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Nutrient management influenced phytochemical content and biological activities in rhizosphere of broccoli cv. pushpa

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Abstract
Nutrient management is one of the major challenges in organic farming practices. This study assessed how different forms of nutrient management can influence quality and biological activities in rhizosphere of broccoli. Application of VC+FYM and bioinoculants (*Azotobacter* isolate R*azo*B and phosphate solubilizer *Bacillus megaterium* MTCC4714) along with 100% K increased the contents of phytochemicals and soil biological properties significantly (P<0.05) compared to that in all other nutrient management practices. The pair-wise correlation matrix analysis indicated a significant positive correlation between the contents of phytochemicals and soil biological properties. Findings of this study clearly revealed a direct relationship between improved soil biological properties and higher contents of phytochemicals in broccoli head under organic manure with bioinoculants application and this nutrient management practice may be recommended for organically grown broccoli in acidic soils on hill terrace.

Keywords: broccoli, phytochemicals, biofertilzers, rhizosphere, nutrient

Introduction
Broccoli is a significant source of nutritional antioxidants such as ascorbic acid, β-carotene, tocopherols and phenolics. In addition, it has sulphur containing phytochemicals like glucosinolate and also possesses strong anticarcinogenic activity (Pandey and Rai, 2006) [8]. Though, soil nutrient management is an important aspect in achieving high yield and quality of broccoli, the information on nutrient management of broccoli in acidic soils of hill terraces in the northeastern region is lacking. Now-a-days, farmers are frequently advised to apply organic residues, inorganic fertilizers and bio-inoculants in combination to achieve higher crop productivity without deteriorating soil health (Chhonkar, 2003) [3]. The microbial communities that successfully colonize the rhizosphere soil provide benefit to plants in terms of availability of mineral nutrients through the action of mineralization, improved soil structure, etc. Therefore, the rhizosphere microbial community represents the portion of the soil ecosystem, which exerts maximum effect on the above ground community (Reese *et al*., 2005) [10]. Consequently, there is an increasing need to understand the rhizosphere so that we can effectively manage ecosystem to either harness their potential benefits or reverse the negative consequences of anthropogenic interventions. The aim of this investigation was to assess the quality (phytochemicals) of broccoli under various forms of nutrient management practices in comparison to that in recommended inorganic fertilizers practice. Impacts of different nutrient management practices on soil enzymes and microbial biomass in broccoli rhizosphere were analyzed and their relationships with phytochemical content in broccoli curd were studied.

Materials and Methods
Experimental location
The experiment was carried out on hill terrace at the experimental farm of Division of Horticulture, ICAR Research Complex for NEH Region (25° 41`-21`` N, 91° 55`-25`` E and 1010 m above msl) located at Umiam, Meghalaya, India. The crop growing period was September 2009 to February 2010 with relative humidity of 70 to 85% (morning) and 55 to 65% (evening), ambient temperature ranged from 20 to 24°C (max) and 7 to 16° C (min).

Plot layout and nutrient management treatments
The experimental field was divided into three blocks and each block consisted of eight plots (each plot area was 3 m^2). The eight different nutrient management treatments were: (1) control (2) Vermicompost (VC) @ 2 t ha^{-1} + FYM @ 5 t ha^{-1}, (VC + FYM), (3) Recommended NPK fertilizers (80:100:100 kg ha^{-1}), (100%NPK), (4) 50% of recommended dose of NPK +

50% of VC+FYM, (½NPK + ½ VC+FYM), (5) 25% lime + ½NPK (¼L + ½NPK), (6) ¼L + ½NPK + ½VC + FYM, (7) ¼L + VC + FYM and (8) VC + FYM+ R*azo*B + PSB + 100% K. Each treatment was randomly allotted to three plots such that each block received at least one treatment plot.

Phytochemical content of broccoli

The harvested curds were immediately stored at 2°C for determination of phytochemicals *viz.* ascorbic acid, β-carotene, total chlorophyll and total phenol contents. The ascorbic acid content was determined by the method described by Jagota and Dani (1982) [5]. The concentration of ascorbic acid in the sample was calculated from the slope of the ascorbic acid standard curve. β-carotene was determined as per procedure described by Srivastava and Kumar (2002) [12]. Total chlorophyll content was determined by using the colorimetric method described by Singh *et al.* (2006) [11]. The total phenol content was determined by the method described by Malick and Singh (1980) [6]. The concentration of phenols in the test sample was calculated from the standard curve and expressed as mg phenols/100 g material.

Soil biological parameters in rhizosphere soils

Three rhizospheric soil samples at 0-15 cm depth from each plot (collected at the time of harvesting of broccoli curds) were analysed immediately for soil biological properties. Soil dehydrogenase activity (DHA) was measured in terms of amount of triphenyl formazan (TPF) produced during incubation of soil sample with 2,3,5- triphenyltetrazolium chloride (TTC) at 37°C for 24 h and was expressed as µg (TPF) g^{-1} soil ha^{-1} (Cassida *et al*., 1964) [2]. Soil phosphomonpesterases activity (PHA) was determined in terms of amont of *p*-nitrophenol produced during incubation (37°C for 1 hour) of soil sample with *p*-nitrophenyl phosphate in presence of modified universal buffer and was expressed as µg $PNPg^{-1}$ soil ha^{-1} (Tabatabai and Bremner, 1969) [13]. Microbial biomass carbon (MBC) in rhizosphere soil was determined using the chloroform-fumigation-extraction procedure described by Brookes and Joergensen (2006) [1]. The difference in C content between fumigated and non-fumigated samples was determined and then, MBC was calculated using a conversion factor, K_{EC} = 0.45. MBC content was expressed in µg g^{-1} (dw) soil.

Statistical analysis

All univariate analysis was performed using SPSS v12.0 (SPSS Inc., Chicago, IL, USA). Each parameter recorded for different nutrient management treatments was normally distributed as determined using Kolmogorov-Smirnov test for univariate normality. Data for each parameter under different nutrient management were analysed for the differences in among treatment means (P<0.05) by performing two-way analysis of variances (ANOVA) with replications (incorporating the Levene's statistic to test for the equality of group variances). Duncan multiple range test (P<0.05) was performed to analyse the pair-wise comparisons among the means of a parameter under different nutrient managements. The data matrix was tested for multivariate normality test by performing simple scatter plots of all pair wise combinations of variables (draftsman plot analysis).

Result and Discussion

The combined use of organic residues and inorganic fertilizers or organic residues and biofertilizers improved quality of broccoli head in terms of the content of phytochemicals significantly (P<0.05) compared to that in broccoli head obtained from organic residues or inorganic fertilizers alone applied pots (Table 1). Broccoli curds contained significant higher amount of ascorbic acid, β-carotene, chlorophyll and total phenol in VC+FYM+RazoB+PSB+100% K plot in comparison to that in other nutrient management plots. Previous study reported that inoculation of sweet pepper with *Azospirillum brasiliense* and *Pantoea dispersa* increased the concentration of citric acid, ascorbic acid in green fruits compared with non-inoculated fruits under limited N supply (Del Amor *et al*., 2008) [4].

Application of organic residues (VC+FYM) might augment the activities of soil microbiota that may increase availability of plant essential nutrients like N and P through better mineralization process. This was evident because of higher MBC coupled with higher DHA and PHA in soils under integrated nutrient management plots (Table 1). DHA and PHA were significantly (P<0.05) higher in rhizosphere soils of VC+FYM+RazoB+PSB+100%K plot compared to that in other nutrient management plots. Previous studies reported that higher MBC content in soils along with higher DHA and PHA are good indices of enhanced C mineralization in soils along with higher availability of N and P for plant uptake (Nannipieri, 1994) [7]. Ramesh *et al.* (2010) [9] also reported that DHA, PHA and MBC were higher in organic residue amended soils by 52.3%, 28.4% and 34.4%, respectively compared to that in soils of conventional farms.

The results of pair-wise correlations of seven different variables (phytochemicals and biological activities in the rhizosphere) are presented in Table 2. DHA, PHA and MBC in rhizosphere soils showed significant positive correlation with ascorbic acid, β-carotene, chlorophyll and total phenol contents in broccoli head (r = 0.39, n= 24, *P*<0.05). These results clearly indicated that higher biological activities in rhizosphere soils enhance the quality of broccoli head. Results confirmed the common people belief that organically produce foods contain more phytochemicals compared to that in foods produced by conventional practices.

Table 1: Content of phytochemicals in broccoli head and soil biological properties in rhizosphere soils as influenced by nutrient management practices in acidic soils of hill terraces

Nutrient management regimes	AA mg/100g [fw] head	BC mg/100g[fw] head	CC mg/g [fw] head	TP mg/100g [fw] head	DHA µg TPF $hr^{-1}g^{-1}$ [dw] soil	MBC µg g^{-1} [dw] soil	PHA µg PNP hr^{-1} g^{-1} [dw] soil
Control	67.2	21.6	0.25	78.0	4.0	452.8	1.1
VC+FYM	68.1	25.6	0.32	79.4	6.8	469.7	1.8
100%NPK	71.0	28.6	0.33	80.9	7.3	596.6	2.2
½ NPK+ ½ VC+FYM	73.3	29.6	0.35	81.4	9.5	632.6	3.1
¼ L+ ½ NPK	72.0	22.5	0.32	80.7	8.0	512.0	2.0
¼ L+ ½ NPK+ ½ VC+FYM	73.7	30.2	0.33	81.5	10.3	679.1	3.2
¼ L+VC+FYM	69.4	25.4	0.32	79.4	7.2	581.8	2.1

VC+FYM+R*azo*B+PSB+100%K	75.0	32.4	0.35	83.1	10.8	742.6	3.3
SE(m) ±	0.6	0.2	0.008	0.7	0.2	15.1	0.0
$CD_{0.05}$	1.3	0.4	0.02	1.5	0.5	32.3	0.1

[AA- Ascorbic acid, BC- β-carotene, CC- Chlorophyll content, TP- Total phenol, DHA- Dehydrogenase activity, MBC- Microbial biomas s carbon, PHA- Phosphatase activity.]

Table 2: Pair-wise correlation matrix generated using variables (content of phytochemicals and soil biological activities) recorded for broccoli grown under various nutrient management practices

Parameters	AA	BC	CC	TP	DHA	MBC	PHA
AA	1.0						
BC	0.76**	1.0					
CC	0.66**	0.71**	1.0				
TP	0.87**	0.77**	0.66**	1.0			
DHA	0.90**	0.82**	0.83**	0.84**	1.0		
MBC	0.85**	0.89**	0.69**	0.76**	0.86**	1.0	
PHA	0.91**	0.89**	0.79**	0.83**	0.98**	0.92**	1.0

AA – Ascorbic acid content; BC− β-carotene content; CC− Chlorophyll content; TP−Total phenol; DHA−Dehydrogenase activity; MBC− Microbial Biomass Carbon; PHA− Phosphatase activity. Correlation coefficient values superscripted by single and double stars (* and **) are statistically significant at $P_{0.05}$ and $P_{0.01}$, respectively

Conclusion

The study has confirmed that the combined use of the bioinoculants *i.e. Azotobacter* (isolate R*azo* B) and phosphate solubilizing bacteria (MTCC4714) with organic manure and 100% K is an optimum nutrient management practice for broccoli cultivation in obtaining better quality and improving soil quality in acidic soil of hill terrace in the northeastern region. Findings provided a clear indication that improved soil biological activities in the rhizosphere of broccoli lead to higher contents of phytochemicals in the broccoli head. This statement may hold true for other vegetable crops grown on hill terraces. Knowing the fact that a direct relationship existed between contents of phytochemicals in broccoli head and soil biological activities in the rhizosphere, it is now important to understand the mechanism that how the soil biological activities linked with higher contents of phytochemicals through future research at molecular level.

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