CONTEMPORARY RESEARCH IN INDIA
ISSN 2231 - 2137

A MODIFIED METHOD FOR MASS MULTIPLICATION OF THE FUNGAL BIOCONTROL AGENT, *TRICHODERMA VIRIDE*

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Received: 31/08/2017	Edited: 06/09/2017	Accepted: 13/09/2017

Abstract: *The currently used method for the in situ mass multiplication of the bio-control agent Trichoderma involves use of neem cake and farmyard manure in the ratio 1:10. Adulteration of neem cake with common salt leading to poor growth and multiplication of Trichoderma, and often the multiplied inoculum was found to be contaminated by the dreaded fungus, Aspergillusflavus. Unavailability of quality neem cake led to search for other alternate substrates for mass multiplication of the fungus. Oven dried coconut cakeshowed best result, when efficacy was tried. Oven dried coconut cake and farm yard manure at different proportions were tried for mass multiplication of Trichodermain field level.Trichoderma was initially multiplied in coconut cake for 5 days and the multiplied inoculum was later mixed with farmyard manure and allowed to grow for 10 more days. Coconut cake and farmyard manure at the ratio 2:8 yielded the best results.*

Introduction

Biological control of fungal pathogens offers environmentally safe, durable and cost-effective alternativeto chemicals. Among the different bioagents, *Trichoderma* is most exploited. *Trichoderma viride* and *Trichoderma harzianum* have curved a niche for themselves in India as important biocontrol agents for management of various plant diseases.These beneficial microorganisms need somesuitable carrier for their delivery, which can support their life duringstorage and transportation. Also there is a need to reduce the cost of biopesticide byemploying cheaper substrates like agricultural waste products as mass multiplying agents. Many agro-industrial by-products like de-oiled cakes such as Neem, coconutetc. are in use since centuries, being more cheaper and usable products. These de-oiled cakes contain several chemicals, micronutrients, and many more constituentsmaking them a good source of nutrition for beneficial microorganisms in crop cultivation and hence could be exploited for mass multiplication of *Trichoderma*. As per the Package of Practices Recommendations of Kerala Agricultural University, neemcake mixed with FYM is recommended for farmers for the field level mass multiplication of Trichoderma. But adulteration of neem cake with common salt, which has become a common malpractice nowadays, results in inhibition of growth of Trichoderma. In addition to the poor growth, the mass multiplied product was found to be contaminated by the dreaded fungus, *Aspergillusflavus*. Due to the similarity of growthof *Trichoderma* and *Aspergillus* in the initial stages, farmers can not differentiate the contamination. The above constraints lead to the search for other alternate substrates such as coconut cake as different treatmentsin combination with FYM in different proportions for mass multiplication of the *Trichodermaviride*.

Materials and methods

1. Efficiency of different de-oiled cakes for mass multiplication of Trichoderma under *in vitro* conditions

Two 5mm mycelial discsof *Trichoderma viride* (KAU culture) was inoculated in to 250 ml conical flasks containing 150 ml potato dextrose brothand were incubated at room temperature (29 ± 1^0 C) for

10 days. The mycelial mats along with spores collected from the flaskswere grinded in a sterile pestle and mortar using sterile water. The mycelial mat was filtered and the spores suspension collected in a beakerwasmade up to 400ml. The spore count was determined by dilution method with the aid of haemocytometer and was adjusted to 6 x 10^6spores per ml.Coconut cake and neem cake used in the present study were collected from local markets. The different treatments and the quantity taken were as follows.

Table-1

Sl.No.	Treatments	Quantity(g)
1	Untreated coconut cake	250
2	Sun dried coconut cake (7 hours)	250
3	Oven dried coconut cake (60^0C for 7 hours)	250
4	Untreated coconut cake + untreated neem cake	125 g each
5	Untreated neem cake	250

Each treatment was replicated thrice. 250 g each of oilcakeswere taken in 20 cm diameter pertriplates separately andwere inoculated with 60 ml (6 x 10^6spores per ml) of spore suspension and incubated at room temperature (29±1^0 C). 30 % moisture was maintained for each treatments.The observations on fungal growth wasrecorded daily. The population dynamics in terms of colony forming units of *T. viride*in different treatments per gram were recorded 5 days after inoculation by serial dilution plate technique.

2. Efficiency of coconut cake for mass multiplication of Trichoderma under *in vivo* conditions

The treatment which showed the best sporulation was selected for next experiment. The Trichoderma enriched oil cake, from the above experiment, was mixed with farm yard manure (cowdung) in different proportions and kept in the separate plastic basins (30 x 60 cm) for mass multiplication. The different proportions of Trichoderma enriched oil cake and farm yard manure tried were 1:9, 2:8 and 1:10 respectively. Six replications were maintained for each treatment. The moisture content was maintained up to 30% by sprinkling water at alternate days and the basins were covered with moistened news paper for providing suitable humid conditions for fungal growth. These basins were incubated at normal temperature for 10 days.The visual observation on fungal growth were made daily andthe mean population in terms of colony forming units of *T. viride* was recorded after 10 days of incubation by serial dilution - plate technique.The data were subjected to analysis of variance (ANOVA) and the Duncan's Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel and Torrie, 1980).

Results and discussion

The results of the first experiment, after 6 days of incubation showed that the maximum mean population of *T. viride* was produced on oven dried coconut cake (40 x 10^6cfu/g) and sun dried coconut cake (39 x 10^6cfu/g) followed by untreated coconut cake (27 x 10^6cfu/g)(Table-2). The least population was recorded from untreated neem cake (4 x 10^6cfu/g).From this experiment we could find that oven dried coconut cake treatment was the best in terms of mean population and hence was selected for the*invivo* experiment.

The results of the mean population of *T. viride* on different proportions of Trichoderma enriched oven dried coconut cake and FYM revealed that the highest mean population was supported by 2:8 (coconut cake : FYM) proportion (71 x 10^8cfu/g) which was significantly superior over all other treatments (Table-3). The second best proportion was 1:9 (coconut cake : FYM) with a mean population of 55 x 10^8cfu/g.

Table 2: Mean Population of *T. viride* on coconut cake and neem cake with different treaments

Sl. No.	Different treatments	Mean population per gram (x 10^6 cfu)
1.	Un treated coconut cake	27
2.	Sun dried coconut cake	39
3.	Oven dried coconut cake	40
4.	Coconut cake + Neem cake	18
5.	Neem cake	4
	CD(0.05)	6.1

Table 3: Mean Population of *T. viride* on Trichoderma enriched coconut cake and FYM with different proportions

Sl.No.	Treatments	Mean population per gram(x 10^8cfu)
1	*T. viride* enriched oven dried coconut cake and FYM (1:9)	55.000
2	*T. viride* enriched oven dried coconut cake and FYM (2:8)	71.667
3	*T. viride* enriched oven dried coconut cake and FYM (1:10)	43.000
	CD(0.05)	11.02

The results of the above experiments revealed that oven dried or sun dried coconut cake was the best substrate for initial multiplication of *T. viride* and for further mass multiplication in field level, 2:8 proportion of coconut cake : FYM (cowdung) was better. During the incubation period, faster colonization and sporulationof *T. viride* was observed on oven and sun dried coconut cake, whereas in the untreated neem cake no colonization and sporulation was observed. The colonization and sporulation was less in the untreated coconut cake and untreated coconut-neem cake mixture and in these treatments, colonization and sporulation *Aspergillus* spp. was more. In the untreated coconut cake also *Aspergillus* contamination was present. This may be the reason for the poor performance of these treatments. These experiments showed that drying of coconut cake will avoid contaminations and will promote the growth of *T. viride* inabetter way. In the field level, Jahagirdar*et al.* (1998) and Chakrabarty*et al.* (2014) have reported maximum sporulation of *T. viride* on FYM and cowdung respectively which support the present finding that FYM can promotegrowth of *Trichoderma* sp.

In the present study, least mean population of *T. viride* was observed in the untreated neem cake. This finding is in line with observations made by earlier workers (Prakash*et al.*, 1999; Niranjana*et al.*, 2009; Chakrabarty*et al.*, 2014) but in contrast to the studies by Singh *et al.*(2015) andJahagirdar*et al.* (1998) which reportedgood sporulation of *Trichoderma* spp. in neem cake. The least colonization *T. viride*on neem cake may be due to presence of salt as adulterant and it was confirmed by measuring the EC of the neem cake collected, which was found to be 22dS/m.

Several workers also tried mass multiplication of *Trichoderma* spp. on many other locally available substrates like wheat bran (Jahagirdar*et al.*,1998), tea waste (Prakash*et al.*, 1999), rice bran (Niranjana*et al.*, 2009), sugarcane baggase (Subash*et al.*, 2014). Perusal of available literature does not find studies using coconut cake for mass multiplication of *T. viride*and hence this modified method is novel. More over the byproducts of oil industry can be effectively utilized by the farmers for field level multiplicationof Trichoderma at a lesser cost. Therefore, the present findingsrecommend farmers theuse of sundried coconut cake instead of the salt contaminated neem cake for the initial multiplication *T. viride*and further mass multiplication of the multiplied inoculum in combination with FYM in the field.

References

Chakrabarty, R., Acharya, G.C. and Sarma, T. C. 2014.Evaluation of substrates for multiplication of bio agents, *Trichoderma viride. Afr. J. Agric. Res.* **9**: 1938-1940.

Jahagirdar, S., Siddaramaiah, A.L. and Narayanaswamy, H.1998.Screening of substrates for mass multiplication of *Trichoderma viride. Karnataka J.Agri.sci.***11**: 233-236.

Niranjana, S.R., Lalitha, S. and Hariprasad, P. 2009. Mass multiplication and formulations of biocontrol agents for use against fusarium wilt of pigeonpea through seed treatment. *International Journal of Pest Management*.**55**: 317–324.

Prakash, M.G., VinayaGopal, K., Anandaraj, M. and Sarma, Y. R.1999.Evaluation of substrates for mass multiplication of fungal biocontrol agents *Trichoderma harzianum*and *T. virens.Journal of Spices and Aromatic Crops*.**8**: 207-210.

Singh, R., Kumar, A., and Tomer, A. 2015. De-oiled Cakes of Neem, Jatropha, Mahua and Karanja: A New Substrate for Mass Multiplication of *T.harzianum*. *J. Plant PatholMicrob* 6: 288. doi:10.4172/2157-7471.1000288

Subash, N., Meenakshisundaram, M., Sasikumar, C. and Unnamalai, N. 2014. Mass cultivation of *Trichoderma harzianum* using agricultural waste as a substrate for the management of damping off disease and growth promotion in chilli plants (*Capsicum annuum* l.). *Int. J. Pharm. Pharm. Sci*. **6**: 188-192a