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RESEARCH ARTICLE

COMBINING *PSEUDOMONAS* AND *TRICHODERMA* STRAINS WITH ORGANIC AMENDMENTS TO ENHANCE SUPPRESSION OF ROOT ROT DISEASE INCIDENCE IN COLEUS

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ABSTRACT

The fungal and bacterial biocontrol agents and organic amendments were tested individually and in combinations for their efficacy against root rot pathogen, *Macrophomina phaseolina* under *invitro*, glass house and field conditions. Among the fungal and bacterial (*Pseudomonas*) antagonists screened, *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* (Pf7) isolates exhibited maximum inhibition compared to other isolates. Among the organic amendments, tested *invitro* against the pathogen, neem cake and coir pith compost were most effective in reducing the growth of the pathogen. The compatibility studies revealed the isolate of *T. viride*, *T. harzianum*, *P. fluorescens* (Pf7) were compatible with each other and also with eight organic amendments. Of the various treatments tested, soil application of *T. viride* and neem cake recorded least incidence of disease when compared to individual treatments both under glass house and field conditions. Furthermore, the same treatment also increased the yield compared to control.

INTRODUCTION

Coleus forskohlii Briq. [syn: *Coleus barbatus* (Andr.) Benth.] is a plant of Indian origin (Valdes *et al*., 1987) belonging to mint family Lamiaceae and grows perennially over tropical and subtropical regions of India, Pakistan, Sri Lanka, East Africa and Brazil at 600-1800 m elevation. In India, the crop is cultivated in the parts of Gujarat, Maharashtra, Rajasthan, Karnataka and Tamil Nadu and is being grown in an area of more than 2500 ha for its tuberous roots. Traditionally, the roots have been used as condiments in pickles, and also for medicinal purposes by the ayurvedic schools of medicines (Ammon and Muller, 1985). Root juice is given to children suffering from constipation (Singh *et al.*, 1980). Kothas, the native tribes of Trichigadi in Nilgiri, South India consider the decoction of tuberous roots as tonic (Abraham,1981). The therapeutic properties of forskolin are utilized in treating cardiac insufficiency, hypertension, glaucoma, thrombosis, cancer, asthama and metastatic conditions (Seamon, 1984). The novel feature of forskolin is its unique mechanism of generating cyclic adenosine monophosphate (AMP) in the cells through the direct activation of the catalytic unit of adenylate cyclase enzyme, which made the pharmaceutical industry to recognize the plant as most medicinally and economically important. The crop is subjected to diseases caused by fungi, bacteria and viruses. Of these diseases, root rot caused by *M. phaseolina* (Tassi.) Goid causes considerable losses (Kamalakannan *et al*., 2005).

Several effective fungicides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, health and environmental hazards, residue persistence and development of tolerance. There is a vital need for alternative methods of control for root rot. So far, effective and ecologically sound management practices have not been developed for this disease. Therefore, the objective of the current study was to develop a biological control strategy for this disease that is durable and is an alternative to agrochemicals.

Several antagonistic organisms have been successfully used as biocontrol agents for controlling soil borne pathogens (Deacon, 1991). In most of the research, to date, biocontrol agents are applied singly to combat the growth of the pathogens. Although the potential benefits of a single biocontrol agent application has been demonstrated in many studies, it may also partially account for the reported inconsistent performance because a single biocontrol agent is not likely to be active in all kinds of soil environment and all agricultural ecosystems (Raupach and Kloepper, 1998). These have resulted in inadequate colonization, limited tolerance to changes in environmental conditions and fluctuations in production of antifungal metabolites (Weller and Thomashow, 1994). Thus, more emphasis was laid on the combined use of two or more strains of biocontrol agents, which turned out to be more successful than either of them alone, as reported by several workers (Thilgavathi *et al*., 2007; Senthilraja *et al*., 2010). Mixtures of biocontrol agents will also have the

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advantage of exercising a broad spectrum activity, enhancing the efficacy and reliability of biological control generally and ensuring greater induction of defence enzymes over individual strains (Latha *et al*., 2009). Hence, the present investigation was undertaken to study the effectiveness of combinations of fungal and bacterial biocontrol agents with organic amendments against root rot of Coleus and to develop ecofriendly management practices to control the disease.

MATERIAL AND METHODS

Isolation of pathogen

Coleus plants showing the typical symptoms of root rot disease were collected from Agricultural College and Research Centre, Madurai. The pathogen was isolated from the roots of these infected plants by tissue segment method (Rangaswami, 1972) on potato dextrose agar (PDA) medium. The isolates of the pathogen were purified by single hyphal tip method (Rangaswami, 1972) and the auxenic cultures were maintained on PDA slants for further studies.

Source of antagonists and organic amendments

Six species of *Trichoderma viz. T. viride, T. harzianum T. longibrachiatum, T. koningii, T. pseudokoningi* and *T. hamatum* and *Chaetomium* sp. maintained at the Department of Plant Pathology, Madurai were obtained and used in the study. Eight isolates of *P. fluorescens* were isolated from rhizosphere soil of Coleus collected from different areas using King's B medium (KB) (King *et al*., 1954) and pure cultures were maintained on respective agar slants at 4 ^{o}C. Various organic amendments *viz*., neem cake, groundnut cake, poultry manure, castor cake, farm yard manure, gingelly cake, coir pith compost and coconut cake were purchased from the pesticide market at Madurai.

Invitro screening of antagonists and organic amendments

Efficacy of fungal and bacterial antagonists against *M. phaseolina* under *invitro*

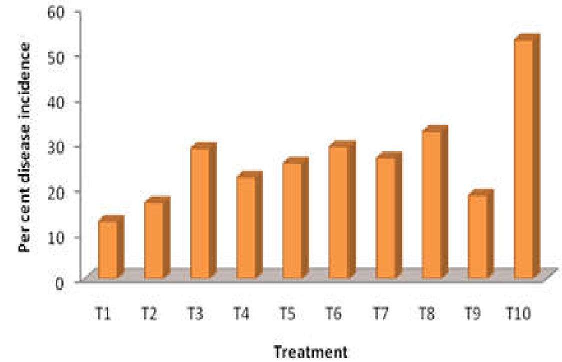
The antagonistic potential of six species of *Trichoderma viz. T. viride, T. harzianum T. longibrachiatum, T. koningii, T. pseudokoningi* and *T. hamatum* and *Chaetomium sp*. and also isolates of *P. fluroscens* was tested *invitro* through dual culture method (Dennis and Webster, 1971). The per cent inhibition in mycelial growth and sclerotial number were calculated using the formula suggested by Pandey *et al.* (2000).

$$\text{Per cent Inhibition} = \frac{Dc - Dt}{Dc} \times 100$$

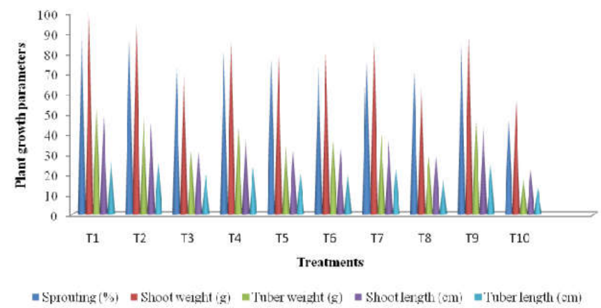
Dc : Average diameter of fungal growth in control
Dt : Average diameter of fungal growth in treatment

Efficacy of organic amendments against *M. phaseolina* under *invitro*

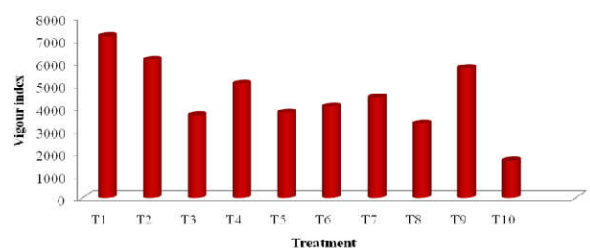
The effect of various organic amendments *viz.,* neem cake, groundnut cake, poultry manure, castor cake, farm yard manure, gingelly cake, coir pith compost and coconut cake at 10 per cent concentration were tested under *invitro* condition against *M. phaseolina* through poision food technique. The oil cakes and other organic amendments were powdered and soaked in sterile distilled water @ 1 g/ml and kept overnight, and filtered through What man No. 1 filter and finally passed through Seitz filter to eliminate bacterial contamination. The filtered oil cakes and other organic amendment extracts formed the standard solution (100 %). These extracts were further diluted to ten per cent concentration and were used for poison food technique.



a. Root rot disease incidence



b. Plant growth parameters



C. vigour index

T1 -	*T. viride* (SA)	T6 -	*T. hamatum* (SA)
T2 -	*P. fluorescens* (SA)	T7 -	*T. reesei* (SA)
T3 -	*T. longibrachiatum* (SA)	T8 -	*Chaetomium* sp (SA)
T4 -	*T. harzianum* (SA)	T9 -	Carbendazim (0.1%)(SD)
T5 -	*T. koningii* (SA)	T10 -	Control

Fig1. Influence of antagonists against root rot disease incidence, plant growth parameters and vigor index under pot culture conditions

Effect of antagonists and organic amendments on biology of *M. phaseolina*

Sclerotial number

One sq. cm area was marked on each Petri plate containing the sclerotia of *M. phaseolina* in dual culture plates containing antagonists and organic amendments was removed intact. It was placed in a beaker containing 10 ml of sterile distilled water and stored for 30 min so as to separate the sclerotia from the medium. The entire contents were squeezed through cheese cloth and washed in several changes of distilled water and then transferred to a glass vial containing 2.5 ml of 2.5 per cent ammonium sulphate. The sclerotia floated after 10 min filtered through a 9 mm filter paper and rinsed with distilled water. The number of sclerotia in filter paper was counted under a stereo zoom microscope (Dhingra and Sinclair, 1978).

Sclerotial size

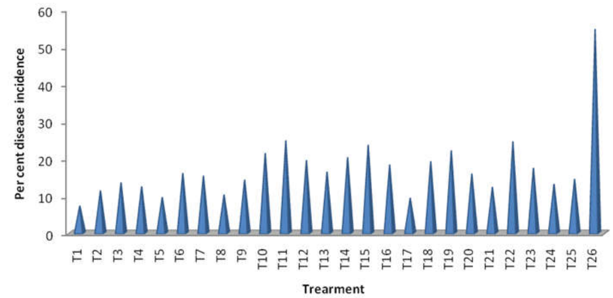
Fifty sclerotia were harvested from each dual culture plate after seven days, dried for two hours in shade and the size was measured with an ocular micrometer in a calibrated microscope.

Sclerotial germination

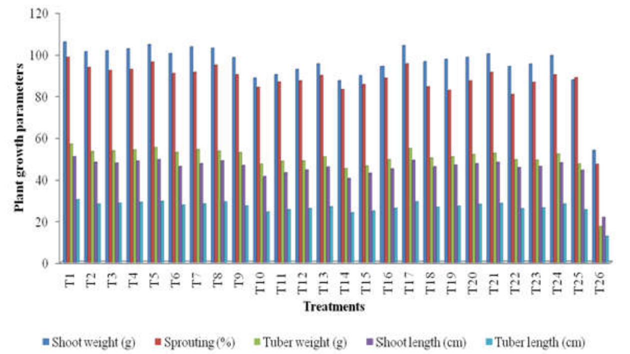
Fifty matured sclerotia were taken from each dual plate and placed in a drop of sterile water in a cavity slide. They were incubated in moist chamber for 72 hours. The number of germinated sclerotia and the number of germ tubes put forth from each sclerotium were counted (Montgomery and Monre, 1938).

Compatibility studies

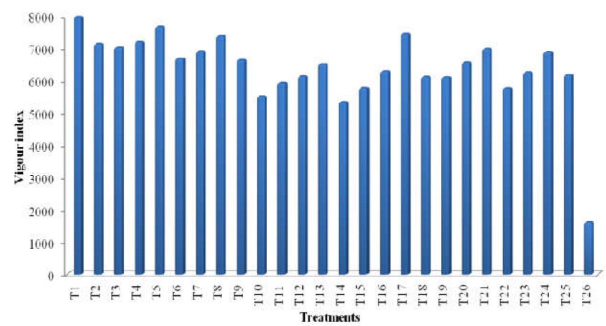
The individual strains with each other and with organic amendments were first assessed for compatibility under *invitro* and then investigated under glass house and field conditions. The compatibility of the fungal biocontrol agent with the PGPR strains was tested by their mycelial overgrowth on the PGPR strains without any inhibition zone, using the dual culture technique (Dennis and Webster, 1971). The compatibility of organic amendments with bacterial and fungal antagonists was also tested by poisoned food technique. Ten per cent concentration of extracts of eight organic amendments was prepared and tested for their compatibility with *P. fluorescens*, *T. viride* and *T. harzianum*. The Petri dishes containing PDA and KB medium were inoculated with 9-mm disc of TV1 and streaked with Pf7, respectively. The growth of fungal and bacterial antagonists was recorded on incubation at room temperature and expressed as compatible (+) or incompatible (−).



a. Root rot disease incidence



b. Plant growth parameters



C. Vigour index

T1 - *T. viride* (SA) + Neem cake (SA)
T2 - *T. viride* (SA) + Groundnut cake (SA)
T3 - *T. viride* (SA) + Castor cake (SA)
T4 - *T. viride* (SA) + Poultry manure (SA)
T5 - *T. viride* (SA) + Coirpith compost (SA)
T6 - *T. viride* (SA) + Coconut cake (SA)
T7 - *T. viride* (SA) + Gingelly cake (SA)
T8 - *T. viride* (SA) + FYM (SA)
T9 - *T. harzianum* (SA) + Neem cake (SA)
T11 - *T. harzianum* (SA) + Castor cake (SA)
T12 - *T. harzianum* (SA) + Poultry manure (SA)
T13 - *T. harzianum* (SA) + Coirpith compost (SA)
T14 - *T. harzianum* (SA) + Coconut cake (SA)
T15 - *T. harzianum* (SA) + Gingelly cake (SA)
T16 - *T. harzianum* (SA) + FYM (SA)
T17 - *P. fluorescens* (SA) + Neem cake (SA)
T18 - *P. fluorescens* (SA) + Groundnut cake (SA)
T20 - *P. fluorescens* (SA) + Poultry manure (SA)
T21 - *P. fluorescens* (SA) + Coirpith compost (SA)
T22 - *P. fluorescens* (SA) + Coconut cake (SA)
T23 - *P. fluorescens* (SA) + Gingelly cake (SA)
T24 - *P. fluorescens* (SA) + FYM (SA)
T25 - Carbendazim (0.1%) (SD)
T26 - Control

Fig. 2 Influence of combined application of antagonists and organic amendments on root rot disease incidence, plant growth parameters and vigour index under pot culture conditions

Mass multiplication of fungal and bacterial antagonists

Mass multiplication of the fungal antagonists

The mass multiplication of the fungal antagonists on Molasses yeast medium was done as per the procedure described by Jeyarajan *et al.* (1994). Nine mm PDA culture discs of the antagonists cultures were inoculated into 250 ml flasks containing 70 ml of Molasses yeast broth. After 15 days of incubation, the mycelial mat of the fungus was homogenized and the content was mixed with talc powder @ 1:2 (v/w) and shade dried for a day. For each kg of the talc powder, 5 g of carboxy methyl cellulose was added as adhesive material and the talc-based preparation was stored in polythene bags and used for further studies.

Mass multiplication of *P. fluorescens*

P. fluorescens was multiplied in sterilized King's broth (King *et al*. 1954) for 48 hours. The pH of the talc powder was adjusted to seven by adding calcium carbonate @ 150 g/kg. The substrate was then sterilized at 1.4 kg cm^{-2} pressure for 30 min for two successive days. A quantity of 400 ml of *P. fluorescens* suspension was added to one kg of substrate mixed with 5 g of carboxy methyl cellulose and mixed well.

The talc-based preparation was stored in polythene bags and used for further studies.

Mass multiplication of pathogenic inoculum

The root rot pathogen *M. phaseolina* was multiplied in sand – maize medium (Riker and Riker,1936). Sand and ground maize grains were mixed at a ratio of 19:1, moistened with water and autoclaved at 1.04 kg per cm^{-2} pressure for two hours on three successive days. The sterilized medium was inoculated with mycelial disc of *M. phaseolina* and incubated at room temperature for 21 days. Sieved garden soil was sterilized in an autoclave at 1.04 kg per cm^{-2} pressure for three hours on two consecutive days. The pathogen multiplied on sand maize medium was applied at a rate of 50 g/kg of soil.

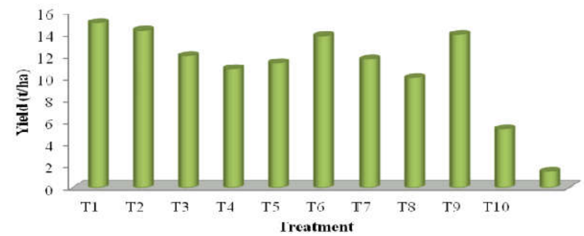
Influence of talc based bioformulations and organic amendments on root rot incidence and plant growth parameters under pot culture conditions

Effect of talc based bioformulations

Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 hour for two consecutive days and filled in pots. The talc based bioformulation was applied to the planting hole at following doses. The culture of *M. phaseolina,* mass multiplied in sand maize medium (sand and Maize powder at the ratio of 19: 1) was incorporated with potting medium at the rate of 50 g/kg of soil. The Coleus cuttings were planted in the holes. The pathogen alone inoculated served as control. Three replications (10 pots per replication) were maintained and the pots were arranged in a randomized manner. The root rot incidence of *M. phaseolina* was recorded on 45 days after planting and expressed as percentage of disease incidence. In the same experiment various biological parameters like sprouting, shoot length, root length, tuber weight, shoot weight and vigour index were recorded. The treatments were as below T1- *T. viride* @ 2.5 kg/ha, T2- *P. fluorescens* @ 2.5 kg/ha, T3- *T. longibrachiatum* @ 2.5 kg/ha, T4-*T. harzianum* @ 2.5 kg/ha, T5- *T. koningii* @ 2.5 kg/ha, T6- *T. hamatum* @ 2.5 kg/ha, T7 - *T. reesei* @ 2.5 kg/ha, T8-*Chaetomium* sp @ 2.5 kg/ha, T9-Carbendazim (0.1 %) and T10- Control. All the treatments were delivered as soil application except Carbendazim which was delivered through soil drenching.

Effect of organic amendments

A pot culture experiment was laid out in completely randomized block design to test the efficacy of organic amendments in controlling the root rot of coleus. Potting medium (red soil: cow dung: manure at 1:1:1 w/w/w) was autoclaved for 1 hour for two consecutive days. The treatment consists of, neem cake, groundnut cake, poultry manure, castor cake, gingelly cake, coconut cake, coir pith compost and farmyard manure. The amendments were applied basally at the following rate of the soil and allowed to wither for 10 days. The virulent strain of *M. phaseolina* mass multiplied in sand maize medium (sand and maize powder at the ratio of 19: 1) was incorporated in the soil at the rate of 50 g/kg of soil. The Coleus cuttings were planted in the inoculated pots. Three replications (10 pots per replication) were maintained and the pots were arranged in a randomized manner. The root rot incidence was recorded on 45 days after planting and expressed as percentage of disease incidence. In addition to this various biological parameters were also recorded. The treatments of the experiment are T1- Neem cake @ 150 kg/ha, T2 - Groundnut cake @ 150 kg/ha, T3 - Castor cake @ 150 kg/ha, T4 - FYM @ 12.5 t/ha, T5 - Coconut cake @ 150 kg/ha, T6 - Poultry manure @ 1 t/ha, T7 - Gingelly cake @150 kg/ha, T8 - Coir pith compost @ 6.25 t/ha, T9 - Carbendazim (0.1%) and T10 – Control.



T1 - *T. viride* (SA) + Neem cake (SA)
T2 - *T. viride* (SA) + Coir pith compost (SA)
T3 - *T. viride* (SA) + Groundnut cake (SA)
T4 - *T. viride* (SA) + Poultry manure (SA)
T5 - *T. viride* (SA) + FYM (SA)
T6 - *T. harzianum* (SA) + Neem cake (SA)
T7 - *P. fluorescens* (SA) + Coir pith compost (SA)
T8 - *P. fluorescens* (SA) + FYM (SA)
T9 - Carbendazim (0.1%) SD
T10 - Control

Fig. 3. Effect of combined application of antagonists and organic amendments on yield under field conditions

Effect of combined application of antagonists and organic amendments

Two kg of soil was taken in 30 cm earthen pots and autoclaved at 1.4 kg/cm^{-2} for two hr. The culture was multiplied in sand maize medium and added to the sterilized pot culture soil at 1:19 ratio and thoroughly mixed. Talc based formulation of the antagonists organisms were used as soil application. *Trichoderma* spp. and *P. fluorescens* were applied @ 2.5 kg/ha in the pots. The organic amendments *viz.,* neem cake @ 150 kg/ha, groundnut cake@ 150 kg/ha, poultry manure @ 1 t/ha, coir pith compost @ 6.25 t/ha, gingelly cake @ 150 kg/ha, castor cake @ 150 kg/ha, coconut cake @ 150 kg/ha and FYM@ 12.5 t/ha were added to each pot and mixed thoroughly. Three replications (10 pots/replication) were maintained for each treatment. The pots were maintained in the glass house with judicious, uniform and regular watering. Coleus cuttings were planted in each pot. The number of sprouted cuttings were counted 30 DAP and expressed as per cent sprouting. The disease incidence was recorded on 45 DAP and expressed as per cent disease incidence. Observations on shoot length, tuber length, shoot weight, tuber weight, and vigour index were also recorded. The treatments were as follows. T1- *T. viride* @ 2.5 kg/ha + Neem cake @150 kg/ha, T2 - *T. viride* @ 2.5 kg/ha + Groundnut cake @ 150 kg/ ha, T3 -*T. viride* @ 2.5 kg/ha + Castor cake @150 kg/ha, T4 - *T. viride* @ 2.5 kg/ha+Poultry manure @ 1 t/ha, T5-*T.viride* @ 2.5 kg/ha+Coir pith compost@6.25 t/ha, T6- *T. viride* @ 2.5 kg/ha + Coconut cake @ 150 kg/ha,T7- *T. viride* @2.5 kg/ha+ Gingelly cake @ 150 kg/ha,T8- *T. viride* @ 2.5 kg/ha+ FYM@ 12.5 t/ha, T9-*T. harzianum* @ 2.5 kg/ ha+Neem cake@150 kg/ha, T10-*T. harzianum* @ 2.5 kg/ha+Groundnut cake @ 150 kg/ ha,T11-*T. harzianum*@2.5 kg/ha+Castor cake @ 150 kg/ha, T12- *T. harzianum* @ 2.5 kg/ha+Poultry manure @ 1 t/ha, T13-*T.harzianum* @ 2.5 kg/ha + Coir pith compost @ 6.25 t/ha, T14- *T. harzianum* @ 2.5 kg /ha+Coconut cake @ 150 kg/ha, T15- *T. harzianum* @ 2.5 kg/ha+Gingelly cake @ 150 kg/ha, T16- *T. harzianum* @ 2.5 kg/ha+FYM @ 12.5 t/ha,T17- *P. fluorescens* @ 2.5 kg/ha+Neem cake @ 150 kg/ha,T18- *P. fluorescens* @ 2.5 kg /ha+Groundnut cake @ 150 kg/ha,T19- *P. fluorescens* @ 2.5 kg/ha+Castor cake@ 150 kg/ha, T20- *P. fluorescens* @ 2.5 kg/ha+Poultry manure @ 1 t/ha,T21- *P. fluorescens* @ 2.5 kg/ha+Coir pith compost @ 6.25 t/ha, T22- *P. fluorescens* @2.5 kg/ha+Coconut cake @ 150 kg/ha,T23- *P. fluorescens* @ 2.5 kg/ha+Gingely cake @ 150 kg/ha, T24- *P. fluorescens* @ 2.5 kg/ha+FYM @12.5 t/ha, T25-Carbendazim (0.1%) and T26-Control.

Evaluation of organic amendments and antagonists against root rot incidence under field conditions

Based on the results of pot culture experiments eight effective treatments were selected and their efficacy was evaluated under field conditions using Carbdendazim as standard. The various treatments were as follows. T1-*T.viride* @ 2.5 kg/ha+Neem cake @ 150 kg/ha, T2-*T.viride* @ 2.5 kg/ha+Coir pith compost@6.25 t/ha, T3-*T. viride* @ 2.5 kg/ha+ Groundnut cake @ 150 kg/ ha, T4- *T. viride* @ 2.5 kg/ha+Poultry manure @ 1 t/ha, T5- *T. viride* @ 2.5 kg/ha+ FYM@ 12.5 t/ha, T6-*T. harzianum* @ 2.5 kg/ ha+Neem cake@150 kg/ha, T7- *P. fluorescens* @ 2.5 kg/ha+Coir pith compost @ 6.25 t/ha, T8-*P. fluorescens* @ 2.5 kg/ha+FYM @12.5 t/ha, T9-Carbendazim (0.1%) and T10-Control. The disease incidence, plant growth parameters and yield was recorded in each case and expressed as per cent decrease or increase.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Prior to statistical ANOVA the percentage values of the disease index were arcsine transformed. Data were subjected to ANOVA at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's multiple range test (DMRT).

RESULTS

Fungicidal property of antagonists and organic amendments

Fungal antagonists

Among the various fungal antagonists tested under *invitro* conditions, *T. viride* recorded a maximum growth inhibition of 55.70 per cent which was on par with *T. harzianum* (48.06 per cent) and the least inhibition of 38.72 per cent was recorded by *T. pseudokoningii* (Table 1).

Bacterial antagonists

Among eight isolates of *P. fluorescens* assessed for mycelial inhibition of *M. phaseolina* by dual culture method, Pf7 significantly showed maximum inhibition of 53.05 per cent inhibition over control and with mean mycelial growth of 42.25 mm under *invitro.* It was on par with Pf8 and Pf3 with 48.33 mm and 46.11 mm per cent inhibition over control and mycelial growth of 46.25 and 48.50 mm, respectively. Lowest per cent inhibition over control of 36.94 was recorded by Pf6 with mycelia growth of 56.75 mm (Table 2).

Organic amendments

Eight organic amendments were evaluated for their efficacy against the mycelial growth of *M. phaseolina* under *invitro*. The results revealed that neem cake extract exerted maximum mycelial growth of 19.90 mm followed by coir pith compost (38.50 mm). The next best effective treatment was FYM compost extract at 10 per cent concentration which recorded a colony diameter of 42.60 mm (Table 3).

Effect of antagonists and organic amendments on biology of *M. phaseolina*

Number of sclerotia

All the antagonists and organic amendments tried were found to reduce the number of sclerotia produced by the pathogen per unit area and they differed significantly. However, among the antagonists, *T. viride* caused appreciable reduction in number of sclerotia produced by the pathogen (63.86) followed by *P. fluorescens* (74.16) accounting 57.24 and 50.34 per cent reduction over control while in control the number of sclerotia produced by the pathogen was 149.35 per 9 mm disc. Of the organic amendments maximum reduction of 59.99 per cent with mean sclerotial number of 61.33 was noticed in neem cake and was followed by coir pith compost (69.00) accounting 54.96 per cent reduction as against control recording mean number of 153.20 (Table 4 and 5).

Size of sclerotia

Variation in size of sclerotia was observed in all antagonists as well as organic amendments. In general, all the antagonists and organic amendments tried were found to reduce the size of sclerotia produced by the pathogen and they differed significantly. However, among antagonists, *T. viride* caused appreciable reduction in size of sclerotia produced by the pathogen (68.42 μm) accounting 16.52 per cent reduction, which followed by *P. fluorescens* documenting 71.28 μm with 13.11 per cent reduction, while in control the size of sclerotia produced by the pathogen was 82.04 μm.

Among the organic amendments tested for their efficiency in reducing the size of sclerotia neem cake recorded highest reduction (27.71 %) with sclerotium size of 59.90 μm and followed by coir pith compost (25.28 %) with sclerotium size 61.30 μm as against control recording 82.04 μm. The least reduction in size of sclerotium (81.00) was noticed in castor cake with 1.26 per cent reduction (Table 4 and 5).

Sclerotial germination

The germination per cent of sclerotium obtained from dual culture plates involving antagonists as well as organic

Table 1. *Invitro* antagonism of fungal antagonists against *M. Phaseolina*

S. No.	*Trichoderma* spp.	* Mycelial growth (mm)	Per cent inhibition over control
1.	Trichoderma viride	39.87	55.70
2.	*T. harzianum*	46.79	48.06
3.	*T. koningii*	54.54	39.40
4.	*T. pseudokoningii*	55.15	38.72
5	*T. longibrachiatum*	50.57	43.81
6.	*T. hamatum*	48.43	46.18
7.	*Chaetomium* sp.	49.32	46.20
8.	Control	90.00	-
	CD (P=0.05)	8.01	-

*Mean of three replications

Table 2. *Invitro* antagonism of *P. fluorescens* against *M. phaseolina*

S. No.	Isolate	* Mycelial growth (mm)	Per cent inhibition over control
1.	*P. fluorescens* (Pf1)	47.25	47.50
2.	*P. fluorescens* (Pf2)	53.25	40.83
3.	*P. fluorescens* (Pf3)	48.50	46.11
4.	*P. fluorescens* (Pf4)	49.75	44.72
5.	*P. fluorescens* (Pf5)	55.25	38.61
6.	*P. fluorescens* (Pf6)	56.75	36.94
7.	*P. fluorescens* (Pf7)	42.25	53.05
8.	*P. fluorescens* (Pf8)	46.25	48.33
9.	Control	90.00	-
	CD (P=0.05)	8.10	-

*Mean of three replications

Table 3. *Invitro* antagonism of organic amendments against *M. phaseolina*

S.No.	*Treatment	**Mycelial growth (mm)	Per cent inhibition over control
1.	Neem cake	19.90	77.88
2.	Groundnut cake	52.60	41.55
3.	Castor cake	59.40	34.08
4.	Farm yard manure	42.60	52.66
5.	Coconut cake	76.20	15.33
6.	Poultry manure	43.40	51.77
7.	Gingelly cake	58.70	34.77
8.	Coir pith compost	38.50	57.22
9.	Control	90.00	-
	CD (P=0.05)	8.31	-

**Mean of three replications; *10 %Concentration

Table 4. Effect of antagonists on sclerotial production, size and germination

S.No.	Treatment	*Sclerotial number	Per cent reduction over control	*Size of the sclerotia (μm)	Per cent reduction over control	*Sclerotial germination (%)	Per cent reduction over control
1.	*Trichoderma viride*	63.86	57.24	68.42	16.52	38.72	58.16
2.	*T. harzianum*	80.34	46.20	73.36	10.58	42.48	54.10
3.	*T. hamatum*	108.15	27.58	75.54	7.92	44.20	52.24
4.	*P. fluorescens*	74.16	50.34	71.28	13.11	39.34	57.49
5.	Control	149.35	-	82.04	-	92.56	-
6.	CD (P=0.05)	10.75		3.51		5.09	

*Mean of three replications

Table 5. Effect of organic amendments on sclerotial production, size and germination

S.No	Treatment	*Sclerotial number	Per cent reduction over control	*Size of the sclerotia (μm)	Per cent reduction over control	*Sclerotial germination (%)	Per cent reduction over control
1.	Neem cake	61.30	59.99	59.30	27.71	34.72	62.48
2.	Groundnut cake	103.00	32.76	74.34	09.38	59.34	35.89
3.	Castor cake	132.00	13.88	81.00	01.26	81.00	12.48
4.	Farm yard manure	78.16	48.98	73.00	11.01	54.12	41.52
5.	Coconut cake	110.31	27.99	76.00	07.36	58.30	37.01
6.	Poultry manure	108.30	29.30	78.00	04.92	61.00	34.09
7.	Gingelly cake	123.85	19.15	79.10	03.58	78.00	15.73
8.	Coir pith compost	69.00	54.96	61.30	25.28	44.48	51.94
9.	Control	153.20	-	82.04	-	92.56	-
	CD (P=0.05)	1.70	-	1.65	-	1.70	-

*Mean of three replications

Table 6. Root rot disease incidence influenced by organic amendments under pot culture conditions

S. No.	Treatment	*PDI	Per cent reduction over control
1.	Neem cake (SA)	15.26 (22.34)	72.11
2.	Groundnut cake (SA)	30.12 (33.27)	44.95
3.	Castor cake(SA)	34.47 (35.94)	37.01
4.	Farm yard manure (SA)	22.67 (28.41)	58.57
5.	Coconut cake (SA)	32.64 (34.82)	40.35
6.	Poultry manure (1t/ha)	25.41(30.25)	53.36
7.	Gingelly cake (SA)	27.35(31.52)	50.02
8.	Coir pith compost (SA)	21.78 (27.10)	60.20
9.	Carbendazim (0.1%)(SD)	18.56 (24.00)	66.08
10.	Control	54.72 (47.43)	-
	CD (P=0.05)	2.18	

*Mean of three replications; Figures in parantheses are arcsine – transformed values; SA – Soil Application; SD – Soil Drenching

Table 7. Effect of organic amendments on plant growth parameters under pot culture conditions

S.No.	Treatment	*Sprouting (%)	*Shoot length (cm)	*Tuber length (cm)	*Shoot weight (g)	*Tuber weight (g)	Vigour index
1.	Neem cake (SA)	90.37 (73.97)	46.08	26.87	96.34	48.23	6592.49
2.	Groundnut cake (SA)	74.28 (59.67)	33.64	19.26	76.19	36.75	3929.41
3.	Castor cake(SA)	67.42 (55.27)	26.81	17.84	70.48	32.92	3010.30
4.	Farm yard manure (SA)	82.16 (65.42)	38.53	23.45	84.72	44.18	5092.28
5.	Coconut cake (SA)	63.18 (52.59)	24.14	16.78	68.51	29.68	2585.32
6.	Poultry manure (1t/ha)	79.54 (63.40)	35.43	21.37	80.63	40.34	4517.87
7.	Gingelly cake (SA)	77.25 (61.73)	31.59	18.13	74.93	34.83	3492.83
8.	Coir pith compost (SA)	85.73 (68.48)	41.62	24.66	85.12	43.51	5682.18
9.	Carbendazim (0.1%)(SD)	86.62 (70.35)	42.86	25.32	87.37	45.62	5973.93
10.	Control	48.31(44.03)	21.75	13.64	49.26	17.37	1709.69
	CD (P=0.05)	3.08	1.41	0.73	2.22	1.58	-

*Mean of three replications; Figures in parantheses are arcsine – transformed values; SA – Soil Application SD – Soil Drenching

Table 8. Efficacy of combined application of antagonists and organic amendments against root rot disease incidence under field conditions

S. No	Treatment	*PDI	Per cent reduction over control
1	*T. viride* (SA) + Neem cake (SA)	8.15 (16.58)	61.19
2	*T. viride* (SA) + Coir pith compost (SA)	10.86 (19.24)	48.28
3	*T. viride* (SA) + Groundnut cake (SA)	12.34 (20.56)	41.23
4	*T. viride* (SA) + Poultry manure(SA)	12.68 (20.82)	39.61
5	*T. viride* (SA) + FYM (SA)	12.00 (20.26)	42.85
6	*T. harzianum* (SA) + Neem cake (SA)	15.13 (20.89)	27.95
7	*P. fluorescens* (SA) + Coir pith compost (SA)	13.89 (21.88)	33.85
8	*P. fluorescens* (SA) + FYM (SA)	14.85 (22.66)	29.28
9	Carbendazim (0.1%) (SD)	14.00 (21.97)	33.33
10	Control	21.00 (27.27)	-
	CD (P=0.05)	1.185	

*Mean of three replications SA – Soil Application SD – Soil Drenching

Table 9. Plant growth parameters as influenced by combined application of antagonists and organic amendments under field conditions

S. No	Treatment	*Sprouting (%)	*Shoot length (cm)	*Tuber length (cm)	*Shoot weight (g)	*Tuber weight (g)	Vigour index
1	*T. viride* (SA) + Neem cake (SA)	96.83 (79.18)	48.64	27.60	107.43	55.30	7382.31
2	*T. viride* (SA) + Coir pith compost (SA)	95.38 (77.59)	45.64	26.80	106.43	54.00	6909.32
3	*T. viride* (SA) + Groundnut cake (SA)	91.00 (72.54)	44.31	25.30	103.38	53.64	6334.51
4	*T. viride* (SA) + Poultry manure(SA)	90.00 (71.56)	46.67	27.30	101.54	56.30	6657.30
5	*T. viride* (SA) + FYM (SA)	93.52 (75.25)	45.78	28.00	108.10	58.00	6899.90
6	*T. harzianum* (SA) + Neem cake (SA)	89.13 (70.75)	44.93	27.00	100.00	51.80	6411.12
7	*P. fluorescens* (SA) + Coir pith compost (SA)	89.17 (70.78)	47.78	29.00	103.15	52.00	6846.47
8	*P. fluorescens* (SA) + FYM (SA)	91.00 (77.34)	46.50	27.95	101.36	52.30	6477.15
9	Carbendazim (0.1%) (SD)	87.00 (68.86)	42.10	24.30	90.31	55.00	5776.8
10	Control	45.13 (72.20)	19.00	10.95	53.00	19.25	1351.64
	CD (P=0.05)	1.612	1.24	1.50	1.72	1.08	162.04

*Mean of three replications; SA – Soil Application SD – Soil Drenching

amendments was recorded and presented in table 4 and 5. The result clearly indicated that among the antagonists least sclerotial germination was noticed in case of *T. viride* (38.72 %) accounting 58.16 per cent reduction over control. This was on par with *P. fluorescens* with 39.34 per cent germination documenting 57.49 per cent reduction as against control recording 92.56. In case of organic amendments, neem cake recorded maximum reduction (62.48 %) of sclerotium germination and was followed by coir pith compost with 51.94 per cent reduction over control. The least inhibition of sclerotial germination (81.00 %) was documented in case of castor cake with 12.48 per cent reduction.

Compatibility studies

Compatibility among biocontrol agents

Strains of *P. fluorescens* (Pf7), and *T. viride* (Tv1) and *T. harzianum* were tested under *invitro* for compatibility. Strains that overgrew each other were compatible with each other, whereas strains that were separated by an inhibition zone were incompatible. No inhibition zone formed between *P. fluorescens* (Pf7), *T. viride* (Tv1) and *T. harzianum* indicating that these strains were compatible.

Compatibility of biocontrol agents with organic amendments

The result of the compatibility studies of fungal and bacterial antagonists with organic amendments revealed that there was no growth inhibition of biocontrol strains. This indicated that biocontrol strains (Pf7, Tv1 and *T. harzianum*), and eight organic amendments in study were compatible with each other.

Influence of antagonists and organic amendments on root rot disease incidence and plant growth parameters under pot culture conditions

Antagonists

Various fungal and bacterial antagonists were evaluated for their efficacy against root rot disease incidence under pot culture conditions using 0.1 per cent Carbendazim as standard check. The disease incidence was varied across the treatment imposed from 12.64 to 52.86 PDI. Among bioagents, *T. viride* recorded least disease incidence of 12.64 accounting 76.08 per cent reduction over control and was followed by *P. fluorescens* with 16.75 PDI documenting 68.31 per cent reduction over control as against control recording 52.86 PDI. The highest disease incidence was noticed in case of *Cheatomium* sp. accounting to a least reduction of 38.47 per cent (Fig. 1a). In the same study further, the effect of antagonists on plant growth parameters was recorded and presented in Fig.1b and c. The result clearly depicted that antagonists in addition to disease reduction had a pronounced effect on plant growth parameters. Among the treatments soil application with *T. viride* recorded the highest sprouting of cuttings (94.28 %) followed by soil application with *P. fluorescens* recording 86.52 per cent sprouting as against 46.72 per cent in control. In addition to this the plants grown from the treatment *viz.,* soil application with *T. viride* recorded maximum shoot length (48.71 cm), tuber length (27.24 cm), shoot weight (99.54 g), tuber weight (52.43 g) and vigour index (7160.56) and was followed by soil application with *P. fluorescens* recording shoot length of 45.23 cm, tuber length of 25.36 cm, shoot weight (93.71 g) and tuber weight (48.15 g) and vigour index of 6107.44 when compared to other treatments. There was pronounced reduction in shoot length, root length and sprouting of cuttings in pathogen inoculated control.

Organic amendments

Eight organic amendments were assessed for their efficiency reducing root rot incidence under pot culture conditions using 0.1 per cent Carbendazim as check. Neem cake recorded least disease incidence 15.26 PDI with 72.11 per cent reduction. It was followed by coir pith (21.78 PDI) and FYM (22.67 PDI) accounting 60.00 and 58.57 per cent reduction, respectively. Maximum disease incidence was noticed in case of castor cake with 34.47 PDI documenting 37.01 percent reduction (Table 6). As in case of antagonist variation in sprouting with different organic amendment also noticed. Maximum sprouting (90.37 %) was noticed in case of neem cake and was followed by coir pith compost (85.73 %). The least sprouting was noticed in case of coconut cake (63.18 per cent) as against control recording 48.31 per cent. Furthermore, the treatment neem cake recorded maximum shoot length (46.08 cm), tuber length (26.87 cm), shoot weight (96.34 g), tuber weight (48.23 g) and vigour index (6738.39) and was followed by coir pith compost with the shoot length 41.62 cm, tuber length 24.66 cm, shoots weight 85.12 g, tuber weight 44.18 g and vigour index of 5682.18. There was a pronounced reduction in all biological parameters in case of pathogen inoculated control. The observations suggested that the application of neem cake at 150 kg/ha was found to be effective and significantly supported in increasing the sprouting and also other biological parameters apart from reducing disease incidence (Table 7).

Combined application of organic amendment and antagonists

Two antagonistic fungi namely *T. viride* and *T. harzianum* and one antagonistic bacterium, *P. fluorescens* (Pf7) were combined with eight organic amendments in different combinations and disease incidence was assessed. Among the different treatments, maximum reduction of disease incidence (86.61 %) was noticed in treatment *T. viride* combined with neem cake with least disease incidence of 7.32 PDI. It was followed by *T. viride* combined with coir pith compost (9.63 PDI) with 82.75 per cent reduction as against the control documenting 54.68 PDI. The least control of disease was noticed in *T. harzianum* combined with castor cake (24.84 PDI) with 54.57 per cent reduction over control (Fig. 2a). The variation in biological parameters was documented in the study and presented in Fig. 2b and c. The treatment soil application of *T. viride* with neem cake recorded maximum sprouting (98.46 %), shoot length (50.76 cm), tuber length (30.13 cm), shoot weight (105.79 g) tuber weight (56.82 g) and vigour index 7964.42 and was followed by treatment soil application of *T. viride* with coir pith compost documenting sprouting (96.17 %), shoot length (49.34 cm), tuber length (29.36 cm), shoot weight (104.58 g), tuber weight (55.17 g) and vigour index 7660.65 when compared to control. The lowest biometric observations were recorded in pathogen inoculated control.

Evaluation of antagonists and organic amendments against root rot disease incidence and plant growth parameters under field conditions

Root rot disease incidence

Eight effective treatments from pot culture studies were further subjected to evaluation under field conditions using carbendazim as chemical check. The results of field studies were more consistent with that of pot culture experiments. Among the selected eight treatments from soil application of

T. viride and neem cake recorded least disease incidence (8.15 PDI) accounting 61.19 per cent reduction over control. It was followed by soil application of *T. viride* and coir pith compost with 10.86 PDI documenting 48.28 per cent reduction over control. The least reduction of disease was noticed in case of soil application *P. fluorescens* and FYM with 14.85 PDI accounting 29.28 per cent reduction over control (Table 8).

Plant growth parameters

In a field study conducted to evaluate the efficacy of antagonists and organic amendments various plant growth parameters were documented and presented in table 9. The result indicated that there exists a marked variation among the treatments with respect to sprouting, shoot length, tuber length, shoot weight, tuber weight and vigour index. Among the various treatments soil application of *T. viride* and neem cake recorded maximum sprouting (96.83 per cent), shoot length (48.64 cm), tuber length (27.60 cm), shoot weight (107.43 g), tuber weight (55.30 g) and vigour index (7382.31). This was followed by soil application of *T. viride* and coir pith compost with 95.38 per cent sprouting, shoot length of 45.64 cm, tuber length of 26.80 cm, shoot weight 106.43 g, tuber weight 54 g and vigour index of 6909.32.

Yield

Regarding the tuber yield, all the treatments were significantly superior to the control. The treatments soil application of *T. viride* and neem cake recorded the maximum tuber yield of 15.01 t ha^{-1} accounting to the 64.42 per cent increase in yield as against the minimum of 5.34 t ha^{-1} in the control. It was on par with soil application of *T. viride* and coirpith compost recording 14.32 t ha^{-1} accounting 62.70 per cent increase over control. The least yield of 10.00 t ha^{-1} was recorded *P. fluorescens* and FYM accounting 46.60 per cent increase in yield (Fig. 3).

DISCUSSION

The biological control of soil-borne pathogens with mixture of biocontrol agents, and organic amendments is a new approach in crop protection to reduce the disease damage level in economically important crops (Bharathi *et al*., 2004; Senthilraja *et al*., 2010). In the current study, the isolates of *T. viride*, *P. fluorescens* (Pf7), and organic amendments, such as neem cake and coir pith compost showed greater antagonistic activity against *M. phaseolina* under *invitro*. In addition to this antagonists as well as organic amendments reduced the number of sclerotia produced by pathogen. The results are consistent with the findings of several research workers who demonstrated the use of antagonistic microorganisms (*T. viride* and *P. fluorescens*), organic amendments against various soil borne fungal pathogens (Thilgavathi *et al*., 2007; Karthiba *et al*., 2011). Several authors have suggested that combinations of introduced biocontrol agents have to be compatible with each other for better and more consistent disease suppression (Raaijmakers *et al.,* 1995). In the present study, the isolates of *P. fluorescens* (Pf7), and *T. viride* (Tv1) and *T. harzianum* were compatible with each other and with eight organic amendments. These results are in conformity with those reported earlier by several investigators. *T. viride* and *Gliocladium virens* were compatible with 10 per cent concentration of gingelly cake, groundnut cake and neem leaf extracts (Dubey and Patel, 2000). Krishnamoorthy and Bhaskaran (1993) reported the enhanced population of *Trichoderma* spp. due to addition of neem cake into the soil. The organic manure, FYM was the best substrate for mass multiplication of *Trichoderma* spp. (Panicker and Jayarajan, 1993).

In our study *T. viride* and *P. fluorescens* in talc based formulation gave maximum disease reduction under galss house conditions compared to other antagonists. In addition to the disease reduction the same treatments also increased sprouting and also the yield parameters. The results are consistent with findings of Paramasivan *et al*. (2005) who reported that the soil application of *T. viride* was found to be more effective among all other treatments tested. The inhibitory effect of *Trichoderma* and *Pseudomonas* was due to production antimicrobial metabolites from both antagonists. The growth factors produced by the antagonists in the vicinity of the roots might have favoured the root growth which helped in tapping nutrients more actively to give higher results in the various growth parameters and thereby increasing the yield (Ebenezar, 1996). Addition of organic amendments to soil reduced the inoculum density of the soil-borne plant pathogens through changes in the general microbial balance by different mechanisms (Lukade, 1992). In our study neem cake showed fungi toxicity against *M. phaseolina* under as glass house and field conditions when compared to other amendments. The possible reason for the suppression of disease both under glass house as well as field conditions could be production of inhibitory metabolites from amendments and also increase the population of native antagonists. Rukmani and Mariappan (1990) reported that organic amendments reduced sclerotial population of *M. phaseolina.* Lumsden *et al.* (1982) stated that soil amendment with the composted sludge led to the stimulation of germination followed by the lysis of the propagules. The stimulated saprophytic microbial activity depleted the nitrogen level or its form in the soil resulting in imparing the infection process by the pathogen. The decline in the inoculum potential of *M. phaseolina* by incorporation of organic amendments might be due to the release of toxicants (Smith and Ashworth 1965). Soil amendment with neem cake was reported to favour the multiplication of *Trichoderma* spp. (Krishnamoorthy and Bhaskaran, 1991).

Furthermore, the efficacy of bioagents increased when they are mixed with organic amendments when compared with individual application. Among the combination *T. viride* combined with neem cake was proved to be the best combination for suppressing the disease incidence and also increasing the yield and other biological parameters. The findings are in agreement with outcomes of Sethuraman (1991) and Ramamoorthy (1998) who reported the combination of *Trichoderma* with neem cake provided added efficacy when compared with individual application. Thus, in conclusion soil application of *T. viride* and neem cake was efficient in reducing the disease incidence apart from increasing the biometric parameters and yield.

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