academicJournals

Vol. 9(15), pp. 1110-1117, 15 April, 2015
DOI: 10.5897/AJMR2014.7357
Article Number: 7ED6A7752651
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Bacterial inoculation effect on soil biological properties, growth, grain yield, total phenolic and flavonoids contents of common buckwheat (*Fagopyrum esculentum* Moench) under hilly ecosystems of North-East India

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Received 31 December, 2014; Accepted 25 March, 2015

Field experiments were carried out at Research Farm, ICAR Sikkim Centre, Tadong during two consecutive *Rabi* seasons of 2012 and 2013 to determine the effect of different microbial inoculants on selected soil biological properties, growth, yield, and quality of common buckwheat, and then identify the best inoculant for application for local common buckwheat production in hilly ecosystem of North-East India. The results indicated that seed inoculants applied to common buckwheat effectively increased plant growth, chlorophyll content (SPAD), yield attributing characters, total phenolic and flavonoid content, grain yield, and soil biological properties. Among the different inoculations, combined application of *Azotobacter* spp. and *Azospirillum* spp. was found most efficient and resulted in maximum values of plant growth parameter, yield attributing characteristics, grain yield (1.23 Mg/ha), soil microbial biomass carbon (SMBC) and dehydrogenase activities at all the growth stages of common buckwheat.

Key words: Buckwheat, dehydrogenase activities, flavonoid content, phenolic content, microorganisms, yield.

INTRODUCTION

Buckwheat (*Fagopyrum* spp.) is a unique traditional food crop of tribes of Himalayan region of North East India. It occupies about 90% of cultivated lands in the higher Himalayas with a solid stand. It is a short duration crop (2-3 months) and fits well in the high Himalayan ecosystems where a crop's growing season is limited period because

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of early winter and heavy snow fall. In the higher Himalayas, up to 4500 m, buckwheat is the only crop grown (Joshi and Paroda, 1991). Buckwheat seems able to use insoluble phosphorus and potassium in soil and produces good seed yields, even on less fertile soils (Kontturi et al., 2004). Buckwheat possesses tolerance ability against drought, poor soil and extreme environments and has wide potential for adapting to climate change (CGIAR, 2013). Among the buckwheat genotypes cultivated in North East India, common buckwheat locally known as *Meethey Phapar* (*Fagopyrum esculentum*) is gaining more popularity due its taste and shorter growth period. North East region of India is designated as natural economic zone and opportunity zone for organic farming. Buckwheat concerns to dietary food crops with high nutritional value in respect of protein content (Krzysztof et al., 2012) with optimum combination of irreplaceable amino acids, vitamins, macro- and micro-elements, and enzymes. Buckwheat is a unique crop, which contains vitamin P (Pirogovskaya et al., 2004). Recently, its cultivation area has gradually decreased, largely because of low yield and profit to farmers. There are several reasons for low productivity of buckwheat in the region, among them proper nutrition to the crop is most the important one. There is ample scope for increasing production of buckwheat with the use of good agronomic practices as well as proper fertility management. Hence, there is an urgent need to conduct research to allow for an increase in buckwheat production in the region. Most of the studies on buckwheat have focused on breeding and cultivation; research on buckwheat fertilization has mostly concentrated on chemical fertilizers and their effect (Zhang et al., 2001). However, fertilizer application sometimes causes crop lodging in results in yield reduction. Some research has shown that the application of microorganisms could increase soil nutrient supply and stimulate plant growth (Tao et al., 2004). There is very limited or no information available on the effect of micro-organism inoculation on growth, yields, quality and soil biological properties of common buckwheat under hilly ecosystems. Therefore, the present investigation was carried out to find out the best inoculant of common buckwheat for enhancing the productivity under the Himalayan region of North East India.

MATERIALS AND METHODS

Details of experimental field

Field experiments were carried out during two consecutive *Rabi* (Winter) seasons of 2012 and 2013 at experimental block of Research Farm, ICAR Research Complex for NEH Region Sikkim Centre, Tadong, situated at a latitude of 27°32' N and longitude of 88°60' E, altitude of 1300 m above mean sea level (amsl). The average rainfall received during the period of investigation was 143.5 mm and the region hardly receives any rainfall during cropping period (winter season). Soils of experimental field were clay loam and belongs to Inceptisol and had soil pH 5.7 (1: 2.5 soil and water ratio), 226.3 kg/ha alkaline permanganate oxidizable N, 23.40 kg/ha Brays P_1, 199.7 kg 1 *N* ammonium acetate exchangeable K and 1.93% organic carbon.

Experimental design and treatments

In order to evaluate the effects of selected inoculants on the growth, productivity, quality and soil biological properties of common buckwheat, 100 g of buckwheat seed for each plot was treated one day before sowing with 25 ml of different inoculants or a mixture as designed ($3x10^9$ cfu/ml in case of microorganism). In plots of 3.0 m x 4.0 m, the seeds were sown in four replications at spacing of 30 cm between rows, 10 rows per plot in a completely randomized block design. Seed sown was 25 g/plot. The experiment comprises six treatments *viz.,* control, cow urine, *Azospirillum* spp., *Azotobacter* spp., *Azotobacter* spp. + *Azospirillum* spp. and *Azotobacter* spp. + *Azospirillum* spp. + cow urine. Sowing was done in shallow furrows made with the help of wooden plough (*Desi* country plough made of woods and a shovel) /and the seeds were sown in line, prior to sowing, 1.8 kg/plot of vermicompost were applied irrespective of treatments, and no other manure were applied during the experiment. Thinning was done at 15 days after sowing (DAS) to maintained optimum plant population. The crop was sown on 5th and 8thNovember in 2012 and 2013, respectively as per the recommended practices and harvested on 24th and 28th February during 2013 and 2014, respectively. Observation on growth viz. plant height (cm), stem girth (cm), leaves/plant plant, root length (cm), root dry weight (g/plant) and top dry weight (g/plant) and yield parameters were recorded as per the standard procedure. Similarly, chlorophyll content in leaves of buckwheat was determined by using SPAD (CCM-200) at 30, 60 and 90 DAS in morning hour, during both years.

Quality analysis

Preparation of extracts

Buckwheat flour (2 g) from raw samples was homogenized with 20 ml of 80% ethanol. The mixture was kept in agitation for 30 min at 160 rpm in an orbital shaker. Then, the homogenate was centrifuged for 10 min at 11000 rpm and the supernatant was removed, filtered (0.45 µm) and stored at -18°C for analysis.

Estimation of total phenolic content (TPC)

TPC in extracts was determined using Folin-Ciocalteau reagent, following the method described by Singleton et al. (1999). The liquid extracts were diluted and mixed with Folin-Ciocalteau reagent (2 ml) and 20% sodium carbonate solution. The mixture was incubated in the dark for 1 h at room temperature (25ºC). After incubation, absorbance was measured at 525 nm using spectrophotometer. The results were expressed as mg equivalent of Gallic acid (GAE) per 100 g of dry matter (QE).

Estimation of total flavonoid content (TFC)

TFC were measured by method of Zhishen et al. (1999) using Quercetin standard. Briefly, 0.5 mL of aliquot of extract was added to 75 µL of 5% $NaNO_2$ solution. After 6 min, 150 µL of 10% $AlCl_36H_2O$ solution was added and the mixture was allowed to stand another 5 min. Then, 0.5 mL of 1 mol/l NaOH and 2.5 mL of distilled water was added. The solutions were mixed and absorbance was measured at 510 nm using spectrophotometer. Total flavonoid content of extracts was expressed as mg of quercetin/100 g of dry matter (QE).

Estimation of soil biological properties

Soil sample were taken from crop root (0-15 cm soil depth) by core sampler at 30, 60 DAS and at harvest of buckwheat. The soil samples were air dried and kept in freezer (-20ºC) until the analysis of soil biological properties. Estimation of soil biological properties such as dehydrogenase activity and soil microbial biomass carbon were done per the procedures describe below.

Dehydrogenase activity

Dehydrogenase activity of soil samples was estimated by the method described by Casida et al. (1964).

Reagents

Triphenyl-tetrazolium chloride (TTC): TTC (3.0 g) was dissolved in 100 ml distilled water and stored in an amber coloured bottle at 4°C; methanol (AR grade); Standard triphenyl formazan (100 μg/ml): 10 mg triphenyl (TPF) dissolved in 100 ml distilled water.

Procedure of estimation

Fresh air-dried soil sample (6 g) was saturated with 1.0 ml freshly prepared TTC (3% w/v) solution in a screw capped test tube to which pinch (0.1 g) of $CaCO_3$, was added. Care was taken that no air bubble remained during packing of soil sample and rotated gently by shaking. These test tubes were incubated at 28±1°C (28-30°C) for 24 h. After 24 h, TPF was extracted (pink layer). 10 ml Methanol was added to these test tubes and rotated it well for 1 min /sample. The supernatant was taken out carefully after allowing standing for 10 minutes. Absorbance of supernatant was recorded by Spectrophotometer at 485 nm. A standard curve was prepared with TPF (0-50 μg/ml). Concentration of TPF in sample was calculated with standard curve. Dehydrogenase activity was calculated and expressed in terms of μg TPF liberated g/soil/h or μg TPF g/soil/day.

$$\text{Dehydrogenase activity } (\mu \text{ TPF g/soil/day}) = \frac{\text{Concentration reading of spectrophotometer}}{6}$$

Microbial biomass carbon (MBC)

Microbial biomass carbon in soil samples was estimated by the method described by Vance et al. (1987) and Numan et al. (1998) derived a method for estimation of microbial biomass C.

Reagent

Chloroform; 0.5 M K_2SO_4: Prepared by adding 87.135 g of K_2SO_4 in 1 L distilled water.

Procedure of estimation

Soil sample (17.5 g) was taken in a closed-capped bottle and 1.0 ml of chloroform was added and fumigated these samples and one non fumigated set was also prepared in a 250 ml flask. After that, these incubated samples were kept in dark for 24 h. After 24 h of incubation, chloroform was evaporated at 50°C in BOD that is the caps were opened for next 20-24 h. After that 70 ml 0.5 M K_2SO_4 was added to samples and shaken for 30 min. Supernatant was taken out by filtering the samples with Whatman No. 42 filter paper. Absorbance of supernatant was recorded immediately for both fumigated and non-fumigated at 280 nM. Soil microbial biomass carbon (SMBC) was calculated and expressed as mg kg/soil.

Statistical analysis

All the data obtained was statistically analysed using the *F*-following Gomez and Gomez (1984). CD values at $P = 0.05$ were used to determine the significance of difference between treatment means.

RESULTS

Effect of inoculation on growth of common buckwheat

Mean data of two years showed that in general, plant height (Table 1a), root length, root dry and top dry weight accumulation (Table 1b) increased with the age of crop and achieved to the maximum at maturity except leaves/plant and stem girth, which recorded increase only up to 90 DAS. Initially, plant growth in terms of plant height, stem girth, leaves/plant, root length, root and top dry weight accumulation was slow up to 30 DAS. Thereafter, the rate of increase reached a peak between 30 and 60 days and declined towards maturity. Inoculation has significant effect on all growth parameters of common buckwheat under study. Among the inoculations, combined application of *Azospirillum* spp. and *Azotobacter* spp. resulted in significant higher values of plant height (cm), stem girth (cm), leaves/plant, root length (cm), root dry and top dry weight accumulation (g/plant) at all the growth stages of crop. However, it remained statistically at par with single application of *Azospirillum* spp. at 90 DAS in terms of plant height, at 30 DAS in terms of stem girth and leaves/plant, at 30 DAS and 60 DAS in terms of root length root and aerial part dry weight accumulation during the course of study.

Effects of inoculation on chlorophyll content (SPAD), yield attributes and yields of common buckwheat

In general, irrespective of treatments, chlorophyll content in leaves of common buckwheat increased linearly from 30 to 60 DAS declined thereafter. Seed inoculation with different substrate showed the significant effect on chlorophyll content in common buckwheat at all the growth stages (Figure 1). Among the inoculations, combined application of *Azospirillum* spp. and *Azotobacter* spp. recorded significantly higher SPAD values at all the growth stages except at 60 DAS; at this stage, it remained statistically at par with the single application of *Azospirillum*. With respect to yield attributes and yield, seed inoculation showed significant effect over control. Among the treatments, combined inoculation of *Azospirillum* spp. and *Azotobacter* spp. resulted in

Table 1a. Inoculation effect on growth of common buckwheat (Mean data of 2 years).

Treatment	Plant height (cm)				Stem girth (cm)				Leaves/plant			
	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
Control	15.37	55.8	73.4	109.7	0.21	0.56	0.63	0.62	2.75	5.25	12.25	11.10
Cow urine	14.97	61.6	72.7	116.2	0.22	0.57	0.65	0.64	4.50	6.50	14.75	13.50
Azospirillum	15.22	64.4	84.9	121.2	0.26	0.60	0.71	0.65	6.00	7.25	17.75	15.25
Azotobacter	16.90	62.6	86.7	121.0	0.25	0.59	0.70	0.66	4.75	6.75	19.00	16.50
Azospirillum+*Azotobacter*	17.72	66.9	90.0	126.2	0.28	0.63	0.75	0.71	6.25	8.25	21.75	20.00
Azospirillum+*Azotobacter*+Cow urine	16.40	62.7	84.0	116.5	0.23	0.57	0.68	0.62	4.00	5.50	18.25	15.00
SEM±	0.99	1.14	1.81	1.37	0.01	0.005	0.01	0.012	0.44	0.32	0.63	0.43
CD (*P*=0.05)	2.98	3.45	5.60	4.13	0.02	0.015	0.03	0.037	1.34	0.97	1.89	1.32

Table 1b. Inoculation effect on growth of common buckwheat (Mean data of 2 years).

Treatment	Root length (cm)				Root dry weight (g/plant)				Top dry weight (g/plant)			
	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest	30 DAS	60 DAS	90 DAS	At harvest
Control	1.99	8.75	10.18	11.00	0.106	0.255	0.375	0.825	0.620	2.29	3.50	5.52
Cow urine	2.05	9.80	11.63	12.60	0.110	0.271	0.405	0.870	0.683	2.35	3.58	5.81
Azospirillum	2.94	10.80	12.40	13.00	0.120	0.284	0.423	0.945	0.866	2.41	3.70	6.15
Azotobacter	2.81	9.68	11.98	12.75	0.115	0.262	0.428	0.908	0.782	2.40	3.60	5.73
Azospirillum+*Azotobacter*	3.05	11.10	12.97	14.50	0.125	0.297	0.480	0.968	0.890	2.60	3.82	6.71
Azospirillum+*Azotobacter*+Cow urine	2.66	10.15	11.43	12.50	0.112	0.265	0.415	0.875	0.761	2.31	3.52	6.17
SEm±	0.03	0.12	0.18	0.45	0.002	0.004	0.008	0.009	0.010	0.06	0.02	0.13
CD (*P*=0.05)	0.10	0.36	0.54	1.34	0.005	0.013	0.024	0.027	0.030	0.17	0.07	0.40

maximum number of seeds/plant (135), seed yield/plant (2.99 g), test weight (23.65 g), and grain yield (1.23 Mg/ha) over other treatments (Table 2).

Effect of inoculation on total phenolic and flavonoid contents of buckwheat seed

Mean data of two years pertaining to total phenolic and flavonoids content is depicted in Figure 2. Seed inoculation had significant effect on total phenolic and flavonoids content in seed of common buckwheat. All the treatments significantly enhanced the total phenolic and flavonoids content in seed over control. Among the treatments, combined application of *Azospirillum* spp. and *Azotobacter* spp. recorded the highest in total phenolic (17.20 mg GAE/100 g) and flavonoid (5.28 mg QE/100 g) contents were in tune of 19.77 and 26.31% increment over the control (no inoculation).

Effect of inoculation on dehydrogenase activities and soil microbial biomass carbon

Mean data of two years presented in Figure 3 showes that soil microbial biomass carbon (SMBC) and dehydrogenase activity registered marked increase with the advancement in crop growth stages up to harvest. During the experiments it was found that among the treatments, significantly higher value of soil SMBC and dehydrogenase activity recorded with the

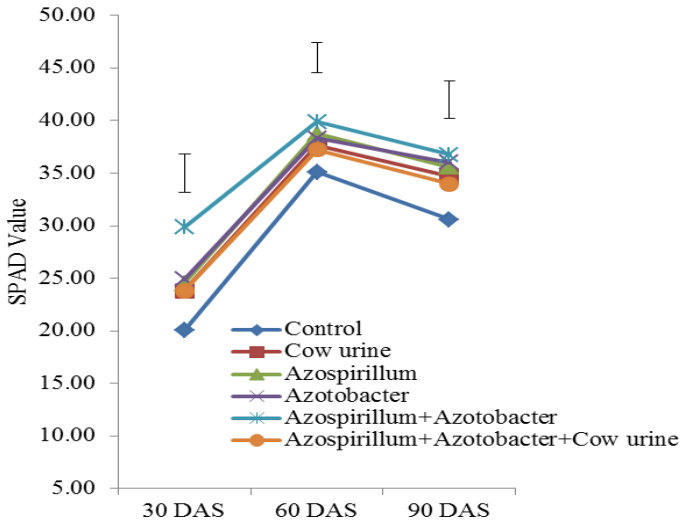

Figure 1. Inoculation effect on leaf chlorophyll content (SPAD) of common buckwheat (Mean Data of 2 years). The vertical bars indicate C.D. at *P* = 0.05.

Table 2. Inoculation effects on yield attributes and yield of common buckwheat (Mean data of 2 years).

Treatment	Seeds/plant	Seed yield/plant (g)	Test weight (g)	Yield (Mg/ha)
Control	101	2.02	21.5	0.95
Cow urine	102	2.23	22.4	0.99
Azospirillum	128	2.45	23.0	1.17
Azotobacter	111	2.13	22.4	1.04
Azospirillum+Azotobacter	135	2.99	23.7	1.23
Azospirillum+Azotobacter+Cow urine	121	2.65	22.6	1.05
SEm±	1.2	0.13	0.12	0.05
CD (*P*=0.05)	3.6	0.40	0.37	0.14

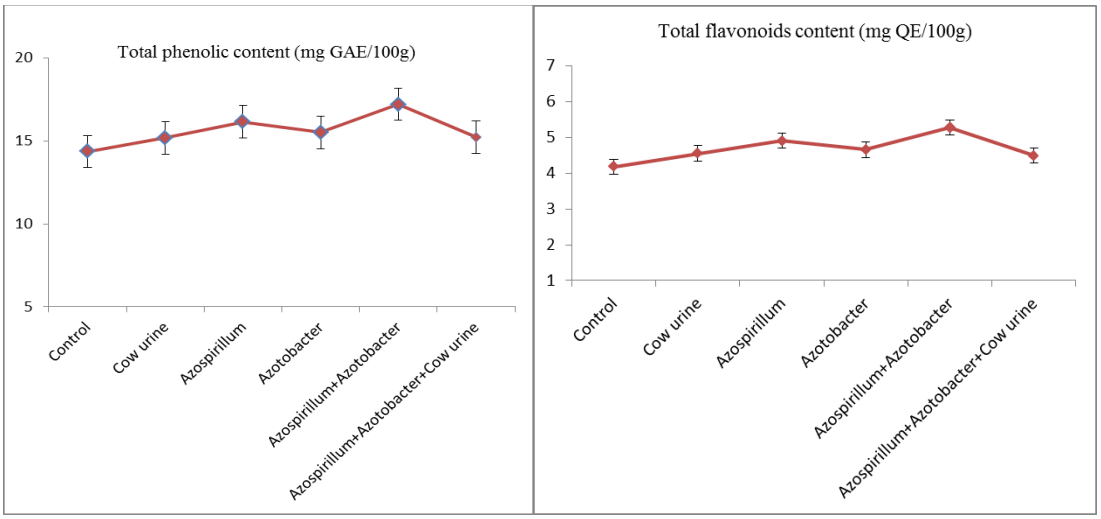

Figure 2. Inoculation effect on total phenolic (mg GAE/100g of seed) and total flavonoids content (mg QE/100 g seed) of common buckwheat (mean data of 2 years).The vertical bars indicate C.D. at *P* = 0.05.

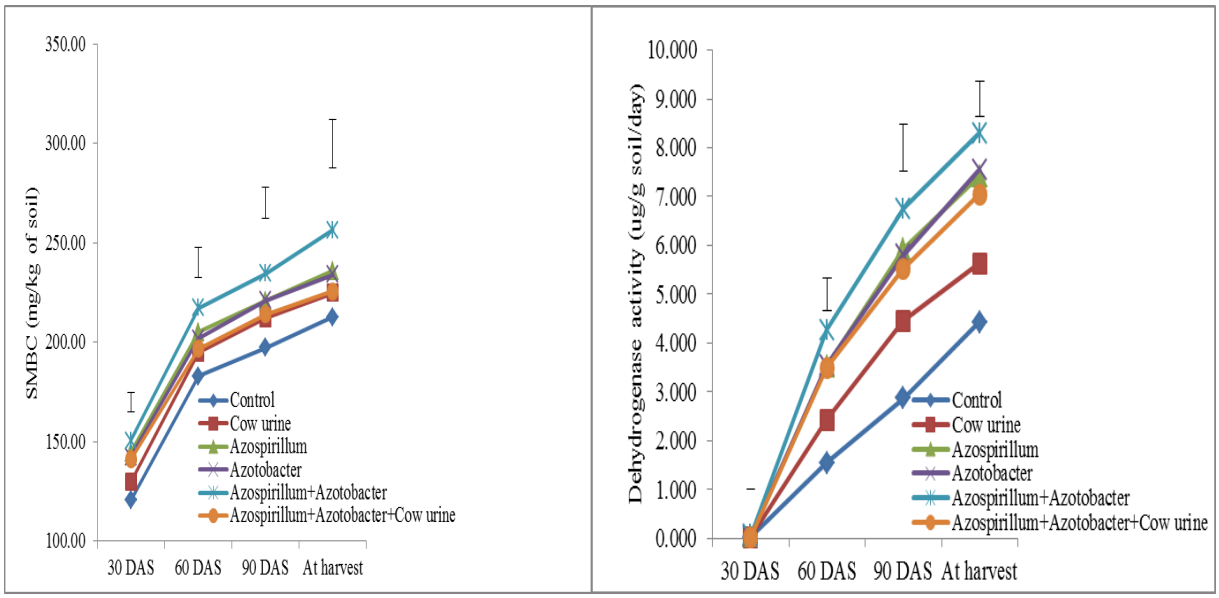

Figure 3. Inoculation effect on soil microbial biomass carbon (mg/kg of soil) and soil dehydrogenase activity (µg/g soil/day)of common buckwheat (mean data of 2 years). The vertical bars indicate C.D. at *P* = 0.05.

combined application of over the control, and other treatments during at all the growth stages *viz.,* 30, 60, 90 DAS and at harvest.

DISCUSSION

Growth of common buckwheat

Significant response in plant growth characteristic of common buckwheat plants was observed under inoculated plots compared to un-inoculated ones. Inoculation of *Azospirillum* spp. and *Azotobacter* spp. exerted the significant effect on all the growth parameters at all the growth stages of buckwheat over control. This was due to *Azospirillum* spp. and *Azotobacter* spp. playing pivotal role in nitrogen fixation which may improve the nitrogen fixation. In addition, they provide growth promoting substances, such as indole acetic acid and gibberellins (Fayez et al., 1985). Poor growth characteristics in control plots and higher growth in treated plots could be due to poor and higher nutrients supply, respectively. The positive effects of seed inoculation reflects on plant growth in this study have also been reported by Nwangburuka et al. (2012). They observed that inoculated plants grown with organic amendments produced higher growth characteristics than un-inoculated ones. Increase nutrients availability in soil due to biofertilizers were reported by several workers (Sridevi and Ramakrishnan, 2010; Geeta et al., 2013). In the study combined application of *Azospirillum* spp. +*Azotobacter* spp. resulted in maximum plant height, stem girth, leaves/plants, root length, root and top dry weight at all the stages of plant growth over the others. Better plant growth might be due to proper supply of nitrogen and growth promoting hormones by *Azospirillum* spp. + *Azotobacter* spp. and enhanced uptake of phosphorus and other nutrients due to mycorrhizal colonization (Zaidi et al., 2004). Enhanced nutrients availability could also be attributed to the decomposition of organic manure or transforming of inorganic substances to available form by microorganisms. These results are supported by the findings of Tao et al. (2004).

Chlorophyll content (SPAD), yield attributes and yields of common buckwheat

The results (Figure 2) show that the chlorophyll contents of common buckwheat are relatively lower in seeding stage but with time the chlorophyll contents increase, and reached the maximum when they are in full bloom stage (60 DAS), and thereafter the chlorophyll contents decline gradually. Seed inoculation recorded higher SPAD values at all the stages of crop growth over control. Across the growth stages, the combined application of by *Azospirillum* spp. + *Azotobacter* spp. recorded about 13-49% higher chlorophyll content (SPAD) over control. The beneficial effects of bacterial inoculation on increased chlorophyll content might be due to the higher amount of nitrogen supplied to the growing tissue and organs supplied by N_2 fixing *Azospirillum* spp. and *Azotobacter* spp.. When nitrogen levels in plant tissues are low, plants do not metabolize nutrients efficiently (Conley et al., 2002). According to Haboudane et al. (2002), the higher the SPAD value, the greater the chlorophyll and nitrogen

content of the leaves (Swiader and Moore, 2002). The increase in chlorophyll content with increasing nitrogen has also been reported by Seneweera et al. (2011). All yield attributes were found superior with seed inoculation as compared to control and this could be assigned to better growth and development of plants with higher dry matter accumulation, robust growth and increased photo-synthetic activity which resulted in higher accumulation of photosynthates. The number of leaves is an important factor, because the leaves are structures bearing photosynthetic machinery and an increase in leaf number may promote better root development, better translocation of water uptake and deposition of nutrients and yield (Chandrasekhar et al., 2005). Combined application of *Azospirillum* spp. and *Azotobacter* spp. resulted in maximum value of yield attributes among the treatments. Plant growth regulating substance such as indole acetic acid (IAA), gibberellic acid ($GA_{3)}$ and cytokines produced by *Azospirillum* spp. and *Azotobacter* spp. are known to promote better growth (Tiwary et al., 1998). The yield of the crop is final product of various yield attributing characters. The effect of any treatment on yield attributes is directly reflected in the yield. In this study, Combined inoculation of *Azospirillum* and *Azotobacter* recorded 29.5, 24.2, 5.1, 18.3 and 17.1 per cent higher yield over the control, cow urine treatment, *Azospirillum* spp., *Azotobacter* spp., *Azospirillum* spp. +*Azotobacter* spp. and *Azospirillum* spp. +*Azotobacter* spp. + Cow urine, respectively. The higher grain yield due to biofertilizers inoculation might be due to increase in plant height and total chlorophyll content and yield component. Similar findings were also reported by Tao et al. (2004) and Babu et al. (2014).

Total phenolic (TPC) and flavonoid contents (TFC) of buckwheat seed

Genotype is the primary determinant of the composition of secondary plant metabolites (TPC and TFC), although their expression is strongly influenced by environmental pressures of climate. Seed inoculation with *Azospirillum* spp. and *Azotobacter* spp. had a significant ($P \leq 0.05$) impact on the production of total phenolics and flavonoid production (Figure 2). These microorganisms can fix atmospheric nitrogen and supply it to plants as they synthesize several different phytohormones that can act like growth regulators and may have mechanisms for the solubilization of minerals, such as phosphorus which may become more readily available for plant growth and they may synthesize some less well characterized low molecular mass compounds or enzymes that can modulate plant growth and development (Glick, 1995; Hanan et al., 2008) and resulted in great enhancement effect on total phenolics, total flavonoids, compared to their conventionally grown counterparts. This might be mainly due to better nitrogen supply by the microorganisms. When nitrogen supply was better, improvements in both phenol and flavonoids content were also reported by Sene et al. (2001). They also found positive correlation in grain yield and the phenol pool of aerial parts.

Soil biological properties

The data on microbial activity in terms of dehydrogenase activity and soil microbial biomass carbon during crop growth period was recorded at 30, 60, 90 DAS and at harvest and presented in Figure 3, respectively. These activities provide the information about the microbial growth and development. Dehydrogenase activity was chosen as an index of microbial activity as it refers to group of mostly endo cellular enzymes, which catalyze oxidation of soil organic matter (Pascual et al., 1998). In the present study, higher values of dehydrogenase activity and soil microbial biomass carbon were observed with microbial inoculants. The combined inoculation of *Azospirillum* spp. and *Azotobacter* spp. in buckwheat seed resulted almost in double activities of dehydrogenase enzyme in soil. Similarly, across the growth stages about 18-25% higher SMBC was observed due to the same treatment over control. This might be due to better establishment of inoculated microorganism, which stimulates the indigenous microorganisms. Our results suggest that seed inoculation should also improve the soil fertility by increasing the biological activity of soil, which in turn reduce the fertilizer requirements. Indeed, these results are very desirable from economic and ecological point of view (Piotrowska et al., 2012). These results are in close conformity with those reported by Abdullahi et al. (2013).

Conclusion

Azospirillum spp. and *Azotobacter* spp. thrives well in acidic soils of Sikkim and their combined application resulted in better buckwheat productivity and positively influenced the soil biological properties. Hence, this combination may be recommended for obtaining good crop yield and sustaining soil health.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors are thankful to the Director of ICAR Research Complex for NEH Region, Barapani, India and NICRA (National Initiatives of Climate Resilient Agriculture) for providing necessary facilities and financial support to conduct this research.

REFERENCES

Abdullahi R, Sheriff H H, Lihan S (2013). Combine effect of bio-fertilizer and poultry manure on growth, nutrients uptake and microbial population associated with sesame (*Sesamumindicum*L) in North-eastern Nigeria. IOSR J. of Envi. Sci. Toxi. Food Tech. 5: 60-65.

Babu Subhash, Kalita H, Singh Raghavendra, Gopi R, Kapoor C, Das SK (2014).Buckwheat (*Fagopyrum* spp..).*In:* Handbook on organic crop production in Sikkim. (*Eds.* R. K. Avasthe, YashodaPradhan and Khorlo Bhutia).Sikkim Organic Mission, Govt. of Sikkim and ICAR Research Complex, Sikkim Centre, Tadong, Gangtok, Sikkim. pp. 47-52.

Casida LEJ, Klen DA, Santro T (1964). Soil dehydrogenase activity. Soil Bio. Biochem.98: 371-376.

CGIAR (2013). Annual report of programme on climate change, agriculture and food security http://ccafs.cgiar.org/

Chandrasekhar B R, Ambrose G, Jayabalan N (2005). In fluence of biofertilizers and nitrogen source levels on growth and yield of Echinochloa frumentacea (Roxb.) Link. J. Agric. Tech. 1:223-234.

Conley ME, Paparozzi ET, Stroup WW (2002). Leaf anatomical and nutrient concentration responses to nitrogen and sulfur applications in poinsettia. J. Plant Nutr. 25:1773-1791.

Fayez M, Eman NF and Makbol HE (1985). The possible use of nitrogen fixing *Azospirillum* as biofertilizer for wheat plants. Egypt. J. Microbiol. 20: 199-206.

Geeta B, Patil HC, Lakshman Romana, Mirdhe, Agadi BS (2013). Effect of co- inoculation of AM fungi and two beneficial microorganisms on growth and nutrient uptake *Eleusiencoracana* Gaertn. (Finger millet). Asian J. P. Sci. Res. 3: 26-30.

Glick BR (1995). The enhancement of plant growth by free bacteria.Can. J. Microbl. 41: 109-117.

Gomez K A, Gomaz A A (1984). *Statistical procedures for Agricultural Research.* John Wiley & Sons, Singapore

Haboudane D, Miller JR, Tremblay N, Zarco-Tejada P, Dextraze L (2002). Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. Remote Sens Environ. 81:416-426.

Hanan AA, Taie R, El-Mergawi, Radwan S (2008) Isoflavonoids, Flavonoids, phenolic acids profiles and antioxidant activity of soybean seeds as affected by organic and bioorganic fertilization. American-Eurasian J. Agric. Environ. Sci. 4:207-213.

Joshi BD, Paroda RS (1991).Buckwheat in India. NBPGR, Shimla Sci. Monogr, 2:l17.

Kontturi M, Marjo K, Ketoja E (2004). Buckwheat cultivars in the north***In:*** Proceedings of the 9th International Symposium on Buckwheat, Prague 2004. pp. 496-498.

Krzysztof D, Danuta G, Monika K, Barbara P (2012). Influence of technological process during buckwheat groats production on dietary fibre content and sorption of bile acids. Food Res Inter. 47:279-283.

Numan N, Morgan, MA, Helihy M (1998). Ultraviolet absorbance (280 nm) of compounds related from soil during chloroform fumigation as an estimate of the microbial biomass. Soil Biol. Biochem. 30:1599-1603.

Nwangburuka CC, Olawuyi OJ, Oyekale K, Ogunwenmo KO, Denton OA, Nwanko E (2012). Growth and yield response of *Corchorusolitorius* in the treatment of Arbuscularmychorrhizae (AM), poultry manure (PM), combination of AM-PM and inorganic Fertilizer (NPK). Adv. in App. Sci. Res. 3:1466-1471.

Pascual JA, Hernandez T, Garcia C, Ayuso M (1998). Enzymatic activities in an arid soil amended with urban organic wastes: Laboratory experiment. Biol. Tech. 64*:* 131-138.

Piotrowska A, Dlugosz J, Zamorski R, Bogdanowicz P (2012). Changes in soil biological and chemical properties of an arable soil treated with the microbial biofertilizer UGmax. Pol. J. Environ. Stud. 21(2):455-463.

Pirogovskaya GV, Rusalovitch AM, Soroko VI, Sazonenko OP, Shakovets OE (2004). Efficiency of new forms of mineral fertilizers for field grown buckwheat on light textured soils Proceedings of the 9th International Symposium on Buckwheat, Prague 2004. pp. 470-74.

Sene M, Dore T, Christiane Gallet Jan (2001).Relationships between biomass and phenolic production in grain sorghum grown under different conditions. Agron. 93:49-54.

Seneweera S, Makino A, Hirotsu N, Norton R, Suzuki Y (2011). New insight into photosynthetic acclimation to elevated CO_2: The role of leaf nitrogen and ribulose 1,5-bisphosphate carboxylase/oxygenase content in rice leaves. Environ. Exp. Bot. 71:128-136.

Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent.Meth.inEnzymol. 299:152-178.

Sridevi S, Ramakrishnan K (2010). Effects of combined inoculation of am fungi and Azospirillum 0n the growth and yield of onion (Allium cepa L.). J. Phyt.Phytophysio. 2:88-90.

Swiader JM, Moore A (2002). SPAD - chlorophyll response to nitrogen fertilization and evaluation of nitrogen status in dryland and irrigated pumpkins. J. Plant Nutr. 25:1089-1100.

Tao Y, Shf Q, Zhangl X, Zhou Y (2004). Inoculation effect on growth and flavonoid content of tartary buckwheat in a field experiment. Fagopyrum 21:45-50.

Tiwary DK, Abuhasan MD, Chattopadhyay (1998). Studies on effect of inoculation with Azotobacter and Azospirillum on growth, yield and quality of Banana. Indian J. Agric. Sci. 42:235-240.

Vance ED, Brooks PC, Jenkinson DS (1987). An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem.19: 703-707.

Zaidi A , Khan M S, Aamil M (2004). Bioassociative effect of rhizospheric microrganisms on growth , yield and nutrient uptake of green gram. J. Plant Nutr. 27:599-610.

Zhang X, Chai Y (2001). Effects of rates and combinations of applied nitrogen and phosphorus fertilizers on kernel protein components of buckwheat.Proc. 8th IntI. Symp. Buckwheat at Chunchon: 90-98.

Zhishen J, Mengcheng T, Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64:555-559.