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EFFECT OF MICROBIAL CONSORTIUM ON PLANT GROWTH PROMOTION, BIOCHEMICAL ATTRIBUTES AND NUTRIENT UPTAKE OF CABBAGE (*BRASSICA OLERACEA L VAR. CAPITATA*)

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ABSTRACT
An experiment was conducted to evaluate consortium of PGPRs including *Trichoderma harzianum* (UBT-18), *Pseudomonas fluorescens* (Vpf-1), Phosphate solubilizing bacteria (UBPS-9) and *Azotobacter* sp (UBAZ-1) on seedling health of bio-enriched transplants of cabbage with the aim of primary intervention in organic production of the crop. Germination, protein content, poly phenol oxidase activity of the seedlings, dehydrogenase activity of nursery mix and uptake of nitrogen, phosphorus and potassium at heading stage were obtained maximum in consortium of UBT-18 + UBPS-9 + UBAZ-1 and lowest was obtained in control treatment. Total chlorophyll, root and shoot length, fresh and dry biomass of root and shoot, vigor index and phenol content of the seedlings and available phosphorus and potassium of soil were found maximum in consortium of Vpf-1 + UBPS-9 + UBAZ-1 and lowest were found in control treatment. Available nitrogen of soil was maximum in consortium comprising of all four bioinoculants and lowest was found in control treatment. Hence from these results it was concluded that application of consortium of *Trichoderma harzianum* or *P. fluorescens* along with phosphate solubilizing bacteria and *Azotobacter* may be applied through seed treatment as one of the primary approach in organic production system of cabbage.

INTRODUCTION

Vegetable crops are grown worldwide as a source of nutrients and fiber in the human diet. Among the vegetables, cabbage belong to the genus *Brassica* of the family cruciferae are cultivated all over the world. Cabbages are rich source of Vitamin A, B and C and contain minerals like P, K, Ca, Na and Fe. The production is mostly depending upon inorganic inputs leads to human health hazards, environmental pollution and deterioration of soil health with respect to nutritional imbalance and microbial diversity. To cope up with the situation, encouraging the activity of beneficial microorganisms in the crop rhizosphere is the need of the day. Hence establishment of the Plant growth promoting microbes (PGPMs) in the rhizosphere immediately after germination of seeds is required to be taken care of. Use of PGPMs enriched seedling is one of the prime criteria in organic vegetable cultivation. Plant growth promoting rhizobacteria (PGPR) reside in the root and rhizosphere soil and have the capability to increase plant growth (Kloepper *et al.,* 1989). Application of biofertilizer increase plant growth and yield of chili (Khan and Parari, 2012). Application of PGPR along with manures increased nutrient content in soil (Das and Singh, 2014). Consorted application of plant growth promoting microorganisms increased plant growth (Maiyappan *et al*., 2010) However, the activity of the microorganisms is highly location and crop specific. Therefore, it is presumed that only native isolates of the microbes can play a pivotal role in this approach and to acquire maximum benefit rigorous screening is required to select appropriate microbial consortium before field application in a particular zone. With this background the present study was carried out under green house condition as well as in a field to investigate the effect of single and consortium inoculation of *Trichoderma harzianum, Pseudomonas fluorescens,* Phosphate solubilizing bacteria and *Azotobacter* on plant growth promotion, biochemical attributes at seedling stage and nutrient uptake at maximum fruiting stage and fertility status of the soil after harvest of the crop.

MATERIALS AND METHODS

Bio agents used

The investigations were carried out with the help of four bioagents, *Trichoderma harzianum* (UBT-18), *Pseudomonas fluorescens* (Vpf-1), Phosphate Solubilizing Bacteria (UBPS-9) and Azotobacter sp (UBAZ-1). The isolates of these bioagents were collected from the repository Dept. of Plant Pathology, UBKV, Pundibari, Coochbehar.

Bio-inoculation of Seed, sowing and transplanting

Seeds of cabbage (cultivar. Green Express), were first surface sterilized with 0.1% $HgCl_2$ solution then repeatedly washed in sterile distilled water. Seed encapsulation with bio agent(s) was carried following the pellet formulation technology suggested by Fravel et al. (1985). Homogenized cell suspension of the bio agent(s) containing viable propagules of about $1x10^{-9}$cfu/ml-1 was mixed in bentonite solution and then 3% sodium alginate solution was mixed to make slurry.

Seeds were then soaked in the slurry for 15-30 min and then immediately transferred to 2.5% $CaCl_2$ solution to make seed encapsulation with bio-agent(s). Pro-trays were used to develop the seedlings of cabbage. Potting mixture was prepared with sterilized soil and farm yard manure at 3:1 v/v. Potting mixture was inoculated with talc based formulation of the bio-inoculants @ 5g/kg of potting mix. Equal volume of homogenized cell suspension and equal quantity of talc based formulation were mixed for combined treatments. The seeds of different treatments with three replications were sown in pro-trays. The bio enriched seedlings of cabbage were developed in the pro-trays were transplanted in the field to evaluate the nutrient uptake ability at heading stage and fertility status of the soil after harvesting of the crop. The plots of 2X4 m2 were prepared and the bio-enriched seedlings were transplanted in the field by making holes at desired spacing and in each holes 500 g incubated soil + FYM mix was applied in a randomized block design with three replications for each treatment. The initial available NPK status of the experimental field was 193, 10, 62 kg ha^{-1}, respectively. Balanced fertilizer @ 20:20:20 was applied through spray @ 5g/ lit of water two times during active vegetative phase of the crop. Need based spray of contact insecticides was done to protect the plants from invasion of insects. The total no of 10 treatments were arranged as T1: *Trichoderma harzianum* (UBT-18) T2: *Pseudomonas fluorescens* (Vpf-1) T3: Phosphate solubilizing bacteria (UBPS-9) T4: *Azotobacter* (UBAZ-1) T5 *T.harzianum* (UBT-18) + *P.fluorescens* (Vpf-1) T6: Phosphate solubilizing bacteria (UBPS 9)+ *Azotobacter* (UBAZ 1) T7: *T. harzianum* (UBT-18) + Phosphate solubilizing bacteria (UBPS-9) + *Azotobacter* (UBAZ-1) T8: *P.fluorescens* (Vpf -1) + Phosphate solubilizing bacteria (UBPS-9) + Az*otobacter* (UBAZ-1) T9: *T. harzianum* (UBT-18) + *P. fluorescens* (Vpf-1) + Phosphate solubilizing bacteria (UBPS-9) + *Azotobacter* (UBAZ-1) T10: Control.

Germination percentage

Germination of the seeds was recorded on 4-5 days after sowing. Total germination was computed by using the following formula.

Germination percentage = (Total nos. of seeds germinated/ Total nos. of seeds sown) x100.

Evaluation of seedling growth

Ten randomly selected transplants of 21 days age from each treatment were taken for evaluation of seedling health. The length, fresh wt. and dry wt. of root and shoot of individual plants were recorded. The total chlorophyll of leaves was measured by using SPAD 502.Vigor index (VI) was computed from the following formula as suggested by Abdul-Baki and Anderson (1973).

Vigor index = Germination (%) × (root length + shoot length)

Biochemical analysis of bio inoculated seedlings

Biochemical studies were conducted to estimate total protein, total phenol, and poly phenol oxidase in leaves and dehydrogenase activity in nursery mix at transplanting stage as per methods described below.

Estimation of total protein was analyzed following the method described by Lowry *et al*. (1951). The total phenol content was estimated using Folin - ciocalteau reagent as per the procedure given by Malick and Singh (1980). Estimation of Poly Phenol Oxidase (PPO) was analyzed by the method given by Mayer *et al*. (1965). Dehydrogenase activity was estimated by the method of Rossel *et al*. (1997).

Analysis of nitrogen, phosphorus and potassium content in plants at heading stage

Estimation of total nitrogen, phosphorus and potassium was analyzed by the method given by Jackson (1967).

Analysis of Available nitrogen, phosphorus and potassium of soil after harvesting of bio-primed plants

Estimation of available N was analyzed by the method given by Jackson (1967), available P was analyzed by the method given by Bray and Krutz (1945) and available K was analyzed by the method given by Jackson (1967).

RESULTS AND DISCUSSION

Effect of microbial consortia on physical attributes of cabbage seedlings at transplanting stage

The effect of microbial consortium on physical attributes of cabbage seedlings at transplanting stage (Table 1) revealed that chlorophyll content was significantly high with consorted inoculation of VPf-1, UBPS-9 and UBAZ-1 compared to control but did not showed any significant differences among bio-inoculated seedlings. Germination was significantly higher with consorted inoculation of UBT-18, UBPS-9 and UBAZ-1 (67.83%) and it was statistically at par with consorted inoculation of VPf-1, UBPS-9 and UBAZ-1 (67.76%) and consortium of UBT-18 and VPf-1 (66.70%). Root length, shoot length, fresh shoot weight, root fresh weight , dry shoot weight and dry root weight were significantly higher with consortium inoculation of VPf-1, UBPS-9 and UBAZ-1 (8.30 cm, 16.08 cm, 3633.33 mg, 637.33 mg, 328.00 mg and 107.93 mg respectively) followed by consortium inoculation of UBT-18, UBPS-9 and UBAZ-1 (7.60 cm, 15.56 cm, 3543.33 mg, 591.00 mg, 318.33 mg and 94.40 mg respectively).The highest vigor index was recorded in the seedling grown with consortium of VPf-1, UBPS-9 and UBAZ-1 (2023.37) and it was statistically at par with consortium of UBT-18, UBPS-9 and UBAZ-1(1983.15) treatment. Consortium application of three PGPR increased germination percentage, seedling growth, vigor index, dry weights, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid contents higher than single and dual application of bio-agent(s) (Mathivanan *et al., 2014)*.

The results of biochemical analysis of cabbage seedlings showed that protein content and dehydrogenase activities were significantly high where consorted inoculation of UBT-18, UBPS-9 and UBAZ-1 was made (Fig. 1 and 3) and it was statistically at par with consorted inoculation of VPf-1, UBPS-9 and UBAZ-1. Total phenol content was significantly high in the seedlings produced by consorted inoculation of VPf-1, UBPS-9 and UBAZ-1 (Fig.2) compared to control treatment. Polyphenol oxidase activity was significantly high where consorted inoculation of UBT-18, UBPS-9 and UBAZ-1 (Fig. 4) was made compared to control treatment. Singh *et al*. (2015) was found higher phenol concentration and protein content on cluster bean due to inoculation of seed with PGPR compared to un-inoculated seed. Consortium application of

Table-1: Effect of microbial consortia on seedling health of cabbage at transplanting stage

Treatment	Chlorophyll (SPAD Value)	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh shoot wt (mg)	Fresh Root wt (mg)	Dry shoot wt (mg)	Dry Root wt (mg)	Vigor index
T1	37.67	74.67 (59.79) *	12.58	5.23	2690.00	370.67	263.00	65.13	1329.81
T2	37.00	74.00 (59.35)	12.24	4.63	2653.33	322.67	252.67	64.23	1248.85
T3	36.00	74.00 (59.35)	12.44	4.83	2653.33	344.33	256.00	66.20	1278.32
T4	37.50	74.67 (59.79)	12.31	4.83	2626.67	367.00	247.67	68.20	1280.49
T5	37.83	84.33 (66.70)	13.73	6.53	3116.67	510.33	281.67	81.03	1709.37
T6	37.67	81.00 (64.16)	14.23	5.57	3176.67	444.00	292.00	69.97	1603.13
T7	39.40	85.67 (67.83)	15.56	7.60	3543.33	591.00	318.33	94.40	1983.15
T8	39.60	83.00 (67.76)	16.08	8.30	3633.33	637.33	328.00	107.93	2023.37
T9	38.33	78.33 (62.29)	14.52	5.30	3273.33	411.33	293.33	65.43	1552.47
T10	31.67	58.67 (49.99)	8.59	3.81	1933.33	286.00	176.67	31.67	727.63
SEm ±	1.99	0.75	0.06	0.20	19.47	1.20	2.13	1.50	24.17
LSD(P = 0.05)	4.19	2.22	0.13	0.43	40.91	2.51	4.46	3.14	71.82

*Figures in parenthesis are arcsine transformed prior to the analysis

T1 = Seed bio priming with UBT-18 ,T2 = Seed bio priming with VPf 1 ,T3 = Seed bio priming with UBPS-9 ,T4 = Seed bio priming with UBAZ-1, T5 = Seed bio priming with UBT-18 + VPf-1 ,T6 = Seed bio priming with UBPS-9 + UBAZ-1 ,T7 = Seed bio priming with UBT-18 + UBPS-9 + UBAZ-1 ,T8 = Seed bio priming with VPf-1 + UBPS-9 + UBAZ-1 , T9 = Seed bio priming with UBT-18 + VPf-1 + UBPS-9 + UBAZ-1, T10 = Control

Table 2: Effect of microbial consortia on NPK uptake by bio-primed cabbage plants and subsequent fertility status of soil.

Treatment	Plant uptake N (kg/ha)	P (kg/ha)	K (kg/ha)	Soil fertility status available N (kg/ha)	available P (kg/ha)	available K (kg/ha)
T1	22.84	12.66	51.72	218.67	21.33	75.67
T2	18.88	10.97	50.98	215.00	21.00	90.67
T3	24.23	15.11	46.22	214.33	22.67	98.67
T4	27.47	10.20	38.39	227.00	17.33	82.67
T5	22.68	11.82	60.02	216.00	28.33	83.33
T6	34.76	14.94	59.77	227.33	28.00	101.33
T7	40.81	27.76	90.00	232.00	30.33	108.00
T8	36.38	23.16	78.87	230.33	31.67	114.67
T9	37.26	20.84	68.04	232.33	28.67	105.00
T10	12.05	6.81	30.55	211.00	17.67	70.33
SEm ±	1.52	0.88	2.16	1.61	1.39	3.73
LSD(P = 0.05)	4.52	2.63	6.42	4.80	4.14	11.08

T1 = Seed bio priming with UBT-18 ,T2 = Seed bio priming with VPf 1 ,T3 = Seed bio priming with UBPS-9, T4 = Seed bio priming with UBAZ-1, T5 = Seed bio priming with UBT-18 + VPf-1 ,T6 = Seed bio priming with UBPS-9 + UBAZ-1 ,T7 = Seed bio priming with UBT-18 + UBPS-9 + UBAZ-1, T8 = Seed bio priming with VPf-1 + UBPS-9 + UBAZ-1, T9 = Seed bio priming with UBT-18 + VPf-1 + UBPS-9 + UBAZ-1, T10 = Control

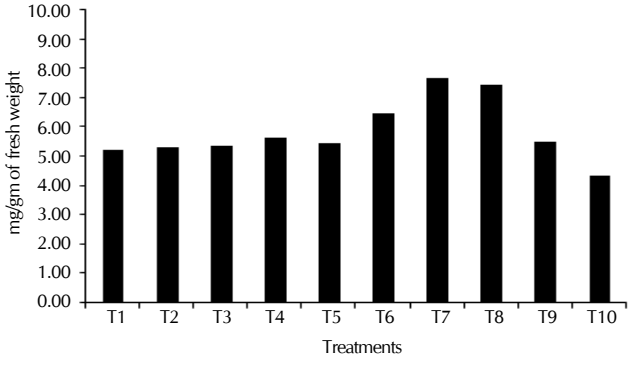

Figure 1: Effect of microbial consortin on protein concentration in cabbage seedlings

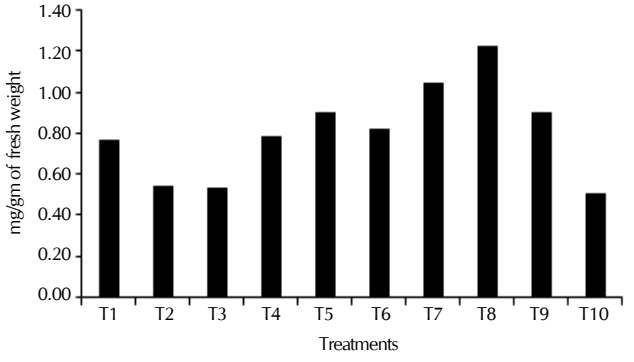

Figure 2: Effect of microbial consortia on phenol concentration in cabbage seedlings

three PGPR increases protein content and poly phenol oxidase activity higher than single and dual application of bio-agent(s) (Mathivanan *et al.,2014)*. Consortium application of *Glomus aggregatum + Bacillus coagulants + Trichoderma harzianum* increased dehydrogenase activities in the root zone soil of *Solanum viarum* seedlings reported by Hemashenpagam *et al* 2011.

Nutrient uptake by bio-primed cabbage plants at heading stage and fertility status of soil after harvest of the crop

The results on nutrient uptake by bio-primed cabbage plants and soil fertility status after crop harvest (table 2) revealed that

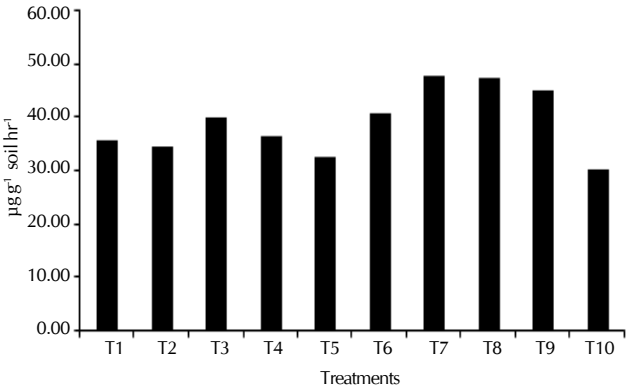

Figure 3: Effect of microbial consortin on soil microbial activity in cabbage seedlings rhizosphere

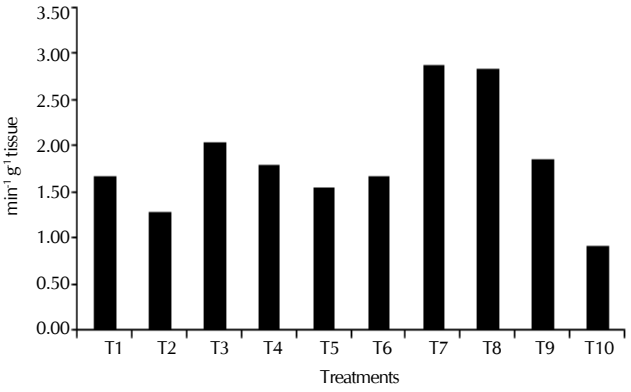

Figure 4: Effect of microbial consortin on polyphenol oxidase activity in cabbage seedlings

uptake of nitrogen, phosphorus and potassium was maximum in consorted application of UBT-18, UBPS-9 and UBAZ-1 (40.81, 27.76 and 90.00 kg/ha, respectively). Nitrogen uptake was significantly at par with consortium of UBT-18, VPf-1, UBPS-9 and UBAZ-1 (37.26 kg/ha) followed by consortium of VPf-1, UBPS-9 and UBAZ-1 (36.38 kg/ha). Available nitrogen in soil was significantly higher in consortium comprising of UBT-18, VPf-1, UBPS-9 and UBAZ-1 (232.33 kg/ha) treatment and it was statistically at par with consortium of UBT-18, UBPS-9 and UBAZ-1 (232.00 kg/ha) followed by consortium of VPf-1, UBPS-9 and UBAZ-1 (230.33 kg/ha). Available phosphorus was significantly high in consortium of VPf-1, UBPS-9 and UBAZ-1(31.67 kg/ha) and it was statistically at par with T5, T6, T7 and T9 treatments. Available potassium was significantly high in consortium of VPf-1, UBPS-9 and UBAZ-1 (114.67 kg/ha) and it was statistically at par with T7 and T9 treatments. The higher nutrient uptake of *Azadirecta indica* seedlings was obtained by Chandra in 2013 due to combined application of AMF + PSB + Azotobacter. The highest NPK contents of tomato were recorded for consortium application of *Pseudomonas +Azotobacter + Azosprillum (*Sharafzadeh 2012).

Hence from the study it is concluded that application *of Trichoderma harzianum or Pseudomonas fluorescens* along with *Azotobacter sp* and phosphate solubilizing bacteria had a significant effect on better seedling health which even after transplanting helped the plants to uptake of nutrients vis a vis the greater microbial activity in the rhizosphere leading towards higher influx of nutrients in soil. The consortium of the isolates used in the investigation may be utilized in this area and in allied crops also whenever organic cultivation is planned for.

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