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Evaluation of different native *Streptomyces* spp. for effective management of rhizome rot of turmeric

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(Submitted: February 19, 2020; Accepted: October 15, 2020)

Summary

The efficacy of talc based bioformulations containing various biocontrol agents against rhizome rot disease caused by *Pythium aphanidermatum* in turmeric plants was evaluated under field condition. Indigenous biocontrol agents such as *Streptomyces lydicus*, *Streptomyces griseus* and *Streptomyces sannanensis* belonging to actinomycetes group, *Pseudomonas fluorescens* (bacterial) and *Trichoderma atroviride* (fungal) were selected for the biological control of rhizome rot of turmeric. The results indicated a significantly stronger reduction in disease severity in trial plots treated with *Bacillus subtilis* based commercial fungicide 'Companion' when compared to plants treated with indigenous biocontrol agents. However, it was reverse in trial plots in terms of turmeric rhizome yield potential, yield attributes, physiological components, biochemical constituents and quality characteristics of rhizomes. Among 17 treatments, a dual mixture of *S. griseus* and *T. atroviride* achieved the best disease control as well as plant growth improvement when compared to single and triple combinations of biocontrol agents. The present study confirms that exploration of microbial formulations containing *Streptomyces* spp. as soil inoculant to turmeric plants exhibited some benefits to turmeric plant growth as well as controlling rhizome rot disease, which ultimately enhance the overall quality characteristics of rhizomes. Further, our results suggest that a dual combination of biocontrol agents represent a promising method for effective management of rhizome rot of turmeric.

Keywords: Turmeric, Rhizome rot, Companion, Biocontrol agents, *Streptomyces*, *Pythium aphanidermatum*, Curcumin.

Introduction

Turmeric (*Curcuma longa* L.) is an herbaceous annual plant belonging to the family Zingiberaceae that is a potential cash crop in India. It has a large oval rhizome with sessile cylindrical tubers containing curcumin and oleoresin contents and commonly used as a flavouring, colouring agent and preservatives including biomedical applications (CHATTOPADHYAY et al., 2004). Turmeric is a tropical rhizomatous crop cultivated in a wide variety of soil (PARTHASARTHY et al., 2007) and climatic conditions in India. It prefers to grow in the range of soil pH 6.5 to 8.5, electrical conductivity 0.50 to 1.85 dS/m, total organic carbon 2.5 to 8.5%, Clay/silt content 145.5 to 275.5 g kg⁻¹, water holding capacity 64.3 to 75.5%, temperature 25 to 37 °C, relative humidity 60 to 75%, annual rainfall 1500 to 2000 mm and sunshine duration of 5.3 to 7.0 hrs/day (AKPAN, 2018). The important turmeric varieties grown in India are Alleppey Finger, Salem turmeric, Raja pore, Erode local, BSR-1 and 2, PTS-10, Roma, Suguna, Sudarsana, Sangli turmeric and Nizamabad Bulb (SUMATHI et al., 2008).

Indian turmeric is considered the best in the world in terms of quality, especially in high curcumin content. India produced 6,62,200 tons of turmeric from an area of 1,86,000 ha during 2017-18 (CHOUDHARY and RAHI, 2018). The major turmeric exporting countries are India, China, Myanmar and Bangladesh. India has occupied around 60% of the world trade in turmeric production (PONMURUGAN et al., 2017). The rhizome rot disease caused by the fungal pathogen *Pythium graminicolum* f.sp. *aphanidermatum* is more prevalent in turmeric plantations of India (RAMARETHINAM and RAJAGOPAL, 1999). It is a serious problem in certain pockets of southern India and there was a significant reduction in turmeric export since 2010 due to this disease severity. In advanced stages of the disease, the rhizomes get decomposed; decayed and rotten rhizome emits foul smell. Symptoms are stunted growth of the plant, pseudo-stem at the base and yellowing of leaves and necrotic spots. In advanced stages, the bright orange colour of the tissues of rhizomes changes to different shades of brown. In severe cases, rotting of rhizomes with white fungal filaments on the rhizome surface, complete dryness and death of the whole plant are the symptoms (PONMURUGAN et al., 2016). In India, all turmeric cultivars available today are highly susceptible to rhizome rot and no resistance or tolerant source has been identified yet. The quality of turmeric rhizomes is being affected by a number of root and leaf diseases in which rhizome rot is considered to be very important in terms of capital loss and poor quality of rhizomes. Biocontrol agents, especially actinomycetes and *Streptomyces* spp. in particular, have been proven as efficient biocontrol agents in controlling various plant diseases as well as enhancing the plant growth significantly by producing a wide spectrum of growth promoting and antibiotics like substances (ETEBARIAN, 2006). *Streptomyces* spp. are having high G+C content (80%) in the genome and they are potential secondary metabolite producer with substantial antagonistic properties (SANGLIER et al., 1993). *Streptomyces* spp. are able to suppress the growth of pathogens by parasitizing the mycelium and degrading the spores (MORENO et al., 2003). With the reported exploration of various PGPRs against rhizome rot in turmeric (CHENNIAPPAN et al., 2019) in mind, efforts were made to evaluate the bioefficacy of *Streptomyces* spp. consortia along with various other biocontrol agents for their efficacy against rhizome rot and for improvement of plant growth and metabolism.

Material and Methods

Field experiments

Fields with history of rhizome rot disease in turmeric plantations were selected for the present study. Field experiments were conducted for four consecutive years with three cycle of the crop cultivation at Thondamuthur turmeric fields located in Coimbatore district of Tamil Nadu, India during 2015-2018 (latitude - 10.9753 and longitude - 76.8586). Erode turmeric variety was selected for planting which carried out during May-June with the receipt of pre-monsoon

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showers. The field experiments were devised as completely randomized block design with different treatments implemented and the plots were also analyzed randomly using quadrat method to study the consequence. Each experimental block contained 100 plants, replicated three times. The sampling timeline is given in Fig. 1. The field layout is provided as supplemental material.

Effectual indigenous *Streptomyces* strains such as *S. lydicus* (BU 013), *S. griseus* (KSR 348) and *S. sannanensis* (BU 254) and other biocontrol agents such as *Pseudomonas fluorescens* (Pf 017) and *Trichoderma atroviride* (UTA 9041) were identified by screening the PGPR traits tests like, HCN, indole, Phosphate solubilization, siderophore production and confirmed species level by sequencing were applied (PONMURUGAN et al., 2017). The treatment details are represented in Tab. 1.

Selection of fungicides

The systemic fungicide Companion, which contains the spore forming bacteria *Bacillus subtilis* GB03 with 5.5×10^6 CFU/ml. was preliminarily screened under *in vitro* condition (Data not shown). 0.05% of Companion was applied at 100 ml/plant dosage to the trial plots two times per month as per the recommendations of Indian Institute of Spices Research, Kozhikode, Kerala, India.

Preparation of bioformulations

Actinomycetes (*S. lydicus*, *S. griseus* and *S. sannanensis*), bacterial (*P. fluorescens*) and fungal (*T. atroviride*) antagonists were grown on casein nitrate agar (KUSTER and WILLIAMS, 1964), nutrient agar and *Trichoderma* selective media (ELAD and CHET, 1983), respectively. All the biocontrol agent strains were formulated using talc powder as carrier for delivery as per the protocol made by ELANGO et al. (2015). For field application, each antagonist was mixed with sterilized talcum powder at a concentration of 400 ml broth (8×10^8 CFU/ml) per kg of talc and incubated for 3 days under shade condition. Later, this talc formulation was further mixed with dried farm yard manure with 60-70% moisture content before application to trial plots. It was applied to the soil around the plant (at a depth of 10 cm) at 100 gm/plant at two months intervals (initiated from the vegetative stage of turmeric where the establishment of bulb occurred at the 4-5 months from planting) for a period of six months. The pretreatment data was taken between the planting until the initiation of treatments in the trial plots. Periodically soil samples were collected from the experimental plots for the enumeration of biocontrol agents with specific medium following the dilution plate technique to estimate the survival rate of each antagonist in the soil samples.

Tab. 1: Treatment details and rates of application of biocontrol agents

| Treat-ment | Treatment details | Dosage | Mixture Ratio |
|-----------------|--|--------------|---------------|
| T ₁ | Companion | 100 ml/plant | 0.05% |
| T ₂ | <i>S. lydicus</i> | 100 gm/plant | 100% |
| T ₃ | <i>S. griseus</i> | 100 gm/plant | 100% |
| T ₄ | <i>S. sannanensis</i> | 100 gm/plant | 100% |
| T ₅ | <i>P. fluorescens</i> | 100 gm/plant | 100% |
| T ₆ | <i>T. atroviride</i> | 100 gm/plant | 100% |
| T ₇ | <i>S. lydicus</i> + <i>P. fluorescens</i> | 100 gm/plant | 50+50% |
| T ₈ | <i>S. lydicus</i> + <i>T. atroviride</i> | 100 gm/plant | 50+50% |
| T ₉ | <i>S. griseus</i> + <i>P. fluorescens</i> | 100 gm/plant | 50+50% |
| T ₁₀ | <i>S. griseus</i> + <i>T. atroviride</i> | 100 gm/plant | 50+50% |
| T ₁₁ | <i>S. sannanensis</i> + <i>P. fluorescens</i> | 100 gm/plant | 50+50% |
| T ₁₂ | <i>S. sannanensis</i> + <i>T. atroviride</i> | 100 gm/plant | 50+50% |
| T ₁₃ | <i>P. fluorescens</i> + <i>T. atroviride</i> | 100 gm/plant | 50+50% |
| T ₁₄ | <i>S. griseus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 100 gm/plant | 50+25+25% |
| T ₁₅ | <i>S. lydicus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 100 gm/plant | 50+25+25% |
| T ₁₆ | <i>S. sannanensis</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 100 gm/plant | 50+25+25% |
| T ₁₇ | Infected Control | 100 gm/plant | - |

Disease assessment

The disease incidence was recorded in all the trial plots periodically by partial removal, 25 plants from each block were observed carefully without disturbing root establishments by digging the soil around the rhizosphere and are completely removed if infection arises severely. This has ensured number of plant reduction in that blocks. Percentage of disease incidence (PDI) was calculated as proposed by ELANGO et al. (2015) for disease assessment.

Soil analysis

The soil samples were collected periodically in two-week intervals from the experimental fields and air dried at room temperature. Various soil edaphic parameters like soil pH, electrical conductivity (EC), total organic matter (OM) as carbon content (WALKLEY and BLACK, 1934), available nitrogen (AOAC, 1990), available phosphorous (JACKSON, 1973), exchangeable potassium (MURPHY and RILEY, 1962), calcium, magnesium and sodium (BHARGAVA and RAGHU-

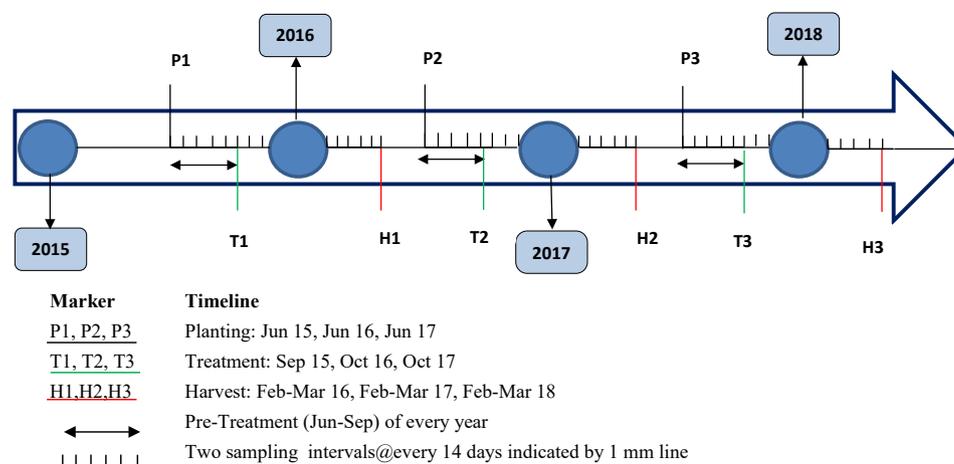


Fig. 1: Timeline chart for the field trial conducted in the turmeric fields

PATHI, 2001) were estimated at the maturity stage of turmeric. The biocontrol agents *S. lydicus*, *S. griseus*, *S. sannanensis*, *P. fluorescens* and *T. atroviride* were enumerated in soil samples periodically as mentioned above and were reported at two-month intervals for enumeration of microflora using suitable basal medium following the dilution plate technique to know their survival rate in soil (KUSTER and WILLIAMS, 1964; ELAD and CHET, 1983).

Determination of physiological and biochemical constituents

Net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Sc) were measured using an infrared gas analyzer (ADC LCA- 3, UK) and an open type Parkinson leaf chamber (ADC PLC-3). Water use efficiency (WUE) was calculated from the ratio between net Pn rate and rate of Tr (PREMKUMAR et al., 2008). The biochemical composition of leaf samples such as chlorophyll, carotenoid (WELBURN, 1994), total sugar (DUBOIS et al., 1956) nitrogen (AOAC, 1990), amino acids (MOORE and STEIN, 1948) and protein (LOWRY et al., 1951) contents were analyzed. The study was carried out throughout the stages of turmeric plant and were reported during the maturation stage of the plants that exhibited the trends of treatment schedules.

Analysis of quality parameters of turmeric rhizomes

The crop was harvested after maturity during the experimental period; rhizomes were separated carefully and cleaned; and the fresh weight was recorded. Mean yield and yield attributes in terms of productivity index (PI) were calculated from the yield data recorded with individual trial plots. According to SHARMA and SATHYANARAYANA, (1990) PI = yield of the treated plot/mean yield of the field. The number of primary, secondary and tertiary finger rhizomes originated from the mother rhizomes and its dry weight was enumerated carefully. The extraction and quantification of curcumin and oleoresin contents from the turmeric rhizomes of different treatments were carried out according to the methods of BAGCHI (2012) and KUMAR et al. (2014).

Statistical analysis

All the data collected for three cropping cycles with 25 plants per block (total 100 plants) in three replicates for all parameters in each block were subjected to analysis of variance (ANOVA) and Newman Keuls test (at P = 0.05) using SPSS 17.0 statistical package (SPSS, Inc. Chicago, IL). If the data did not fulfill the assumptions of normal distribution and homogeneity of variances, non-parametric test were carried out using Kruskal-Wallis test and when the significant differences were obtained Mann-Whitney U test (at P = 0.05) was used for pair's comparison (GOMEZ and GOMEZ, 1984).

Results

Rhizome rot disease protection in turmeric

In response to soil application of Companion (commercial fungicide) and indigenous biocontrol agents, there was a significant reduction in rhizome rot disease incidence in turmeric plants (Tab. 2). The results revealed that the former was significant in disease protection when compared to later treatments. Among the 17 treatments, the actinomycete was found to be better than fungal and bacterial antagonists in protecting the plants against the rhizome rot. Systemic fungicide, Companion showed a maximum disease protection of 60.3%. Among the *Streptomyces* spp. tested against *P. aphanidermatum*, *S. griseus* (42.7%) was found better. Other biocontrol agents, *T. atroviride* showed nearly 42.4% disease reduction when compared to *P. fluorescens* (40.1%). Dual combination of *S. griseus* and *T. atroviride* strains achieved the best disease control (50.8%) against *P. aphanidermatum*. The results clearly indicated that all the triple combination of biocontrol agents were found to be inferior to dual combinations in terms of disease reduction that ranged between 33.9 and 34.3%. The untreated control plants exhibited lowest disease protection (-13.85%), with all finger and mother rhizomes rotten and decayed.

Rhizome yield and their attributes

A significant difference in rhizome yield and their attributes as productivity index was recorded after imposing various treatments

Tab. 2: Effect of various biocontrol agents and fungicide on rhizome rot disease incidence, yield and yield attributes in turmeric plants.

| Treatment details | Disease incidence (%) | | Disease Protection | Rhizome Yield (t ha ⁻¹) | Productivity Index (%) |
|--|-------------------------|--------------------------|--------------------------|-------------------------------------|---------------------------|
| | Pre treatment | Post treatment | | | |
| Companion [#] | 57.8 ± 0.7 ^a | 22.3 ± 0.3 ^a | 60.3 ± 7.0 ^g | 22.3 ± 1.5 ^b | 0.48 ± 0.08 ^b |
| <i>S. lydicus</i> * [*] | 57.9 ± 0.3 ^a | 35.0 ± 0.3 ^f | 41.0 ± 0.8 ^c | 44.2 ± 1.7 ^f | 1.09 ± 0.02 ^{cd} |
| <i>S. griseus</i> * [*] | 58.7 ± 0.5 ^a | 32.2 ± 0.6 ^e | 42.7 ± 2.4 ^{cd} | 45.8 ± 1.4 ^f | 1.61 ± 0.31 ^e |
| <i>S. sannanensis</i> * [*] | 58.7 ± 0.4 ^a | 35.2 ± 0.2 ^f | 41.8 ± 2.1 ^c | 45.0 ± 2.5 ^f | 1.51 ± 0.37 ^e |
| <i>P. fluorescens</i> ** ^{**} | 57.4 ± 0.5 ^a | 33.5 ± 0.2 ^e | 40.1 ± 3.0 ^c | 40.8 ± 2.0 ^e | 0.93 ± 0.21 ^c |
| <i>T. atroviride</i> *** ^{**} | 57.2 ± 0.0 ^a | 32.7 ± 0.6 ^e | 42.4 ± 2.7 ^c | 42.8 ± 2.9 ^e | 1.95 ± 0.28 ^e |
| <i>S. lydicus</i> + <i>P. fluorescens</i> | 57.8 ± 0.5 ^a | 29.6 ± 0.2 ^{cd} | 48.3 ± 2.1 ^{de} | 52.7 ± 0.8 ^{gh} | 2.12 ± 0.02 ^f |
| <i>S. lydicus</i> + <i>T. atroviride</i> | 59.3 ± 0.3 ^a | 27.1 ± 0.3 ^c | 47.1 ± 1.9 ^d | 51.0 ± 1.4 ^g | 2.43 ± 0.36 ^f |
| <i>S. griseus</i> + <i>P. fluorescens</i> | 58.9 ± 0.2 ^a | 26.0 ± 0.4 ^b | 48.7 ± 0.8 ^{de} | 52.7 ± 0.7 ^{gh} | 2.21 ± 0.34 ^f |
| <i>S. griseus</i> + <i>T. atroviride</i> | 58.0 ± 0.3 ^a | 25.0 ± 0.6 ^b | 50.8 ± 0.7 ^f | 55.7 ± 0.6 ⁱ | 2.93 ± 0.06 ^g |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> | 58.5 ± 0.2 ^a | 29.4 ± 0.3 ^{cd} | 48.0 ± 1.4 ^{de} | 50.2 ± 0.7 ^g | 2.50 ± 0.01 ^{fg} |
| <i>S. sannanensis</i> + <i>T. atroviride</i> | 58.0 ± 1.0 ^a | 27.8 ± 0.4 ^c | 46.6 ± 2.3 ^d | 50.6 ± 1.4 ^g | 2.78 ± 0.15 ^{fg} |
| <i>P. fluorescens</i> + <i>T. atroviride</i> | 57.1 ± 1.2 ^a | 29.8 ± 0.5 ^{cd} | 45.3 ± 1.5 ^d | 51.4 ± 0.7 ^g | 2.12 ± 0.02 ^f |
| <i>S. griseus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 58.0 ± 0.5 ^a | 35.2 ± 0.4 ^f | 34.3 ± 2.3 ^b | 33.7 ± 1.5 ^c | 0.81 ± 0.39 ^c |
| <i>S. lydicus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 59.2 ± 1.0 ^a | 34.5 ± 0.3 ^f | 35.0 ± 2.2 ^b | 35.9 ± 0.8 ^{cd} | 0.87 ± 0.37 ^c |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 59.2 ± 0.4 ^a | 33.5 ± 0.2 ^e | 33.9 ± 3.8 ^b | 32.9 ± 2.2 ^c | 0.98 ± 0.23 ^c |
| Untreated control | 58.5 ± 0.5 ^a | 66.6 ± 0.7 ^g | -13.5 ± 1.1 ^a | 11.4 ± 3.1 ^a | 0.04 ± 0.31 ^a |

[#] Systemic fungicide, *Actinomycete biocontrol agents, ** Bacterial biocontrol agent, *** Fungal biocontrol agent

Identical superscript letters denote that values are not significantly different at P < 0.05. A higher letter denotes improvements acquired due to treatment and lower alphabet denotes decline acquired due to treatment.

in the trail plots (Tab. 2). Among 17 different treatments, the highest rhizome yield of 55.7 t/ha was recorded in T10, *S. griseus* and *T. atroviride* with productivity index of 2.93%. This was followed by the treatment containing *S. lydicus* with *P. fluorescens*, *S. griseus* with *P. fluorescens*, *S. lydicus* with *T. atroviride* *S. sannanensis* with *T. atroviride* and *S. sannanensis* with *P. fluorescens* combinations in which the rhizome yield was in the range of 50.2-52.7 t/ha and productivity index was in the range of 2.12-2.78%. There was a striking difference in the rhizome yield potential between single and triple combination mixture of biocontrol agents treated plants which ranged from 40.8-45.8 t/ha to 32.9-35.9 t/ha; respectively. The Companion treatment registered least rhizome yield of 22.3 t/ha with the productivity index of 0.48%. Wherein the rhizome yield of the Companion treatment was 22.3 and the mean yield of all the plot in that field was 46.5. Hence for T1 (Companion treatment) $22.3/46.5 = 0.48\%$. Untreated control plants registered the yield of 11.4 t/ha with the productivity index of 0.04%. Among the individual treatments, *S. griseus* treated plots produced a higher rhizome yield of 45.8 t/ha with the productivity index of 1.6%. Similarly, among triple combination mixtures tested, the highest rhizome yield of 35.9 t/ha was recorded in a treatment containing *S. lydicus* + *P. fluorescens* + *T. atroviride* with productivity index of 0.87% (Tab. 2).

Rhizome quality characteristics of treated plants to fungicide and biocontrol agents

It is evident from the Tab. 3 that turmeric rhizome quality characteristics like number of primary, secondary and tertiary finger rhizomes were influenced by various bioinoculant treatments. The rhizome quality characteristics like curcumin and oleoresin contents were also shown in the Tab. 3. Improvement with increased yield and their attributes was reflected in rhizome quality characteristic parameters of treated plants. In the case of biocontrol agents treated plants, the maximum number of primary, secondary and tertiary finger rhizomes was noticed in *S. griseus* and *T. atroviride* mixtures with 5.3, 4.9 and 5.8 followed by other treatments combinations. And no significant increase was observed between single and triple com-

bination treatments as per the Mann-Whitney U test (at $P = 0.05$) method of statistical analysis. In case of biocontrol treated plants, there was significant increase in curcumin and oleoresin contents of 8.7% and 9.5%; respectively in plants treated with *S. griseus* and *T. atroviride* mixtures with no appreciable changes in single and triple combination of biocontrol agents. Both curcumin and oleoresin contents were least in untreated control plants (1.7 and 1.5%; respectively) but these were superior to Companion treatment (4.0 and 4.4%; respectively).

Physiological and biochemical response of treated plants to fungicide and biocontrol agents

Both physiological and biochemical traits were appreciably enhanced in turmeric plants treated with biocontrol agents, especially in combination treatments (Tab. 4 and 5). Among 17 treatments imposed against rhizome rot of turmeric, combination of two biocontrol agents ($T_7 - T_{13}$) performed better than single ($T_2 - T_6$) and triple combinations ($T_{14} - T_{16}$) in terms of enhancement in physiological and biochemical response. The physiological parameters such as photosynthetic rate, transpiration rate, water use efficiency and stomatal conductance were improved maximum in *S. griseus* and *T. atroviride* treated plants with $13.1 \mu\text{m min}^{-2}\text{m}^{-2}$, $4.6 \text{ m s}^{-2}\text{m}^{-2}$, 3.5 WUE and $2.7 \text{ mol s}^{-2}\text{m}^{-2}$; respectively. The minimum Pn and Tr rates was recorded in the Companion treatment which accounted nearly about $7.7 \mu\text{m min}^{-2}\text{m}^{-2}$ and $1.8 \text{ m s}^{-2}\text{m}^{-2}$, respectively. Similarly, water use efficiency and stomatal conductance recorded were 1.6 WUE and $0.9 \text{ mol s}^{-2}\text{m}^{-2}$ in Companion treated plants (Tab. 4). Further the results indicated that there was no significant difference ($P < 0.05$) in physiological traits recorded between single and triple combination treatments.

Biochemical constituents were also increased to a greater extent in biocontrol agents treated plants when compared to Companion schedule (Tab. 5). The highest amount of chlorophyll and carotenoid contents was registered as 6.1 and 3.1 mg g^{-1} fresh weight of leaves in T10 treatment. Similarly, the total sugar (7.3%) and nitrogen (5.8%) contents was found to be highest in the same T10 treatment. The

Tab. 3: Effect of various biocontrol agents and fungicide on quality characteristics of turmeric rhizomes

| Treatment details | No. of primary fingers | No. of secondary fingers | No. of tertiary fingers | Curcumin (%) | Oleoresin (%) |
|--|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Companion [#] | 2.3 ± 0.3 ^b | 2.2 ± 0.9 ^b | 3.5 ± 1.3 ^b | 4.0 ± 0.4 ^b | 4.5 ± 1.1 ^b |
| <i>S. lydicus</i> * | 3.0 ± 0.1 ^{cd} | 2.8 ± 0.3 ^c | 4.1 ± 0.4 ^c | 4.9 ± 0.3 ^c | 6.6 ± 0.4 ^c |
| <i>S. griseus</i> * | 3.3 ± 0.2 ^d | 3.4 ± 0.6 ^d | 4.7 ± 0.6 ^{de} | 5.4 ± 0.3 ^{cd} | 6.9 ± 0.1 ^{ef} |
| <i>S. sannanensis</i> * | 3.4 ± 0.2 ^d | 3.7 ± 0.6 ^e | 4.5 ± 0.7 ^d | 6.2 ± 0.3 ^e | 7.4 ± 0.6 ^{efg} |
| <i>P. fluorescens</i> ** | 3.0 ± 0.3 ^{cd} | 3.0 ± 0.3 ^{cd} | 4.0 ± 0.3 ^{cd} | 5.7 ± 0.3 ^{ef} | 7.1 ± 0.5 ^f |
| <i>T. atroviride</i> *** | 3.0 ± 0.4 ^{cd} | 3.1 ± 1.7 ^{cd} | 4.4 ± 0.8 ^d | 6.6 ± 0.4 ^f | 8.6 ± 1.1 ^{gh} |
| <i>S. lydicus</i> + <i>P. fluorescens</i> | 3.6 ± 0.3 ^e | 3.6 ± 0.6 ^d | 4.8 ± 0.3 ^{cde} | 6.1 ± 0.1 ^e | 8.5 ± 0.1 ^h |
| <i>S. lydicus</i> + <i>T. atroviride</i> | 3.8 ± 0.9 ^{ef} | 3.8 ± 0.6 ^e | 5.2 ± 0.6 ^e | 7.1 ± 0.1 ^g | 9.0 ± 0.9 ⁱ |
| <i>S. griseus</i> + <i>P. fluorescens</i> | 4.5 ± 0.2 ^f | 4.0 ± 0.4 ^e | 5.4 ± 0.8 ^e | 7.0 ± 0.5 ^g | 9.0 ± 0.5 ⁱ |
| <i>S. griseus</i> + <i>T. atroviride</i> | 5.3 ± 0.1 ^g | 4.9 ± 0.1 ^f | 5.8 ± 0.6 ^f | 8.7 ± 0.6 ^h | 9.5 ± 0.4 ^j |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> | 3.6 ± 0.1 ^e | 3.6 ± 0.5 ^d | 5.0 ± 0.4 ^e | 6.3 ± 0.2 ^{ef} | 8.1 ± 0.5 ^g |
| <i>S. sannanensis</i> + <i>T. atroviride</i> | 3.6 ± 0.4 ^e | 3.6 ± 1.4 ^d | 4.8 ± 1.3 ^{cde} | 7.4 ± 0.3 ^{fgh} | 7.4 ± 1.8 ^{efg} |
| <i>P. fluorescens</i> + <i>T. atroviride</i> | 3.6 ± 0.1 ^e | 3.4 ± 0.6 ^d | 4.8 ± 0.2 ^{cde} | 6.3 ± 0.3 ^{ef} | 7.1 ± 0.4 ^f |
| <i>S. griseus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 2.8 ± 0.1 ^c | 2.6 ± 0.3 ^c | 3.6 ± 0.6 ^b | 5.0 ± 0.0 ^c | 5.8 ± 0.9 ^d |
| <i>S. lydicus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 2.7 ± 0.2 ^c | 3.2 ± 0.2 ^{cd} | 3.4 ± 1.3 ^b | 6.3 ± 0.4 ^{ef} | 5.3 ± 0.8 ^c |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 2.6 ± 0.1 ^c | 2.8 ± 0.8 ^c | 3.6 ± 1.0 ^b | 5.5 ± 1.1 ^d | 5.9 ± 1.0 ^{cd} |
| Untreated control | 1.5 ± 0.4 ^a | 1.9 ± 1.8 ^a | 2.3 ± 1.8 ^a | 1.7 ± 0.5 ^a | 1.5 ± 1.6 ^a |

[#] Systemic fungicide, * Actinomycete biocontrol agents, ** Bacterial biocontrol agent, *** Fungal biocontrol agent

Identical superscript letters denote that values are not significantly different at $P < 0.05$. A higher letter denotes improvements acquired due to treatment and lower alphabet denotes decline acquired due to treatment.

Tab. 4: Effect of various biocontrol agents and fungicide on physiological variations in rhizome rot disease infected turmeric plant leaves

| Treatment details | Pn rate | Tr rate | WUE | SC |
|--|--------------------------|-------------------------|-------------------------|-------------------------|
| Companion [#] | 07.7 ± 1.4 ^b | 1.8 ± 0.2 ^b | 1.6 ± 0.2 ^b | 0.9 ± 0.3 ^b |
| <i>S. lydicus</i> * | 10.7 ± 0.8 ^e | 2.5 ± 0.3 ^{bc} | 1.8 ± 0.1 ^b | 1.4 ± 0.0 ^c |
| <i>S. griseus</i> * | 10.3 ± 1.8 ^{cd} | 2.7 ± 0.3 ^{bc} | 1.9 ± 0.2 ^c | 2.0 ± 0.4 ^e |
| <i>S. sannanensis</i> * | 12.3 ± 0.3 ^{gh} | 2.9 ± 0.5 ^d | 1.8 ± 0.1 ^{bc} | 2.1 ± 0.3 ^e |
| <i>P. fluorescens</i> ** | 11.1 ± 1.0 ^{ef} | 2.0 ± 0.3 ^{bc} | 1.9 ± 0.3 ^c | 1.5 ± 0.3 ^{cd} |
| <i>T. atroviride</i> *** | 11.1 ± 2.4 ^{ef} | 2.4 ± 0.5 ^c | 1.8 ± 0.3 ^{bc} | 1.6 ± 0.3 ^{cd} |
| <i>S. lydicus</i> + <i>P. fluorescens</i> | 11.3 ± 1.0 ^f | 4.1 ± 0.3 ^{gh} | 1.5 ± 0.1 ^b | 2.2 ± 0.1 ^f |
| <i>S. lydicus</i> + <i>T. atroviride</i> | 12.0 ± 2.4 ^{sh} | 3.5 ± 0.3 ^{ef} | 2.4 ± 0.4 ^d | 2.1 ± 0.2 ^e |
| <i>S. griseus</i> + <i>P. fluorescens</i> | 12.3 ± 0.6 ^{gh} | 4.0 ± 0.8 ^f | 3.0 ± 0.3 ^f | 2.0 ± 0.3 ^e |
| <i>S. griseus</i> + <i>T. atroviride</i> | 13.1 ± 1.2 ⁱ | 4.6 ± 0.4 ^h | 3.5 ± 0.6 ^g | 2.7 ± 0.2 ^g |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> | 11.2 ± 0.3 ^{ef} | 3.6 ± 0.2 ^{ef} | 2.9 ± 0.1 ^e | 2.1 ± 0.1 ^e |
| <i>S. sannanensis</i> + <i>T. atroviride</i> | 10.2 ± 1.7 ^d | 3.4 ± 0.3 ^e | 2.7 ± 0.3 ^{de} | 1.6 ± 0.3 ^{cd} |
| <i>P. fluorescens</i> + <i>T. atroviride</i> | 11.8 ± 0.6 ^g | 4.0 ± 0.2 ^g | 2.7 ± 0.1 ^{de} | 1.9 ± 0.1 ^d |
| <i>S. griseus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 09.0 ± 0.9 ^c | 3.3 ± 0.1 ^e | 2.7 ± 0.7 ^{de} | 1.5 ± 0.1 ^{cd} |
| <i>S. lydicus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 10.2 ± 0.5 ^d | 2.9 ± 0.4 ^d | 3.0 ± 0.3 ^f | 2.0 ± 0.6 ^e |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 09.5 ± 0.6 ^c | 2.7 ± 0.5 ^{bc} | 2.6 ± 0.7 ^{de} | 1.4 ± 0.1 ^c |
| Untreated control | 03.6 ± 2.4 ^a | 1.1 ± 0.5 ^a | 1.2 ± 0.5 ^a | 0.4 ± 0.5 ^a |

[#] Systemic fungicide, *Actinomycete biocontrol agents, ** Bacterial biocontrol agent, *** Fungal biocontrol agent

Pn rate: Photosynthetic rate (per mol min⁻² m⁻²); Tr rate: Transpiration rate (mol s⁻² m⁻²); WUE: Water use efficiency (Ratio of Pn/Tr rates); SC: Stomatal conductance (mol s⁻² m⁻²).

Identical superscript letters denote that values are not significantly different at P < 0.05. A higher letter denotes improvements acquired due to treatment and lower alphabet denotes decline acquired due to treatment.

Tab. 5: Effect of various biocontrol agents and fungicide on biochemical constituents in rhizome rot disease infected turmeric plant leaves

| Treatment details | Chlorophyll (mg g ⁻¹) | Carotenoid (mg g ⁻¹) | Sugar (%) | Nitrogen (%) | Amino acids (%) | Protein (%) |
|--|-----------------------------------|----------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Companion [#] | 3.2 ± 0.4 ^b | 1.3 ± 0.2 ^b | 3.2 ± 0.6 ^b | 2.1 ± 0.4 ^b | 1.6 ± 0.3 ^b | 2.1 ± 0.5 ^b |
| <i>S. lydicus</i> * | 3.4 ± 0.5 ^b | 1.3 ± 0.2 ^b | 4.7 ± 0.3 ^{cd} | 3.3 ± 0.2 ^c | 2.0 ± 0.2 ^b | 2.9 ± 0.5 ^c |
| <i>S. griseus</i> * | 5.6 ± 0.3 ^{de} | 2.5 ± 0.3 ^d | 4.7 ± 0.6 ^{cd} | 4.0 ± 0.5 ^d | 2.3 ± 0.2 ^{bc} | 4.3 ± 0.3 ^f |
| <i>S. sannanensis</i> * | 6.0 ± 0.5 ^e | 2.9 ± 0.5 ^e | 5.1 ± 0.3 ^e | 5.0 ± 0.4 ^g | 2.3 ± 0.2 ^{bc} | 4.2 ± 0.4 ^f |
| <i>P. fluorescens</i> ** | 3.7 ± 0.3 ^b | 1.7 ± 0.3 ^c | 4.2 ± 0.3 ^c | 3.8 ± 0.3 ^{cd} | 2.1 ± 0.1 ^b | 3.0 ± 0.3 ^d |
| <i>T. atroviride</i> *** | 3.5 ± 0.8 ^b | 1.7 ± 0.3 ^c | 5.7 ± 0.5 ^f | 3.4 ± 0.5 ^c | 2.5 ± 0.3 ^c | 3.8 ± 0.4 ^{de} |
| <i>S. lydicus</i> + <i>P. fluorescens</i> | 3.5 ± 0.1 ^b | 1.6 ± 0.2 ^b | 5.9 ± 0.3 ^f | 4.4 ± 0.1 ^{ef} | 2.6 ± 0.5 ^{bc} | 3.8 ± 0.6 ^{de} |
| <i>S. lydicus</i> + <i>T. atroviride</i> | 4.1 ± 0.8 ^c | 1.9 ± 0.3 ^c | 6.1 ± 0.7 ^g | 5.0 ± 0.2 ^g | 2.4 ± 0.7 ^{bc} | 3.2 ± 0.6 ^d |
| <i>S. griseus</i> + <i>P. fluorescens</i> | 5.1 ± 0.5 ^c | 2.4 ± 0.5 ^e | 6.0 ± 0.6 ^g | 5.0 ± 0.3 ^g | 3.0 ± 0.3 ^d | 4.0 ± 0.4 ^f |
| <i>S. griseus</i> + <i>T. atroviride</i> | 6.1 ± 0.5 ^c | 3.1 ± 0.2 ^f | 7.3 ± 0.6 ^h | 5.8 ± 0.3 ^h | 3.5 ± 0.3 ^e | 4.6 ± 0.2 ^g |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> | 3.6 ± 0.2 ^{bc} | 1.4 ± 0.4 ^{bc} | 5.4 ± 0.5 ^{ef} | 4.6 ± 0.3 ^{ef} | 2.4 ± 0.5 ^{bc} | 3.7 ± 0.2 ^{de} |
| <i>S. sannanensis</i> + <i>T. atroviride</i> | 3.4 ± 0.8 ^b | 1.7 ± 0.3 ^c | 5.5 ± 0.7 ^{ef} | 4.5 ± 0.6 ^{ef} | 2.7 ± 0.2 ^{bc} | 3.9 ± 0.5 ^{de} |
| <i>P. fluorescens</i> + <i>T. atroviride</i> | 3.4 ± 0.3 ^b | 1.3 ± 0.2 ^b | 5.5 ± 0.3 ^{ef} | 4.2 ± 0.1 ^e | 2.7 ± 0.4 ^{bc} | 4.1 ± 0.4 ^f |
| <i>S. griseus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 4.6 ± 0.5 ^{cd} | 2.0 ± 0.3 ^e | 4.6 ± 0.7 ^d | 4.0 ± 0.5 ^d | 2.4 ± 0.7 ^{bc} | 3.6 ± 0.4 ^e |
| <i>S. lydicus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 5.2 ± 0.4 ^c | 3.1 ± 0.5 ^f | 4.3 ± 0.3 ^c | 5.0 ± 0.4 ^g | 2.8 ± 0.3 ^d | 2.5 ± 0.3 ^c |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 4.2 ± 0.7 ^c | 2.0 ± 0.3 ^e | 5.7 ± 0.6 ^e | 4.4 ± 0.4 ^{ef} | 2.5 ± 0.3 ^c | 2.8 ± 0.4 ^c |
| Untreated control | 2.2 ± 0.9 ^a | 1.0 ± 0.4 ^a | 2.4 ± 1.1 ^a | 1.6 ± 0.6 ^a | 1.2 ± 0.2 ^a | 1.2 ± 0.5 ^a |

[#] Systemic fungicide, *Actinomycete biocontrol agents, ** Bacterial biocontrol agent, *** Fungal biocontrol agent

Identical superscript letters denote that values are not significantly different at P < 0.05. A higher letter denotes improvements acquired due to treatment and lower alphabet denotes decline acquired due to treatment.

lipids and protein content was increased substantially up to 3.5 and 4.6% in the plants treated with *S. griseus* and *T. atroviride* (T10). All the biochemical parameters were found to be elevated more in dual combinational treatments than in single and triple combinations. The untreated control plants exhibited least chlorophyll and carotenoid contents of 2.2 and 1.0 mg g⁻¹ respectively, with the least other biochemical constituents like total sugar, nitrogen, protein and lipids but superior to Companion treated plants (Tab. 5).

Determination of nutrient status and analysis of soil edaphic parameters in turmeric soils

Turmeric plants prefers between acidic to alkaline pH condition to grow and thrive abundantly. The result on the pH values of turmeric soils showed that it was found to be in the range of 7.5-8.3. Similarly, the results on the estimation of soil electrical conductivity (Ec) indicated that it was in the range between 0.7 and 1.8. The highest amount of organic matter (OM) about 8.1% was recorded in soil

samples collected from the plots amended with the combination of *S. griseus* and *T. atroviride* formulation. Similarly, the estimation of available N, P, exchangeable K, Ca, Mg and Na contents were found to be higher in the same treatment than in other treatments. These results were reflected similar to that of biochemical and physiological traits. The available N and P contents recorded were 3.1% and 381 ppm; respectively in the trail plots treated with *S. griseus* and *T. atroviride* combination. The amount of exchangeable K, Ca, Mg and Na contents recorded was 270.3, 586.7, 97.7 and 76.1 ppm in the same treatment. It was superior to fungicide, single and triple combination treatments of biocontrol agents. Total OM, available N and P contents were found to be 4.5%, 0.8% and 194.3 ppm; respectively to soils of untreated control plants. In the same way, the level of exchangeable K, Ca, Mg and Na contents were found to be 145, 192, 45.3 and 24.9 ppm in the same treatment (Tab. 6).

Survival of biocontrol agents in turmeric soils

The population density of all biocontrol agents increased their population level from June to December and thereafter there was a reduction in the population density with the age of the sample. The enumeration graph strictly showed that there was a general decrease in population density of biocontrol agents after soil delivery in the experimental plots (Fig. 2). At the time of application, the total population density of all the biocontrol agents were 8×10^8 colony forming unit per ml. Finally, towards the end of experimentation (April) the population density of biocontrol agents was declined in varying levels. The mean population level of *S. griseus* was 120×10^5 cfu g⁻¹ soil dry weight followed by *S. lydicus* (95.5) and *S. sannanensis* (95.0) in the month of April. It was about 50.5 and 60.5×10^5 cfu g⁻¹ soil dry weight of *P. fluorescens* and *T. atroviride*; respectively in the same period (Fig. 2).

Discussion

In our studies, there was a significant ($P < 0.05$) reduction in rhizome rot incidence in trial plots due to fungicide and biocontrol agent treatments (Tab. 2). The Companion fungicide protected the maximum disease control (61.55%) followed by the dual combination of *S. griseus* and *T. atroviride* (56.97%) and minimum with the triple combination of biocontrol agents (ranged between 39.31 and 43.41%). The treatments containing the combination of three biocontrol agents ($T_{14} - T_{16}$) were inefficient in disease reduction when compared to single ($T_2 - T_6$) and dual application ($T_7 - T_{13}$) of biocontrol agents. It is due to competition between biocontrol agents in nutrient absorption from the rhizosphere and their growth. A large number of soil-borne plant diseases in plantation crops were successfully controlled through bacterial, fungal and actinomycete antagonists (GNANAMANGAI and PONMURUGAN, 2011).

When compared to biocontrol agents in terms of disease control, Companion fungicide gave a better protection than that of biocontrol agents (Tab. 2). Rhizome rot of ginger can be controlled effectively by the application of various contact and systemic fungicides like Bavistin 50WP, Ridomil Gold MZ-72, Captan, Dithane M-45, Copper oxychloride and Bordeaux mixture. Many researchers worked on the chemical control of the disease and reported to be very promising effect (KAPRATWAR et al., 2016).

Due to the treatment of various biocontrol agents, rhizome yield and their attributes were significantly improved in which the highest rhizome yield of 26.77 t/ha was registered in the treatment of *S. griseus* and *T. atroviride* mixture. The same had been reflected in productivity index also (Tab. 2). There was a remarkable difference in the rhizome yield between single and triple combination mixture of biocontrol agent treated plants. The Companion fungicide treated trial plots registered with the least yield (17.35 t/ha) which is inferior to

Tab. 6: Effect of various biocontrol agents application on nutrient status of turmeric soils

| Treatment | pH | Electric conductivity (dS/m) | Organic matter (%) | N (ppm) | P (ppm) | K (ppm) | Ca (ppm) | Mg (ppm) | Na (ppm) |
|--|-----------------------|------------------------------|------------------------|-----------------------|--------------------------|--------------------------|--------------------------|------------------------|------------------------|
| Companion [#] | 7.8±0.3 ^{bc} | 1.1±0.2 ^{ab} | 5.7±0.3 ^b | 1.3±0.3 ^b | 269.0±18.4 ^b | 178.3±34.3 ^a | 287.3±20.7 ^b | 60.7±2.3 ^b | 36.3±0.1 ^b |
| <i>S. lydicus</i> [*] | 7.5±0.7 ^a | 1.0±0.3 ^b | 6.5±0.6 ^d | 2.3±1.4 ^{cd} | 292.7±15.1 ^{bc} | 199.3±32.7 ^b | 357.7±20.3 ^c | 68.3±3.8 ^b | 61.2±3.6 ^d |
| <i>S. griseus</i> [*] | 7.6±0.4 ^a | 1.2±0.4 ^b | 7.1±0.4 ^c | 1.8±0.2 ^c | 293.3±15.8 ^{bc} | 201.3±23.3 ^b | 373.3±24.3 ^c | 76.7±7.0 ^c | 51.6±4.8 ^c |
| <i>S. sannanensis</i> [*] | 8.0±0.6 ^{cd} | 1.2±0.2 ^b | 5.9±0.2 ^b | 2.6±0.3 ^d | 307.3±11.6 ^c | 203.3±33.3 ^b | 362.7±17.5 ^c | 77.7±9.8 ^c | 63.9±6.2 ^d |
| <i>P. fluorescens</i> ^{**} | 8.3±0.4 ^{de} | 1.1±0.4 ^{ab} | 6.3±0.4 ^c | 1.6±0.4 ^{bc} | 338.3±14.3 ^{cd} | 232.7±26.9 ^{bc} | 432.0±29.1 ^d | 77.3±5.3 ^c | 63.2±5.4 ^d |
| <i>T. atroviride</i> ^{***} | 7.9±0.4 ^c | 1.3±0.3 ^{cd} | 6.6±0.8 ^{de} | 1.6±0.3 ^{bc} | 340.7±15.2 ^{cd} | 234.7±33.2 ^{bc} | 385.0±28.8 ^c | 80.0±3.1 ^d | 58.8±5.3 ^{cd} |
| <i>S. lydicus</i> + <i>P. fluorescens</i> | 7.5±0.3 ^a | 1.1±0.5 ^{ab} | 7.1±0.3 ^c | 2.4±0.2 ^{cd} | 321.0±24.8 ^{cd} | 253.7±25.8 ^c | 586.3±20.0 ^g | 91.3±1.5 ^e | 68.0±2.1 ^{cd} |
| <i>S. lydicus</i> + <i>T. atroviride</i> | 8.2±0.3 ^d | 1.5±0.5 ^{cd} | 7.4±0.5 ^{efg} | 2.6±0.2 ^d | 359.3±11.1 ^d | 266.3±32.3 ^d | 564.7±21.8 ^{ef} | 94.3±1.7 ^{ef} | 71.6±2.9 ^e |
| <i>S. griseus</i> + <i>P. fluorescens</i> | 8.3±0.3 ^{de} | 1.5±0.4 ^{cd} | 7.1±0.4 ^c | 2.6±0.7 ^d | 333.3±17.7 ^{cd} | 255.7±25.3 ^c | 533.3±17.3 ^c | 90.3±2.7 ^e | 72.2±1.9 ^e |
| <i>S. griseus</i> + <i>T. atroviride</i> | 8.2±0.4 ^d | 1.8±0.6 ^{de} | 8.1±0.2 ^{gh} | 3.1±0.5 ^e | 381.0±21.0 ^f | 270.3±20.9 ^d | 586.7±18.8 ^{gh} | 97.7±2.0 ^{ef} | 76.1±1.5 ^f |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> | 8.2±0.1 ^d | 1.7±0.4 ^d | 7.1±0.3 ^c | 2.7±0.3 ^{de} | 365.0±12.5 ^e | 266.3±32.3 ^d | 580.0±27.2 ^g | 90.7±4.2 ^e | 70.4±1.9 ^e |
| <i>S. sannanensis</i> + <i>T. atroviride</i> | 8.2±0.2 ^d | 1.5±0.3 ^{cd} | 7.2±0.5 ^{ef} | 2.6±0.2 ^d | 351.3±13.1 ^d | 240.3±30.7 ^{bc} | 584.0±27.2 ^g | 82.0±8.1 ^d | 71.4±2.0 ^e |
| <i>P. fluorescens</i> + <i>T. atroviride</i> | 8.0±0.3 ^{cd} | 1.8±0.4 ^{de} | 7.8±1.4 ^f | 2.7±0.4 ^{de} | 361.3±19.2 ^e | 236.7±32.8 ^{bc} | 523.7±25.9 ^e | 82.3±8.6 ^d | 70.3±1.0 ^e |
| <i>S. griseus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 7.7±0.6 ^b | 1.8±0.3 ^{de} | 7.5±0.4 ^f | 2.4±0.5 ^{cd} | 356.0±15.3 ^e | 232.7±26.9 ^{bc} | 434.3±25.8 ^d | 80.0±9.2 ^d | 68.8±7.7 ^{cd} |
| <i>S. lydicus</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 7.6±0.4 ^a | 1.4±0.5 ^c | 6.8±1.3 ^{de} | 2.6±0.3 ^d | 373.3±14.7 ^e | 234.7±33.2 ^{bc} | 471.7±25.5 ^d | 83.7±7.8 ^d | 70.3±6.3 ^e |
| <i>S. sannanensis</i> + <i>P. fluorescens</i> + <i>T. atroviride</i> | 7.7±0.3 ^b | 1.2±0.03 ^b | 6.2±0.8 ^c | 2.4±0.5 ^{cd} | 371.0±12.1 ^e | 236.7±33.8 ^{bc} | 465.3±15.1 ^d | 82.7±6.4 ^d | 73.5±5.1 ^e |
| Untreated control | 7.5±0.1 ^a | 0.7±0.03 ^a | 4.5±0.4 ^a | 0.8±0.5 ^a | 194.3±13.3 ^a | 145.0±21.2 ^a | 192.0±12.3 ^a | 45.3±2.8 ^a | 24.9±2.5 ^a |

[#] Systemic fungicide, ^{*} Actinomycete biocontrol agents, ^{**} Bacterial biocontrol agent, ^{***} Fungal biocontrol agent

Identical superscript letters denote that values are not significantly different at $P < 0.05$. A higher letter denotes improvements acquired due to treatment and lower alphabet denotes decline acquired due to treatment.

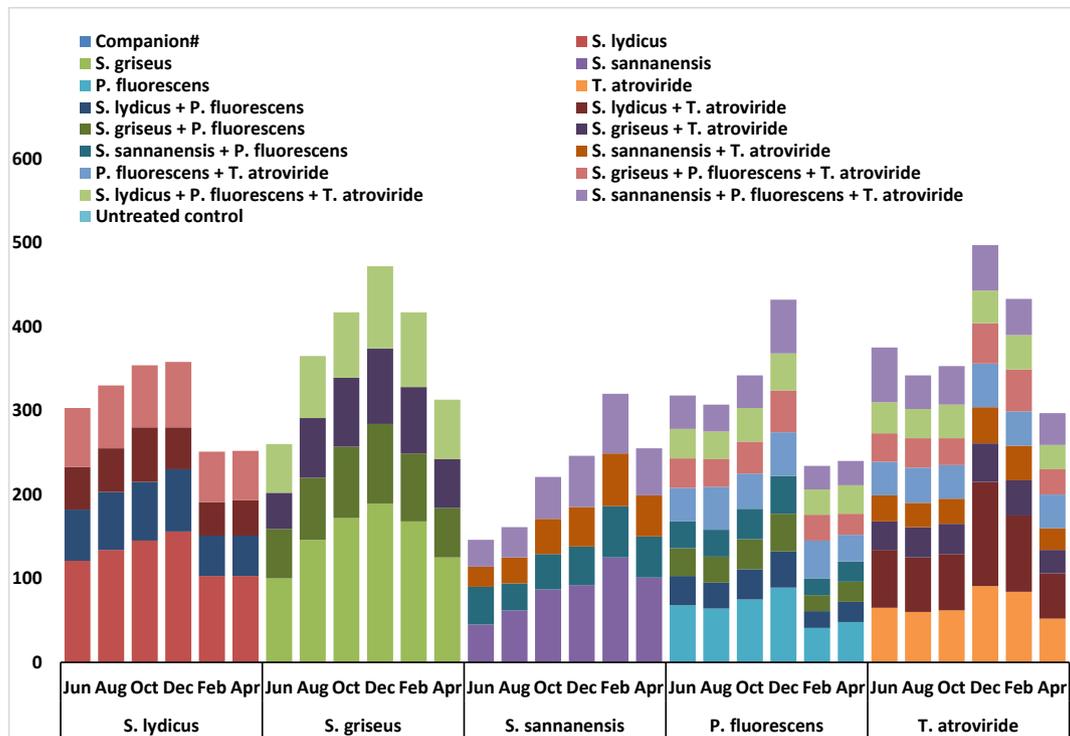


Fig. 2: Population densities of individual biocontrol agents after imposing various treatments in turmeric soils*
*The mean values of each biocontrol agents for a period of four consecutive years with three harvest cycles

triple combination mixture of biocontrol agents (Tab. 2). According to GNANAMANGAI and PONMURUGAN (2011) biocontrol agents are potential microorganisms in soil system to the recovery of plants from infection. It has been reported that biocontrol agents enhance plant growth by producing growth stimulating hormones such as auxins, cytokinins and gibberellins (AJAY et al., 2004). A moderate yield was recorded in the plots treated with Companion fungicide which is not only due to the disease control but also due to their phytotoxic effect (BACMAGA et al., 2016).

Actinomycetes particularly *Streptomyces* spp. inhabiting the rhizosphere, which have the ability to colonize plant root systems can stimulate plant growth by secreting antibiotics like substances, enzymes and phytohormones (MANJUKARUNAMBIKA et al., 2013; ELANGO et al., 2015). Utilization of such *Streptomyces* spp. in turmeric fields is a promising approach for the biological control of rhizome rot as well as for the improvement of plant growth and rhizome yield potential. SABARATNAM and TRAQUAIR (2002) and GOPALAKRISHNAN et al. (2013) reported the plant growth promoting and biocontrol activities of *Streptomyces* spp. in rice, sorghum and tomato.

Turmeric rhizome quality characteristics like number of primary, secondary and tertiary finger rhizomes and amount of curcumin and oleoresin contents were significantly increased due to bioinoculants treatment. A significant improvement was noted in the combination of *S. griseus* and *T. atroviride* treated plants (Tab. 3). There are enormous research reports coincide with significant improvement in yield and productivity in various crops by exploring soil application of biocontrol agents (GUPTA et al., 2000; YAN et al., 2002). Curcumin followed by oleoresin contents are the bioactive compounds of turmeric, which are responsible for the wide spectrum of medicinal properties lead to commercial exploitation.

The present study revealed the increased values in biochemical and physiological constituents, advance in progress of rhizome quality in response to bioformulation. Both physiological and biochemical traits were enhanced in biocontrol agent treatments, especially in combinational treatments (Tab. 4 and 5). The substantial increase

of all biochemical and physiological constituents in biocontrol agent treated plants may be due to their utilization by the pathogen during host-pathogen interaction (PONMURUGAN and BABY, 2007). Increase in protein, nitrogen and amino acid due to biocontrol agent treatments assail to increase in translation process (protein synthesis) and hydrolysis of protein for cellular metabolism of the host plant (REDDY et al., 2003).

In our present study a minor variation of pH and Ec was observed between treatments with the application of biocontrol agents. The values of pH positively coincided with exchangeable calcium level content because high calcium content is required for maintaining turmeric soil at alkaline pH and Ec, which is suitable for turmeric plant growth. In soils with pH above 6.0, accumulation of calcium and the H^+ and Al_3^+ ions are likely to interfere with uptake of potassium which reflected in our results. Due to biocontrol applications, soil nutrients were improved significantly in turmeric soils which could be competent enough with soil-borne pathogens like *P. aphanidermatum* (SRINIVASAN et al., 2016). Periodical monitoring of soil nutrients status revealed the uptake and fertility status of soil due to single and combined biocontrol treatments. Curcumin synthesis in turmeric requires soil nitrogen, since it forms the structural unit of many proteins by undergoing phenyl propanoid pathway (SANDEEP et al., 2015).

The population density of biocontrol agents in turmeric soils was positively coincided with the result of soil nutrient could survive well in the turmeric soils for a prolonged duration (Fig. 1 and Tab. 6). From our field experiments, bioformulations containing *Streptomyces* spp. were found to be superior in terms of rhizome rot suppression and growth promotion in turmeric when compared to *Pseudomonas* and *Trichoderma* spp. Effective biological control of phytopathogens results could be expected from mixtures of antagonists rather than from high populations of a single antagonist due to mimic the natural soil dynamics, broaden the spectrum of biocontrol activity and the efficacy with reliability of disease control (DUFFY and WELLER, 1995). Among *Streptomyces* spp. tested for the biological control of

P. aphanidermatum, *S. griseus* is better than *S. lydicus* and *S. sananensis*.

The present investigation provides information for better management of turmeric plants using biocontrol agents against rhizome rot infection. Hence, biological control might be an alternative, eco-friendly and sustainable technique for turmeric farmers towards the management of rhizome rot.

Acknowledgements

The authors are thankful to University Grant Commission, New Delhi, Government of India (Ref. No. MRP-Major-BIOT-2013-12463) for financial support through major research project. Moreover, the authors are grateful to Mr.T.Rajan, Thontamuthur, Coimbatore District, Tamil Nadu, India for providing turmeric fields to conduct field experiments. We also acknowledge DST-FIST (Ref. No. SR/FST/LS-II/2017/106) and UGC-SAP (Ref. No. F.5-16/2016/DRS-1/SAP-II) grant, Government of India given to the Department of Botany, Bharathiar University for their infrastructural support.

We also acknowledge DBT STAR SCHEME, New Delhi, (BT/HRD/11/09/2018) and Fund for Improvement of S&T Infrastructure (FIST) DST-FIST (SR/FST/College-235/2014) of K.S.Rangasamy College of Technology (Autonomous), Tiruchengode for Laboratory analytical support.

Conflict of interest

No potential conflict of interest was reported by the authors.

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Layout of the conducted field trial in a Turmeric cultivation fields



1-17 - Treatment trial plots

Fa-Fd series - Fields with treatment trial plots

Ca-Cd series - Fields with control plot

* For every cropping cycles the alternative fields and the opposite plots were selected