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Research Article

In vitro studies on the development of microbial consortia for the management of major diseases in coconut and citrus

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ABSTRACT: Microbial consortia for disease suppression involve combining multiple beneficial microorganisms to enhance their effectiveness in plant disease management. In present study, development of microbial consortia for the management of major diseases in coconut and citrus was carried out using bacteria *Pseudomonas fluorescens*, *Pseudomonas putida (striata*), *Bacillus subtilis* and fungi – *Trichoderma reesei*, *T. harzianum*, *T. asperellum* against major pathogens *viz*. *Ganoderma lucidum, Thielaviopsis paradoxa, Phytopthora palmivora, Lasiodiplodia theobromae* isolated from the coconut rhizosphere, and *Fusarium solani* isolated from the citrus rhizosphere. The promising fungal and bacterial antagonists were identified and studied for compatibility. Non-volatile compounds of consortia inhibited the test pathogens with an increase in concentration from 10 % to 75% with fungal consortia and bacterial consortia and also with mixed consortia which is composed of bacterial consortia + fungal consortia. Superior growth suppression was recorded with mixed consortia even at 10% concentration (59.44% to 65.83%) against the test pathogens in the ascending order of *L. theobromae* (59.44%) *T. paradoxa* (63.89%), *G. lucidum* (65.83%), *P. palmivora* (63.61%) and *F. solani* (62.78%). A similar trend was observed in 75% concentration where inhibition observed in the order of *Thielaviopsis paradoxa* (90.28%), *G. lucidum* (89.44%), *F. solani* (82.50%), *L. theobromae* (81.94%) and *P. palmivora* (81.39%). Volatile effect by bacterial consortia recorded the superior inhibition on test pathogens in the order of *Ganoderma* (85.28%), *F. solani* (75.28%), *T. paradoxa* (71.94%), *P. palmivora* (71.67%) and *L. theobrome* (67.50%) compared to the individual bioagents. Similarly, the fungal consortia showed the superior inhibitory effect on test pathogens in the order of *G. lucidum* (83.25%), *P. palmivora* (82.50%), *L. theobromae* (83.06%), *F. solani* (80.56%) and *T. paradoxa* (73.61%). Since there was no zone of inhibition between the strains, the interactions between *Pseudomonas* and *Bacillus* strains of *Trichoderma* spp. were compatible with one another. Neem cake recorded superior CFU population from 9.43 X 10^6 CFU at seven days by *T. asperellum*. Shelf life study on mixed consortia with bacterial + fungal bioagents in talc formulation indicated that all the bacterial and fungal CFU count recorded in 10^6 dilution for 90 days.

KEYWORDS: Basal stem rot, bud rot, coconut and citrus diseases, microbial consortia, leaf blight

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INTRODUCTION

The South Indian states of Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh are the locations where coconuts are grown in India. East Godavari, West Godavari, Visakhapatnam, and Srikakulam are significant coastal districts in Andhra Pradesh that are known for farming coconuts. Damage from diseases and insect pests is one of the main causes of the decline in coconut yield and productivity. The most devastating disease to affect coconuts is called basal stem rot (*Ganoderma* wilt), which is brought on by *G. applanatum* (Pers.) Pat. and *G. lucidum* (Leys) Kant. The infection ultimately causes the palms to die (Snehalatharani *et al*., 2016). The other important diseases are stem bleeding caused by *T. paradoxa* and bud rot caused by *Phytophthora palmivora* can also cause severe yield losses. All three diseases are caused by fungal pathogens which are soil-borne in nature. Soil-borne pathogens have a broad host range and persist for longer periods in soil by resistant resting structures (Gaitan *et al.*, 2021). Leaf blight disease caused by *L. theobromae* (Syn: *Botryodiplodia theobromae*) (Pat.) Griffon and Maubl is an airborne disease which causes serious damage in seedlings, leaves and nuts of adult palms and maximum incidence is observed during summer months. Spores and the resting structures on the affected portion of the leaves served as inoculum for further spread through the wind (Ramjegathesh *et al.*, 2019).

Sweet orange and acid lime are the two chief commercial citrus fruits grown in Andhra Pradesh, which holds the first rank in acid lime production. Dry root rot is reported from

old and neglected orchards of sweet orange, acid lime and mandarin besides mango, jackfruit, gulmohur, acacia, coconut and areca nut. Sweet orange and acid lime are most commonly affected with dry root rot disease. The disease starts in the soil, in one or more of the lateral roots and the symptoms are the appearance of wilting followed by dieback until the complete dieback of the tree (Lakshmi *et al.,* 2014).

An intriguing and promising substitute for chemical techniques for protecting plants against the growing hazards of plant diseases is biological plant protection. This strategy focuses on using Biological Control Agents (BCAs) to inhibit the growth of important plant diseases. Microbial BCAs interact with pathogens or plant hosts to increase their resistance, which may be a useful way to control the development of agricultural diseases. The development of microbial consortia, which may be the best substitute, is one way to create new research prospects for sustainable plant cultivation management, which will progress the current biological approaches. Microbial consortia, which are composed of various microorganisms with distinct modes of action, have the potential to enhance the strength and durability of the overall antipathogen impact (Maciag *et al*., 2023).

Consortia of antagonists used for disease suppression involve the strategic combination of multiple beneficial microorganisms to enhance their effectiveness in controlling plant diseases and also expand their benefits like enhanced efficacy through diverse modes of action, expanded spectrum of activity against multiple pathogens, Synergistic interactions for increased effectiveness, improved persistence and stability in the environment, adaptability to diverse environmental conditions and crops, reduced risk of resistance development and also compatibility with Integrated Pest Management (IPM) practices (Kohl, 2019).

Potential microbial inoculants in the rhizosphere may be improved by the creation and use of microbial consortia based on compatibility. One dependable way to lower variability and boost biological control reliability is to apply multiple biocontrol agents. In a variety of environmental circumstances, the employment of multiple antagonists with distinct mechanisms of action may increase the effectiveness of biocontrol (Grosch *et al*., 2011). The three-way mixture of plant growth-promoting rhizobacteria strains *viz. Bacillus pumilus*, *B. subtilis* and *Curtobacterium flaccumfaciens* as a seed treatment showed increased plant growth promotion and reduction of cucumber diseases (Raupach & Kloepper, 1998) under field conditions. Use of different bacterial species of *P. aeruginosa, Serratia marcescens, B. subtilis, P. fluorescens* and *P. striata* in biological control of plant pathogens and PGPR activities have been reported by several workers (Asaka & Shoda, 1996; Someya *et al*., 2000; Kim *et al*., 2009; Suryadi *et al*., 2011; Rajeela *et al*., 2017). Moreover, *B. subtilis* and *P. fluorescens* have been successfully formulated and are commercially available for the biological management of crop diseases (Jayaraj *et al*., 2005; Mathivanan *et al*., 2005). Hence, the present investigation has been carried out with different biocontrol agents having different modes of action to develop an affordable, intuitive and beneficial strategy for the farmers and to reduce the hazardous effects of pesticides against major diseases.

MATERIALS AND METHODS

Plant pathogens

Pure culture of major disease-causing pathogens of coconut *viz*. *G. lucidum, T. paradoxa, P. palmivora, L. theobromae* from coconut*,* and *F. solani* from citrus were procured from the university's Plant Pathology laboratories of Ambajipeta (A) and Tirupathi (T) which were isolated from disease-infected coconut and citrus, respectively.

Rhizosphereic microbial bioagents

Promising bacterial isolates *viz.* Pf-A: *P. flourescens*-Ambajipeta. Pst-A: *P. striata*-Ambajipeta. Pf-T: *P. flourescens*-Tirupati. Bs-T1: *B. subtilis*-Tirupati1. Bs-T2: *B. subtilis*-Tirupati2 and fungal microbial isolates *viz.* Tr-A: *T. reesei*-Ambajipeta. Tr-T: *T. reesei*-Tirupati. T. h-A: *T. harzianum*-Ambajipeta. Ta-A: *T. asperellum*-Ambajipeta were procured from the university Plant Pathology laboratories of Ambajipeta (A) and Tirupathi (T) which were isolated from coconut and citrus rhizosphere, respectively. These isolates were further purified and maintained on selective media *viz*. King's B for *Pseudomonas* spp., *Bacillus* Medium (M 1383) for *Bacillus* sp, *Trichoderma* Specific Media (TSM) for *Trichoderma* spp.

In vitro screening of fungal and bacterial antagonists against fungal pathogens

The effectiveness of the bacterial and fungal antagonists viz. *P. fluorescens, B. subtilis* and *T. asperellum*, *T. reesei* and *T. harzianum* isolates against mycelial growth of test pathogens *viz. G. lucidum, T. paradoxa, P. paradoxa, L. theobromae* and *F. solani* were tested by dual culture technique *in vitro* (Dennis & Webster, 1971). For each test, an 8 mm diam. Mycelial disc from a 7-day-old culture of test pathogen was placed on the agar surface of a 90 mm Petri dish 1 cm from the edge of the dish. Streaking of *P. fluorescens/B. subtilis* strains or an 8 mm diameter mycelial disc from an actively growing *Trichoderma* sp. culture was done on the agar surface opposite the target pathogen. Three replicated plates were maintained for each treatment. The plates were incubated together with the experimental controls (pathogen without antagonists) at 28±2°C for 7 days, and the

radial growth (mm) of the pathogen mycelium was recorded. Inhibition of mycelia growth of the pathogen percentage was calculated.

Compatibility of fungal and bacterial bio-control agents

An *in vitro* compatibility test was conducted using the Dual culture plate method, as described by Siddiqui and Shaukat (2003), between *P. fluorescens/P. striata* and *T. harzianum*, or *B. subtilis* and *T. harzianum*. As a result, P. *fluorescens/P. striata* and *B. subtilis* were cultured overnight on nutrient broth and streaked on one side of a Petri dish that contained PDA. A 5 mm *T. harzianum* disc that was 9 days old was used to inoculate the opposite side of the Petri dish. Subsequently, the plates were incubated at 25±1°C, and any zone of inhibition was measured.

Compatibility between *P. fluorescens/P. striata and B. subtilis*

The Fukui *et al.* (1994) method was used to examine the compatibility of the isolates of *P. fluorescens/P. striata* and *B. subtilis* with one another. Using a nutrient agar medium, the compatibility between *P. fluorescens* and *B. subtilis* strains was ascertained. Both vertically and horizontally, the bacterial strains were streaked next to one another. After being incubated for 72 hrs at room temperature (28±2°C), the inhibitory zone was noted on the plates. An inhibitory zone's absence showed compatibility with the corresponding bacterial strains, whereas its presence (if any) indicated incompatibility.

Development of compatible fungal mixed culture

The interactions were studied by dual direct opposition mycelial cultures. Interactions between the opposition colonies were visually assessed by following the methodology developed by Molla *et al*. (2001).

Antagonistic assay of Volatile Organic Compounds (VOC) emitted from fungal and bacterial antagonists against test pathogens mycelium growth and conidia germination

The approach of Raza *et al.* (2015) was followed with minor adjustments for testing the VOCs generated by bacterial and fungal antagonists. The antifungal properties of the VOCs generated by bacterial and fungal antagonists on PDA and NA medium against test pathogens were assessed using a dual-plate experiment. Petri plates containing PDA/NA were cultured for 7 days at 28°C with bacterial and fungal antagonists. Then, to create a double-plate chamber, each antagonist plate was covered with a Petri plate containing PDA/NA that had been cultured with a 5 mm diameter plug of test pathogen. The two plates were then sealed with parafilm and kept at 28°C for seven days. Petri plates with only PDA/NA media were placed over the control plates that had been treated with test pathogen plugs. Every experiment was carried out three times. During incubation, the diameters of the mycelia were measured every 24 hrs. To perform an antagonistic assay against the germination of conidia, 100 µL 10^2 cfu/Ml was substituted for the test pathogen plug.

***In vitro* antagonistic activity of the cell-free culture extracts/non-volatile compounds**

Cell-free culture extracts and non-volatile substances have been developed using the Santra and Banerjee (2023) approach. After screening the bioagents *in vitro*, three fungal hyphal/bacterial antagonists that showed potential were chosen, and their efficacy as consortia was then tested. The 0.5 cm colony blocks were removed from fully grown PDA/NA plates containing fungi and bacteria isolates. The blocks were then inoculated collectively into 100 ml of Potato Dextrose Broth medium (PDB)/Nutrient Broth (NB) that had been pre-sterilized and cooled (to a temperature of 28°C) in a 250 ml Erlenmeyer flask. In a BOD shaker incubator, inoculated PDB/NB flasks were incubated for 8 to 10 days at 28°C with gentle shaking at 120 rpm. Each broth's cell-free culture extracts were made by filtering.

Each broth was centrifuged at 10,000 rpm for 20 min after the cell mass was removed using Whatman No. 4 filter paper to create cell-free culture extracts. The cell-free culture extract is subsequently applied in different concentrations – 10%, 25%, 50% and 75% – in the poisoned food method. The efficacy of the cell-free extracts was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the formula.

$$I = (C-T)/C \times 100$$

where, I = per cent inhibition, C = growth in control, T = growth in treatment

Results and Discussions

The present studies on *in vitro* evaluation of microbial consortia for the management of major diseases in coconut and citrus were conducted during 2021–22 with the main emphasis on screening of microbial bioagents, compatibility and evaluation of shelf life of bioagents in talc formulation. The observations of all aspects were analyzed and interpreted in the following subheadings. All the *in vitro* studies were conducted in the Department of Plant Pathology, Dr. YSRHU - Horticultural Research Station, Ambajipeta, Dr. B. R. Ambedkar Konaseema District. The results are presented under different subheadings.

***In vitro* evaluation of microbial consortia against coconut and citrus pathogens**

Four *Trichoderma* isolates and five bacterial isolates from the coconut and citrus rhizosphere were tested against

test pathogens of coconut and citrus. Out of four fungal (Tr-A, Tr-T, T. h-A, Ta-A) and five bacterial (Pf-A, Pst-A, Pf-T, Bs-T1, Bs-T2) isolates screened against pathogens of coconut (*G. lucidum, T. paradoxa, P. palmivora* and *L. theobromae*) and citrus (*F. solani*). Tr-A, T. h-A, Ta-A, Pf-A, Pst-A and Bs-T_1 were found to be effective under *in vitro* conditions (Table 1).

The data on *in vitro* evaluation of bioagents against *G. lucidum* revealed that Pf-T showed maximum inhibition of 84.72% followed by Ta-A (79.44%), Bs-T1 (78.33%), Tr-A (73.61%), T. h-A (70.83%), Pf-A (68.33%, Pst-A (64.72%), Bs-T2 (62.22%) and Tr-T (60.00%), respectively (Plates 1-9). In the case of the second pathogen *T. paradoxa* it is observed that Ta-A showed the maximum inhibition of 79.17% followed by Tr-A (78.33%), T. h-A (70.83%), Pst-A (68.33%), Pf-A (68.33%), Bs-T1 (49.44%), Tr-T2 (47.22%) and Pf-T stand in last with 42.22% inhibition. Another pathogen *P. palmivora* is inhibited by Pf-T with 88.33% followed by Tr-A (84.72%), Tr-T (78.33%), Ta-A (76.94%), T. h-A (73.89%), Pst-A (71.11%), Pf-A (70.28%), Bs-T1 (59.72%) and Bs-T2 (48.61%) whereas in citrus pathogen *F. solani* inhibited maximum by Pf-T with 81.11% followed by Ta-A (79.17%), T. h-A (75.28%), Tr-T (69.44%), Bs-T (65.56%), Tr-A (65.28%), Pst-A (43.06%), Pf-A (42.78%) and lowest by Bs-T2 (25.28%). Influence of bioagents against *L. theobromae* resulted highest inhibition by Pf-T (78.61%) followed by T. h-A (73.89%), Ta-A (72.22%), Tr-T (67.22%), Bs-T1 (65.28%), Pf-A (41.67%), Pst-A (38.89%) and Bs-T2 (24.17%) (Table 1, Plates 1-9).

As per the findings of Srinivasulu and Raghavarao (2009), the major pathogens of leaf rot can be killed or inhibited by certain bacteria *(P. fluorescens, B. subtilis*) and fungi (*T. viride*), which means that the disease can be ameliorated through the use of biocontrol agents. Plant diseases are commonly controlled through the use of bacteria and fungi (Ramjegathesh *et al*., 2013).

Higher mycelial growth % inhibition of test pathogens was seen in the *in vitro* antagonistic assay of both *Trichoderma* and Pseudomonas strains as compared to the control in dual culture procedures. This may be related to its capacity for mycoparasitism. According to an earlier work by You *et al.* (2016), *Trichoderma* species often employ a variety of biocontrol strategies to inhibit fungi that are detrimental to plants.

Table 1. *In vitro* evaluation of bioagents against major pathogens of coconut and citrus crops

Tr.	**Bioagents**	***G. lucidum***	**% inhibition over control**	***T. paradoxa***	**% inhibition over control**	***P. palmivora***	**% inhibition over control**	***F. solani***	**% inhibition over control**	***L. theobromae***	**% inhibition over control**
T1	Tr-A	23.75 (29.13)	73.61	19.50 (26.18)	78.33	13.75 (21.71)	84.72	31.25 (33.9)	65.28	25.00 (29.93)	72.22
T2	Tr-T	36.00 (36.81)	60.00	47.50 (43.54)	47.22	19.50 (26.18)	78.33	27.5 (31.60)	69.44	29.50 (32.88)	67.22
T3	T. h-A	26.25 (30.77)	70.83	26.25 (30.77)	70.83	23.50 (28.96)	73.89	22.25 (28.11)	75.28	23.25 (28.72)	74.17
T4	Ta-A	18.50 (25.45)	79.44	18.75 (25.63)	79.17	20.75 (27.02)	76.94	18.75 (25.61)	79.17	23.50 (28.94)	73.89
T5	Pf-A	28.50 (32.24)	68.33	28.75 (32.40)	68.06	26.75 (31.06)	70.28	51.50 (45.85)	42.78	52.50 (46.42)	41.67
T6	Pst-A	31.75 (34.27)	64.72	28.50 (32.24)	68.33	26.00 (30.63)	71.11	51.25 (45.70)	43.06	55.00 (47.84)	38.89
T7	Pf-T	13.75 (21.54)	84.72	52.00 (46.13)	42.22	10.5 (18.87)	88.33	17.00 (24.13)	81.11	19.25 (25.88)	78.61
T8	Bs-T1	19.50 (26.18)	78.33	45.50 (42.39)	49.44	36.25 (36.97)	59.72	31.00 (33.76)	65.56	31.25 (33.93)	65.28
T9	Bs-T2	34.00 (35.61)	62.22	90.00 (71.53)	0.00	46.25 (42.82)	48.61	67.25 (55.10)	25.28	68.25 (55.69)	24.17
T10	Control	90.00 (71.53)	0.00	90.00 (71.53)	0.00	90.00 (71.53)	0.00	90.00 (71.53)	0.00	90.00 (71.53)	0.00
	SEm+	1.06		0.87		0.98		1.43		1.14	
	CD (1%)	3.08		2.52		2.84		4.17		4.1	

***ROC**-Reduction over control (% inhibition over control)

Note: Tr-A: *Trichoderma reesei*-Ambajipeta. Tr-T: *T. reesei*-Tirupati. T. h-A: *Trichoderma harzianum*-Ambajipeta. Ta-A: *Trichoderma asperillum*-Ambajipeta. Pf-A: *Pseudomonas flourescens*-Ambajipeta. Pst-A: *Pseudomonas striata*-Ambajipeta. Pf-T: *Pseudomonas flourescens*-Tirupati. Bs-T1: *Bacillus subtilis*-Tirupati1. Bs-T2: *Bacillus subtilis*-Tirupati2.

In vitro efficacy of non-volatile compounds of fungal consortia

The data (Table 2 and Plate 10) on the antagonistic effect of non-volatile compounds of fungal consortia against test pathogens revealed that the efficacy of non-volatile compounds showed an increasing trend with an increase in concentration of 10% to 75% in poison food technique. At 10% concentration, the efficacy was recorded from 6.67% to 18.33% against the test pathogens in the ascending order of *T. paradoxa* (6.67%), *L. theobromae* (10.56%), *G. lucidum* (16.94), *P. palmivora* (18.33) and *F. solani* (18.33). A similar trend was observed in 25%, 50% and 75%, also. However, at 75% concentration, maximum inhibition was recorded against test pathogens in the order of *P. palmivora* (67.22%), *F. solani* (65.83%), *G. lucidum* (58.06%), *L. theobromae* (36.39%) and *T. paradoxa* (27.78%).

In vitro efficacy of non-volatile compounds of Bacterial (Pf-A, Pst-A and Bs-T_1) consortia

The data (Table 3 and Plate 11) on the effect of non-volatile compounds of bacterial consortia against test pathogens revealed that the efficacy of non-volatile compounds showed an increasing trend with an increase in concentration from 10% to 75% in poison food technique. At 10% concentration, the efficacy was recorded from 6.67% to 59.44% against the test pathogens in the ascending order of

Table 2. Antagonistic effect of non-volatile compounds from fungal (Tr-A, T. h-A, Ta-A) consortia against *G. lucidum, T. paradoxa, P. palmivora* and *L. theobromae* (coconut) and *F. solani* (citrus)

Tr.	% con	*T. paradoxa*	ROC*	*G. lucidum*	ROC	*P. palmivora*	ROC	*F. solani*	ROC	*L. theobromae*	Reduction over control
T1	10%	84.10 (66.56)	6.67	74.75 (59.83)	16.94	73.5 (59.02)	18.33	73.50 (59.02)	18.33	80.50 (63.77)	10.56
T2	25%	83.25 (65.82)	7.50	66.50 (54.63)	26.11	68.25 (55.69)	24.17	66.50 (54.61)	26.11	72.50 (58.35)	19.44
T3	50%	75.25 (59.99)	16.67	50.50 (45.26)	43.89	41.5 (40.08)	53.89	42.75 (40.81)	52.50	62.75 (52.36)	30.28
T4	75%	65.01 (53.72)	27.78	37.75 (37.88)	58.06	29.5 (32.88)	67.22	30.75 (33.65)	65.83	57.25 (49.15)	36.39
T5	control	90.00 (71.53)	0.00	90.00 (71.53)	0.00	90.00 (71.53)	0	90.00 (71.53)	0	90.00 (71.53)	0
	Sem+	1.01		0.68		0.65		0.59		0.43	
	CD (1%)	3.09		2.09		1.98		1.80		1.30	

*ROC-Reduction over control

Table 3. Antagonistic effect of non-volatile compounds from bacterial consortia against phytopathogenic fungi

Tr.	% con	*T. paradoxa*	ROC*	*G. lucidum*	ROC	*P. palmivora*	ROC	*F. solani*	ROC	*L. theobromae*	ROC
T1	10%	84.00 (66.56)	6.67	51.25 (45.69)	43.06	36.50 (37.14)	59.44	39.50 (38.92)	56.11	78.00 (62.01)	13.33
T2	25%	79.25 (62.82)	11.94	42.25 (40.51)	53.06	31.25 (33.96)	65.28	35.75 (36.70)	60.28	64.50 (53.42)	28.33
T3	50%	68.25 (55.69)	24.17	30.500 (33.50)	66.11	21.50 (27.60)	76.11	26.25 (30.70)	70.83	54.75 (47.71)	39.17
T4	75%	56.75 (48.86)	36.94	21.25 (27.42)	76.39	35.50 (23.16)	82.78	20.50 (26.90)	77.22	46.25 (42.83)	48.61
T5	control	90.00 (71.53)	0.00	90.00 (71.57)	0.00	90.00 (71.57)	0.00	90 .00 (71.57)	0.00	90.00 (71.53)	0
	SEM	0.75		0.55		0.56		0.47		0.73	
	CD(1%)	2.29		1.67		1.71		1.44		2.24	

*ROC-Reduction over control

T. paradoxa (6.67%), *L. theobromae* (13.33%), *G. lucidum* (43.06%), *P. palmivora* (59.44%) and *F. solani* (56.11%). A similar trend was observed in 25%, 50% and 75%, also. At 75% concentration, the pathogens were inhibited in the order of *P. palmivora* (82.78%), *F. solani* (77.22%), *G. lucidum* (76.39%), *L. theobrmae* (48.61%) and *T. paradoxa* (36.94%).

In vitro efficacy of non-volatile compounds of bacterial (Pf-A, Pst-A and Bs-T_1) consortia + fungal (Tr-A, T. h-A, Ta-A) consortia

The data (Table 4) on the effect of non-volatile compounds of bacterial consortia + fungal consortia against test pathogens revealed that the efficacy of non-volatile compounds showed an increasing trend with an increase in concentration from 10% to 75% in poison food technique. At 10% concentration, the efficacy was recorded from 59.44% to 65.83% against the test pathogens in the ascending order of *L. theobromae* (59.44%), *T. paradoxa* (63.89%), *G. lucidum* (65.83%) *P. palmivora* (63.61%) and *F. solani* (62.78%). A similar trend was observed in 25%, 50% and 75% also.

At 75% concentration, the pathogens were inhibited in the order of *T. paradoxa* (90.28%), *G. lucidum* (89.44%), *F. solani* (82.50%), *L. theobromae* (81.94%) and *P. palmivora* (81.39%).

Table 4. Antagonistic effect of non-volatile compounds from fungal + bacterial consortia against phytopathogenic fungi

Tr.	Concentration (%)	*T. paradoxa*	ROC*	*G. lucidum*	ROC	*P. palmivora*	ROC	*F. solani*	ROC	*L. theobromae*	ROC
T1	10%	32.50 (34.73)	63.89	30.75 (33.50)	65.83	32.75 (34.89)	63.61	33.50 (35.34)	62.78	36.50 (37.15)	59.44
T2	25%	25.50 (30.27)	71.67	24.25 (29.61)	71.94	27.25 (31.44)	69.72	26.75 (31.12)	70.28	26.50 (30.96)	70.56
T3	50%	15.25 (22.95)	83.06	16.50 (23.94)	81.67	20.75 (27.04)	76.94	21.50 (27.60)	76.11	21.25 (27.42)	76.39
T4	75%	8.75 (17.18)	90.28	9.50 (17.91)	89.44	16.75 (24.12)	81.39	15.75 (23.33)	82.50	16.25 (23.73)	81.94
T5	control	90.00 (71.53)	0.00	90.00 (71.53)	0	90.00 (71.53)	0	90.00 (71.53)	0	90.00 (71.53)	0
	Sem±	0.69		0.64		0.52		0.53		0.51	
	CD (1%)	2.1		1.96		1.58		1.62		1.56	

Table 5. Antagonistic effect of volatile compounds from microbial bioagents against phytopathogenic fungi

Tr.	Treatment	*G. lucidum*	ROC	*T. paradoxa*	ROC	*P. palmivora*	ROC	*L. theobromae*	ROC	*F. solani*	ROC
T1	*T. asperellum* (Ta-A)	41.25 (39.95)	54.17	86.25 (68.29)	21.94	41.25 (39.95)	54.17	89.75 (71.33)	0.28	40.25 (39.37)	55.28
T2	*T. harzianum* (T. h-A)	68.75 (56.03)	23.61	35.75 (56.98)	4.17	85.75 (67.84)	4.72	87.5 (69.39)	2.78	56.75 (48.88)	36.94
T3	*T. reesei* (Tr-A)	21.5 (27.57)	76.11	35.75 (36.71)	60.28	36.50 (37.18)	59.44	28.75 (32.42)	68.06	35.75 (36.67)	60.28
T4	Fungal consortia	15.25 (22.96)	83.06	23.75 (29.14)	73.61	15.75 (23.22)	82.50	15.25 (22.28)	83.06	17.50 (24.71)	80.56
T5	*Bacillus subtilis*-1 (Bs-T1)	26.25 (30.81)	70.83	46.50 (42.98)	48.33	36.5 (37.18)	59.44	79.75 (63.94)	11.39	29.25 (32.73)	67.50
T6	*P. fluorescens* (Pf-A)	16.5 (23.90)	81.67	48.5 (44.13)	46.11	34.5 (35.94)	61.67	83.25 (66.09)	7.50	27.25 (31.42)	69.72
T7	*P. striata* (Pst-A)	19.5 (26.16)	78.33	18.75 (25.64)	79.17	35.75 (36.70)	60.28	38.00 (38.02)	58	29.5 (32.88)	67.22
T8	Bacterial consortia	13.25 (21.310	85.24	25.25 (30.14)	71.94	25.5 (30.30)	71.67	29.25 (32..73)	67.50	22.25 (28.08)	75.28
T9	control	90.00 (71.57)	0.00	90.00 (71.56)	0.00	90.00 (71.56)	0.00	90.00 (71.56)	0.00	90.00 (71.56)	0.00
	SEM	0.87		0.94		1.02		0.92		0.94	
	CD (1%)	3.42		3.69		4.12		2.63		3.68	

In vitro efficacy of volatile compounds

The bioagents were exposed against various pathogens and recorded the volatile effect. The data (Table 5 and Plates 12, 13) on the volatile effect of bioagents against test pathogens showed that bacterial consortia T_8 (Pf-A, Pst-A and Bs-T_1) and fungal consortia T_4 (Tr-A, T. h-A, Ta-A) have shown the superior inhibition when compared to the individual bioagents. The bacterial consortia T8 recorded the superior inhibition on test pathogens in the order of *Ganoderma* (85.28%), *F. solani* (75.28%), *T. paradoxa* (71.94%), *P. palmivora* (71.67%) and *L. theobromae* (67.50%) compared to the individual bioagents. Similarly, the fungal consortia showed the superior inhibitory effect on test pathogens in the order of *G. lucidum* (83.25%), *P. palmivora* (82.50%), *L. theobromae* (83.06%), *F. solani* (80.56%) and *T. paradoxa* (73.61%).

In the study by Srinivasulu (2008), the incidence of bud rot was decreased by spraying the coconut nuts and crown region twice at 30-day intervals with a 10- to 15-day-old *P. fluorescens* culture filtrate. This volatile's ability to change the cellular membrane's structure and promote potassium ion and other cellular component leaks is thought to be the cause of its antifungal activity (Zhang *et al*., 2017). According to Ma *et al*. (2019), this volatile has an inhibitory effect on *Aspergillus flavus*, causing early apoptosis to be activated, mitochondrial malfunction, disruption of pyruvate metabolism, reduction of acetyl-CoA synthesis, changes to ATP synthesis, and activity of dehydrogenases in the mitochondrion.

Testing of compatibility between fungal and bacterial antagonists

Based on results obtained from the experiments of *in vitro* antagonism, 3 bacterial and 3 fungal bioagents were

Table 6. Compatibility

Bioagent	growth of *Trichoderma* sp. (mm)	% inhibition of *Trichoderma* spp.	Reaction
***B. subtilis* vs *Pseudomonas* sp. and *Trichoderma* sp.**			
B. subtilis (Bs-T1) + *T. reesei* (Tr-A)	90	0	Compatible
B. subtilis (Bs-T1)-+ *T. asperellum* (Ta-A)	90	0	Compatible
B. subtilis (Bs-T1)-+ *T. harzianum* (T. h-A)	90	0	Compatible
B. subtilis (Bs-T1)-+ *P. flourescens* (Psf-A)	90	0	Compatible
B. subtilis (Bs-T1)-+ *P. striata* (Pst-A)	90	0	Compatible
Control-*T. reesei*-A	90	0	-
***P. fleuroscens* vs *Trichoderma* sp.**			
P. flourescens (Psf-A) + *T. reesei* - (Tr-A)	90	0	Compatible
P. flourescens (Psf-A) + *T. asperelum* - (Ta-A)	90	0	Compatible
P. flourescens (Psf-A) + *T. harzianum* - (*T.h*-A)	90	0	Compatible
P. flourescens (Psf-A) + *P. striata* (Pst-A)	-	0	Compatible
T. reesei - (Tr-A)	90	0	-
***Pseudomonas striata* vs *Trichoderma* sp.**			
Bioagent	**growth of *Trichoderma* sp. (mm)**	**% inhibition of *Trichoderma* sp.**	**Reaction**
P. straita (Pst-A) + *T. reesei* - (Tr-A)	90	0	Compatible
Ps. Straita (Pst-a) + *T. asperellum* - (Ta-A)	90	0	Compatible
P. straita (Pst-A) + *T. harzianum* - (T. h-A)	90	0	Compatible
T. reesei - (Tr-A)	90	0	
***Trichoderma* spp.**			
Bioagent	**growth of *Trichoderma* sp. (mm)**	**% inhibition of *Trichoderma* sp.**	**Reaction**
T. reesei (Tr-A), + *T. asperellum* (Ta-A) + *T. harzianum* (T. h-A)	90	0	Compatible

evaluated in various combinations like bacteria-fungi, bacteria-bacteria and fungi-fungi for testing compatibility.

Compatibility between *Bacillus subtilis* vs *Pseudomonas* sp. and *Trichoderma* sp.

In vitro study on compatibility between *B. subtilis* vs *P. fluorescens*, fungal bioagents – *T. reesei* (Tr-A), *T. asperellum* (Ta-A) and *T. harzianum* (T. h-A) indicated that the absence of inhibition zone between two species indicated that these biocontrol agents were compatible with each other (Table 6).

Compatibility between bacterial bioagents

In vitro study on compatibility between *B. subtilis* vs *P. fluorescens*, fungal bioagents – *T. reesei* (Tr-A), *T. asperellum* (Ta-A) and *T. harzianum* (T. h-A) indicated that the absence of inhibition zone between two species indicated that these biocontrol agents were compatible with each other (Table 6).

Compatibility between *P. striata* vs *Trichoderma* spp.

In vitro study on compatibility between *P. striata* vs, fungal bioagents – *T. reesei* (Tr-A), *T. asperellum* (Ta-a) and *T. harzianum* (T. h-A) indicated that the absence of inhibition zone between two species indicated that these biocontrol agents were compatible with each other (Table 6).

Compatibility between *Trichoderma* spp. and *Pseudomonas*

In vitro, a study on compatibility between fungal bioagents – *T. reesei* (Tr-A), *T. asperellum* (Ta-a) and *T. harzianum* (T. h-A) indicated that the absence of inhibition zone between two species and found to be compatible with each other (Table 6 and Plate 14, 15).

Since there was no zone of inhibition among the strains, this investigation showed that interactions between *Pseudomonas* and *Trichoderma* spp. were compatible with one another. According to (Harshita *et al.*, 2018; Khadeejath *et al*., 2018; Rajeswari & Kapoor, 2017), *Pseudomonas* and *Trichoderma* species are compatible in *in vitro* experiments. Our results corroborated their findings. The *in vitro* compatibility of the potentially antagonistic strains of *Trichoderma* and *Pseudomonas* was demonstrated in our investigation. *T. reseei, T. harzianum* and *T. asperellum* exhibited favourable interactions with *P. fluorescens* and *B. subtilis*. This may be explained by the fact that the metabolites produced by the strains of *Trichoderma* and *Pseudomonas* work in concert with one another. It is generally anticipated that applying compatible antagonists in combination will improve plant pathogen management (Skidmore & Dickson, 1976).

Screening of substrate for multiplication

Substrate screening for multiplication on various substrates indicated that neem cake recorded a superior CFU population from 9.43 x 10^6 CFU at seven days by *T. asperellum* followed by vermicompost (6.36 x 10^6 CFU), coir pith (6.30 x 10^6 CFU), farm yard manure (4.21 x 10^6 CFU) by and lowest population in press mud (3.16 x 10^6 CFU) (Table 7).

Visual observations of substrates for mass multiplication on neem cake, farm yard manure, vermicompost, coir pith and press mud indicated that thick spur of green colour mycelia growth observed on neem cake ranged which was indicated as excellent grade followed by FYM (average),

Table 7. Screening of substrate for multiplication of microbial consortia (7 days)

	***Trichoderma* sp. CFU @10^6**		
Substrate/Bioagents	***T. reesei***	***T. asperellum***	***T. harzianum***
Neem cake	8.14	9.43	8.46
FYM	4.21	5.76	5.67
Vermicompost	5.69	6.36	4.37
Coir pith	5.33	6.30	4.62
Press mud	1.56	2.66	3.16

Table 8. Visual identification of substrate for mass multiplication

Substrate	**Bioagents- mycelial growth (7 days)**				**CFU @ 10^6**	**Grading**
	T. reesei	***T. asperellum***	***T. harzianum***	**Fungal consortia**		
Neem cake	***	***	***	***	3.63-8.46	excellent
FYM	**	**	**	**	1.30-5.76	average
Vermicompost	**	**	**	**	2.61-6.36	good
Coir pith	**	**	**	**	1.56-6.30	average
Press mud	*	*	*	*	0-3.16	poor

vermicompost (good), coir pith (average) and press mud (poor) (Table 8 and Plate 16).

Furthermore, rhizosphere bacterial populations and crop quality can be significantly impacted by the kind and rate of organic amendment (Allison *et al.*, 2011).

SHELF-LIFE STUDY

Shelf-life study of fungal antagonists in talc formulations

Shelf life study of promising fungal bioagents in talc formulation indicated that *T. reesei* recorded superior CFU with 33.33 x 10^6 followed by *T. asperellum* (31.66 x 10^6) and *T. harzianum* (27.66 x 10^6) at initial days. After 30, 60 and 90 days CFU count was recorded in the same order with reduced number at the same dilution, *i.e.*, 10^6. The dilution was reduced to 10^4 after 120 days and the same count was maintained up to 160 days in the same order. However, 10^6 is only the recommended standard for field application (Table 9 and Plate 17).

Shelf-life study of bacterial antagonists in talc formulations

A shelf life study of promising bacterial bioagents in talc formulation indicated that all the bacterial sp. recorded too many numbers to count (TNTUC) x 10^8. At 60 days *B. subtilis* recorded a superior number of CFU with 61.33 x 10^6 followed by *P. fluorescens* (58.66 x 10^6) and *P. striata* (48.66 x 10^6). When the storage period at 90 days also same trend was observed with 10^6 dilutions. During 120 - 180 days, it descended to 10^5 - 10^4 dilution, respectively (Table 10).

Shelf-life study of bacterial sp. and fungal sp. in mixed consortia talc formulations

A shelf life study of promising bacterial bioagents + fungal bioagents in mixed consortia talc formulation indicated that all the bacteria and fungi recorded CFU count in 10^6 dilutions for 90 days. At 120 days bacteria CFU declined to 12.66 x 10^5 followed by 150 days (8.91 x 10^4) and 180 days (5.33 x 10^6). In the case of fungi, it was below the benchmark 10^6 dilution at 120 days (Table 11 and Plate 18-20).

Shelf-life of bacterial bioagents in bacterial consortia and *Trichoderma* spp. in fungal consortia of talc formulations

A shelf life study of promising bacterial bioagents mixture as consortia in talc formulation indicated that all the bacteria recorded to numbers to count at 10^8 dilutions for 30 days. At 60 days bacteria recorded CFU with 51.66 x 10^6 later declined by 90 days (42.33 x 10^5), 120 days (24.66 x 10^4), 150 days (16.66 x 10^4) and 180 days (11.66 x 10^4). In the case of fungi, when the storage period at 120 days CFU count is below 10^6 (Table 12).

Table 9. Shelf-life of fungal antagonists in talc formulations

CFU	Storage period (days)	*T. reesei*	*T. asperellum*	*T. harzianum*
10^6	0	33.33	31.66	27.66
10^6	30	28.66	26.33	23.66
10^6	60	19.66	18.33	17.66
10^6	90	15.33	13.33	11.33
10^4	120	13.33	12.66	10.33
10^4	150	10.33	11.66	8.33
10^4	180	9.66	10.33	7.33

Table 10. Shelf-life of bacterial antagonists in talc formulations

CFU	Storage period (days)	*P. fluroscens*	*P. striata*	*B. subtilis*
10^8	0	TNTC*	TNTC	TNTC
10^7	30	TNTC	TNTC	TNTC
10^6	60	58.66	48.66	61.33
10^6	90	44.66	39.33	57.33
10^5	120	29.66	26.66	36.66
10^5	150	18.66	18.33	25.33
10^4	180	15.33	8.66	13.33

***** Too Numbers To Count

Table 11. Evaluation of shelf-life of mixed consortia

CFU	Storage period (days)	Bacteria	*Trichoderma* spp.
10^6	0	21.56	22.66
10^6	30	20.21	17.33
10^6	60	19.33	10.66
10^6	90	16.33	6.33
10^5	120	12.66	0
10^4	150	8.91	0
10^4	180	5.33	0

Table 12. Shelf-life of bacterial consortia and fungal consortia separately

CFU	Storage period (days)	bacterial consortia	fungal consortia
10^8	0	TNTC *	25.66
10^7	30	TNTC	11.33
10^6	60	51.66	19.66
10^6	90	42.33	15.33
10^5	120	24.66	13.33
10^4	150	16.66	10.33
10^4	180	11.66	9.66

* Too Numbers To Count

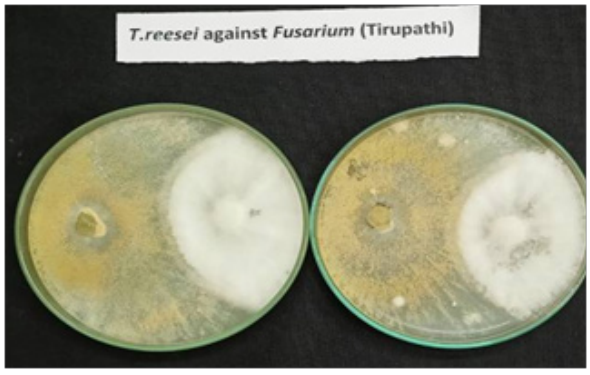


Plate 1. Dual culture study with fungal bioagent *Trichoderma reesei* against test pathogen *Fusarium solani*.

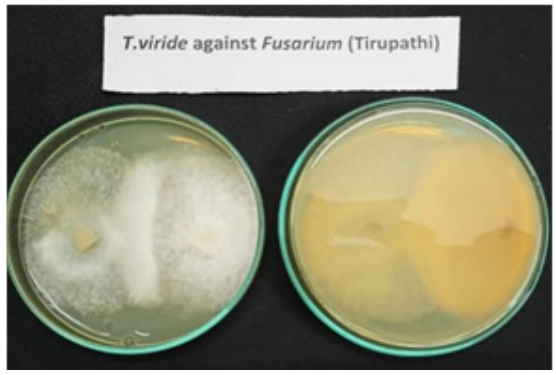


Plate 2. Dual culture study with fungal bioagent *T. asperellum* against test *pathogen Fusarium solani*.



Plate 3. Dual culture study with fungal bioagent *Pseudomonas fluorescens* against test pathogen *Fusarium solani*.

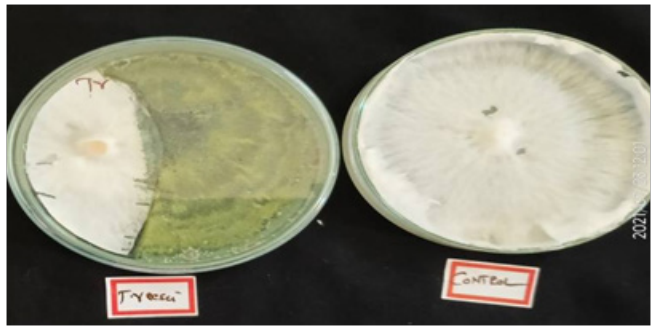


Plate 4. Dual culture study with fungal bioagent *Trichoderma asperellum* against test pathogen *Ganoderma lucidum*.

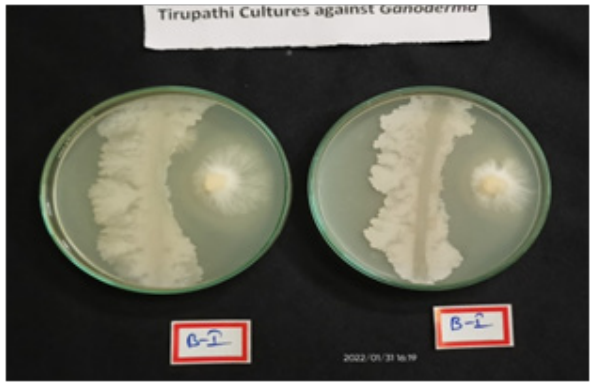


Plate 5. Dual culture study with bacterial bioagent *Bacilus subtlis - I* and II against test pathogen *Ganoderma lucidum*.



Plate 6. Dual culture study with bacterial bioagent *T. reesei* against

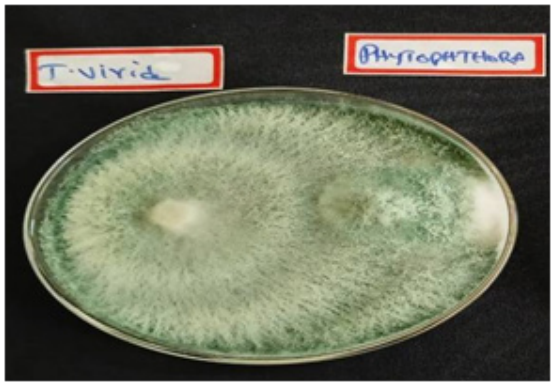


Plate 7. Dual culture study with fungal bioagent *Trichoderma asperellum* against test pathogen *Phytopthora palmivora*.

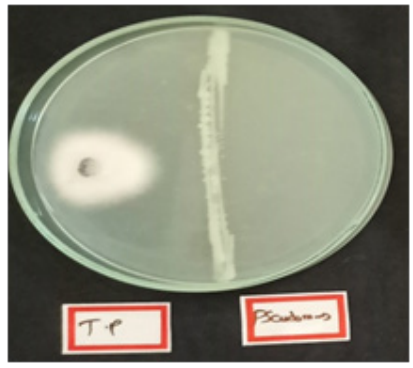


Plate 8. Dual culture study with bacterial bioagent *Pseudomonas fluoresens* against test pathogen *Thielaviopsis paradoxa*.

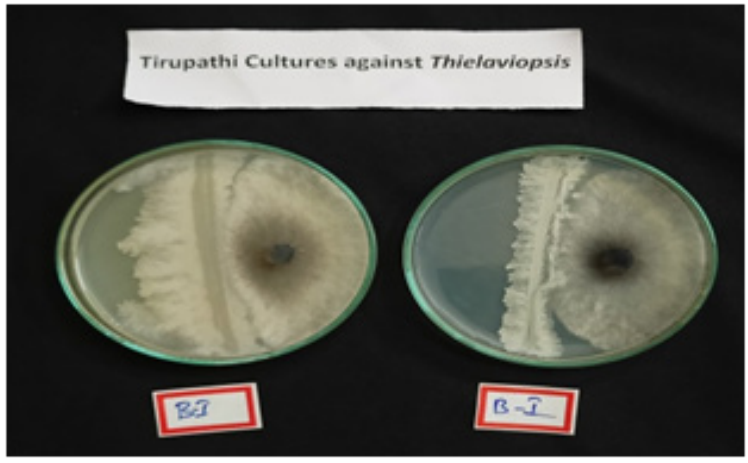


Plate 9. Dual culture study with bacterial bioagent *Bacillus subtilis - I* against test pathogen *Thielaviopsis paradoxa*.

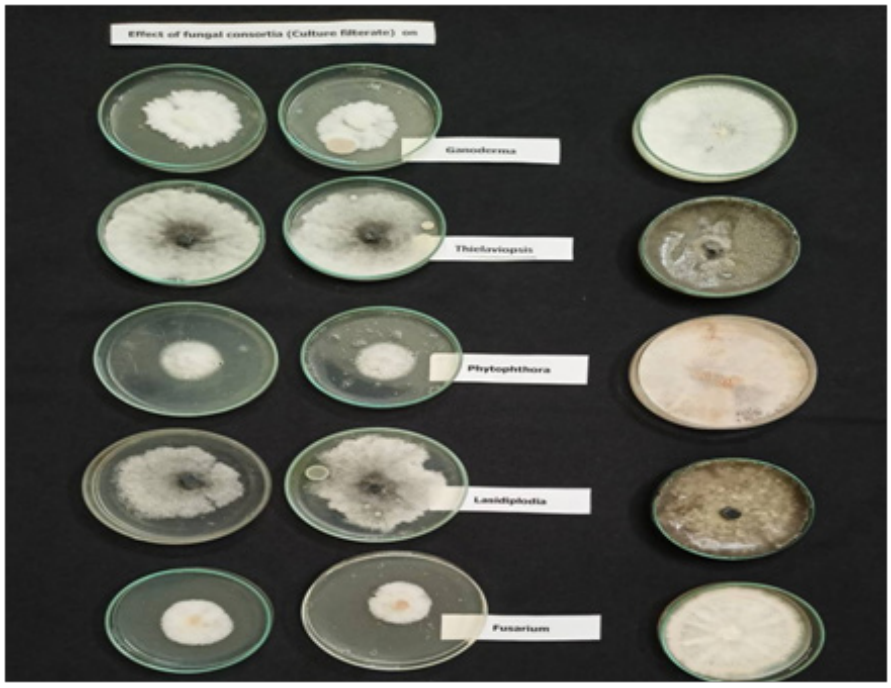


Plate 10. Antagonistic effect of non - volatile compounds from fungal consortia against phytopathogenic fungi.

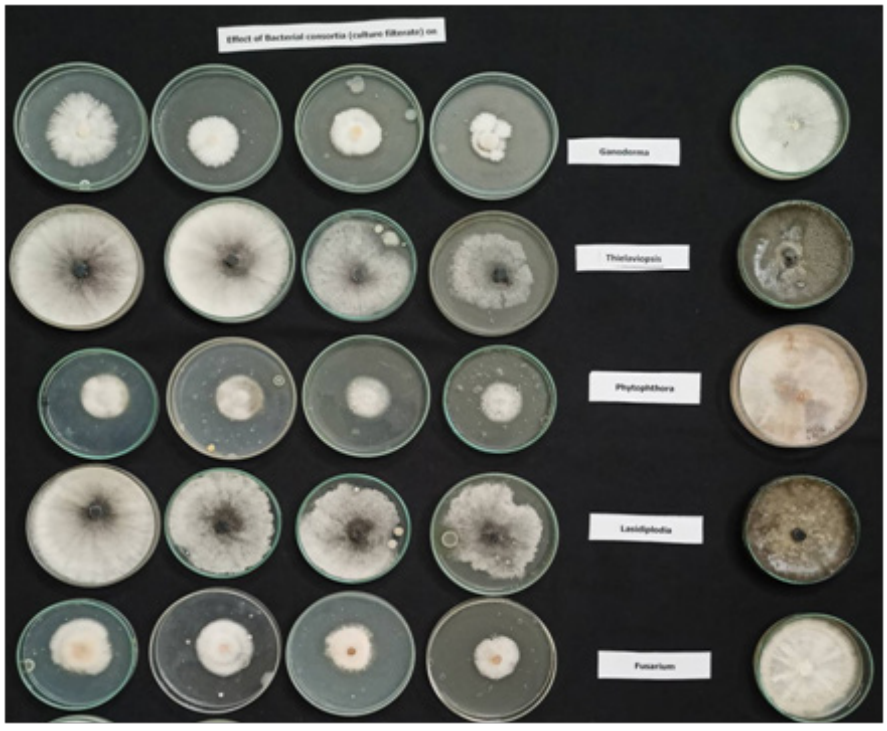


Plate 11. Antagonistic effect of non - volatile compounds from Bacterial consortia against phytopathogenic fungi.

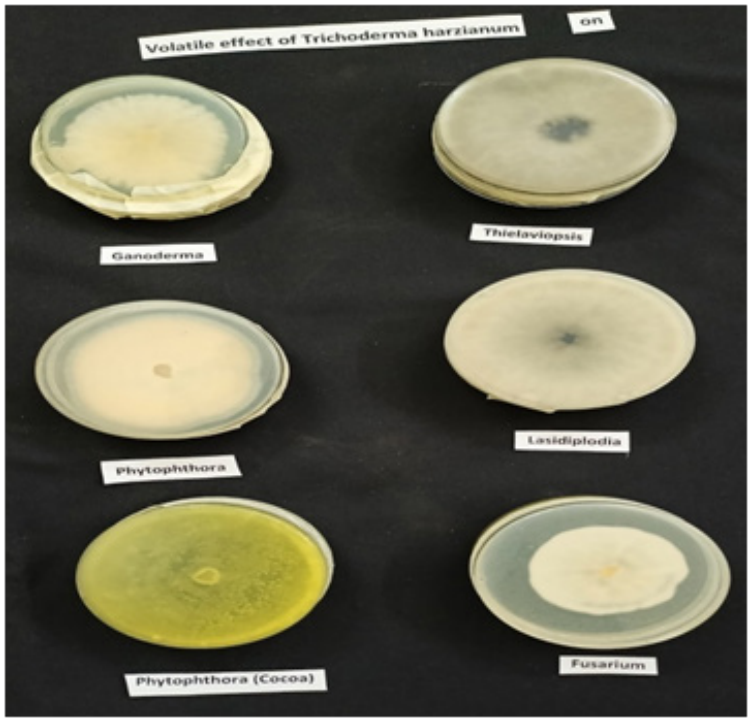


Plate 12. Antagonistic effect of volatile compounds from microbial bioagents *T. harzianum* against phytopathogenic fungi.

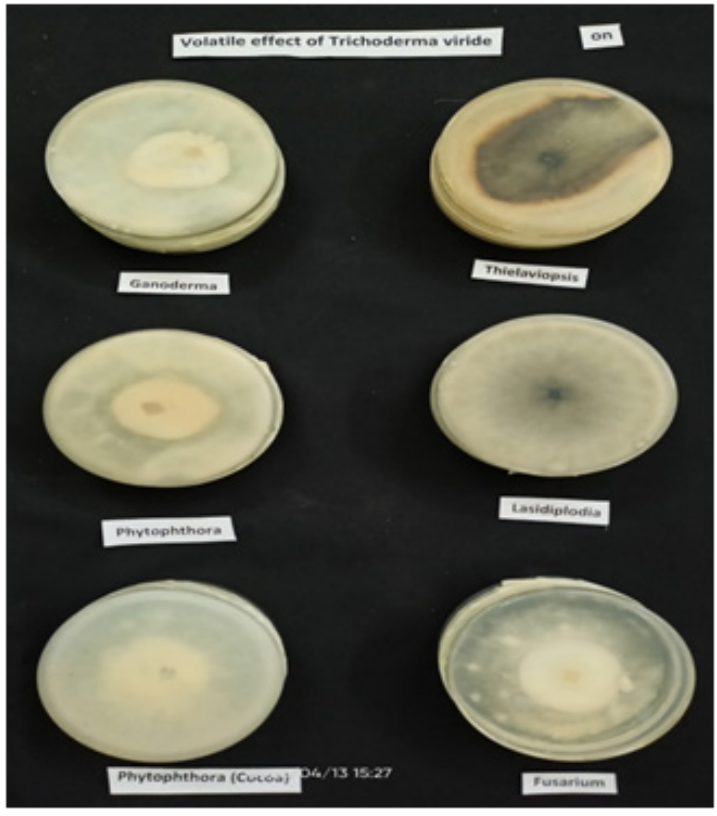


Plate 13. Antagonistic effect of volatile compounds from microbial bioagents *T. asperellum* against phytopathogenic fungi.

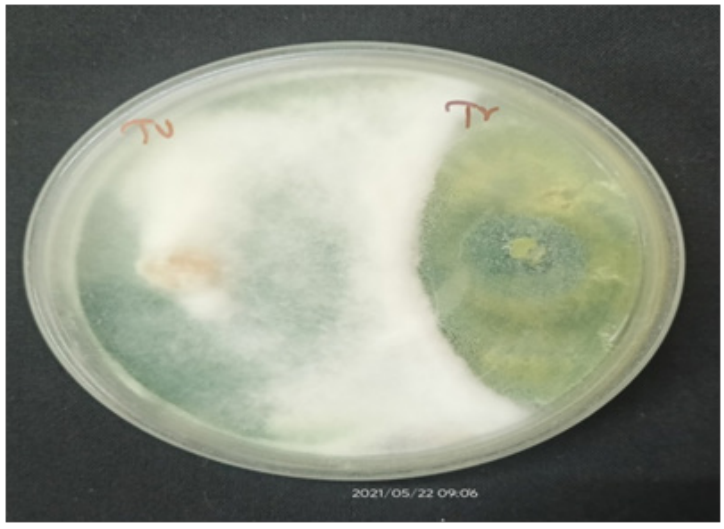


Plate 14. Studies on compatibility between effective fungal antagonists *T. asperellum* (vs) *T. reesei*.

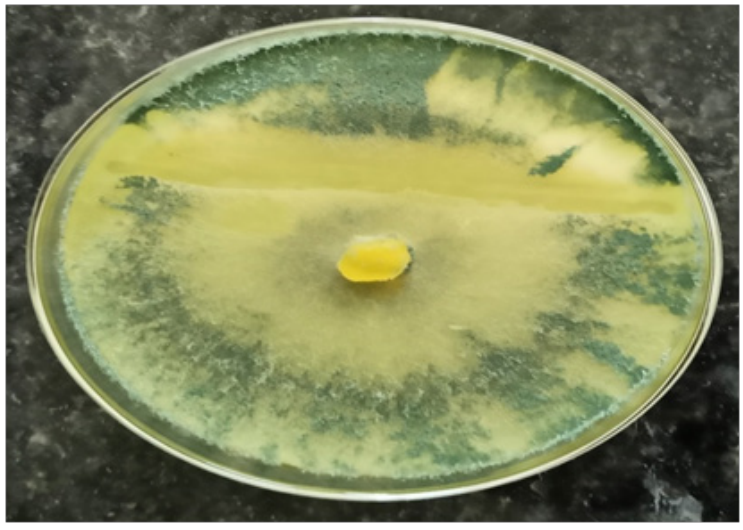


Plate 15. Studies on compatibility between effective fungal vs Bacterial antagonists *T. reesei* vs *Bacillus subtils-I*.



Plate 16. Screening of substrates for mass multiplication of bacterial consortia.

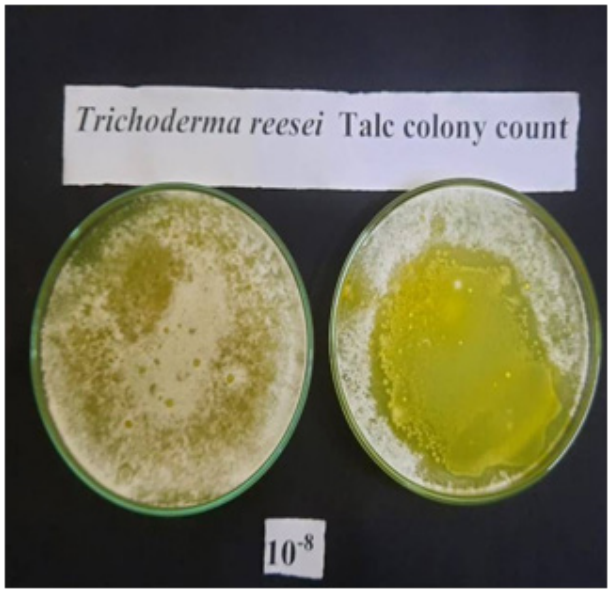


Plate 17. Evaluation of Shelf life of *T. reesei* in fungal consortia on PDA.

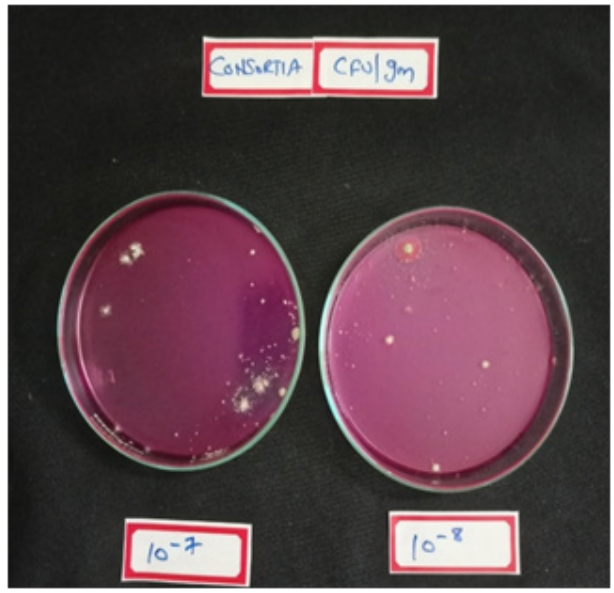


Plate 18. Evaluation of shelf life of fungal bioagents in mixed consortia on TSM.

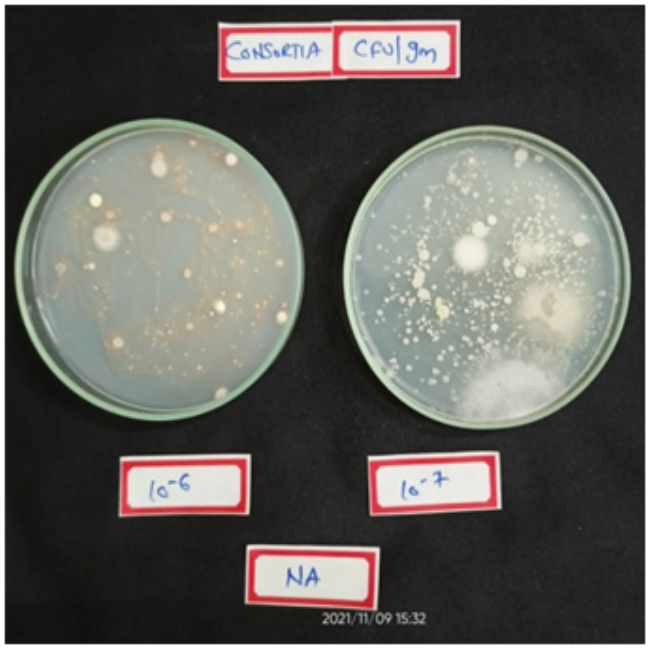


Plate 19. Evaluation of shelf life of Bacterial bioagents in mixed consortia on NA.

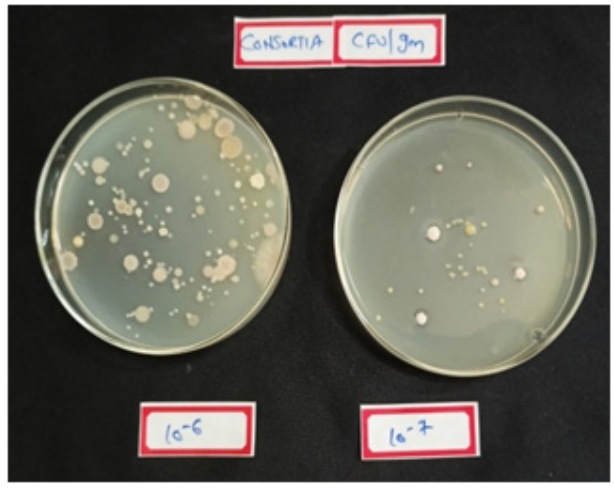


Plate 20. Evaluation of shelf life of bioagents mixed consortia on NA+PDA.

CONCLUSIONS

Based on *in vitro* studies three bacterial and three fungal antagonists were found promising against various fungal pathogens of coconut and citrus crops and were also found compatible with each other. These antagonists showed significant positive effects witnessed with volatile and non-volatile compounds against test pathogens. Neem cake was found best substrate for multiplication for fungal and bacterial antagonists. Shelf life studies of talc formulations indicated that consortia of bacteria and fungi can be mixed and served best before 120 days. The mixed consortia may be tested further in field studies so as to forward the product for recommendation.

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