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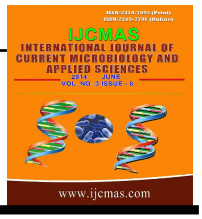

Original Research Article

Role of consortia of biocontrol agents and PGPR's in the production of cabbage under nursery condition

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A B S T R A C T

Greenhouse studies were undertaken to assess the effectiveness of selected biocontrol agents in combination with PGPR's against damping-off and wilt pathogens of cabbage crops. The wilt pathogens of Cole crops from infected plants and soil samples were identified as *Pythium*sp and *Fusarium*sp. In pathogen city test conducted under greenhouse condition they were found to cause 50-85 per cent seedling loss of cabbage crops. Under greenhouse condition the biocontrol agents and PGPR's were evaluated for their growth promoting and disease suppressing abilities. The combination of *Azotobacterchroococcum* + *Bacillusmegaterium* + *Pseudomonas fluorescens* + *Bacillus subtilis* + *Trichodermaharzianum* showed enhanced seedling vigour, total biomass, least disease incidence, and more biocontrol efficiency.

Keywords

Biocontrol Agents, PGPR'S and Cabbage

Introduction

Cabbage is an important source of vegetables consumed all over the world. They belong to the genus *Brassica* of the family Cruciferae. Among the cole crops, Cabbage (*Brassicaeoleraceae* var. capitata Linn.), is grown all over India.

It is rich in vitamin A, B and C and contains minerals like P, K, Ca,Na, and Fe. Cabbage and cauliflower is grown around the year in Kolar, Bangalore urban and rural districts Bidar, Haveri, Hassan, Belgaum and Kodagu districts. It is affected by several diseases of fungal, bacterial and viral origin. Among the fungal diseases, damping off caused by *Pythium,Phytophthora,Fusarium,Rhizoctonia, Sclerotium,etc*is the most devastating, and results in 50-60 per cent seedling loss in the nursery (Singh and Srivastava, 2000). As these are essentially soil borne, they consequently affect the establishment of seedlings in the main field.Plant disease control, therefore, has now become heavily dependent on fungicides to combat the wide variety of fungal diseases that threaten the agricultural crops. But accumulation of chemical residues causes serious ecological problems. A land-mark study published by the Environment Protection Agency (EPA) indicates that, in the U.S. alone 3000-6000 cancer cases are

induced annually by pesticide residues on foods and another 50-100 by exposure to pesticides during application (Anon, 2002).

The increased concern for environmental awareness of chemical hazards has evoked a worldwide interest in microbial control of pathogens. In this context, many microorganisms like *Azotobactoer*sp, *Bacillus*sp, *and Pseudomonas*sp, *Tricoderma*sp have been exploited as biocontrol agents for the management of soil borne pathogens and a number of them have been registered and are commercially available for use against pathogens (Baker, 1987; Bennett, 1997; Tewwari and Mukhopadhyay, 2001). The microbial consortium is a "group of species of microorganisms that act together as a community". In a consortium, the organisms work together in a complex and synergistic way. A new approach in sustainable production systems is PGPR's microorganisms to minimize the use of mineral fertilizers and maximize plant growth. The positive effect of a single microbial species on plant growth in low-fertility soils was improved by the application of mixtures of different microbial inocula.

Microbial consortia are much more efficient than single strains of organisms with diverse metabolic capabilities. Many of these biocontrol agents and PGPR's are known to produce amino acids, vitamins and growth promoting substances like IAA, GA and cytokinins which help in better growth of crop plants (Ponmurugan and Gopi, 2006).Traditionally seedlings are raised in soil beds. However in recent years, seedlings are raised in the seedling trays with the help of coir pith compost as a substrate. Farmers either raise seedlings on their own or they purchase the seedlings from the commercial nurseries where the seedlings of different crops are raised. The environment in the commercial nurseries is entirely different from the natural condition therefore the disease management practices including the efficiency of the biological control agents may vary. Thus it needs a thorough investigation.It is raised in nurseries before transplanting to the main field, the management of disease and nutrient needs of the crop in nursery stage plays an important role in maintaining the health and vigor of the seedlings which in turn affects the quality of the final product (Shiau*etal*., 1999).

Materials and Methods

Isolation of pathogen

Collection of diseased specimens

The commercial nurseries around Kolar and Bangalore were regularly visited for recording the different diseases prevailing in the commercial nurseries. Such diseased specimens were collected for further studies. The infected seedlings were collected in butter paper bag and later kept in polythene bags and brought to the laboratory.

Isolation of pathogens

Infected plant part was washed in running tap water and cut into small bits (2-3 mm). These bits were surface sterilized with Sodium hypochlorite (20 %) solution for 60 sec subsequently they were washed in sterile distilled water to remove traces of sodium hypochlorite. The bits were picked up and placed using a sterilized needle and forceps in the centre of the petriplates containing carrot extract agar (Appendix) and potato dextrose agar in separate plates.

The inoculated plates were incubated in incubator at 24 ±1° C and plates were observed at regular intervals for the development of the fungal colonies. Loopful of fungal culture from the inoculated plate was picked and observed under microscope and pure culture was made on PDA.

Maintenance of the culture

The pathogen was sub cultured on PDA slants and allowed to grow at 24 ± 1^0C temperature for 10 days. The culture so obtained was stored in refrigerator at 4ºC and were sub cultured periodically once in a month.

Evaluation of Pathogenicity

Pathogenicity tests were carried out to evaluate the ability of fungal isolates to produce typical symptoms of damping off under artificial condition on cabbage in seedling crates.Pathogenicity of different fungi was carried out in glass house using a sterilized soil on cabbage and cauliflower respectively. The soil was autoclaved two times at three days interval at 15 psi for 60 minutes in autoclavable polybags. The autoclaved soil was moistened to 60 % with sterile water and was supplemented with 15 agar discs (1cm) of the pathogen cut with a sterile cork borer (Lim and See, 1982). The soil was incubated for seven days before the seeds were sown. The soil was filled into seedling crates and healthy seeds were selected and treated with 1 % sodium hypo chloride solution for two minutes, subsequently they were washed in sterile distilled water to remove traces of sodium hypo chloride and fifty seeds were sown in each crate approximately at a depth of 1.5 cm, totally three replicates were maintained along with three uninoculated crates as control in each crop. Each pot was observed for seed germination and survival of seedlings upto 30 days. The pathogens were reisolated from infected seedlings and soil and compared with the original pathogens inoculated.

Identification and characterization of the fungal isolates

The isolates that were showing high incidence of disease from the pathogen city test were selected and were identified based on the growth pattern and the morphological characteristics on PDA plates and the structure of the conidiophores under light microscope (Singh and Srivastava, 1953; Nethravathi, 2001a).

Collection and maintenance of biocontrol agents and PGPR's

The biocontrol agents collected from National Bureau of Agriculturally Important Insects (NBAII), Bangalore were as follows:

1. Bacterial biocontrol agents: *Bacillus subtilis* and *Pseudomonas fluorescens,*
2. Fungal biocontrol agents: *Trichodermaviride, T. virens* and *T. harzianum.*

The beneficial microorganisms: *Azotobacter chroococcum and Bacillus megaterium* collected from Department of Agricultural Microbiology.

Glasshouse evaluations

A seedling tray experiment was conducted to evaluate the antagonistic and growth promotion effect of the microbial consortia enriched substrate under greenhouse condition in the Department of Agricultural microbiology.

Preparation of pathogen inoculums

Pathogens were first grown on PDA plates. A mixture of 940 gram sand and 60 gram crushed sorghum (sand: sorghum: 94 %: 6 % w/w) were mixed by adding tap water till the sand could form a ball. Then the mixture was filled to autoclavable poly bags and the opening of the bag was sealed using rubber band with cotton plug and autoclaved. Mycelial discs of five mm size were cut from the margin of actively growing pathogen on the PDA plate and transferred aseptically to the polybags containing sterilized sorghum and sand mixture and were incubated at 27 ± 1°C for 15 days. The bags were carefully shaken periodically in order to permit uniform growth(Rini and Sulochana, 2007).

Mass multiplication of Biocontrol agents and PGPR's

Mass multiplication of *Trichoderma harzianum*

Five mm disc of the *Tricoderma harziaum* grown on PDA plates and was transferred to sterile potato dextrose broth aseptically and incubated at 27 ± 1°C potato dextrose broth as a stationary culture at room temperature for eight days. After eight days. After incubation the mycelial mat was separated, macerated using homogenizer and the fungal mass was obtained. The inoculum containing 7 x 10^5cfu/ml was added at the rate of 10 ml/kg of substrate.

Mass multiplication of bacterial biocontrol agents and PGPR's

The bacterial biocontrol and beneficial microorganisms were first grown on their nutrient agar plate and then aseptically transferred to sterile nutrient broth and incubated at 27 ± 1º C on a rotating shaker at 150 rpm for five days, then shaking was stopped and allowed till good turbidity was formed plate (Plate 2d), then the biocontrol and beneficial microorganisms culture was thoroughly mixed and the inoculum containing 2× 10^8 cfu /ml was addedthe rate of 10 ml Kg/substrate.

Enrichment of substrate for green house studies

6.5 kilograms of coir pith compost was mixed with 2.5 kilograms of Soilrite (25 %) and amended with one kilogram of pongamia cake (10 %) (Plate 3a, 3b and 3c) and was autoclaved two times at three days interval at 15 psi for 60 minutes in autoclavable polybags and filled into large polythene bags. Fungal and biocontrol agents and PGPR's *viz*., *Pseudomonas fluoroscens, Bacillus Subtilis, Tricodermaha rzianum, Azotobacter chroochoccum*and *Bacillus megaterium,* were mixed with the substrate @ 100 ml individually and in combinations, and the substrate was manually mixed and the polybags were covered properly and were incubated for 30 days before taking up the sowing. The substrate was regularly mixed for proper aeration and multiplication of the consortia.

The mass multiplied pathogen inoculum viz., *Pythium*sp and *Fusarium* sp, was added to substrate mixture @ 100 grams to each polybags and mixed properly. The bags were incubated for one week and the mass multiplied for with pathogen study. A green house studies were conducted to evaluate the performance of method of application of *Pseudomonas fluorescens* (commercial formulation) under direct seeded wet sowing rice (ADT 43) in randomized block design with fourteen

treatments and three replicationsA green house studies were conducted to evaluate the performance of biocontrol agents and PGPR's on growth of cabbage in complete randomized design with fourteen treatments in T_{1-} Pathogens +*Azotobacter chroococcum*(Az), T_{2-} Pathogens + *Bacillus megaterium* (Bm),T_{3-} Pathogens + *Pseudomonas fluorescens (*Pf),T_{4-} Pathogens+*Bacullus subtilis (*Bs), T_{5-} Pathogens +*Trichoderma harzianum (*Th) , T_{6-} Pathogens+ Az+ Bm +Pf,T_{7-}, Pathogens+ Az+ Bm+Bs,T_8, Pathogens+ Az+ Bm+ThT_{9-}, Pathogens+ Az+ Bm+Pf +Bs,T_{10-} Pathogens+ Az+ Bm+Pf +Th,T_{11}, Pathogens+ Az+ Bm+ Bs +Th, T_{12-}Az+ Bm +Pf +Bs+ Th,T_{13-} Pathogens only , T_{14-}Uninoculated checkand three replication.

Statistical analysis

The data collected in this study were subjected to completely randomized statistical analysis for greenhouse study and Randomized complete block design statistical analysis for field study and comparison between treatment means was made using Duncan's multiple range test (DMRT) for drawing conclusions (Littly and Hills, 1978).

Results and Discussion

The experimental results obtained from the studies on isolation of pathogen from damping off seedlings collected from commercial nurseries and different microbial biocontrol agents for their efficacy against isolated plant pathogens, biocontrol agents for their compatibility with PGPR microorganisms, enrichment of tray mixture with promising microbial agents for nursery study and evaluation of the microbial consortia for their efficacy with respect to biocontrol activity and plant growth promoting activities under nursery are presented below.

In cabbage, the results presented in Table 1 revealed that treatment combinations recorded heihest germination per centage , took minimum days for their fifty per cent germination over the pathogens inoculated treatment.The results presented in Table 2 revealed that treatment combinations recorded heihest, Biocontrol efficiency, and least disease incidence compared to pathogen control. In Table 3 treatment combinations recorded heihest root and shoot length, and number of leaves over the pathogens inoculated treatment. In table 4 revealed that treatment combinations recordedheihest root and shoot biomass and seedling vigourover the untreated control.

Germination and disease incidence

The data revealed that all the treatments with consortia of PGPR's and biocontrol agents significantly reduced the disease incidence and also enhanced the growth of the cabbage seedlings over the control and single inoculation treatment and also increased the seedling growth. The effect of biocontrol agents and PGPR's on disease incidence of pre-emergence and post-emergence, days for 50 per cent germination, and germination per cent was evaluated in nurseries under green house condition (Table 1&2).

Several workers have reported the use of biocontol agents and PGPR's in enhancing the seedling growth and reducing the disease incidence in major vegetable and field crops. Similar result obtained by srinivasan and Mathivanan (2011), due to the induction of systemic resistance shows promising results against multiple plant diseases. In cabbage and cauliflower

seedlings treated with Pathogens + Az+ Bm +Pf +Bs+ Th significantly reduced the pre-and post- emergence disease incidence and increased the germination per cent. Similar result obtained by Manoranjitham *et al*. (2001), who reported the reduction in population of *Pythiumsp* and *Fusarium*sp in the soil might be the reason for lesser incidence of pre- and post- emergence disease incidence of tomato in antagonists applied to pots either alone or in combination. Karthikeyan (2001) who reported the use of a consortia of *Trichodermaviride* and *T. harzianum* and *Paecilomyceslilacinus* against damping off incidence of brinjal and Mohan (2006) who reported a decrease in the disease incidence of brinjal seedlings when treated with a consortia of biocontrol agents and PGPR's.

Root and shoot biomass

All the treatments recorded the enhanced root and shoot biomass over the control. In both cabbage seedlings treated with Pathogens+ Az+ Bm +Pf +Bs+ Th was effective in increase the shoot and root biomass and followed by the followed by the Pathogens+ Az+ Bm + Pf + Th compared to other treatments(Table 4).

These results are in accordance with the findings of Several workers reported the use of biocontol agents and PGPR's in enhancing the seedling growth and reducing the disease incidence in major vegetable and field crops.Kloepper*et al*., 2004 reported the use of plant-growth-promoting rhizobacteria (PGPR) colonize the rhizosphere of many plant species and confer beneficial effects, such as increased plant growth and reduced susceptibility to diseases caused by plant pathogenic fungi, bacteria, viruses. The similar result were obtained in the crops like green gram (Thilagavathi., 2007), in tomato Muthuraju*et al*., 2002). Such increased root and shoot biomass production could attributed to the good seedling growth due to PGPR's strain compressing effective combinations would be mutually non-inhibitory because of overlapping niche in the rhizosphere and the proven ability of PGPR's strains to produce secondary metabolites.

Seedling vigour

The seedling vigor, root length and shoot length of cabbage (Table 3).and was maximum in the treatment Pathogens + Az + Bm + Pf + Bs + Th. which was on par with Pathogens +.Az+ Bm + Pf + Th(Table 2&5).

The result obtained was on par with the result obtained by Siddiquiand Akhtar (2009) who studied the studied the effect of consortia of antagonistic fungi (*Paecilomyce slilacinus, Pochonia chlamydosporia and Trichoderma harzianum*) and plant growth-promoting rhizobacteria (PGPR), namely *Bacillus subtilis, Paenibacillu spolymyxa and Burkholderia cepacia*, alone and in combination in glasshouse experiments on the growth of tomato and concluded that the application of antagonistic fungi and PGPR caused a significant increase in growth of tomato seedlings. Chandanie*et al*. (2009) reported the co-inoculation of *Glomous. mosseae* with *Tricoderma harzianum* synergistically enhanced plant growth of cucumber.

Table.1 Biocontrol efficiency of consortia of promising microbial agents on cabbage crop in nursery stage under greenhouse condition

Treatments	Germination percentage	Days for 50% germination
T1	75.55^{e}	12.22^{ij}
T2	77.70^{e}	11.00^{gh}
T3	82.14^{cde}	12.00^{ij}
T4	79.99^{d}	10.33^{fg}
T5	82.22^{cde}	11.33^{hi}
T6	84.44^{cd}	10.00^{ef}
T7	82.22^{cde}	8.66^{cd}
T8	86.67^{bc}	8.33^{bc}
T9	84.44^{cd}	7.66^{b}
T10	91.11^{b}	6.04^{a}
T11	86.66^{bc}	8.00^{bc}
T12	91.11^{b}	8.33^{bc}
T13	63.26^{f}	13.00^{j}
T14	95.00^{a}	6.00^{a}
CD (P≤0.05%)	6.42	0.88

Table.2 Biocontrol efficiency of consortia of promising microbialagents on cabbage crop in nursery stage under greenhouse condition

Treatments	Pre-emergence disease incidence (%)	Post emergence disease incidence (%)	Biocontrol efficiency (per cent)
T1	25.01^{g}	24.00^{i}	12.66^{g}
T2	22.81^{f}	26.19^{h}	20.13^{f}
T3	18.27^{d}	19.05^{e}	39.16^{e}
T4	20.46^{e}	19.05^{e}	35.5^{e}
T5	18.18^{d}	21.43^{f}	35.4^{e}
T6	15.91^{c}	14.29^{d}	50.57^{d}
T7	18.18^{d}	9.52^{b}	54.84^{c}
T8	13.63^{b}	9.52^{b}	62.28^{b}
T9	15.91^{c}	11.90^{c}	54.66^{cd}
T10	9.09^{b}	6.54^{b}	74.52^{a}
T11	13.64^{b}	9.52^{b}	62.24^{b}
T12	9.09^{b}	6.14^{b}	75.17^{a}
T13	37.35^{h}	28.57^{g}	0.00^{g}
T14	0.00^{a}	0.00^{a}	-
CD (P≤0.05%)	1.49	1.39	4.00

Table.3 Efficacy of consortia of promising microbial agents on enhancing seedling vigour of cabbage crop in nursery

Treatments	Root length (cm)	Shoot length (cm)	No. of leaves
T1	3.33^{g}	5.12^{h}	2.30^{g}
T2	4.43^{f}	6.26^{g}	2.33^{g}
T3	6.93^{e}	8.08^{f}	2.53^{g}
T4	5.67^{e}	7.86^{f}	2.43^{g}
T5	7.12^{e}	9.22^{e}	3.02^{f}
T6	7.43^{cde}	9.65^{de}	3.98^{de}
T7	7.33^{de}	9.43^{de}	3.76^{e}
T8	8.65^{abc}	10.12^{bcd}	4.42^{bc}
T9	8.52^{bcd}	9.75^{cde}	4.24^{cd}
T10	9.66^{a}	10.59^{ab}	4.57^{ab}
T11	9.56^{ab}	10.40^{abc}	4.45^{bc}
T12	9.87^{ab}	10.86^{a}	4.82^{a}
T13	1.98^{h}	2.98^{j}	1.00^{i}
T14	2.14^{gh}	4.22^{i}	1.83^{h}
CD (P≤0.05%)	1.28	0.71	0.28

.

Table.4 Efficacy of consortia of promising microbial agents on enhancing seedling vigour of cabbage crop in nursery

Treatments	Shoot dry weight (g)	Root dry weight (g)	Seeding vigour
T1	0.015^{fg}	0.012^{i}	638.40^{i}
T2	0.017^{f}	0.020^{h}	830.61^{h}
T3	0.019^{f}	0.038^{f}	1232.92^{f}
T4	0.06^{ef}	0.027^{g}	1082.26^{g}
T5	0.121^{d}	0.042^{e}	1343.47^{ef}
T6	0.126^{cd}	0.044d^{e}	1442.24^{e}
T7	0.123^{cd}	0.046^{cd}	1378.01^{e}
T8	0.130^{bc}	0.059^{b}	1542.72^{c}
T9	0.128^{bcd}	0.048^{c}	1626.80^{c}
T10	0.136^{ab}	0.066^{a}	1818.56^{ab}
T11	0.134^{ab}	0.062^{b}	1754.87^{b}
T12	0.139^{a}	0.069^{a}	1888.71^{a}
T13	0.007^{g}	0.002^{j}	313.77^{j}
T14	0.012^{g}	0.009^{i}	604.20^{i}
CD (P≤0.05%)	0.008	0.003	111.78

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