soil enhanced all plant growth and biochemical parameters. The highest plant growth response was obtained in the treatment SS + R + Gb. The proline content (an indicator of plant stress) was highest in chickpea raised in soil amended with sewage sludge.

***Key words*:** *Funneliformis caledonius, Glomus bagyarajii, Rhizobium,* Sewage sludge, Chickpea

The agriculture sector is the mainstay of the Indian economy; more than half of India's population is wholly or significantly dependent on agriculture and allied activities for their livelihood [1]. Pulses, a rich source of protein are grown in places with unique agro-climatic conditions and are essential dietary supplement for vegetarian Indians. Chickpea (*Cicer arietinum* L.) is India's oldest and most widely cultivated pulse crop. It is distinctive due to its high protein content to about 40% of its weight [2].

Plant-growth-promoting symbiotic rhizobacteria, mainly *Rhizobium*, invade root cells of legumes and forms root nodules where in the bacteria fix atmospheric nitrogen into the form available to the host [3], while arbuscular mycorrhizal fungus (AMF) also forms symbiotic association with the host plant and produce highly convoluted projections termed as arbuscules within the root cortex, that serve as a nutrient exchange site (mainly phosphorus) for the plant [4]. The interaction between the two symbiotic species with the legume provides sustenance and defence from soil-borne pathogens to the host plant [5]. The tripartite symbiotic associations between *Rhizobium*, AMF and legumes show the intricacy of microbial interactions, leading to increased plant tolerance to environmental stress [6-7].

In agricultural lands, the application of sewage sludge has become a widely recognized practice for soil protection and residual disposal. Sewage sludge, a by-product of treated wastewater, is a source of organic matter, nitrogen, phosphorus and other nutrients. If handled properly and used in appropriate amounts, the sewage sludge application can enhance organic fertility of degraded lands [8]. Judicious application of sewage sludge may reduce the economy burden of synthetic fertilizers [9] in addition to environment threats of unsafe sewage sludge disposal and otherwise adverse effects of fertilizers on soil properties. Overuse of sewage sludge is injurious to plant health and food chain organisms due to presence of heavy metals like As, Cd, Cr, Cu, Hg, Ni, Pb and Zn in it [10-11]. These toxic elements enter the food chain and cause poisoning in humans and other food chain organisms [12-13]. The present study was designed to evaluate the co-inoculation potential of sewage sludge, AMF (*Funneliformis caledonius* and *Glomus bagyarajii*) and *Rhizobium* to improve growth, and physiology, nodulation, nutrient uptake and root colonization by AM fungi in chickpea crop.

MATERIALS AND METHODS

Preparation of soil-sewage sludge mixture

The garden soil for the experiment was collected from the Department of Botany, AMU and divided into two equal sections. One section of the soil was autoclaved at 121°C for 20 minutes, the other half was amended with the 20% dried sewage

* **Mudassara Hasan**
✉ mudassarahasan93@gmail.com

[1] Department of Botany, Aligarh Muslim University, Aligarh - 202 001, Uttar Pradesh, India

[2] Botany Section, Women's College, Aligarh Muslim University, Aligarh - 202 001, Uttar Pradesh, India

sludge obtained from AMU's sewage treatment plant. The sewage sludge collected was air-dried, powdered, and sieved through a 2 mm mesh. The soil and sludge mixture (80:20 w/w) was then autoclaved.

Pot experiment

The pots were filled with sterilized garden soil and equal number of pots with sewage sludge amended soil and placed in a glasshouse. The pure cultures of two AMF (*F. caledonius* and *G. bagyarajii)* obtained from the CNBRCD's Bangalore culture collections were multiplied separately prior to the experiment. Seeds of chickpea (*Cicer arietinum* L.) obtained from authentic seed seller, Aligarh were surface-sterilized for 15 minutes with 0.5 percent sodium hypochlorite and then rinsed with distilled water.

The certified and viable *Rhizobium* culture-specific for chickpea was obtained from Krishi Vigyan Kendra, Aligarh. The surface sterilized chickpea seeds were treated with *Rhizobium* culture mixed with jaggery and then dried in the shade before sowing. 10g of each AMF inoculum was filled separately in the potting hole for treatments with two separate AMF. The experiment thus had the following 12 treatments (6 with garden soil alone and 6 with soil amended with 20% sewage sludge). Treatments included:

i) Control Soil (S)	vii) S with sewage sludge (SS)
ii) S + *Rhizobium* (R) (S+R)	viii) SS + *Rhizobium* (SS + R)
iii) S + *Funneliformis caledonius* (Fc) (S + Fc)	ix) SS + *Funneliformis caledonius* (SS + Fc)
iv) S + *Glomus bagyarajii* (Gb) (S + Gb)	x) SS + *Glomus bagyarajii* (SS + Gb)
v) S + R + Fc	xi) SS + R + Fc
vi) S + R + Gb	xii) SS + R + Gb

Each of these treatments were replicated three times. The experiment was terminated 60 days after sowing of chickpea seeds to determine the factor resulting in optimal plant growth, physiological parameters, microbial population and nutrient contents. The plant height was determined using a metric scale. Plants were weighed separately to record their fresh weight. The dry weight of plants was determined after the samples were dried at 60°C to a constant weight. The photosynthetic pigments in leaves were estimated following the standard protocols given by Mackinney [14], while the nitrate reductase activity, protein content and proline (indicator of plant stress) in leaves was determined by the methods of Jaworski [15], Lowry *et al.* [16] and Bates *et al.* [17] respectively. Root nodules per plant were counted, and percent root colonization by AM fungi was determined. The roots were cut into segments of 1 cm, stained with trypan blue [18], and observed under the microscope to determine percent mycorrhizal root colonization by the method of Giovannetti and Mosse [19]. Plant samples were powdered and were digested following the standard procedures outlined by Lindner [20] for the estimation of N, P and K.

The statistical analysis was performed by one-way ANOVA using SPSS version 17 (SPSS, Chicago, IL, USA). Duncan's multiple range test analyzed the significant difference among treatments at $p < 0.05$.

RESULTS AND DISCUSSION

The amendment of soil with *Rhizobium* or either of the selected species of AM fungi increased the chickpea plant length, fresh weight, dry weight and N, P, K contents significantly. These findings established that symbiotic association with any of these three micro-organisms enhanced the uptake of N, P, K in the host plant and in turn, increased the total length, fresh and dry weight (Table 1). Soil microbes play a significant role in increasing the uptake of nutrients beneficial for plant growth and yield [21-22]. There was no significant difference ($p > 0.05$) in the growth of chickpea plant in soil inoculated singly with either of three symbionts (*Rhizobium*, *F. caledonius* and *G. bagyrajii*). The growth responses on combined application of *Rhizobium* and either of AM fungi in garden soil was relatively better than on application of a single microbe in 60 days old chickpea plant (Table 1).

Table 1 Impact of inoculation of AMF and/or *Rhizobium* on growth of 60 days old chickpea grown in garden soil with or without sewage sludge application

Treatments	Total length (cm)	Fresh weight (g)	Dry weight (g)	Nitrogen (mg/g)	Phosphorus (mg/g)	Potassium (mg/g)
Soil	15.50±1.36^{g}	11.77±1.00^{h}	2.70±0.12^{h}	1.76±0.09^{d}	0.15±0.02^{g}	1.02±0.08^{f}
Soil + *Rhizobium*	20.10±1.39^{f}	15.80±1.64^{g}	3.20±0.17^{g}	2.09±0.09^{cd}	0.18±0.02^{fg}	1.15±0.08^{ef}
Soil + *Funneliformis caledonius*	20.50±0.83^{f}	17.47±1.05^{fg}	3.90±0.12^{f}	2.11±0.09^{cd}	0.18±0.01^{fg}	1.18±0.09^{ef}
Soil + *Glomus bagyarajii*	21.90±1.07^{ef}	18.53±1.03^{efg}	3.30±0.12^{g}	2.16±0.15^{cd}	0.19±0.01^{efg}	1.24±0.10^{def}
S + R + Fc	25.00±1.59^{e}	20.47±1.56^{def}	4.60±0.12^{de}	2.16±0.15^{cd}	0.21±0.02^{cdef}	1.32±0.07^{cdef}
S + R + Gb	25.50±0.92^{e}	21.20±1.53^{cdef}	4.30±0.12^{ef}	2.07±0.15^{cd}	0.22±0.01^{cdef}	1.37±0.09^{cde}
20% sewage sludge in garden soil	30.30±1.25^{d}	22.40±1.25^{cde}	4.83±0.15^{d}	2.28±0.14^{bc}	0.20±0.01^{def}	1.35±0.09^{cdef}
SS + R	33.27±1.17^{cd}	23.53±1.66^{cd}	5.77±0.18^{c}	2.30±0.11^{bc}	0.24±0.02^{bcde}	1.42±0.10^{cde}
SS + Fc	35.53±1.26^{bc}	23.33±1.19^{cd}	5.83±0.15^{c}	2.27±0.12^{bc}	0.25±0.02^{bcd}	1.52±0.14^{bcd}
SS + Gb	35.47±1.26^{bc}	25.17±1.09^{c}	7.10±0.17^{b}	2.40±0.12^{bc}	0.26±0.02^{bc}	1.60±0.11^{bc}
SS + R + Fc	38.27±1.01^{b}	29.27±1.65^{b}	6.70±0.12^{b}	2.60±0.10^{ab}	0.29±0.01^{ab}	1.78±0.12^{ab}
SS + R + Gb	43.1±1.55^{a}	33.37±1.30^{a}	7.63±0.18^{a}	2.90±0.11^{a}	0.33±0.02^{a}	2.04±0.09^{a}

Mean ± SE

S=soil, R= *Rhizobium,* Fc= *Funneliformis caledonius*, Gb= *Glomus bagyarajii* and SS= 20% sewage sludge in garden soil.

Different alphabets in each stage show statistically significant variation at $p < 0.05$ as per DMRT

The addition of 20% sewage sludge in soil even without AMF enhanced the total plant length, fresh and dry weight and N, P, K content significantly than the growth performance of chickpea plant grown either in garden soil with or without selected symbiotic microbes (Table 1). The increase in plant biomass on sewage sludge amendment is in conformity with the findings of Dar *et al.* [23]. The nutrient rich sewage sludge has been found beneficial for plant growth [24-25]. It was suggested that low amounts of sewage sludge in the soil increased the plant length [26]. The growth response of chickpea in sewage sludge amended soil was better on inoculation with either of the selected AM fungi as compared to inoculation of *Rhizobium* alone. The tripartite symbiosis (chickpea, *Rhizobium* and either of selected AM fungi) increased the plant growth and N, P, K uptake to a relatively greater extent than on symbiosis with anyone of the microbes. The higher growth of chickpea plant was recorded in sewage sludge amended soil and combined application of *Rhizobium* and *Glomus bagyarajii* (Table 1). Higher N, P, K uptake in symbiotically associated host plants has also been reported earlier [27-28].

Table 2 Impact of inoculation of AMF and/or *Rhizobium* on biochemical parameters of 60 days old chickpea grown in garden soil with or without sewage sludge application

Treatments	Total chlorophyll (mg/g FW)	Carotenoid (mg/g FW)	NRA ($\mu mh^{-1}g^{-1}$)	Protein (mg/g FW)	Proline (µmol/g FW)
Soil	1.68 ± 0.14^{d}	0.16 ± 0.03^{h}	0.39 ± 0.03^{g}	20.40 ± 1.59^{f}	15.13 ± 1.13^{cde}
Soil + *Rhizobium*	1.98 ± 0.16^{cd}	0.25 ± 0.03^{gh}	0.42 ± 0.02^{fg}	23.90 ± 2.08^{ef}	13.23 ± 1.27^{de}
Soil + *Funneliformis caledonius*	2.18 ± 0.17^{bc}	0.22 ± 0.03^{h}	0.44 ± 0.02^{efg}	25.80 ± 1.95^{def}	12.57 ± 0.97^{e}
Soil + *Glomus bagyarajii*	2.26 ± 0.12^{bc}	0.28 ± 0.04^{fgh}	0.45 ± 0.02^{efg}	27.20 ± 2.01^{de}	11.93 ± 1.36^{e}
S + R + Fc	2.37 ± 0.19^{abc}	0.39 ± 0.04^{efg}	0.48 ± 0.01^{def}	30.90 ± 1.97^{bcd}	16.37 ± 1.07^{bcde}
S + R + Gb	2.38 ± 0.15^{abc}	0.41 ± 0.06^{ef}	0.50 ± 0.02^{cde}	31.43 ± 1.63^{bcd}	16.10 ± 0.98^{bcde}
20% sewage sludge in garden soil	2.46 ± 0.18^{abc}	0.45 ± 0.02^{de}	0.46 ± 0.02^{def}	28.60 ± 2.25^{cde}	23.23 ± 1.27^{a}
SS + R	2.49 ± 0.15^{abc}	0.51 ± 0.03^{cde}	0.52 ± 0.02^{bcd}	31.67 ± 2.15^{bcd}	17.67 ± 1.82^{bcd}
SS + Fc	2.50 ± 0.16^{abc}	0.55 ± 0.05^{cd}	0.53 ± 0.02^{bcd}	34.10 ± 1.82^{abc}	18.97 ± 1.46^{abc}
SS + Gb	2.47 ± 0.14^{abc}	0.64 ± 0.07^{bc}	0.55 ± 0.02^{abc}	35.07 ± 1.62^{ab}	18.83 ± 1.22^{abc}
SS + R + Fc	2.60 ± 0.14^{ab}	0.74 ± 0.05^{b}	0.58 ± 0.02^{ab}	37.17 ± 1.83^{ab}	20.40 ± 1.62^{ab}
SS + R + Gb	2.84 ± 0.15^{a}	0.93 ± 0.05^{a}	0.60 ± 0.02^{a}	39.03 ± 2.05^{a}	19.97 ± 1.99^{ab}

Mean ± SE

S=soil, R= *Rhizobium,* Fc= *Funneliformis caledonius*, Gb= *Glomus bagyarajii* and SS= 20% sewage sludge in garden soil.

Different alphabets in each stage show statistically significant variation at $p < 0.05$ as per DMRT

Besides beneficial effects of the selected microbes on the growth of chickpea, some plant metabolism indicators viz. total chlorophyll, carotenoids, nitrate reductase activity and protein content also increased in host plants on inoculation of *Rhizobium* and selected AM fungi (Table 2). Samar and Kumar [29] reported the beneficial effects of co-inoculation of symbiotic microbes increased the photosynthetic activity and nutrient uptake in pulses. The significant increase in chlorophyll content on inoculation of AM fungi are in agreement with the results of Lin *et al.* [30] as an outcome of increased growth [31-32]. The improvement in nitrate reductase activity and protein content in the host plant with a tripartite association have been reported earlier [33-34].

The proline content in chickpea grown in garden soil alone increased as compared to plants inoculated with either *Rhizobium* or any other selected AM fungi (Table 2). This finding indicates that plant stress reduced in association with any one of these microbes. But, the combination of *Rhizobium* and either of AM fungi increased proline content as compared to their single inoculation (Table 2). The increase in proline content was high in chickpea grown in sewage sludge amended in garden soil. The proline accumulation in host plants grown in sewage sludge amended soil decreased when inoculated with any one of the selected microbes (Table 2). This response further establishes that all three microorganisms including *Rhizobium* or either of selected species of AM fungi reduced plant stress caused by sewage sludge application (Table 2). Some plant species under different environmental stresses shows proline accumulation [35]. Proline accumulation play a vital role in stressed plants [36]. Microbial inoculation in plants indicates altered efficacies in combating the stress among mycorrhizal plants and proline content act as a dynamic factor to assess stress tolerance [37]. Alternatively, there are also reports which show that AMF inoculation considerably decreased proline accumulation [38] while some studies demonstrate the increase [39-40].

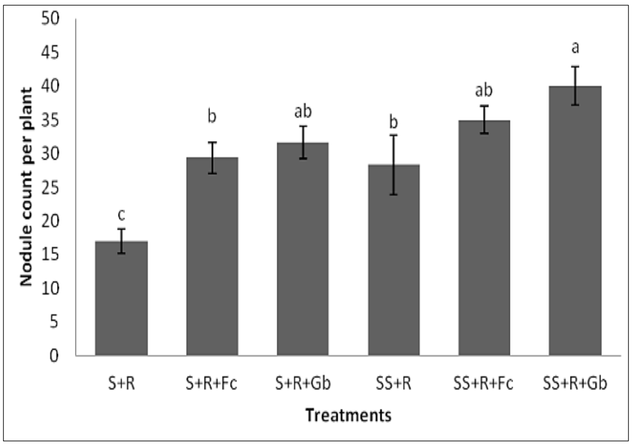

Fig 1 Impact of inoculation of AMF and/or *Rhizobium* on nodule numbers of 60 days old chickpea grown in garden soil with or without sewage sludge application. Different alphabets in each stage show statistically significant variation at $p < 0.05$ as per DMRT. Legend as in Table 1 and 2.

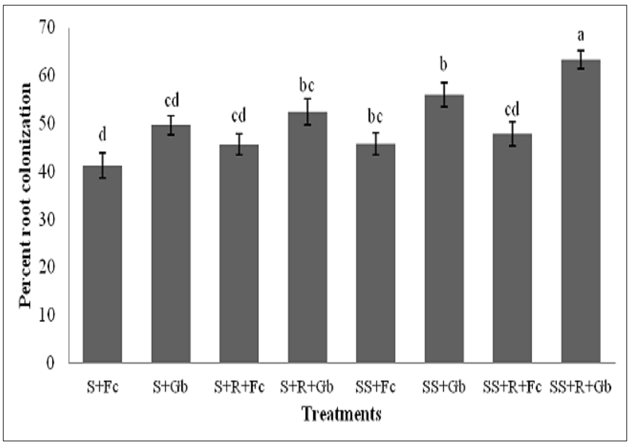

Fig 2 Impact of inoculation of AMF and/or *Rhizobium* on percent root colonization of 60 days old chickpea mycorrhizal population grown in garden soil with or without sewage sludge application. Different alphabets in each stage show statistically significant variation at $p < 0.05$ as per DMRT. Legend as in Table 1 and 2.

The nodule count in *Rhizobium* inoculated plant with or without AMF increased in the order as- S+R < SS+R < S+R+Fc < S+R+Gb < SS+R+Fc <SS+R+Gb (Fig 1). A negative correlation between symbiotic nitrogen fixation and the available soil nitrogen has been reported in legumes [41]. Low nodule count in sewage sludge treated soil inoculated with *Rhizobium* might be because sewage sludge is nutrient rich and studies have revealed that nitrogen fixers works better in nitrogen deficient soils [42]. Nitrogen application reduced symbiotic nitrogen fixation in beans [43]. The per cent root colonization by AM fungi also increased in order as- S+Fc < S+R+Fc < SS+Fc < SS+R+Fc < S+Gb < S+R+Gb < SS+Gb < SS+R+Gb (Fig 2). *G. bagyarajii* treated plants show better root colonization than *F. caledonius* in both single or dual inoculation with *Rhizobium*. Similar studies of symbiosis with increased nodulation and mycorrhizal root colonization on other leguminous crops have been reported [44-46].

CONCLUSION

The findings have shown synergistic effects of AMF, *Rhizobium* and sewage sludge amendments, when applied either singly or in combinations, significantly increased length, fresh and dry weight, chlorophyll, carotenoid, NRA and protein content and nutrient uptake in 60 days old chickpea plants However, the most significant values were obtained from all three. The beneficial impact of microbes was relatively lesser in soil alone. The nodulation was affected in soil and soil amended with sewage sludge but co-inoculation with AM fungi increased the nodulation in addition to root colonization particularly in response to *G. bagyarajii* while proline content increases in soil amended with 20% sewage sludge alone.

Acknowledgements

Authors are thankful to Department of Botany, Aligarh Muslim University, Aligarh, for providing laboratory facilities and encouragement during the course of investigation and Dr. D. J. Bagyaraj for providing necessary AMF culture for the study.

Conflict of interest

The authors declared no conflict of interest.

LITERATURE CITED

1. Government of India. 2011. Faster, sustainable and more inclusive growth: An Approach to the 12th Five Year Plan (Draft). Planning Commission, Government of India, New Delhi.
2. Merga B, Haji J. 2019. Economic importance of chickpea: Production, value, and world trade. *Cogent Food and Agriculture* 5(1): 1615718.
3. Baral B, Teixeira da Silva JA, Izaguirre-Mayoral ML. 2016. Early signaling, synthesis, transport and metabolism of ureides. *Journal of Plant Physiology* 193: 97-109.
4. Sharma MP, Grover M, Chourasiya D, Bharti A, Agnihotri R, Maheshwari HS, Pareek A, Buyer JS, Sharma SK, Schütz L, Mathimaran N. 2020. Deciphering the role of trehalose in tripartite symbiosis among rhizobia, arbuscular mycorrhizal fungi, and legumes for enhancing abiotic stress tolerance in crop plants. *Frontiers in Microbiology* 11: 509919.
5. Venant N, Pascal K, Ernest S. 2013. Isolation of *Bacillus* strains from the rhizosphere of tomato and their in vitro antagonistic effects against phytopathogenic fungi. *Global Advanced Research Journal of Microbiology* 2: 65-71.
6. Schädler M, Ballhorn DJ. 2016. Beneficial soil microbiota as mediators of the plant defensive phenotype and aboveground plant-herbivore interactions. *Progress in Botany* 78: 305-343.
7. Goss MJ, Carvalho M, Brito I. 2017. Functional diversity of mycorrhiza and sustainable agriculture: management to overcome biotic and abiotic stresses. Academic Press.
8. Chew KW, Chia SR, Yen HW, Nomanbhay S, Ho YC, Show PL. 2019. Transformation of biomass waste into sustainable organic fertilizers. *Sustainability* 11(8): 2266.
9. Seleiman MF, Santanen A, Mäkelä PS. 2020. Recycling sludge on cropland as fertilizer–Advantages and risks. *Resources, Conservation and Recycling* 155: 104647.
10. Dar MI, Khan FA, Green ID, Naikoo MI. 2015. The transfer and fate of Pb from sewage sludge amended soil in a multi-trophic food chain: a comparison with the labile elements Cd and Zn. *Environmental Science and Pollution Research* 22(20): 16133-16142.
11. Devkota B, Schmidt GH. 2000. Accumulation of trace metals in food plants and grasshoppers from the Taigetos mountains, Greece. *Agric Ecosyst. Environ*. 78: 85-91.
12. Green ID, Diaz A, Tibbett M. 2010. Factors affecting the concentration in seven-spotted ladybirds (*Coccinella septempunctata* L.) of Cd and Zn transferred through the food chain. *Environmental Pollution* 158(1): 135-141.
13. Dar MI, Naikoo MI, Khan FA, Green IA. 2018. Assessing the Feasibility of sewage sludge applications for the cultivation of *Brassica Juncea* L.: Metal accumulation, growth, biochemical and yield responses. *Journal of Environmental Science and Renewable Resources* 1(1): 104.
14. Mackinney G. 1941. Absorption of light by chlorophyll solutions. *Journal of Biological Chemistry* 140: 315-319.
15. Jaworski EG. 1971. Nitrate reductase assay in intact plant tissue. *Biochemical and Biophysical Research Communications* 43: 1274-1279.
16. Lowry OH, Rosenberg NJ, Farr AL, Randall RJ. 1951. Protein measurement with Folin phenol reagent. *The Journal of Biological Chemistry* 193: 265-257.
17. Bates LS, Waldren RP, Teare ID. 1973. Rapid determination of free proline for water-stress studies. *Plant and Soil* 39(1): 205-207.
18. Philips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society* 55(1): 158-160.
19. Giovannetti M, Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *The New Phytologist* 84: 489-500.
20. Lindner RC. 1944. Rapid analytical methods for some of the more common inorganic constituents of plant tissue. *Plant Physiology* 19: 76-89.

21. Shah KK, Tripathi S, Tiwari I, Shrestha J, Modi B, Paudel N, Das BD. 2021. Role of soil microbes in sustainable crop production and soil health: A review. *Agricultural Science and Technology* 13(2): 119-128.
22. Kumar A, Verma JP. 2019. The role of microbes to improve crop productivity and soil health. *In*: Ecological wisdom inspired restoration engineering. Springer, Singapore. pp 249-265
23. Mirko S, Sofia M, Andrea S, Claudio C, Annalisa T. 2021. Trace metal accumulation and phytoremediation potential of four crop plants cultivated on pure sewage sludge. *Agronomy* 11(12): 2456.
24. Muhammad B, Adnan M, Munsif F, Fahad S, Saeed M, Wahid F, Arif M, Amanullah J, Wang D, Saud S, Noor M. 2019. Substituting urea by organic wastes for improving maize yield in alkaline soil. *Journal of Plant Nutrition* 42(19): 2423-2434.
25. Alves BSQ, Zelaya KPS, Colen F, Frazao LA, Napoli A, Parikh SJ, Fernandes LA. 2021. Effect of sewage sludge and sugarcane bagasse biochar on soil properties and sugar beet production. *Pedosphere* 31(4): 572-582.
26. Mackay JE, Cavagnaro TR, Stöver DSM, Macdonald LM, Grønlund M, Jakobsen I. 2017. A key role for arbuscular mycorrhiza in plant acquisition of P from sewage sludge recycled to soil. *Soil Biology and Biochemistry* 115: 11-20.
27. Soliman AS, Shanan NT, Massoud ON, Swelim DM. 2012. Improving salinity tolerance of *Acacia saligna* (Labill.) plant by Arbuscular Mycorrhizal Fungi and *Rhizobium* inoculation. *African Journal of Microbiology* 11: 1259-1266.
28. Ashrafi E, Zahedi M, Razmjoo J. 2014. Co-inoculations of arbuscular mycorrhizal fungi and rhizobia under salinity in alfalfa. *Soil Science and Plant Nutrition* 60(5): 619-629.
29. Samar S, Kumar A. 2020. Co-inoculation potential impact of PSB and Rhizobium on physico-chemical properties of soil and legume crop growth. *Research Journal of Agricultural Sciences* 11(1): 01-09.
30. Lin J, Wang Y, Sun S, Mu C, Yan X. 2017. Effects of arbuscular mycorrhizal fungi on the growth, photosynthesis and photosynthetic pigments of Leymus chinensis seedlings under salt-alkali stress and nitrogen deposition. *Science of the Total Environment* 576: 234-241.
31. Sampath KG, Ganesh KA. 2003. Effect of AM fungi and *Rhizobium* on growth and nutrition of *Vigna mungo* L. and *Vingna unguiculata* L. *Mycorrhiza News* 14(4): 15-18.
32. Rajasekaran S, Nagarajan SM, Arumugam K, Sravanamuthu R, Balamurugan S. 2006. Effect of dual inoculation (AM fungi and *Rhizobium*) on chlorophyll content of *Arachis hypogaea* L. CV. TMV-2. *Plant Archives* 6(2): 671-672.
33. Massa N, Cesaro P, Todeschini V, Capraro J, Scarafoni A, Cantamessa S, Copetta A, Anastasia F, Gamalero E, Lingua G, Berta G. 2020. Selected autochthonous rhizobia, applied in combination with AM fungi, improve seed quality of common bean cultivated in reduced fertilization condition. *Applied Soil Ecology* 148: 103507.
34. Xie MM, Chen SM, Zou YN, Srivastava AK, Rahman MM, Wu QS, Kuča K. 2021. Effects of *Rhizophagus intraradices* and *Rhizobium trifolii* on growth and N assimilation of white clover. *Plant Growth Regulation* 93(3): 311-318.
35. Dar MI, Naikoo MI, Rehman F, Naushin F, Khan FA. 2016. Proline accumulation in plants: roles in stress tolerance and plant development. (Eds) by N Iqbal, R Nazar, NA Khan in Osmolytes and Plants Acclimation to Changing Environment: Emerging Omics Technologies, (Springer, New Delhi). pp 155-166.
36. Gontia-Mishra I, Sapre S, Sharma A, Tiwari S. 2016. Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. *Plant Biology* 18(6): 992-1000.
37. Echeverria M, Sannazarro AI, Ruiz OA, Menendez AB. 2013. Modulatory effects of *Mesorhizobium tianshanense* and *Glomus intraradices* on plant proline and polyamine levels during early plant response of *Lotus tenuis* to salinity. *Plant Soil* 364: 69-79.
38. Wu QS, Xia RX. 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology* 163: 417-425.
39. Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM. 2008. Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. *Microbial Ecology* 55: 45-53.
40. Elhindi KM, El-Din AS, Elgorban AM. 2017. The impact of arbuscular mycorrhizal fungi in mitigating salt-induced adverse effects in sweet basil (*Ocimum basilicum* L.). *Saudi Journal of Biological Sciences* 24: 170-179.
41. Schipanski ME, Drinkwater LE, Russelle MP. 2010. Understanding the variability in soybean nitrogen fixation across agroecosystems. *Plant and Soil* 329(1): 379-397.
42. Salvagiotti F, Cassman KG, Specht JE, Walters DT, Weiss A, Dobermann A. 2008. Nitrogen uptake, fixation and response to fertilizer N in soybeans: A review. *Field Crops Research* 108(1): 1-13.
43. Reinprecht Y, Schram L, Marsolais F, Smith TH, Hill B, Pauls KP. 2020. Effects of nitrogen application on nitrogen fixation in common bean production. *Frontiers in Plant Science* 11: 1172.
44. Veselaj E, Sallaku G, Balliu A. 2018. Tripartite relationships in legume crops are plant-microorganism-specific and strongly influenced by salinity. *Agriculture* 8(8): 117.
45. Gough EC, Owen KJ, Zwart RS, Thompson J P. 2021. Arbuscular mycorrhizal fungi acted synergistically with Bradyrhizobium sp. to impro.ve nodulation, nitrogen fixation, plant growth and seed yield of mung bean (*Vigna radiata*) but increased the population density of the root-lesion nematode *Pratylenchus thornei*. *Plant and Soil* 465(1/2): 431.
46. Lata K, Sharma TK, Dassani S. 2021. Effect of mycorrhiza and *rhizobium* inoculation on the growth and yield of mung (*Vigna radiata*) plant. *Plant Archives* 21(1): 1847-1850.