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INFLUENCE OF FOLIAR SPRAY OF BIO-INOCULANT AND MICRONUTRIENT ON SOIL BIOLOGY AND YIELD IN BROCCOLI

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***Key words**: Broccoli, Foliar spray, Bio-inoculants (Azotobacter and Azospirillum), Ammonium molybdate, and Soil biology*

Abstract- The experiment was carried out in the years 2021-22, at the Department of Agricultural Microbiology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). The study involved application of foliar spray of bio-inoculants and micronutrient; Ammonium Molybdate. Bio-inoculants; *Azotobacter* and *Azospirillum* inoculation strains were collected from Microbiology repository and were revived for biochemical characteristics on Jensen's and Okon's media respectively. Both the isolates showed positive reaction for starch hydrolysis test, catalase test, urease test and oxidase test. Plant parameters evaluated include biomass accumulation and fruit weight per plant. The experimental results revealed that significantly maximum fruit weight per plant (359.0g), dry shoot biomass (16.6g/ plant), dry root biomass (8.6g/ plant) were recorded under T4 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%). Besides higher microbial population, *Azotobacter* (44.3 x 10^3cfu/ g of soil), *Azospirillum* (28.5x 10^3cfu/ g of soil) were also observed to be significant under T4. Dehydrogenase activity in rhizosphere soils was found maximum 27.3 µg TPF/g soil/h under T4 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%) at 60DAT. Thus application of Bio-inoculants along with Ammonium molybdate (0.2%) as foliar spray along with 50% inorganic fertilizer proved to be the most effective and may be adopted in Broccoli cultivation.

INTRODUCTION

Broccoli (*Brassica oleracea* L.var *italica Plenck*) is an important winter season exotic vegetable of Brassicaceae family. It contains vitamins, antioxidants, glucosinolates, and other anti-cancer chemicals (Parente *et al.*, 2013). It is consumed as a cooked vegetable as well as soup, salad, and as a mixed vegetable. Our country having diverse climatic conditions and well distinct cropping season, offers a great scope to grow these unconventional vegetables commercially. India rank second in area and production for cauliflower and Broccoli. World area and production is 1.21 million hectare and 20.88 Million tonne and production and area in India are 6745 thousand tonnes and 369 thousand hectares (Annonymous, 2015b). Plant nutrition is one of the prime considerations for getting higher yield of any crop and broccoli is a heavy feeder of plant nutrients. Mineral fertilizer improves growth and yield of broccoli, but addition of chemical fertilizer results in pollution of the environment. Instead we can use biofertilizer i.e., free living nitrogen-fixing bacteria such as *Azotobacter* and *Azospirilum* which have the ability not only to fix nitrogen but also to release certain Phytohormones, i.e. GA3, IAA and cytokinins which could stimulate plant growth and increase the availability of nutrients for plant roots by the increase in their dissolution (Siddique *et al*., 2014).

Boron and molybdenum are essential micronutrients required for normal plant growth and development; their deficiencies are very common in Cole crops. Deficiency causes many anatomical, physiological, and biological changes. The affected heads become irregular in shape, smaller in size and bitter in taste which adversely affects the market demand of the crop.

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Micronutrient deficient plant shows the symptoms of hollow stems, browning of heads, and sword like leaves (whiptail). Many researchers observed that boron and molybdenum in cole crops, increased growth and yield of the crops. No systematic work so far has been done in production technology of sprouting broccoli in relation to response of nutrients in Chhattisgarh plains. Keeping this perspective in view, the following experiment was undertaken to evaluate the response of Ammonium molybdenum along with bio-inoculants (*Azotobacter* and *Azospirillum* strains) applied as foliar spray on growth and yield of Broccoli and also to study the rhizosphere biological properties.

MATERIALS AND METHODS

The experiment was carried out during the year 2021-22, in the poly-house at Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). The study was laid out in CRD with 07 treatments replicated four times. Treatments consisted of T_1 (Inorganic fertilization (50% RDF) through Fertigation, (IF), T_2 (IF + Foliar Application of Bio-inoculants), T_3 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.1%), T_4 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%), T_5 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.4%), T_6 (IF + Foliar Application of Ammonium molybdate 0.2%) and T_7 (IF + Foliar Application of Ammonium molybdate 0.4%). Foliar spray of Bio-inoculants *Azotobacter +Azospirillum* at 30, 45 and 60 DAT and foliar Application of Ammonium molybdate was given after 06 days gap of spraying bio-inoculants.

Native *Azotobacter* and *Azospirillum* isolates were obtained from the Departmental Culture Collection Bank for this investigation and revived by inoculating the isolates in Jensen's and Okon's media at pH 7.0 respectively, then incubated at 28±2°C. Bacterial colonies were obtained after 24-48 hours of incubation and their colony morphological properties were examined. Isolates were studied for their phenotypic and growth trend. Gram staining was performed to categorize the isolates from *Azotobactor* and *Azospirillum* as gram positive or negative (Aneja, 2003). Pure colonies were chosen and transferred to corresponding agar slants after morphological traits were observed, in order to preserve them for further investigations at 4°C temperature in the refrigerator.

Starch hydrolysis Test: The starch hydrolysis method detects the enzyme amylase, which breaks down starch. Isolates were streaked singly on starch agar media and incubated at 28 °C ± 2 °C for 48 hours. After completing the requisite incubation, the plates were flooded with iodine solution to test the isolates starch digestion capacity. A yellow zone around the colony indicated starch hydrolysis, while a blue/black area indicated starch presence (Hossain *et al*., 2015).

Catalase Test: A drop of bacterial culture was transferred on the surface of a clean, dry glass slide using a loop. A drop of 3% H_2O_2 was dropped on the slide, quick oxygen evolution (within 5-10 seconds) as proven by bubbling indicated a positive result (Aneja 1993).

Urease Test: *Azotobacter* and *Azospirillum* cultures were added to the Urea agar slants. At 37 °C for 24 hours, the slants were incubated. If the bacterial growth zone on the orange-colored urease slant turns pink, urease hydrolysis activity is confirmed (James and Natalie, 1992).

Oxidase Test: *Azotobacter* and *Azospirillum* cultures were added to nutrient agar medium plates and incubated for 24-48 hours at 37 °C. After incubation, oxidase disc was place on plates. A change in color within 10 seconds or deep purple color change was deemed positive.

Effect of inoculation on biomass and yield of Broccoli

At harvest, all fruits from each treatment and replication were weighed. Its weight was recorded, and the average value was calculated. The plant parts like shoot and roots were collected from the growing test plants while sampling at harvest. Dry bio-mass was recorded by oven dried at 65 °C till it reached at steady weight for 3-5 days.

Enumeration of microbial population in rhizosphere soil of broccoli at flowering stage

Microbial population in rhizosphere soil of broccoli at flowering stage was carried out by serial dilution and plating. The plates were incubated at 28 °C and after 24 hours of incubation, colonies were counted. The populations were expressed as cfu g^{-1} of dry soil using the formula below after the colonies were counted (Schmidt and Caldwell, 1967).

Number of *Azotobacter* and *Azospirillum* per gram of oven dried soil:

$$\text{No. of colony forming units (CFU)} = \frac{\text{No. of colony forming units (CFU)} \times \text{dilution}}{\text{Dry wt. of 1 g moist soil} \times \text{aliquot taken}}$$

Dehydrogenase activity in rhizosphere soil of Broccoli

Soil dehydrogenase activity (DHA) was measured in terms of amount of triphenyl formazan (TPF) produced during incubation of rhizosphere soil sample with 2,3,5- triphenyl tetrazolium chloride (TTC) at 37°C for 24 h and was expressed as µg (TPF) g^{-1} soil hr^{-1} (Klein *et al.*, 1971).

RESULTS AND DISCUSSION

Morphological characteristics of Bio-inoculants

Azotobacter isolate produced translucent, nearly round and gummy colonies which varied in size between 1.5 to 2.00 mm and whitish in colour (Table 1). Based on their colony morphology and growth, colonies of *Azospirillum* isolate were found as flat, round, entire margine and off white in colour. Both the isolates were gram (–ve) (Table 1). The isolated bacteria were identified in reference with Bergey's manual of Systematic Bacteriology. The identification studies are also in coordination with the works done by Gadagi and Tongmin (2002) and Kumar *et al.* (2020).

Bio-chemical characteristics of Bio-inocolants

Azotobactor and *Azospirillum* showed positive reaction for starch hydrolysis, catalase, and urease test and oxidase test (Table 1 and Plate 1). Similar result was obtained by Kujur *et al.*, (2020), and A. Muthukumar *et al.*, (2021).

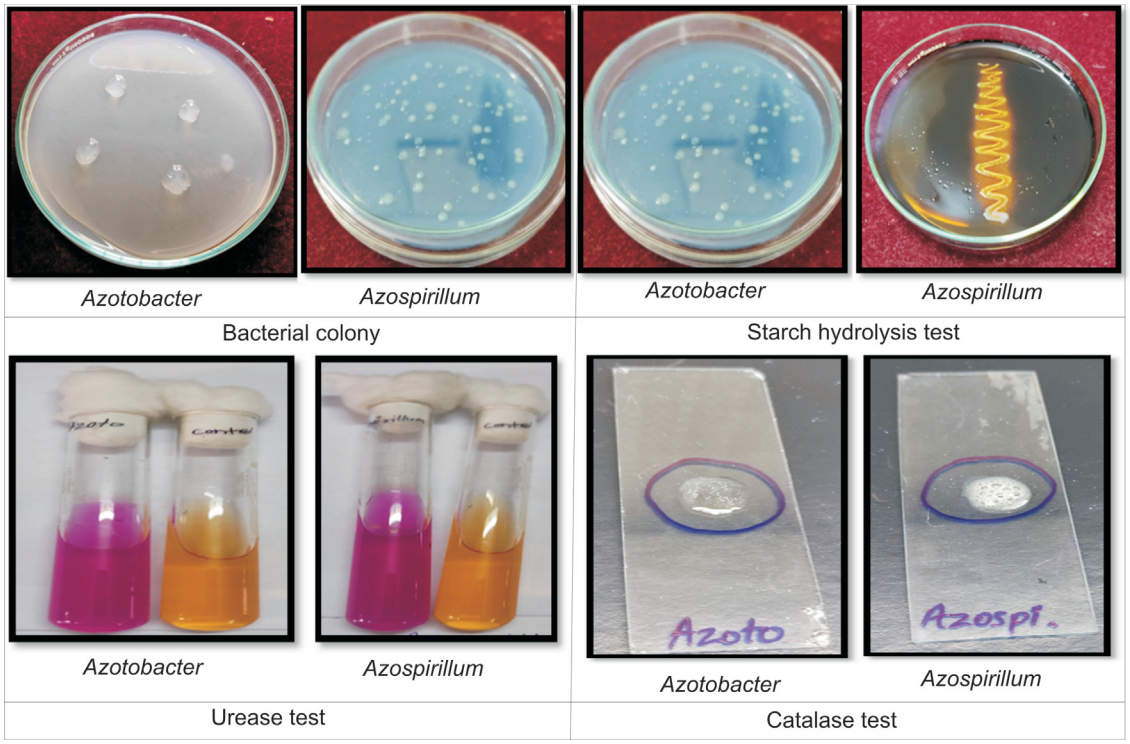

Plate 1. Biochemical characteristics of *Azotobacter* and *Azospirillum* strain

Table 1. Morphological and Bio-chemical characteristics of inoculants used for foliar spray in Broccoli

Biochemical test	*Azotobacter*	*Azospirillum*
Colony morphology	Gummy, round and convex, entire margin, whitish in colour	Flat, round, entire margin, off- white in colour
Gram staining	Gram -ve	Gram -ve
Starch hydrolysis	+ve	+ve
Catalase test	+ve	+ve
Urease test	+ve	+ve
Oxidase test	+ve	+ve

(+ve): Positive response

Yield/plant (g)

The treatment T4 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%) recorded the maximum fruit yield (359.0 g) which was at par with T3 (349.8g) but significantly differs from T2 (343.0g) and other treatments. The significantly minimum yield/plant was recorded in treatment T1 (IF) (268.0g). Application of Bio-inoculants showed significant difference in fruit yield over T1. Applying Molybdenum at 0.2% dose showed significant effect on fruit yield (Table 2). Similar results were obtained by Mohamed and Abdelnaser (2011) on cauliflower, Khare and Singh (2008) on cabbage cv. Golden acre.

Biomass accumulation in Broccoli

Maximum shoot dry weight was recorded in T4 (IF +Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%) (16.6 g/plant) followed by T3 and T2 (14.5g/plant and 13.6 g/plant). Whereas maximum root dry weight was recorded in T4 (IF+ Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%), (8.6 g/plant) followed by T3 (8.2 g/plant) and T2 (8.1 g/plant). Biomass accumulation in Broccoli plant at harvest varied significantly among treatments, being highest with application of bio-inoculants and spray of Molybdenum at moderate dose @ 0.2%. Similar result was obtained by Singh *et al.* (2017).

Soil biological properties

Population density of *Azotobacter* and *Azospirillum* in rhizosphere soil at flowering stage were observed in between 21.5×10^3 to 44.3×10^3 /g of soil and 14.8×10^3 to 28.5×10^3 /g of soil among treatments respectively. The *Azotobacter* and *Azospirillum* populations were recorded significantly maximum i.e. 44.3×10^3/g of soil and 28.5×10^3/g of soil in T4 (IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%). The minimum *Azotobacter* and *Azospirillum* populations i.e. 21.5×10^3/g of soil and 14.8×10^3/g of soil were found in T1 (IF).

The data of dehydrogenase activity (DHA) in rhizosphere soils of broccoli were presented in Table

Table 2. Effect of Bio-inoculants and Ammonium molybdate on Biomass and yield in Broccoli

Treatment	Treatment details	Weight per Curd (in g)	Biomass Accumulation (g plant $^{-1}$)	
			Dry Biomass	
			Shoot	Root
T1	IF	268.0	9.6	7.4
T2	IF + Foliar Application of Bio-inoculants	343.0	13.6	8.1
T3	IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.1%	349.8	14.5	8.2
T4	IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%	359.0	16.6	8.6
T5	IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.4%	298.3	12.9	7.8
T6	IF + Foliar Application of Ammonium molybdate 0.2%	318.0	13.2	7.7
T7	IF + Foliar Application of Ammonium molybdate 0.4%	282.8	12.5	7.3
	SEm±	5.603	0.284	0.146
	CD (0.05 %)	16.591	0.839	0.434

Table 3. Effect of Bio-inoculants and Ammonium molybdate on Dehydrogenase activity in rhizosphere soil at flowering stage of Broccoli

Treatment	Treatment details	Dehydrogenase activity of composite rhizosphere soil sample µgTPF/g soil/h
T1	IF	13.5
T2	IF + Foliar Application of Bio-inoculants	26.0
T3	IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.1%	27.2
T4	IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.2%	27.3
T5	IF + Foliar Application of Bio-inoculants and Ammonium molybdate 0.4%	26.8
T6	IF + Foliar Application of Ammonium molybdate 0.2%	24.5
T7	IF + Foliar Application of Ammonium molybdate 0.4%	22.8
	SEm±	0.421
	CD (0.05 %)	1.247

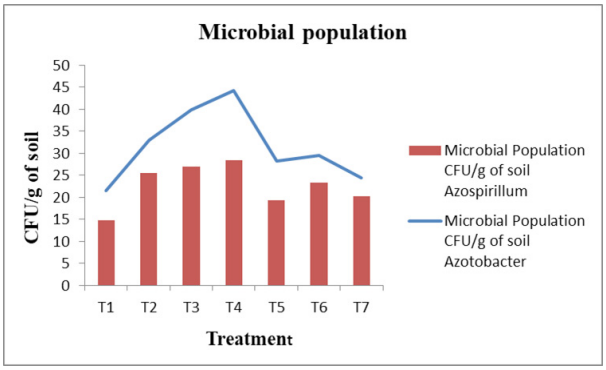

Fig. 1. Effect of Bio-inoculants and Ammonium molybdate on microbial population in rhizosphere soil of Broccoli at flowering stage

3. At 60 DAT the dehydrogenase activity was increased due to application of *Azotobacter* and *Azospirillun* isolates in combination. The treatments which have shown significant maximum in dehydrogenase activity over T1 were T7, T6, T5, T4, T3, T2 and being maximum in T4. Above observations were in close agreement with Nowark (1996) and Wyszkowska and Kucharski (2004) who claimed that dehydrogenase activity is a reflection of the biological status of the soil.

CONCLUSION

Foliar spray of bio-inoculants and micronutrient (Ammonium molybdate) has proven to be the best treatments with regard to the growth performance, fruit yield of broccoli as well as in improving soil biological properties. Broccoli plant cvTSX-2004 responded well to treatments T4 and T3 consisting of foliar application of bio-inoculants and lower doses of Ammonium molybdate (0.1% and 0.2%). It can be recommended for commercial production of broccoli to enhance the yield and quality of fruit. However to arrive at more reliable results, further repeated experimentation is required and also to ensure the long term success in growth of broccoli by inoculation.

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