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Utility of *Trichoderma* Amended Compost to Manage Soil Borne Fungal Diseases of Vegetable Crops in Kota District of Rajasthan (India)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

In the present research article, *Trichoderma* species was isolated and multiplied on a growth medium without contamination for compost preparation. The aim of this study was detecting organic substrates of *Trichoderma* spp. for their antagonistic ability, for this purpose six major agricultural fields of Kota district are selected , from each field about 3-3 samples of soil are collected to detect the percentage of fungal disease incidence and tricho-compost was applied over diseased plants to minimize the fungal attack over vegetable crops. It was observed that the disease incidence is minimized by using tricho-compost mixed with soil in Gopalpura and Balita Village.

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1. INTRODUCTION

Soil - borne fungi are microscopic living cells which grow as minute, thin and long thread-like structures i.e hyphae. A group of hyphae is called mycelium. The mycelium absorbs all essential nutrients of the plants. Common examples of soil borne fungi are *Pythium ,Fusarium, Phytophthora, Rhizoctonia* etc. [1] These fungi enter into the plants directly and indirectly harm the living cells of the plants by making them weak, nutrient deficient , and finally death.

The harmful effect of soil – borne fungi can be controlled by using fungicides and various chemicals , as it is the simplest and quick method applied by farmers for better yield. A huge quantity of chemicals are used to reduce the disease incidence of which is not only detrimental to the environment but are also hazardous to the human health.

There is a need so these harmful chemical control could be minimized or to be replaced by eco- friendly substances over soil – borne fungal diseases [2].

Now a days, In biotechnology and microbiology laboratories fresh and pure culture of *Trichoderma* sp. are used as an amended compost organic material .It was isolated from the soil samples of selected fields to obtain a pure culture of *Trich+oderma.* It worked as biopesticides which protects the plants from harmful fungal diseases [3]. *Trichoderma sp*. are natural competitors against a wide range of harmful fungi; it is added to compost material which work as an antifungal agent to protect crops in the field [4]. *Trichoderma* is a genus of beneficial fungus present in nature. It has been used as bio-control agents against plant pathogens [5].

The systematic position of *Trichoderma* is Division - Ascomycota, Subdivision - Pezizomycotina, Class - Sordariomycetes, Order - Hypocreales, Family – Hypocreaceae. *Trichoderma* is known from 1920 for its capability to function as biocontrol agents (BCA) against plant pathogens [6].

Trichoderma show the multiple interactions with vegetable crop plants like tomato , brinjal, cucurbits , okra etc and soil borne fungal pathogens such as *Pythium ,Fusarium, Phytophthora, Rhizoctonia* etc. [7]. *Trichoderma* is cultured in biotechnical lab, where a specific *Trichoderma* species can be isolated and multiplied on a growth medium without contamination as a biological control agent [8].

2. MATERIALS AND METHODS

2.1 Collection of Soil Samples

Soil samples were collected from different agricultural fields of Kota district namely Girdharpura, Nanta, Manpura, Arjunpura, Badgaon for the isolation of *Trichoderma* spp. (Table 1). These samples are needs to be isolated from the soil to obtain a pure culture of *Trichoderma.*

2.2 Isolation of Soil Samples

Soil Samples were brought to laboratory in a sterilized polythene bag and stored at 5°C until used. Soil -serial dilutions of each preserved samples were prepared in sterilized distilled water. A wide range of media are used for isolation of different groups of fungi that influence the vegetative growth and colony morphology. The pH of the medium was maintained at 5.5 being optimal for the growth and sporulation in a majority of fungi.

Table 1. Disease incidence with fungal diseases observed from different agricultural vegetable crops

S.No.	Name of vegetable crop	Place of collection	Disease incidence	Fungal diseases observed
1.	*Solanum lycopersicum L.*	Girdharpura	26.5%	Wilt of tomato
2.	*Luffa cylindrica*	Nanta	32.7%	*Fusarium* wilt
3.	*Solanum melongena*	Manpura	18.8%	*Phytophthora* blight disease
4.	*Lagenaria siceraria*	Balita	42.4%	Damping - off
5.	*Momordica charantia*	Gopalpura	37.8%	*Fusarium* wilt
6.	*Phaseolus vulgaris L.*	Badgaon	26.4%	Damping - off

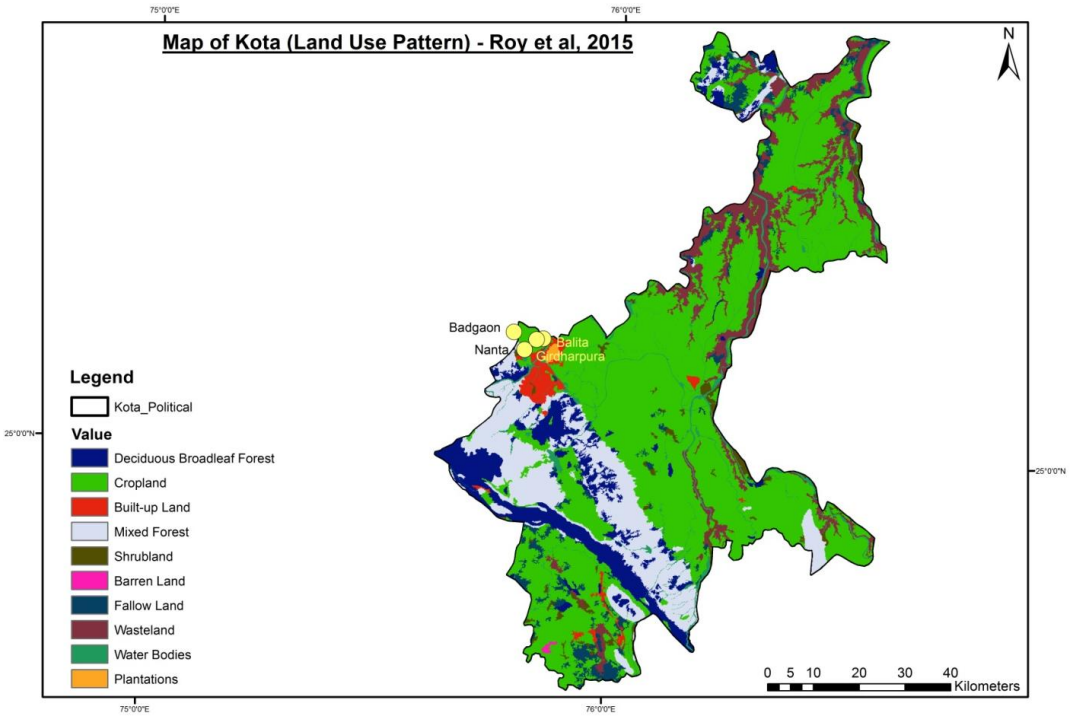

Fig. 1. Map showing different agricultural study sites

Fig. 2. *Trichoderma spp*. isolates in petriplate incubated at 28 ± 2°C after 5 days incubation

Fig. 3. Tricho- compost material after 30 days

Soil samples are diluted up to 38 times in distilled water which is used as inoculums in a sterilized Potato Dextrose Agar (PDA) medium that allow the fungi to grow as organic substrate. All Plates were incubated at 28 ± 2°C for 5 days in BOD.

2.3 Method for Isolation of *Trichoderma (In-Vitro)*

For isolation of fungi *Trichoderma* , pure culture is bought from IMTECH lab , Chandigarh with MTCC no. 793. The growth of pure culture of *Trichoderma* has been screened on culture media PDA for easy identification of *Trichoderma* spores .The mediums used is Nutrient media i.e. Potato Dextrose Agar (extract from 250g of potato boiled and filtered, dextrose 20g, agar 15g and distilled water 1000ml)

There are several methods available for isolation of *Trichoderma* , but the most common and simplest method is Serial Dilution Plate Method [9]. In this method , stock solution of samples is prepared by using dissolved 10 gm of powdered *Trichoderma* pure culture into 90 ml of distilled water. Then, serial dilution of samples were prepared by 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}.

After 5 days, the rhizopheric isolation of *Trichoderma* was observed with yellowish green coloured appearance with fast growth, the conidia are loosely arranged and oval in shape. *Trichoderma* is isolated after identification by counting spores and conidia appears in petriplates [10]. Now 1 ml of each prepared dilution spread on petridish at 28 ± 2°C after 5 days incubation to observe fungal spread. The purified isolates were preserved at 5°C and used for preparation of tricho- compost.

2.4 Preparation of Tricho-compost (Field Experiment)

Tricho – compost is combination of *Trichoderma* inoculum and compositing material found in our surrounding. Following materials are used for preparation of Tricho- compost.

2.4.1 Materials used for making tricho – compost with quantity

- Wood chips – 1 kg
- cow dung (rich in nitrogen, with a carbon & nitrogen ratio C:N of 8) – 4 kg
- Twigs , dry leaves – 2 kg
- Poultry manure – 500 gm
- Sawdust (Source of carbon) – 50 gm
- Vegetable matter- 2kg
- Crop residues – 500gm
- Wood ashes – 100 gm
- Newspapers – 2 kg

2.4.2 Procedure

Now *Trichoderma* inoculum mixed with 0.5 kg of compost (mix up of above listed material) with 2 liter of water. All these ingredients are well mixed together and then placed in the compost bin. Carefully note that the compost and inoculum was mixed up properly before being placed in the compost bin. The compost bin is kept covered for 30 days at a cool place with temperature 25-32^{o} C, so all materials get mixed up and fermented easily. After 30 days, Tricho-compost is ready to applied as a manure in fields. Tricho- compost is applied at a rate of 2 to 2.5 tons/hectare to the crop field for better results.

3. RESULTS AND DISCUSSION

Trichoderma, is a beneficial fungi, as it worked over many plant pathogenic fungi. It worked as antagonistic fungi over soil borne fungal diseases [11]. The identification of the fungal species is based on morphological characteristics of the colony and microscopic examinations [12]. Morphological observations were recorded from cultures grown on PDA plates. Petri plates containing PDA media and incubated at 28 ± 2°C for one week in BOD. The radius of fungal colonial was measured about 22 mm in thickness. Morphological Characters of *Trichoderma* isolates observed in soils of different agricultural sites with presence of pigments, colonial appearance, conidia shape and structure and odour. All the mentioned identified characters are listed below in Table 2.

Table 2. Morphological characters of *Trichoderma* isolates observed in soils of different agricultural sites

S.No	Study site	Presence of pigments	Colony Appearance	Conidia shape and structure	Odour
1.	Girdharpura	Dark green	Highly Branched and compactly tufted	Cylindrical or Globose in shape	Coconut like smell
2.	Nanta	Compute dull green	Frequently branched and long, simple elongations	Obovoid in shape	Coconut like smell

S.No	Study site	Presence of pigments	Colony Appearance	Conidia shape and structure	Odour
3.	Manpura	Dell green to bluish green	Rarely branched and straight elongations	Sub cylindrical	Minute odour
4.	Balita	Dark bluish green	Broad , septate and frequently branched	Ellipsoidal	Less odour
5.	Gopalpura	Dell green to bluish green	Infrequently branched and loosely tufted	Smooth but tuberculate	No odour
6.	Badgaon	White	Less frequent	Rough mass	No Odour

4. CONCLUSION

Tricho-compost works as a natural antifungal agent against harmful fungi which are mostly responsible for soil borne disease like *Fusarium sp , Rhizoctonia sp, Sclerotium sp, Phytophthora sp, Pythium sp, Sclerotonia sp* etc. *Trichoderma sp.* are used in the composting process . Trichoderma spp. are known to have the capability to attack over other fungi. As, they are well known as potential biological control agents among many researchers [13,14], *Trichoderma spp.* are being used to control plant diseases in the sustainable disease management system [15]. Tricho-compost work as growth promoter in many vegetable plants [16]. Trichocompost is primarily used as a soil amendment. It improves soil structure, helpful in maintaining soil pH, improves water holding capacity etc [17]. After applying tricho- compost on the soils of different agricultural fields which contain various fungal diseases ,it was observed that the disease incidence is minimized by using tricho-compost mixed with soil in Gopalpura and Balita Village (as these villages contain highest disease incidence).

It was concluded that amending the soil with tricho-compost as a source of organic matter can be viewed as both a problem-solving activity and as part of ecosystem management. Hence , *Trichoderma sp* are considered safer for the environment and use of tricho-compost as a biological control methods works as a boon and new strategy for disease management and it is environment friendly and pocket friendly method.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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