Journal of Root Crops, 2013, Vol. 39 No. 2, pp. 136-147
Indian Society for Root Crops
ISSN 0378-2409

Worm Power Against Fungal Diseases in Aroids: Prospects and Future Strategies

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Received: 23 October 2013; Accepted: 31 December 2013

Abstract

Organic growers have limited options for plant disease control since most of the effective fungicides are synthetic, toxic and potentially polluting. Vermicompost and vermiwash have been widely explored as eco-friendly options for controlling plant diseases. We explored the possibility of utilizing vermicompost to manage *Phytophthora colocasiae* and *Sclerotium rolfsii* that cause taro leaf blight and collar rot of elephant foot yam, respectively. Microbial diversity was assessed in terms of quality and quantity in 35 vermicompost samples collected from different parts of the country. The distinct isolates were screened against the target pathogens under *in vitro* conditions. Potent organisms were identified by ITS and rRNA sequencing. Induced systemic resistance (ISR) was quantified in terms of phenol content, chitinase and glucanase activities. The variability in disease suppression by various vermicompost samples was studied under *in vitro* conditions. Pot culture studies were conducted in taro and elephant foot yam for two years to assess the potential of vermicompost/vermiwash for disease suppression. A total of 309 culture dependant isolates of vermicompost origin were obtained and 18.9% and 36.4% of these organisms showed >50% inhibition against *S. rolfsii* and *P. colocasiae,* respectively. The disease suppression potential varied with the source of vermicompost. Vermicompost/vermiwash treated plants showed <10% TLB incidence and 0-50% collar rot incidence. Yield increase of 14–70 % was also noted in both crops. There is scope for utilizing vermicompost for eco-friendly management of taro leaf blight and collar rot of elephant foot yam.

Key words: Aroids, vermicompost, *Phytophthora colocasiae, Sclerotium rolfsii, Trichoderma, Bacillus*

Introduction

Agrochemicals which ushered the "green revolution" came as a mixed blessing for mankind. It boosted food productivity at the cost of environment and society. Plant diseases, especially soil-borne pathogens are a serious issue for both greenhouse and field production of many horticultural crops. Organic growers have limited options for managing these diseases since most of the effective fungicides, fumigants and seed treatments are synthetic, toxic and potentially polluting. The increased production of waste in the world is of great concern at different levels of population (Woulters et al., 2005). Crop residues generated from agricultural activities cause serious environmental impact due to accumulation and uncontrolled disposal in the neighbourhood. Various alternatives are exercised to regulate this contamination by elimination, purification and/or recycling. Vermicomposting has been designated as one of the best options for eco-friendly recycling of wastes. Compost and compost tea have been widely explored as an eco-friendly option for controlling diseases. Later it was found that vermicompost can also provide the same effect. It has good biological properties and support microbial populations that are significantly large and more diverse than those in conventional thermophilic compost (Edwards, 1998). It has been documented that

vermicompost can suppress the incidence of plant pathogens such as *Pythium, Rhizoctonia* and *Verticillium* significantly by general or specific suppression mechanisms (Edwards et al., 2004).

Taro leaf blight (TLB) caused by *Phytophthora colocasiae* has become a major concern in all taro growing countries including India causing yield loss of 25-50% (Misra et al., 2010). Present recommendation to combat the disease includes the application of metalaxyl and mancozeb. Evolution/development of metalaxyl resistant strains of *P. colocasiae* is on its course (Nath et al., 2013). The emergence of resistant population of *P. colocasiae* can prove detrimental to taro cultivation in India, particularly with reference to the high genetic variability present among the pathogen (Misra et al., 2010; Nath et al., 2012). *Sclerotium rolfsii* is a devastating soil borne plant pathogenic fungus that causes collar rot in elephant foot yam. Injury to the collar region during intercultural operations and poor drainage acts as predisposing factors for infection causing heavy loss (Misra and Nedunchezhiyan, 2008). The present management strategy includes application of *Trichoderma* and neem cake. With this background information, a study was conducted at Central Tuber Crops Research Institute, Thiruvananthapuram, Kerala, India, to explore the possibility of utilizing vermicompost to manage *P. colocasiae* and *S. rolfsii* that cause taro leaf blight and collar rot of elephant foot yam, respectively. In addition, attempts were made to utilize the microbial population in vermicompost for pathogen suppression and to study the mode of action of suppression offered by vermicompost.

Materials and Methods

Collection of vermicompost and vermiwash

Thirty five vermicompost samples were collected from vermicompost units in the states of Kerala, Andhra Pradesh, Orissa and Tamil Nadu (Table1). Care was taken to collect vermicomposts prepared from different substrates. The substrates used for vermicompost were banana pseudostem, kitchen waste, coconut waste + cow dung, buffalo dung, tissue culture waste, spent mushroom substrate, cassava leaves, cassava thippi (cassava starch factory residue), sweet potato leaves and sweet potato thippi (sweet potato starch factory residue).

Microbial analysis of vermicompost and vermiwash

The culture dependent microorganisms in samples were isolated by dilution plate technique. The sample was mixed thoroughly and 10 g of the sample was weighed and transferred to 90 ml sterile distilled water in conical flasks. The flasks were kept in

Table1. Details of vermicompost samples collected

Sample No.	Place of collection	State	Source
1	Vellayani 1	Kerala	All biodegradable waste
2	Vellayani 2	Kerala	Banana pseudostem
3	Kollam	Kerala	Kitchen waste
4	Kollam	Kerala	Banana pseudostem and cowdung
5	Salem	Tamil Nadu	Coconut waste and cowdung
6	Salem 2	Tamil Nadu	Banana pseudostem and cowdung
7	Kazhakuttam	Kerala	Tissue culture waste
8	Mithranikethan	Kerala	Mushroom (spent mushroom substrate)
9	CTCRI	Kerala	All biodegradable
10	Bhubaneswar	Orissa	Cassava thippy
11	Bhubaneswar	Orissa	Garden residue
12	Bhubaneswar	Orissa	Cassava fresh leaves
13	Bhubaneswar	Orissa	Sweet potato fresh leaves
14	Bhubaneswar	Orissa	Sweet potato thippy
15	Bhubaneswar	Orissa	Sweet potato dry
16	Bhubaneswar	Orissa	Cassava dry
17	Bhubaneswar	Orissa	Vermiwash-cassava fresh leaves
18	Bhubaneswar	Orissa	Vermiwash-sweet potato thippy
19	Bhubaneswar	Orissa	Vermiwash-sweet potato fresh leaves
20	Bhubaneswar	Orissa	Vermiwash-sweet potato dry
21	Bhubaneswar	Orissa	Vermiwash-garden residue
22	Bhubaneswar	Orissa	Vermiwash-cassava thippy
23	Bhubaneswar	Orissa	Vermiwash-cassava dry
24	Peruvannamuzhi	Kerala	All biodegradable
25	Kasaragod	Kerala	Coconut waste
26	Kasaragod	Kerala	Vermiwash-coconut waste
27	Kovvur (ARS)	Andhra Pradesh	Buffalo dung
28	Veerwada	Andhra Pradesh	Buffalo dung
29	CTCRI-Block 5	Kerala	All biodegradable
30	CTCRI-Block 5	Kerala	Vermiwash-all biodegradable
31	CTCRI Block 5	Kerala	Sweet potato waste 1
32	CTCRI Block 5	Kerala	Sweet potato waste 2
33	CTCRI Block 5	Kerala	Cassava waste 1
34	CTCRI Block 5	Kerala	Cassava waste 2
35	Kottayam	Kerala	All biodegradable waste

orbital shaker at 150 rpm for 15 min and 1 ml aliquot was transferred to 9 ml water in screw capped flat bottom tubes (Borosil) and it was further serially diluted to get 10^{-6} concentration. The bacterial population was enumerated on Nutrient Agar (HIMEDIA), Luria Bertani Agar (HIMEDIA) and King's B Agar (HIMEDIA). Fungi were enumerated using Martin's Rose Bengal Agar (HIMEDIA) amended with ampicillin and actinomycetes were enumerated on Ken Knights and Munaiers' Agar (HIMEDIA). Three plates were maintained for each dilution. The plates were incubated for two days at room temperature (28±2°C). The microbial diversity was studied in terms of quality and quantity. The population densities were recorded as colony forming units (cfu) g^{-1} of dry compost. The colonies were purified and maintained on suitable media.

In vitro screening of microbes for antagonistic potential

Isolated microbes were screened for their antagonistic potential against *S. rolfsii,* the pathogen responsible for collar rot disease in elephant foot yam and *P. colocasiae,* the pathogen causing taro leaf blight by adopting (i) direct confrontation/dual culture method (ii) antibiosis test for production of diffusible inhibitory metabolites against pathogen, and (iii) antibiosis test for production of volatile compounds inhibitory to pathogens. Three replications were kept in each case and the radial growth of the pathogen was recorded at an interval of 24 h. The percentage inhibition was worked out as follows:

$$I = \frac{C - Tx100}{C}$$

I - Percentage inhibition of mycelial growth

C - Radial growth of pathogen in control plates

T - Radial growth of pathogen in dual culture plates

Many rounds of screening were done to select the isolates with consistent antagonistic potential against target organisms. The most efficient isolates showing antagonism to both the pathogen were selected and an attempt was made to identify the organism.

Identification of potent organisms

The most potent ten isolates were identified by using biotechnological tools.

Isolation of genomic DNA

The bacterial isolates were incubated overnight in LB broth for the extraction of genomic DNA. A portion of the culture (1.5 ml) was transferred to microfuge tubes and centrifuged at 12,000 rpm for 10 min. The pellets obtained were re-suspended in 450 µl TE buffer and vortexed. To this 45 µl of 10% SDS and 10 µl of 10 mg ml^{-1} proteinase K was added, mixed well by inverting the tubes and centrifuged at 12,000 rpm for 2 min. The upper aqueous layers were then transferred to new tubes and re-extracted by adding equal volume of phenol–chloroform. The mixtures were then centrifuged as above and aqueous layers were transferred to new tubes. To this 50 µl of ammonium acetate was added and mixed well. Finally, the DNA was precipitated by adding 300 µl of isopropanol and centrifuged at 10,000 rpm for 5 min The precipitated DNA was washed using 70% ethanol (10,000 rpm, 1 min, 4^0C), re-suspended in 50 µl TE buffer and stored at – 20°C.

Quality check of DNA

The quality of the DNA isolated was checked by using Agarose Gel Electrophoresis. The sample was prepared by mixing 15 µl of DNA and 3 µl of loading dye and loaded in the well. A single well was loaded with 5 µl of 1kb DNA ladder and electrophoresed at 60 V for 1 h. The gel was placed in the UV Trans illuminator and viewed under UV light.

PCR amplification of 16s rRNA gene

By using 16S rRNA primers, the 16S rRNA was amplified from the genomic DNA. The primer used was forward 8 F as 5AGAGTTTGATCCTGGCTCAG3 and reverse 1492 primer as 5'CGGCTACCTTGTTACGACTT3'. The polymerase chain reaction was performed by using the PCR mix, sterile water–17.25 µl, DNA–2 µl, Taq buffer-2.5 µl, dNTP mix–0.5 µl Primer (forward)-1.0 µl, primer (reverse)– 1.0 µl, Taq polymerase–0.5 µl. The amplification of 16S rDNA was confirmed by running the amplification product in 1% agarose gel electrophoresis in 1x TAE with 1kb marker.

Elution of 16S rDNA

The DNA band amplification were excised from the gel and put into Eppendorf tubes. Added thrice the volume of buffer QG to one volume of gel and incubated at 50°C for 10 min. To help dissolving the gel it was mixed by vortexing the tubes every 2-3 min during the incubation. Added 1 gel volume of iso propanol to the samples and

mixed well and then placed in a QIA quick spin column in 2 ml collection tubes, which were provided along with the kit. To bind DNA, applied 750 µl of the samples to the QIA quick column and centrifuged at 13,000 rpm for 1 min. The supernatant were discarded and the QIA quick columns were placed back in the same collection tubes. Added 0.5ml (500 µl) of buffer QG to QIA quick columns and centrifuged for 1 min. To the wash, added 0.75 ml of buffer PE to QIA quick columns and centrifuged for 1 min. Discarded the supernatant and centrifuged the QIA quick columns for an additional 1 min at 13,000 rpm. Dried it for removing excess of ethanol and then 30 µl of buffer EB (elution buffer) was added to the centre of QIA quick membrane and centrifuged for one more min. This was run in 1% of agarose gel with 1 kb marker in 1 X TAE. The eluted 16S rDNA was stored in refrigerator.

Checking for the re-amplification of the eluted product

This was done using the same primer forward 8F primer 5' AGAGTTTGATCCTGGCTCAG 3' and reverse 1492 R primer 5' CGGCTACCTTGTTACGACTT3'. The polymerase chain reaction was performed by using the PCR mix, sterile water – 17.25 µl, DNA-2 µl, Taq buffer-2.5 µl, dNTP mix–0.5 µl, primer (forward)–1.0 µl, primer (reverse)–1.0 µl, Taq polymerase-0.5 µl. The amplification of 16S rDNA was confirmed by running the amplification product in 1% agarose gel electrophoresis in 1X TAE with 1kb marker.

Sequencing and identification of isolate

The PCR product was eluted and sequenced by outsourcing at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. After sequencing, the sequence obtained was edited and aligned using Bioedit v 7.0.8 tool (Tom Hall Ibis Biosciences). Sequence analysis was performed with sequences available with the National Centre for Biotechnology Information (NCBI) database using Basic Local Alignment Search Tool for nucleotides (Blastn) as well as with the sequence available with Ribosomal Database Project II (RDP II).

Primers ITS1 and ITS4 were used to amplify a fragment of rDNA including ITS1 and ITS2 and the 5.8S rDNA gene of fungal isolates. PCR amplifications were performed in a total volume of 25 µl by mixing 2 µl of the template DNA with 0.4 µM concentrations of each primer, 0.5 µl of dNTP (Merck Genei), 0.5 µl of Taq DNA polymerase dNTP (Merck Genei), 2.5 µl Taq Buffer A dNTP (Merck Genei). These reactions were subjected to an initial denaturation of 2 min at 95°C, followed by 35 cycles of 30s at 95°C, 1 min at 56.7°C, and 1min 30s at 72°C, with a final extension of 8 min at 72°C in a thermal cycler. Aliquots (4 ml) were analyzed by electrophoresis in 1.2% agarose gel in 1X TAE buffer stained with ethidium bromide and photographed over a transilluminator. A single product of '~620bp was obtained and the PCR product was purified using PCR purification kit (Fermentas). This was sequenced at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram and the sequences obtained were analyzed using blastn algorithm in NCBI database.

Pot culture study

A controlled condition experiment was carried out for two years (2011 and 2012) at Central Tuber Crops Research Institute (CTCRI), Thiruvananthapuram, India, in completely randomized design (CRD) to study the effect of vermicompost and vermiwash on the incidence of collar rot in elephant foot yam and taro leaf blight incidence in pots containing 20 kg soil. There were 14 treatments each in taro and elephant foot yam. The treatment details are as follows:

Taro

T_1: Soil application of vermicompost ((@ 375 g plant^{-1} in three splits; at the time of planting, two weeks after sprouting and one month after second application)

T_2: Soil application + vermiwash (10%) spraying at 60 days after planting (DAP)

T_3: Soil application + vermiwash spraying at 90 DAP

T_4: Soil application + vermiwash spraying at 60 and 90 DAP

T_5: Seed treatment with vermiwash (10%)

T_6: Seed treatment with vermiwash + vermiwash spraying at 60 DAP

T_7: Seed treatment with vermiwash + vermiwash spraying at 90 DAP

T_8: Seed treatment with vermiwash+ vermiwash spraying at 60 and 90 DAP

T_9: Soil application of vermicompost + seed treatment with vermiwash

T_{10}: Soil application of vermicompost + seed treatment with vermiwash + vermiwash spraying at 60 DAP

T_{11}: Soil application of vermicompost + seed treatment with vermiwash + vermiwash spraying at 90 DAP

T_{12}: Soil application of vermicompost + seed treatment with vermiwash + vermiwash spraying at 60 and 90 DAP

T_{13}: Present recommendation (POP)-Seed treatment with *Trichoderma viride* + spraying with Mancozeb (0.2%)

T_{14}: Control

Elephant foot yam

T_1: Soil application of vermicompost (@ 800 g plant^{-1} at the time of planting, 400 g plant^{-1}after 45 DAP and 400 g plant^{-1}after one month of second application)

T_2: Soil application + vermiwash (10%) drenching at 90 days after planting (DAP) @ 500 ml plant^{-1}

T_3: Soil application + vermiwash drenching at 120 DAP

T_4: Soil application + vermiwash drenching at 90 and 120 DAP

T_5: Seed treatment with vermiwash (10%)

T_6: Seed treatment with vermiwash + vermiwash drenching at 90 DAP

T_7: Seed treatment with vermiwash + vermiwash drenching at 120 DAP

T_8: Seed treatment with vermiwash + vermiwash drenching at 90 and 120 DAP

T_9: Soil application of vermicompost + seed treatment with vermiwash

T_{10}: Soil application of vermicompost + seed treatment with vermiwash + vermiwash drenching at 90 DAP

T_{11}: Soil application of vermicompost + seed treatment with vermiwash + vermiwash drenching at 120 DAP

T_{12}: Soil application of vermicompost + seed treatment with vermiwash + vermiwash drenching at 90 and 120 DAP

T_{13}: Present recommendation (POP) – Seed treatment with *Trichoderma harzianum*

T_{14}: Control

Taro: Sree Kiran, a released variety of CTCRI, which is susceptible to *P. colocasiae* was used for the experiment. The plants were challenge inoculated with 1cm^2 naturally infected leaf bits. The leaf bit was placed on the upper surface of the plant, covered with a cellophane tape to keep the infected bit in place (Fig.1). Cormel yield was recorded. Disease development was monitored and percentage disease incidence (PDI) was calculated using the formula:

$$PDI = \frac{\text{Sum of all numerical grades}}{\text{Total number of leaves counted x Maximum grade}} \times 100$$

Elephant foot yam: Gajendra was the test variety and it is susceptible to *S. rolfsii*. The plants were challenge inoculated with mycelium colonized elephant foot yam slices (2cm^2). Plants were observed for collar rot incidence, if any, and the PDI was calculated. Corm yield was recorded.

Changes in enzymes in taro plants consequent to vermicompost application

Three month old taro (var. Sree Kiran) plants were used for the study. Vermicompost/vermiwash was applied to the plants as follows:

1. Control
2. Vermicompost soil application (@ 375 g plant^{-1}) (VCSA)
3. Vermicompost soil application + inoculated with pathogen (VCSAP)
4. Vermiwash drenching (10%) (VWD)

Fig.1. Artificial inoculation with naturally infected leaf bits

5. Vermiwash drenching+ inoculated with pathogen (VWDP)
6. Inoculated with pathogen (P)

The virulent isolate of *P. colocasiae,* P-7 collected from West Tripura was used for the challenge inoculation. Plants were kept in a moist chamber to simulate temperature (25±2°C) and humidity (90-95%) required for disease development. Changes in phenylalanine ammonia lyase, β -1-3 glucanase, chitinase and total phenol production were studied at 0, 1, 2, 4 and 8 days after inoculation.

Results and Discussion

Isolation of microorganisms

Microbial population varied significantly among the 35 vermicompost samples used for the study. Bacteria were the most abundant group, followed by actinomycetes. Among the fungal genera, *Aspergillus* was the most predominant. More than 50% of the fungal isolates belonged to this group, followed by *Penicillium*, *Trichoderma, Rhizopus* and *Fusarium. A. niger, A. flavus* and *A. tereus* were the common species of *Aspergillus. Bacillus* spp. and *Streptomyces* spp. were the most abundant bacteria and actinomycetes genus respectively. Compared to fungi and bacteria, less variability was noted with actinomycetes. Studies conducted at Ohio State University revealed that vermicompost had much greater microbial activity and biodiversity than traditional composts (Edwards, 2004). Ashraf et al. (2007) studied fungi, bacteria and actinomycetes associated with different composts and found that the above mentioned organisms were present in vermicompost. According to their study, *Aspergillus* was the most common fungal genus comprising 43% of the total microbial species isolated from various composts followed by *Bacillus* comprising one fifth of the total microbial isolates. Results of this study are in full agreement with the findings of these workers. A total of 309 culture dependent organisms were obtained from 35 vermicompost samples collected from different parts of the country.

In vitro screening of microbes for antagonistic potential

All the 309 culture dependent organisms obtained were screened against *P. colocasiae* and *S. rolfsii.* In the first round of screening, 53 organisms showed more than 50% inhibition to *S. rolfsii.* Similarly, 102 organisms showed more than 50% inhibition to *P. colocasiae.* In the second round of screening, 20 and 30 isolates showed more than 50% inhibition to *S. rolfsii* and *P. colocasiae*, respectively. After three rounds of screening, four fungal and six bacterial isolates were selected based on their consistent high inhibition potential against both the target pathogens (Fig. 2). Vast majority of studies on disease suppression potential of compost demonstrates a relationship between disease suppression and microbial activity (Kavroulakis et al., 2005). The result of the study also clearly indicated the major role played by the microorganisms in pathogen suppression shown by vermicompost.

Identification of potent organisms

Molecular characterization of potent bacterial isolates was done using universal eubacterial 16s rDNA primer (8F/1492R). The nucleotide sequence obtained was initially analysed using NCBI BLAST followed by sequence alignment using Clustal W program of Bio Edit. The isolates were identified as *Bacillus subtilis. B. cereus* and *Providencia rettgeri* (Figs. 3a and 3b). As per

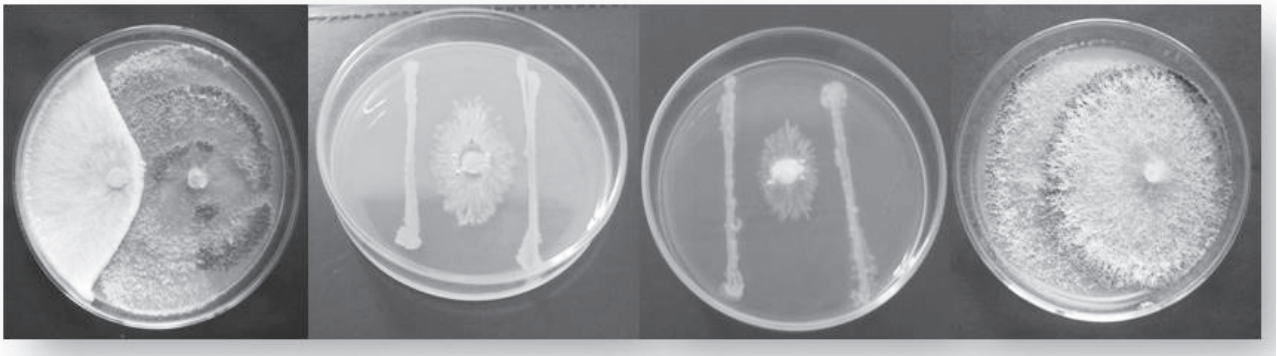

Fig. 2. Mycelial growth inhibition shown by fungal and bacterial isolates

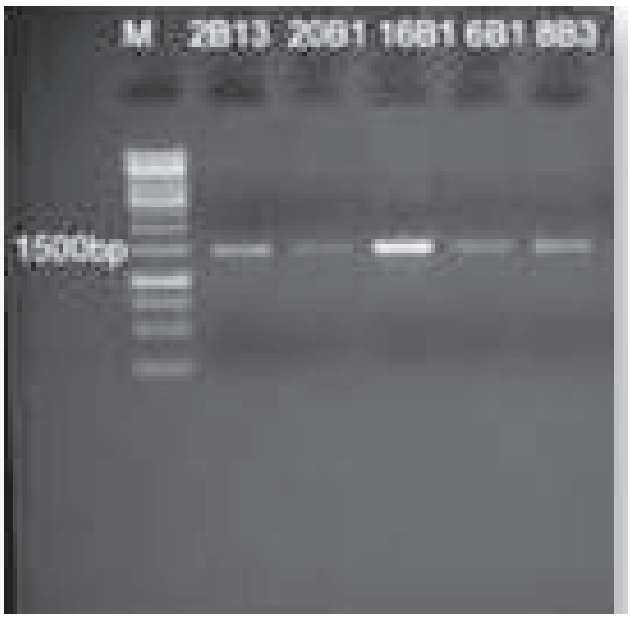

Fig.3a. Amplification of 16s rRNA of bacterial isolates

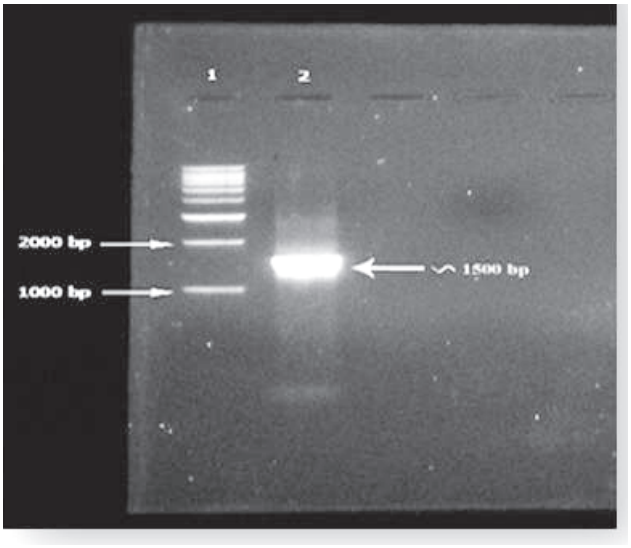

Fig.3b. Amplification of 16s rRNA of bacterial isolate

literature, *P. rettgeri* causes respiratory problems in human hence the bacterium was excluded from further studies. Many reports indicate the extra ordinary antagonistic potential of *Bacillus* spp. Application of *B. subtilis* and *T. harzianum* could check late blight of tomato caused by *P. infestans* (Chowdappa et al., 2013) and seed borne late blight in potato (Wharton et al., 2012).

ITS 1 and ITS 2 region sequencing of the fungal isolates revealed the identity of the organisms as *Trichoderma asperellum* (Fig. 4). All the four potent fungal isolates of vermicompost origin are *T. asperellum* and its

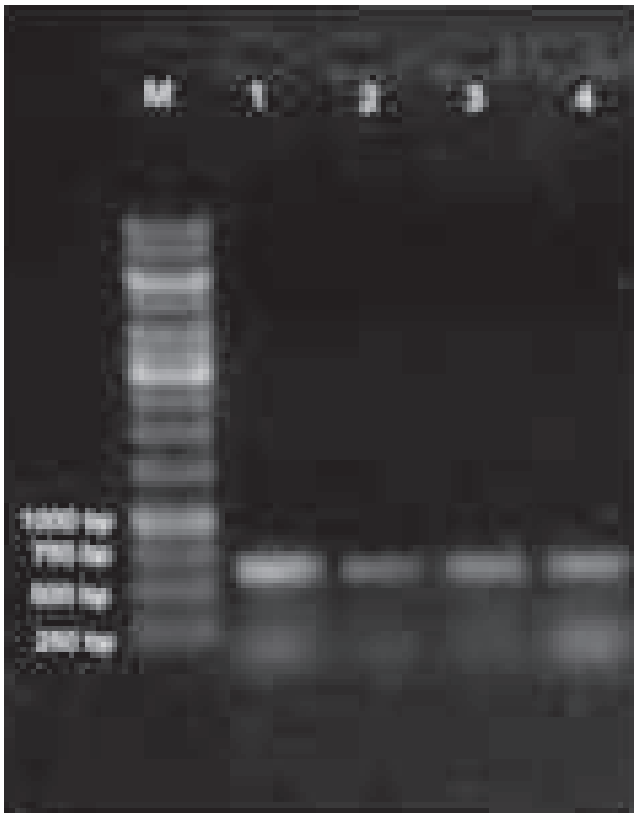

Fig. 4. Amplification of ITS region of fungal isolates

antagonistic potential against different pathogens are reported. *T. asperellum*, a less explored species, is an effective biological control agent against rice seed borne diseases (Watanabe et al., 2006). Mbarga et al. (2012) used four isolates of *T. asperellum* against *Pythium myriotylum* and found that all the four isolates were promising. Potential use of *T. asperellum* as a biological control agent against anthracnose in mango was reported (Villalobos et al., 2013). Wijesinghe (2011) reported antagonistic activity of *T. asperellum* against *Thielaviopsis paradoxa*.*Trichoderma harzianum* was most effective in controlling southern stem rot in artichoke caused by *S. rolfsii* (Sennoi et al., 2013).

Abundance of antagonistic organisms in vermicompost clearly suggests that the disease suppression is biological in nature. All these organisms could be successfully used for the management of taro leaf blight/collar rot of elephant foot yam or could be used for enriching vermicompost. The addition of *T. harzianum* resulted in a high relative abundance of certain chitinolytic bacteria as well as imparted remarkable protection against *Fusarium oxysporum* comparable to that induced by compost alone (Blaya et al., 2013).

Pot culture experiments

Taro

In general, taro leaf blight incidence was less in plants which received vermicompost/vermiwash (Table 2). In 2011, all vermicompost applied plants showed PDI of <10 compared to 16.3 in control. Disease incidence was comparatively less in 2012. Less rainfall and less humidity that existed after challenge inoculation in the second year would have reduced the disease development. Plants which received soil application of vermicompost showed PDI of <5 compared to 8.57 in control. Results clearly indicate that soil application of vermicompost combined with vermiwash spraying could reduce TLB incidence.

In the first year, all the treatments showed significant yield increase over control and it varied from 31.2% - 73.2% in the various treatments. Two treatments, soil application + seed treatment + spraying at 90 DAP and soil application + seed treatment + spraying at 60 and 90 DAP showed significant yield increase over the present recommendation (POP) also and the yield increase varied from 5.93%-26.2% in the various treatments. Yield was also more in the second year due to less disease incidence; high disease incidence in the first year would have reduced the yield. Except three treatments, all the other treatments showed significant yield increment over control and it varied from 14.7% - 71.3%. Two treatments, soil application + spraying at 60 and 90 DAP and soil application + seed treatment + spraying at 60 and 90 DAP showed significant yield increase over the present recommendation (POP). The best treatments are being evaluated under field conditions.

Elephant foot yam

Collar rot incidence was less in treatments with vermicompost/vermiwash application (Table 3). In 2011, no collar rot incidence was noted in three treatments, soil application + spraying at 120 DAP, soil application + spraying at 90 and120 DAP and soil application + seed treatment + spraying at 120 DAP. Compared to the first year, collar rot incidence was less in 2012 and maximum incidence noted was 30% in control. None of the plants in treatments with vermicompost soil application + spraying with vermiwash and present

Table 2. Effect of vermicompost on yield and blight incidence in taro

Notation	Treatments	Per cent disease incidence (PDI)		Cormel yield (g plant^{-1})	
		2011	2012	2011	2012
T_1	Soil application	7.18	4.28	655	752.5
T_2	Soil application + spraying at 60 DAP	2.11	2.80	692	885.0
T_3	Soil application + spraying at 90 DAP	8.97	3.50	675	915.0
T_4	Soil application + spraying at 60 & 90 DAP	4.50	2.96	630	995.0
T_5	Seed treatment	5.26	5.18	625	680.0
T_6	Seed treatment + spraying at 60 DAP	6.38	5.45	660	685.0
T_7	Seed treatment + spraying at 90 DAP	6.15	4.61	685	895.0
T_8	Seed treatment + spraying at 60 & 90 DAP	7.23	4.58	685	802.5
T_9	Soil application + seed treatment	7.14	3.63	650	905.0
T_{10}	Soil application + seed treatment + spraying at 60 DAP	6.47	2.05	625	925.0
T_{11}	Soil application + seed treatment + spraying at 90 DAP	5.52	2.00	745	960.0
T_{12}	Soil application + seed treatment + spraying at 60 & 90 DAP	8.28	1.56	725	1015.0
T_{13}	Present recommendation	12.73	3.14	590	912.5
T_{14}	Control	16.30	8.57	430	592.5
	CD (0.05)			111.8	167.86

Table 3. Effect of vermicompost on yield and collar rot incidence in elephant foot yam

Notation	Treatments	Collar rot incidence (%)		Corm yield (g plant^{-1})	
		2011	2012	2011	2012
T_1	Soil application	25	0.0	787.5	707.5
T_2	Soil application + spraying at 90 DAP	25	0.0	806.3	975.0
T_3	Soil application + spraying at 120 DAP	0	0.0	1006.3	962.5
T_4	Soil application + spraying at 90 & 120 DAP	0	0.0	975.0	1162.5
T_5	Seed treatment	25	20.0	718.8	610.0
T_6	Seed treatment + spraying at 90 DAP	50	10.0	681.3	660.0
T_7	Seed treatment + spraying at 120 DAP	50	10.0	618.8	657.5
T_8	Seed treatment + spraying at 90 & 120 DAP	25	10.0	656.3	707.5
T_9	Soil application + seed treatment	25	0.0	687.5	1030.0
T_{10}	Soil application + seed treatment + spraying at 90 DAP	25	0.0	662.5	1042.5
T_{11}	Soil application + seed treatment + spraying at 120 DAP	0	0.0	937.5	1035.0
T_{12}	Soil application + seed treatment + spraying at 90 & 120 DAP	25	0.0	706.3	1162.5
T_{13}	Present recommendation (POP)	50	0.0	400.0	807.5
T_{14}	Control	100	30.0	350.0	525.0
	CD (0.05)			285.1	261.41

recommendation (POP) was affected by collar rot in the second year. Yield was also more in the second year and it may be due to the less collar rot disease incidence during the period. In 2011, eight treatments showed significant yield increase over the present recommendation (POP) and it varied from 54.7% - 151.5%. In the second year, all the treatments with soil application + spraying out yielded control and the percentage of increase varied from 16.2-121.4%. However, only two treatments, soil application + spraying at 90 and 120 DAP and soil application + seed treatment + spraying at 90 and 120 DAP showed significant yield increase over the present recommendation (POP).

The application of vermicompost/compost tea has shown to significantly suppress several diseases. The application of aqueous compost extracts have shown to reduce diseases caused by necrotrophs as well as biotrophs (Al-Dahmani et al., 2003). Vermicompost treated plants showed lesser *Phytophthora infestans* infection than water sprayed ones (Zaller, 2006). Suppressive composts have been commonly used to control plant diseases caused by soil-borne pathogens, such *as Pythium* spp. (Pascualet al., 2002), *Fusarium* spp. (Ros et al., 2005) and *Phytophthora* spp. (Alfano et al., 2011). Use of compost tea extractions have shown to result in modest to major control of several plant diseases including potato late blight (Al-Mughrabi, 2006). Compost and organic soil amendments have been reported to be effective in the control of *Sclerotium rolfsii* (Danon et al., 2007). Present study indicated the potential of vermicompost for disease management in tropical tuber crops. Even though application of bio-control agents is being recommended for disease management in tuber crops, this was the first attempt to utilize multiple organisms for the control of diseases. Although the potential benefits of a single bio-control agent application has been demonstrated in many studies, it may also partially account for the reported inconsistent performance because a single bio-control agent is not likely to be active in all kinds of soil environment and all agricultural ecosystems (Raupach and Kloepper, 1998).

Changes in resistance inducing enzymes

Taro plants were treated with vermicompost and inoculated with *P. colocasiae*. Enzyme analysis done at 0, 1, 2, 4 and 8 days after inoculation (DAI) indicated that chitinase and β 1, 3 glucanase production increased by the application of vermicompost and maximum

production was noted in the treatment, vermiwash drenching+ pathogen after 24 h followed by vermiwash drenching (Figs. 5 and 6). Total phenol production also increased by the application and maximum production was noted in the treatment, soil application of vermicompost + pathogen after 48 h followed by soil application of vermicompost (Fig. 7). In the pathogen alone treatment also all the three enzymes showed an increase but it was less in quantity and it was at 96 h. Compost would have primed the plants to respond faster or more strongly upon pathogen attack. The effect of composts on biological control of

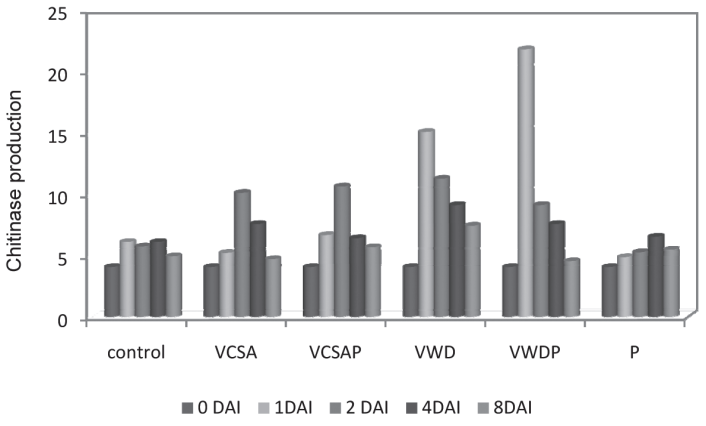

Fig.5. Chitinase production (mg of N acetyl glucosamine released/g of fresh leaves/h) as affected by vermicompost application. VCSA: Vermicompost soil application; VCSAP: Vermicompost soil application + inoculated with pathogen; VWD: Vermiwash drenching; VWDP: Vermiwash drenching + inoculated with pathogen

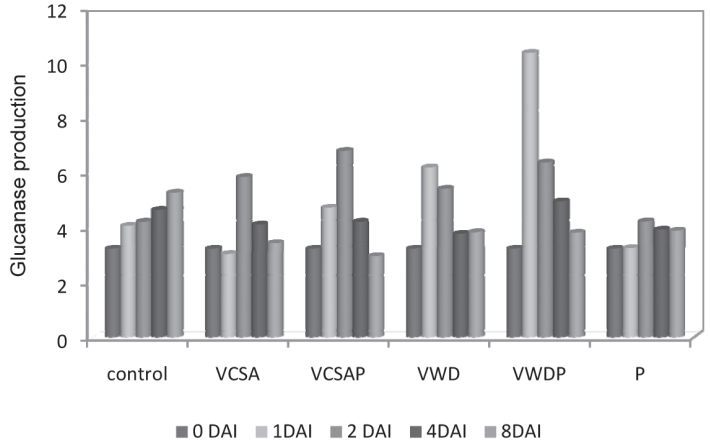

Fig.6. Glucanase production (mg of glucose released/g of fresh leaves/h) as affected by vermicompost application. VCSA: Vermicompost soil application; VCSAP: Vermicompost soil application + inoculated with pathogen; VWD: Vermiwash drenching; VWDP: Vermiwash drenching + inoculated with pathogen

pathogens is likely to include both biological and chemical factors. Vast majority of studies on the disease suppression ability of compost demonstrates a relationship between disease suppression and microbial activity and compost has the ability to trigger plant defense responses that prevent the onset of the disease (Kavroulakis et al., 2005). Pharand et al. (2002) reported that systemic acquired resistance has been recognized as contributing to the bio-control process mediated by compost-amended substrates.

Conclusion

On the basis of this study, it could be concluded that although vermicompost/vermiwash could not completely eradicate the target pathogens, it could bring down the incidence considerably. In addition to promotion of plant growth, vermicompost/vermiwash offers good control against many diseases. Disease suppression by vermicompost can be attributed to the activities of competitive antagonistic microorganisms as well as the antibiotic compounds present in the vermicompost. Many potential bio-control agents were isolated from different samples and these organisms can be utilized for managing taro leaf blight and collar rot of elephant foot yam or can be amended with vermicompost to enhance the activity of vermicompost. Disease management in organic production of tuber crops have limited options since most of the effective fungicides are synthetic, toxic and potentially polluting. Vermicompost and vermiwash can be used as eco-friendly options for managing diseases in tuber crops. In addition to microbial action for disease suppression, concomitant stimulation of several defence mechanisms is a strong possibility. In order to effectively utilize vermicompost and vermiwash as pesticide alternatives, we should be able to predict their effects on plant diseases. For vermicompost and vermiwash to be used as viable disease management options by farmers, it is necessary to establish their disease suppression ability under actual field conditions. Understanding the mode of action of

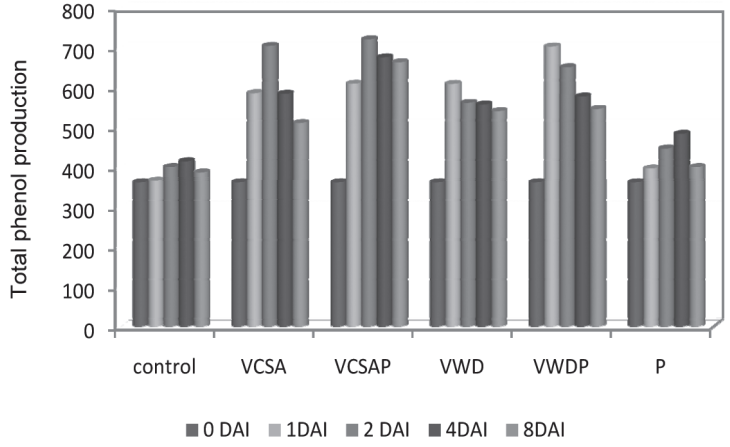

Fig.7. Total phenol production (mg phenols/100 g fresh leaf) as affected by vermicompost application. VCSA: Vermicompost soil application; VCSAP: Vermicompost soil application + inoculated with pathogen; VWD: Vermiwash drenching; VWDP: Vermiwash drenching + inoculated with pathogen

vermicompost by conducting more field studies may help in the formulation of precise application schedule and gain farmer's acceptance as a viable strategy.

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