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Short Research Article

Effect of Biofertilizers on Yield and Biomolecules of Anti-cancerous Vegetable Broccoli

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Abstract

Broccoli is gaining its popularity as an important part of diet all over the world for its anti- oxidant and anti-cancerous properties. Present investigation was carried out to find out the effect of biofertilizersto improve yield and quality of broccoli. The biofertilizers viz. *Azospirillum*, PSB, *Azotobacter* and VAM were applied alone and in combinations,inorganic fertilizer as positive control and without manuring as negative control. It was observed that application of inoculants *Azospirillum*+*Azotobacter* (50% each) significantly increased the curd size (15.17 cm diameter) and curd yield (1.17 kg, 0.93 kg curd with and without guard leaves, respectively) of broccoli found maximum compared to other treatments. The results showed that *Azospirillum* (100%), PSB (100%) and *Azotobacter* (100%) also had better performance than the recommended dose of fertilizers, but other treatment combinations except *Azospirillum*+*Azotobacter* (50% each)performed poor than the recommended dose of fertilizer.Fourier Transform Infra-Red (FTIR) analysis was done to out find the significant presence of potential biomolecules in broccoli. Among the biofertilizer treatments use of *Azospirillum*+*Azotobacte*r (50% each) clearly showed the improvement in protein and lipid profile along with phosphate and sulphate content of broccoli curd.Thus, the study concluded that use of *Azospirillum*+*Azotobacter* (50% each) was found better for improving the curd yield of broccoli and its active biomolecules.

1. Introduction

Broccoli (*Brassica oleracea* var. *italica* Plank) belongs to family Brassicaceae (Cruciferae) is an important high value vegetable and becoming popular very fast worldwide. This cole crop originated from a common ancestor, the wild cabbage, *Brassica oleracea* var. *sylvestris*. Broccoli is herbaceous annual vegetable grown for its green tender curd and biennial in respect of seed production. In sprouting broccoli, a main head is produced terminally on a fleshy, branching, elongated stem consisting of green buds and thick fleshy flower stalkknown as curd. Its production in 2012 was about 7.00 mt in India around double from 2002 (4.89 mt) and China became the leader by producing about 9.59 mt in 2012. Other broccoli producing countries are USA (California), France, Italy, Spain etc. (FAO Database, 2013). Broccoli is rich in nutrients like Ca, Fe, Mg, P, K, Zn and vitamins like B_1, B_2, B_3, B_5, B_6, B_9, C and A. Although, broccoli is known from very ancient time beginning from the time of Roman Empire but got popularity beyond France, Italy after 20th century due to its most potential anti-carcinogenic property. The chemical isothiocyanates are found in broccoli which can produce sulforaphane substrate that manufactures enzymes which are powerful cancer fighters. The bio-active nutrients like β-Carotene, α-Tocopherol, Ascorbate, Glucoraphanin, Aliphatic glucosinolates, Indolylglucosinolates etc.are found in broccoli in abundant (Kurilich et al., 1999). Inclusion of cruciferous vegetables including broccoli in daily diet decreases the risk for a number of different cancers (Verhoeven et al., 1997). Epidemiological studies have revealed that the taking of cruciferous vegetables such as broccoli, brussels sprouts or cabbagesignificantly lowered the risk for prostate cancer (Cohen and Stanford, 2000). It is an excellent dietary source of antioxidant, vitamins and glucosinolates, precursors to a group of isothiocyanates shown to be anti-carcinogenic (Jeffery and Jarrell, 2001). Dietary antioxidants, vitamins and non-nutrient components such as flavonoids present in crucifers like broccoli may decrease the risk for certain cancers (Lindsay and Astley, 2002). Indole-3-carbinol, a hydrolysis product of the glucosinolateglucobrassicin, has proven successful against breast cancer (Telang et al., 1997) and respiratory papilloma (Rosen et al., 1998). As a consequence of continuous use of chemical fertilizers, farmers are facing the problem of soil deterioration, affecting soil health and ultimately yield and quality of crops (Maji and Das, 2008a, b). There is an increased emphasis in organic production of food materials for health and environmental hazards (Reddy et al., 2011 and Maji, 2013). Biofertilizers can be an alternative to crop nutrition and effectively reduce the requirement of synthetic fertilizers in respect to nitrogenous and phosphatic fertilizers. Very little works have been carried out on the efficiency of biofertilizers in yield, quality and response to bio-active components of broccoli and the present experiment was laid down with that aim to improve the yield and bio-moleculespresent in broccoli through application of some biofertilizers.

2. Materials and Methods

The experiment was carried out as pot experiment during October, 2011-February, 2012 at the Horticultural Research Farm (Pragya Vatika), Department of Applied Plant Science, School for Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, India situated at $25^0$56' North latitude and $80^0$52' East longitude at an altitude of 111 m above mean sea level under subtropical climate with extreme summer and winter. The biofertilizers viz. *Azospirillum*, Phosphate Solubilizing Bacteria (PSB), *Azotobacter* and Vesicular Arbascular Mycorrhizae (VAM) (procured from Division of Microbiology, Indian Agricultural Research Institute, New Delhi) were applied alone and in combination (50% each) with recommended dose of inorganic fertilizers as positive control and without manuring as negative controlin complete randomized design (CRD) with 4 replications. The recommended dose of fertilizer (RDF) i.e. 2.4 g nitrogen, 1.2 g P_2O_5 and 1.0 g K_2O plant^{-1} was applied 21 days after application of biofertilizers. Broccoli seedlings were inoculated by dipping the roots of seedlingsin the slurry (10% jaggery solution) of the biofertlizers for at least 30 minutes prior transplanting. VAM was applied in soil near the root zone of seedlings at the time of transplanting. The seedlings were monitored every day and observations were taken on vegetative growth and curd yield parameters. For Fourier Transform Infra-Red (FTIR) spectroscopy study, the florets of broccoli were shade dried at room temperature in a clean environment to avoid contamination for 14 days and powdered in a domestic grinder. After 14 days the florets were kept in oven drier at 50^{o}C for 4-5 hours. The powdered samples were stored in air tight glass bottles at room temperature for further analysis. The samples were again ground in an agate mortar and pestle in order to obtain fine powder and was mixed with completely dried potassium bromide (at a ratio of 1/100) and the mixture was subjected to a pressure of 5×10^6 pa to produce a Kbr pallet for use in a FTIR spectrometer (FTIR 460 plus-Jasco) at University Scientific Instrumentation Centre (USIC) of Babasaheb Bhimrao Ambedkar University, Lucknow. Special care was taken to prepare the pallets at the same thickness by taking the same amount of sample and by applying the same pressure.

3. Results and Discussion

The experimental result (Table 1) revealed that the application of biofertilizers significantly improved the curd yield andits attributes. In general, biofertilizers improved the diameter of curd, number of frauds curd^{-1} and curd weight with or without guard leaves over control (without manuring). Seedlings treated with *Azospirillum*+*Azotobacter* (50% each) significantly increased the curd yield and yield attributes among the

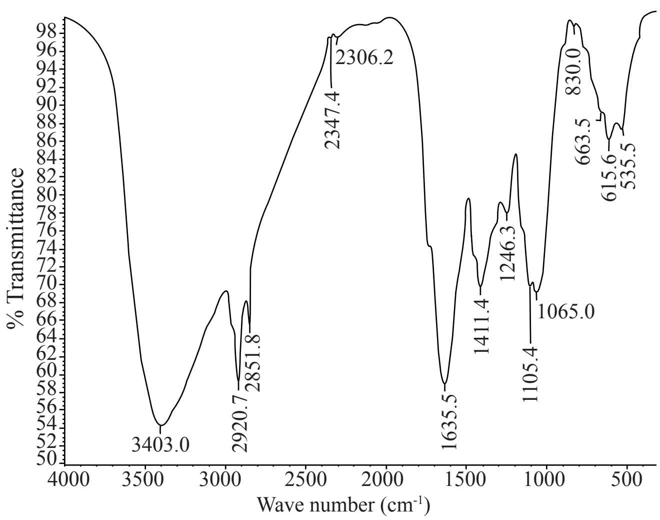
Table 1: Effect of biofertilizers and inorganic fertilizers on broccoli curd yield and its attributes

Treatments	Curd diameter (cm)	No. of frauds head^{-1}	No. of slips curd^{-1}	Weight of the curd with guard leaves (kg)	Weight of the curd without guard leaves (kg)
Azospirillum (100%)	15.05	13.75	30.75	1.02	0.79
Phosphate Solubilizing Bacteria (PSB) (100%)	14.75	12.00	30.50	1.05	0.79
Azotobacter (100%)	14.99	12.50	29.50	1.05	0.84
Vesicular Arbuscular Mycorrhizae (VAM) (100%)	13.23	11.75	30.75	0.97	0.70
Recommended dose of inorganic (NPK) fertilizers (100%)	14.49	11.50	31.25	1.10	0.89
Azospirillum+*Azotobacter* (50%+50%)	15.17	14.00	32.25	1.17	0.93
Azospirillum+VAM (50%+50%)	13.40	12.00	30.25	1.00	0.80
PSB+*Azotobacter* (50%+50%)	13.04	12.75	31.50	1.29	0.86
PSB+VAM (50%+50%)	13.31	13.25	30.75	1.03	0.70
Azotobacter+VAM (50%+50%)	13.37	12.75	30.75	1.00	0.85
Control (Without any manuring)	11.59	11.25	29.00	0.84	0.58
SEd±	0.848	0.762	0.935	0.075	0.025
CD (p=0.05)	1.705	1.532	1.881	0.150	0.051

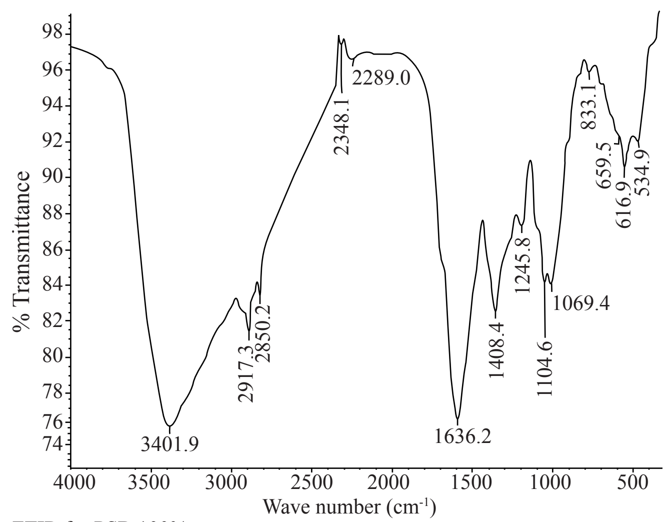
biofertilizer sources even better than the recommended dose of inorganic fertilizers. The result showed that maximum curd diameter (15.17 cm), number of frauds head^{-1} (14.00), number of slips curd^{-1} (32.25), curd weight without guard leaves (0.93 kg) were recorded under treatment combination *Azospirillum+Azotobacter* (50% each) and minimum was always observed under control (without manuring). Though, others biofertilizer treatments improved the yield attributes over negative control but, did not follow any specific pattern. Sometimes, they performed poor compared to positive control (recommended dose of inorganic fertilizers). The number of frauds head^{-1} was not improved by inorganic fertilizers however, the reason behind it is not clear. Interestingly, application of PSB+*Azotobacter* (50% each) resulted the highest curd yield (1.29 kg) with guard leaves but, maximum weight of curd without guard leaves (0.93 kg) was recorded under treatment T_6 (*Azospirillum+Azotobacter* @ 50% each) and it increased actual curd weight almost double (60.34%over the negative control) than control i.e. plants without manuring (0.58 kg curd without guard leaves).

The yield improvement through application of biofertilizers might be due to available nutrients, particularly N and P and micronutrients, increase inmicrobial activity, production of growth promoting substances and plant-soil-microbes interaction asreported by Pathak and Ram (2002) in broccoli, cabbage, tomato andcapsicum. In earlier research, the maximum number of slips and frauds were also recorded by Manivannan and Singh (2004) with the application of *Azospirillum+Azotobacter*. Mehrotra and Lohri (1971) also observed 26-45% increase in yield of cabbage when inoculated with *Azotobacter* as compared to uninoculated control. Similar trend was also reported by Chattoo et al. (1997), Pandey and Kumar (1989), Sable and Bhamare (2007), Sood and Vidyasagar (2007), Gupta et al. (2010) and Chatterjee (2010) in cabbage, cauliflower, knolkhole and broccoli. Similarly, yield improvement through organic nutrition was also showed by Kumar et al., 2014 in radish, Meena et al., 2014 in tomato and Maji and Das (2008 a,b) in fruit crops like guava.

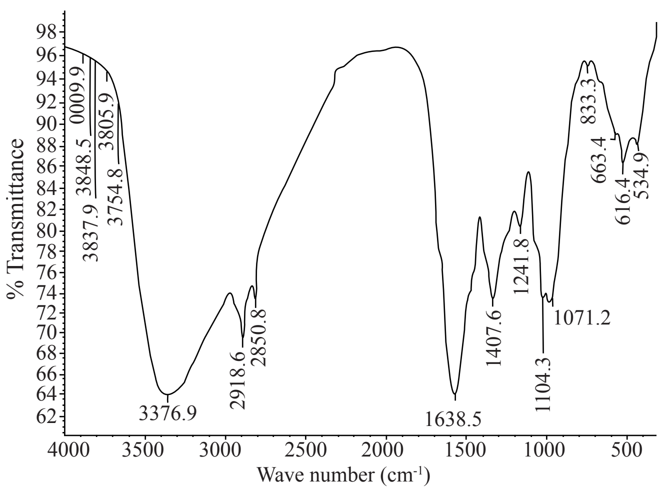
The FTIR analysis (Figure 1 to 10) represented the presence



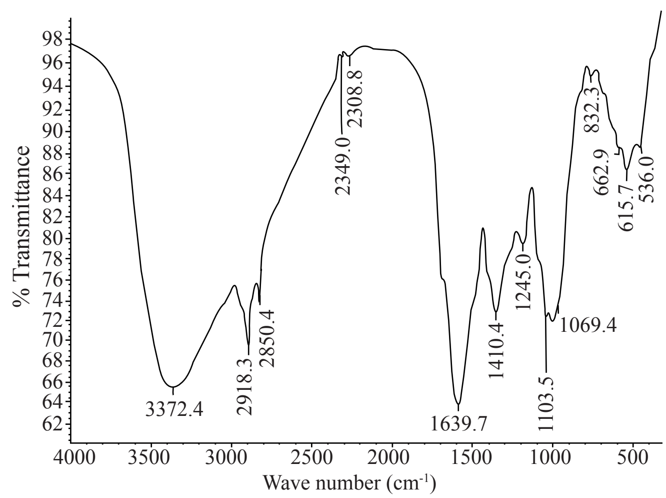
FTIR for *Azospirillum* 100%



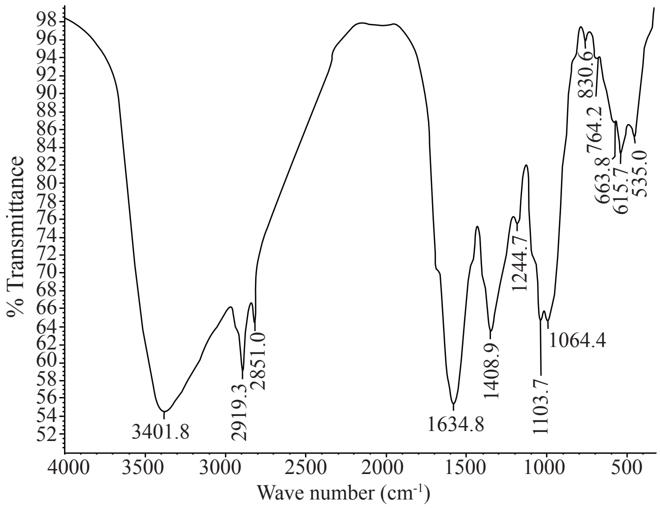
FTIR for PSB 100%



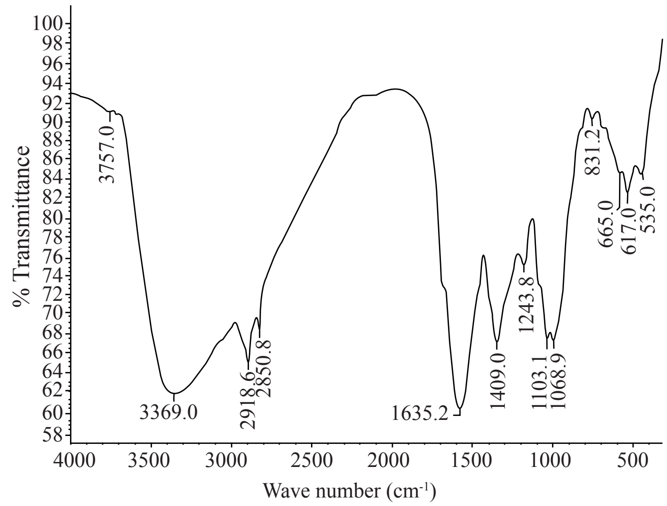
FTIR for *Azotobacter* 100%



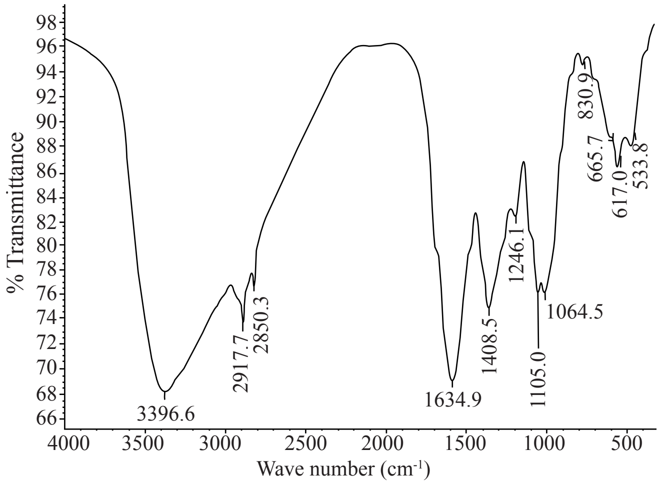
FTIR for VAM 100%



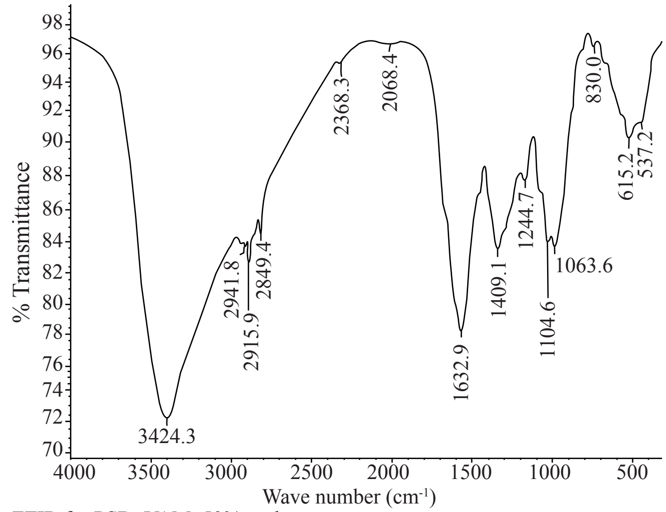
FTIR for RDF 100%



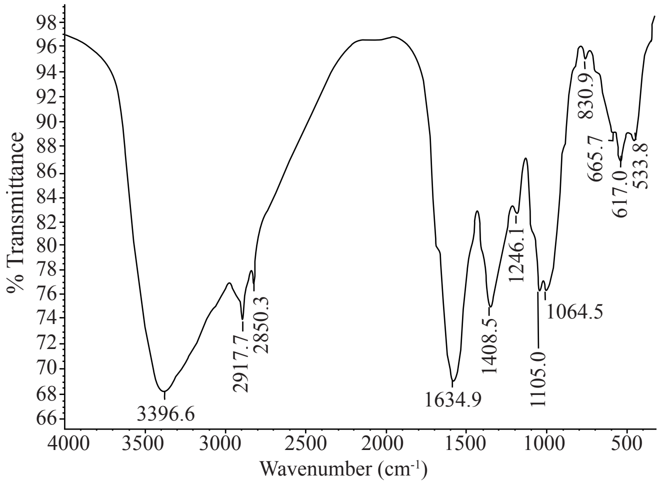
FTIR for *Azospirillum*+*Azotobacter,* 50% each



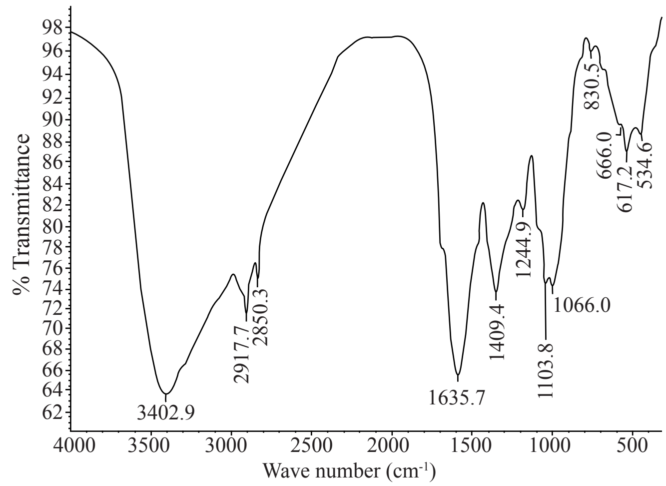
FTIR for PSB+*Azotobacter,* 50% each



FTIR for PSB+VAM, 50% each



FTIR for *Azospirillum*+VAM, 50% each



FTIR for *Azotobacter* 100%

and variations of different active biomolecules of broccoli curd under different treatments. The figures indicated the presence of C=O, C-H, C=C, C-O, C-C and C-X groups and bonding structure responsible for the significant presence of different groups i.e. alkyl, methyl, methylene, alcohols/phenols, ethers (more aromatic), esters, carboxylic acid, anhydrides, dioxyribose,fluoroalkanes, vinyles, nitrile, amines, sulphate and phosphate goups. The O-H groups, carbohydrates, lipid and protein regions were found abundant in all the biofertilizer treatments. The strong and weak peak of bands (absorption peaks found in the present study and respective assignments were presented in Table 2) indicated that the maximum functional groups were found under *Azospirillum*+*Azotobacter* (50% of each) treatment followed by treatment *Azospirillum*+PSB (50% each). However, very little variation was found due to treatment PSB+*Azotobacter,* (50% each) and PSB+VAM, (50% each) (Figure 7 and 8) and *Azospirillum*+VAM (50% each) with *Azotobacter*+VAM, (50% each) in Figure 9 and 10. The information regarding FTIR analysis for biomolecules in broccoli is very less, but the yield and quality improvement was proved by Greenwood et al. (1980), Dufault (1998) and Sarkar et al. (2010) who explained that the yield and other quality attributes were influenced by nutrient management and

Table 2: Peak and assignment found in various treatments under FTIR study

Peak Absorption^{-cm}	Assignment	Peak Absorption^{-cm}	Assignment	Peak Absorption^{-cm}	Assignment	Peak Absorption^{-cm}	Assignment
532.9	C-H Lipid region	615.7	C-H Lipid region	764.2	Amine (Proteins)	1064.4	Carbohydrates
533.8	C-H Lipid region	616.5	Amine (Proteins)	823.3	CH_3 bending (Lipids & proteins)	1064.5	Glycogen
534.6	Amine (Proteins)	616.9	CH_3 bending (Lipids & proteins)	830	PO^{2-} (Phosphate-I)	1065	Deoxyribose
534.9	CH_3 bending (Lipids & proteins)	617	PO^{2-} (Phosphate-I)	830	Carbohydrates	1066	CH bending
534.9	PO^{2-} (Phosphate-I)	617	Carbohydrates	830	Glycogen	1068.9	Sulphide
535	Carbohydrates	617.2	Glycogen	830.5	CH bending	1069.4	Phosphates
535	Glycogen	633.4	CH bending	830.6	Sulphide	1069.4	O-H stretching
535.5	Deoxyribose	659.5	Sulphide	830.9	Phosphates	1103.7	CH_3 bending (Lipids & proteins)
536	CH bending	662.9	Phosphates	831.2	O-H stretching	1103.8	PO^{2-} (Phosphate-I)
537.2	Sulphide	663.2	O-H stretching	833.1	C-H Lipid region	1103.9	Carbohydrates
537.2	Phosphates	663.5	C-H Lipid region	833.3	C-H Lipid region	1104.3	Glycogen
615.2	O-H stretching	663.8	C-H Lipid region	1063.6	CH_3 bending (Lipids & Proteins)	1104.6	Deoxyribose
615.6	C-H Lipid region	665	C-H Lipid region	1063.6	PO^{2-} (Phosphate-I)	1104.6	C-H bending
1104.6	Sulphide	1409.4	Glycogen	2289	CH bending	2850.8	Nitrogen-Ethers
1105	Phosphates	1410.4	Deoxyribose	2306.2	Sulphate	2850.8	Sulphate
1105.4	O-H bonds	1411.4	CH bending	2308.8	Phosphates	2919.3	C-H bending
1241.8	O-H stretching	1408.9	Amines (Proteins)	2347.4	O-H bonds	2920.7	Lipid & proteins
1243.8	C-H Lipid region	1409	(Lipid &protenis)	2349	O-H bonds	2941.6	Phosphate-I
1244.3	C-H Lipid region	1632.9	CH bending	2368.3	O-H bonds	2941.6	Carbohydrates
1244.9	Carbohydrates	1632.9	Sulphate	2368.3	O-H bonds	3369	Carbohydrates
1245	Glycogen	1634.8	Phosphate	2815.9	O-H stretching	3372.4	Deoxyribose
1245.8	Deoxyribose	1634.9	O-H stretching	2815.9	C-H Lipid region	3376.9	C-H bending
1246.1	CH bending	1635.2	C-H Lipid region	2849.4	C-H Lipid region	3396.6	Sulphate
1246.3	Sulphate	1635.5	C-H Lipid region	2849.4	Proteins	3397.5	Phosphate
1407.6	Phosphate	1638.5	Amines (Proteins)	2850.2	Lipid & proteins	3401.8	O-H, stretching
1408.4	O-H stretching	1638.8	CH_3 bending	2850.3	Lipid & proteins	3754.8	PO^{-2} asymmetric phosphate-I
1408.5	C-H Lipid region	1639.7	PO^{2-} (Phosphate - I)	2850.3	Phosphate	3757	Carbohydrates
1409.1	PO^{2-} Phosphate-I	2068.4	Carbohydrates	2850.4	Carbohydrates	3805.9	Carbohydrates
1409.1	Carbohydrates	2068.4	Glycogen	2850.8	Proteins, Amide oligosaccharides	3818.9	Deoxyribose

very specifically nitrogenous nutrition significantly improved the yield and quality attributes. Similarly, improvement in antioxidant with the use of organics in broccoli was also reported by Sanwal et al. (2006). Leclearc et al. (1991) also suggested that application of organic manures could improve active compounds like carotene, antioxidants etc. in carrot. The combine application of *Azospirillum* and *Azotobacter* (50% each) improved the nitrogen availabilityfor the plants by binding the atmospheric nitrogen and thus, improved the bio-active molecules present in broccoli also.

4. Conclusion

Application of biofertlizera combination of *Azospirillum*+ *Azotobacter* (50% of each) through root dipping method during transplanting is beneficial for yield enhancement as well as for the improvement of functional biomolecules present in broccoli.

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