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Effect of different Extracts of de-oiled Cakes and Organic Manures on Mycelial Growth Enhancement of *Trichoderma harzianum in vitro*

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ABSTRACT: Application of talc based formulations of bio-control agents without any nutrient base may yield sometimes disappointing results under field conditions due to lack of nutritional support for the growth of bio-agent thereby quick decline in shelf life and viability of bio-agents apart from incurring extra costs on carrier material. Instead, cheaper alternatives like de-oiled cakes and other organic manures can be utilized as substrates for nutrient base for supporting the growth and multiplication of bio-agents as well as fulfilling the requirement of carrier material. So, in the present study, different aqueous extracts of de-oiled cakes and organic manures at 10 and 15 per cent concentrations were evaluated to check the compatibility as well as growth enhancement effect of organic amendments against fungal biocontrol agent, *T. harzianum* under *in vitro* conditions. Among nine aqueous extracts, neem cake exhibited maximum increase in mycelial growth obtained from the mean of three and five days after inoculation (DAI) by showing an increase of 39.22 percent and 67.88 percent over un-amended control at 10 and 15 percent concentrations respectively followed by Mustard cake showing an increase in mean mycelial growth of about 39.28 percent and 62.59 percent over un-amended control at 10 and 15 percent concentrations respectively which were highly significant to rest all other treatments. The next best treatments were safflower cake showing an increase in mean mycelial growth of 28.51 percent and 57.73 percent followed by groundnut cake (63.48mm) exhibiting the mean mycelia growth of 28.01 percent and 57.17 percent more over un-amended control at 10 and 15 percent concentrations respectively. However, the remaining organic amendments soya cake, cotton cake, Vermicompost, goat manure and poultry manure recorded comparatively less mean mycelial growth enhancement at both the concentrations tested respectively. It was found that increase in the concentration of organic amendment also increased the rate of growth of bio-agent in all treatments of de-oiled cakes and organic manures.

Keywords: *Trichoderma harzianum*, aqueous extracts, de-oiled cakes, organic manures, compatibility.

INTRODUCTION

Trichoderma spp. are cosmopolitan in distribution which are frequently present in all types of soil, manure and decaying plant materials (Alexander, 1978). *Trichoderma* spp. are strong opportunistic invaders, fast growing, prolific producers of spores and powerful antibiotic producers (Singh *et al.*, 2009).

Trichoderma spp. are important in designing effective and safe management strategies. Many species of *Trichoderma* have multiple strategies for fungal antagonism and indirect effects on plant health (such as plant growth promotion and fertility improvements). Some strains are potent antibiotic producers and their suitability for use in biocontrol systems must be carefully assessed. *Trichoderma* as bio-agent have evolved numerous mechanisms for both attacking other fungi and enhancing plant growth.

Trichoderma is used for better management of various foliar and soil borne plant pathogens. Seed treatment with *T. pseudokoningii* and *T. harzianum* reduce the seed mycoflora, enhance the germination and vigour in forest trees such as *Dendrocalamus striuctus, Phyllanthus embtica, Hardwickia binate* and *Dalbrgia latifolia.* These bio control agents (*T. pseudokoningii* and *T. harzianum*) have also been found to be superior over other treatments like chemical, physical and plant extracts (Mamatha *et al.*, 2000). Due to their excellent

effectiveness, species of *Trichoderma* spp. are generally used as biocontrol agents against plant diseases.

Many potential bio control agents could not be moved from the experimental phase to a commercialization phase due to incompatibility with current production methods. Any bio-control agent must be effective and compatible with modern agricultural practices so that its use can be integrated into the production system.. *Trichoderma* spp. are ecofriendly and cheap fungal bio control agents used for suitable management of various foliar and soil borne plant pathogens (Khandelwal *et al.,* 2012). The ability of *Trichoderma* spp. to control plant diseases by mycoparasitism and through production of wide range of antagonistic substances and its role in growth promoters has been known for many years (Harman *et al.,* 2004). The success of bio control agent depends on the clever blending of bio control agent with suitable carrier material or substrate for their multiplication and longevity apart from reducing the cost of carrier material. Most of the bio-formulations available in market were talc based formulations. Instead, cheaper and better alternative to talc based formulations are needed for sustainable agriculture and integrated disease management. Keeping this in view, the present study was under taken by evaluating different extracts of de-oiled cakes and other organic manures for their compatibility with bio-agent by providing support for the growth and multiplication of bio-agent as well as fulfilling the requirement as a carrier material.

MATERIAL AND METHODS

As per the procedure given by Dhingani *et al.* (2013), aqueous extracts of neem seed cake, castor cake, cotton seed cake, vermicompost, Farm Yard Manure (FYM) etc., were prepared as mentioned in below treatments. Forty grams of each organic amendment was suspended in a conical flask in 150 ml of sterilized distilled water and kept for 15 days. Every day, the flasks were shaken to thoroughly mix and dissolve the content. The extract was strained through muslin cloth after 15 days and then filtered in 150 ml using Whatman filter paper No-41. Conical flasks were sterilized for 20 minutes in autoclave at 121°C. The autoclaved extracts were tested against test pathogen using poisoned food technique by mixing in Potato dextrose agar (PDA) medium at concentrations of ten and fifteen percent (Vincent, 1927). For each treatment, three replications were maintained. According to the formula given by Dennis and Webster (1971) percent inhibition of mycelial growth was estimated. The mycelial growth readings were taken after three and five days after inoculation.

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of mycelial growth; C = Colony diameter in control (mm); T = Colony diameter treatment (mm);

Experimental details:

Design: Completely Randomized Design (CRD); Replications: Three; Treatments: Ten.

Treatment details

Tr. No.	Treatments	Tr. No.	Treatments
T_1	Neem seed cake	T_6	Cotton seed cake
T_2	Mustard cake	T_7	Vermicompost
T_3	Safflower cake	T_8	Goat Manure
T_4	Ground nut cake	T_9	Poultry Manure
T_5	Soya cake	T_{10}	Control (Untreated)

RESULTS AND DISCUSSION

The results (Table 1, Fig. 1, and Plate 1) indicated that all the aqueous extracts of oil cakes and organic manure had significant positive effect on *Trichoderma harzianum* at both the concentrations tested. It was observed that enhancement in the growth of *Trichoderma harzianum* was noticed with the simultaneous increase in concentration of extract.

At 10 per cent concentration, aqueous extracts of neem cake and Mustard cake were highly significant in enhancing the mycelial growth of *Trichoderma harzianum* at three days after inoculation (63.51 and 63.73mm) and five days after inoculation (74.57 and 74.41mm) with a mean growth (69.04 and 69.07mm) respectively. Moreover, neem cake (47.22 percent and 33.03 percent) and Mustard cake (47.74 percent and 32.75 percent) shown greatest level of growth enhancement at three and five DAI respectively with a mean growth enhancement of 39.22 and 39.28 per cent over un-amended control. However, these two treatments were at par with each other.

The third and fourth superior treatments were safflower cake (54.44 and 74.21 mm) which accounts for 26.20 percent and 32.40 percent increase in mycelial growth followed by groundnut cake (53.26 and 72.52 mm) with 23.47 percent and 29.38 percent increase in mycelial growth over un-amended control.

The remaining treatments that also exhibited substantial aggravated effect on the growth of *Trichoderma harzianum viz.* were soya cake (52.23 and 73.51mm), vermicompost (51.69 and 66.04mm), goat

manure (51.59 and 66.29mm), poultry manure (49.96 and 66.62mm) and cotton cake (48.98 and 65.49mm) with mean mycelial growth of 62.87, 58.86, 58.94, 58.29 and 57.23 mm respectively over control after three and five DAI. So with these treatments were shown the recognized growth increase as in soya cake (21.08 and 31.14 percent), Vermicompost (19.82 and 17.81 percent), goat manure (19.61 and 18.28 percent), poultry manure (15.83 and 18.85 percent) and cotton cake (13.53 and 16.84 percent) respectively after three and five DAI with a mean growth increase of 26.78, 18.69, 18.85, 17.54 and 15.41 per cent (Table 1, Fig. 1 and Plate 1).

Results obtained from 15 per cent concentration of aqueous extracts of organic amendments were a way forward, where, neem cake and mustard cake were found to be highly significant by encouraging the mycelial growth of *T. harzianum* after three DAI (79.43 and 87.08 mm) and five DAI (76.71 and 84.55 mm) with mean mycelial growths of 83.25 and 80.63 mm respectively. Moreover, neem cake and mustard cake have shown greatest level of growth acceleration after three DAI (84.13 and 77.82 percent) and five DAI (55.36 and 50.84 percent) with a mean increase of 67.88 and 38.49 per cent compared to un-amended control.

Table 1: Compatibility of *Trichoderma harzianum* and organic amendments under *in vitro* conditions.

Tr. No.	Treatments	Mycelial Growth of *Trichoderma harzianum* (mm)											
		10 percent Concentration						15 percent Concentration					
		3 DAI	Per cent increased over control	5 DAI	Per cent increased over control	Mean	Per cent increased over control	3 DAI	Per cent increased over control	5 DAI	Per cent increased over control	Mean	Per cent increased over control
T1	Neem Cake	63.51	47.22 (36.21)	74.57	33.035 (29.86)	69.04	39.22	79.43	84.13 (42.51)	87.08	55.36 (36.63)	83.25	67.88
T2	Musturd cake	63.73	47.74 (36.14)	74.41	32.75 (29.72)	69.07	39.28	76.71	77.82 (41.40)	84.55	50.84 (35.47)	80.63	62.59
T3	Safflower cake	54.44	26.20 (29.02)	74.21	32.40 (29.45)	63.73	28.51	74.37	72.40 (40.32)	82.07	46.43 (34.21)	78.22	57.73
T4	Ground nut cake	53.26	23.47 (27.63)	72.52	29.38 (28.35)	63.48	28.01	72.22	67.42 (39.34)	83.67	49.28 (34.89)	77.94	57.17
T5	Soya cake	52.23	21.08 (26.77)	73.51	31.14 (28.91)	62.87	26.78	71.88	66.64 (39.12)	78.74	40.47 (32.44)	75.31	51.87
T6	Cotton Cake	48.98	13.53 (23.36)	65.49	16.84 (22.19)	57.23	15.41	63.93	48.20 (34.49)	73.32	30.82 (28.97)	68.62	38.37
T7	Vermicompost	51.69	19.82 (26.32)	66.04	17.81 (22.73)	58.86	18.69	68.46	58.71 (37.41)	76.03	35.64 (30.77)	69.98	41.12
T8	Goat Manure	51.59	19.61 (24.76)	66.29	18.28 (23.01)	58.94	18.85	67.92	57.44 (37.13)	74.39	32.72 (29.71)	70.16	41.48
T9	Poultry Manure	49.96	15.83 (26.93)	66.62	18.85 (23.39)	58.29	17.54	65.93	52.83 (35.99)	74.35	32.64 (29.68)	71.40	43.98
T10	Control	43.14	0.00 (0.00)	56.05	0.00 (0.00)	49.59	0.00	43.14	0.00 (0.00)	56.05	0.00 (0.00)	49.59	0.00
	SE(m)±	**3.05**	**3.39**	**1.83**	**1.65**			**1.56**	**1.51**	**1.16**	**1.36**		
	C.D.(P=0.01)	**9.07**	**10.09**	**5.44**	**4.90**			**4.63**	**4.51**	**3.45**	**4.04**		

DAI - Days after inoculation

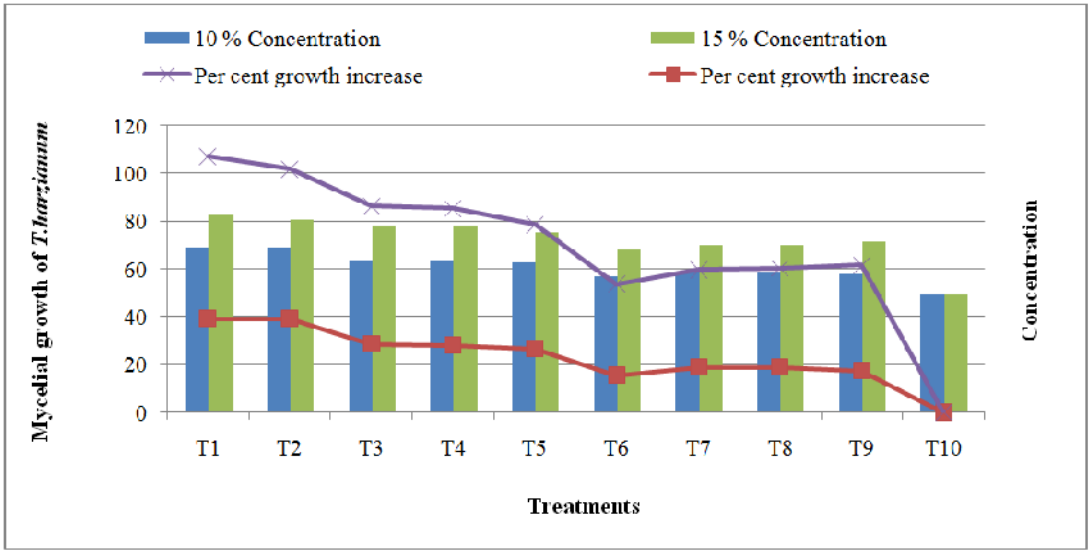
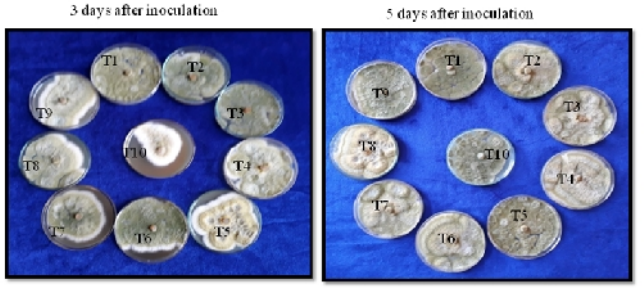


Fig. 1. Compatibility of *Trichoderma harzianum* and organic amendments under *in vitro* conditions.

Compatibility of *Trichodermaharzianum* and organic amendments under *in vitro* conditions.



Tr No	Treatment	Tr No	Treatment
T1	Neem cake	T6	Cotton cake
T2	Mustord cake	T7	vermicompost
T3	Sawflower cake	T8	Goatmanure
T4	Groundnut cake	T9	Poultry manure
T5	Soya cake	T10	Control

Plate 1.

The third and fourth superior treatments at 15 per cent concentration of aqueous extracts were safflower cake (74.37 and 82.07 mm) and groundnut cake (72.22 and 83.67 mm) with a mean of 78.22 and 77.94 mm after three and five DAI respectively. Similarly, growth encouragement showed by safflower cake (72.40 and 46.43 percent) and groundnut cake (67.42 and 49.28 percent) were significant with a mean increase of 57.73 and 57.17 per cent after three and five DAI respectively.

The remaining treatments were shown substantial enhancement effect against the *Trichoderma harzianum*, *viz.*, Soya cake (71.88 and 78.74 mm), Vermicompost (68.46 and 76.03 mm), goat manure (67.92 and 74.39 mm), poultry manure (65.93 and 74.35 mm) and cotton cake (48.20 and 30.82 mm) with a mean increase of 75.31, 69.98, 70.16, 71.40 and 68.62 mm respectively over control after 3 and 5 DAI. Similarly, these treatments were shown the recognized growth increase as in soya cake (66.64 and 40.47 percent), Vermicompost (58.71 and 35.64 percent), goat manure (57.44 and 32.72 percent), poultry manure (52.83 and 32.64 percent) and cotton cake (48.20 and 30.82 percent) after three and five DAI with an increase in mean mycelial growths of 51.87, 41.12, 41.48, 43.98 and 38.37 per cent respectively (Table 1, Fig. 1 and Plate 1).

Findings of the present study suggest that mustard oil cake at the lowest concentration appeared to be compatible with *T. harzianum*. The experimental results were in concurrence with previous reports. Organic amendments enhanced the shelf life of bioagents by increasing spore count due to the continuous food supply and comfortable environment with other physiological parameters (Pan *et al*. 2006; Zaidi and Singh, 2004; Mishra *et al.*, 2009). The Plant based products, organic amendments, crop residues, green manures can dramatically affect soil microbial communities, and are primary drivers of soil microbial dynamics (Rashidul Islam *et al.*, 2013). Peerzada *et al.* 2020 evaluated different types of organic amendments in combination with bio-agent Trichoderma as soil application and found that all organic amendments helped in the multiplication and growth of *Trichoderma* sp. which in turn helped in the reduction of late blight disease in potato. Higher colony forming unit (CFU) count and shelf life was reported with various deoiled cakes and cereal grains when used as substrates (Singh *et al.*, 2020).

Promotive effect of neem aqueous extract may be due to triterpene (azadirachtin) which acts by delaying the transformation of ammonium nitrogen into nitrate nitrogen (Ruben *et al*., 2011). The slow nitrogen conversion led to continuous availability of nitrogen which was required for the organism to grow (Siddiqui *et al.*, 2008). Moreover, Organic amendments like Poultry compost contains NH_4-N and NO_3-N supplied to resident soil micro organisms tend to exploit NH4+ more quickly than plants and then proliferate rapidly (Barakat *et al.*, 2013). The findings were in agreement with other investigators (Dutta and Das 1999; Islam *et al*. 2002; Nahar and Bhuiyan 2003). Sivan *et al.* (1984) also reported that *T. harzianum* can grow on different agricultural waste products.

CONCLUSION

Compatibility test of different aqueous extracts of de-oiled cakes and organic manures at ten and fifteen per cent concentrations against *Trichoderma harzianum* revealed that at both concentrations tested, neem seed kernel cake enhanced the maximum mycelial growth followed by mustard cake. These were followed by safflower cake, groundnut cake and soya cake. Treatments *viz.*, cotton cake, vermicompost, goat manure and poultry manure recorded comparatively less mean growth enhancement at both the concentrations tested. Moreover, it was found that rate of mycelial growth enhancement was increased with

increase in the concentration of de-oiled cakes and organic manures

FUTURE SCOPE

Utilization of de-oiled cakes and organic manures as substrates can be made on a commercial scale for the growth and multiplication of bio-agents instead of special carrier material, which reduces the cost of formulation apart from their increase in effectiveness, shelf life and longevity of bio-agents.

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Conflict of Interest. None.

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