International Journal of Plant Protection | **Volume 5 | Issue 1 | April, 2012 | 154-156**

IJPP

Research Article

Improvement in nutritional status of *Trichoderma* colonized FYM

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ARITCLE INFO

Article Chronicle :
Received : *12.01.2012*
Revised : *15.02.2012*
Accepted : *20.03.2012*

Key words :
Trichoderma, Compost, FYM

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ABSTRACT

This *in vitro* study was conducted to assess the potential of *Trichoderma* in improving the nutritional status of sterilized and unsterilized farm yard manure (FYM). *Trichoderma harzianum* (Th-14) was colonized on FYM and elemental analysis of colonized FYM was carried out. The data indicated that the population of *Trichoderma harzianum* in colonized compost was several folds higher (48.60 x 10^{12} cfu/g) than non-colonized compost (3.00 X10^{4} cfu/g).The per cent macro and micro nutrient was also significantly higher in *Trichoderma* colonized FYM as compared to uncolonized FYM. The increase in nutritional status was retained even after such composts were made free from microbes by autoclaving. The finding is important as it reveals the role of *Trichoderma* in inducing nutritive value of FYM.

How to view point the article **:** Rawat, Laxmi, Singh, Y. and Kumar, J. (2012). Improvement in nutritional status of *Trichoderma* colonized FYM . *Internat. J. Plant Protec*., **5**(1) : 154-156.

INTRODUCTION

Genus *Trichoderma* has gained immense importance since last few decades due to its biological control ability against several plant pathogens. The researchers are interested in this genus because of its novel biological properties and biotechnological applications (Shalini and Kotasthane, 2007). *Trichoderma* species are among the most frequently isolated soil fungi and present in plant root ecosystems (Harman *et al*., 2004). The fungi are opportunistic, avirulent plant symbionts and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from diseases. So far *Trichoderma* sp. are among the most studied fungal biocontrol agents and commercially marked as a potent biopesticides biofertilizers and also used in soil amendments (Harman, 2000; Mohiddin *et al*., 2010). *Trichoderma* can also colonize and decompose dead organic matter. One of the most effective methods for the delivery of *Trichoderma* in soil is through colonized compost like FYM, cow dung, poultry manure etc. In addition to improving N content and the availability of other plant nutrients, these additions help to reduce the composting time considerably. Recent studies have revealed that *Trichoderma* inoculation enhances the decomposition and nutritive value of organic composts like cow dung, poultry manure and press mud. *Trichoderma harzianum* changes the colour of fresh cow dung in five days and enhances the nutritive value of colonized cow dung / farmyard manure that serves as an excellent substrate for its multiplication. It multiplies very well on cow dung /FYM not only under laboratory conditions but also at farmers level in their compost pits (Zaidi, 2006). Farmyard manure has been used successfully as a substrate for developing formulations of *Trichoderma*. However most of the reports are confined to use of FYM for the mass production of fungal antagonists. The present research was conducted to determine the nutritive status of *Trichoderma* colonized FYM.

MATERIALS AND METHODS

The experiment was conducted to assess the increase in the nutritive value of *Trichoderma harzianum* colonized FYM. *Trichoderma harzianum* (Th-14) isolate was collected from the repository of Biocontrol Lab in Department of Plant Pathology, G.B.P.U.A&T, Pantnagar. FYM was collected and air-dried by spreading as an approx. 1" thick layer under open shade for one week. Fifty g of air-dried unsterilized or sterilized (moisture content approx. 30 per cent when estimated by oven drying for 24 h at 40^{0}C) FYM was taken in 250 ml Erlenmeyer flasks. Moisture content of cow dung was adjusted to 10, 20, 30, 40, 50 and 70 per cent by weight of air-dried cow dung by adding water. Two ml suspension of Th-14 spore powder (three

days old culture made in King's B broth (@1%) and was added in each flask and mixed thoroughly. Inoculated flasks were incubated in a BOD incubator at 25±2^{0}C. Flasks were weighed regularly and loss of moisture was replenished by adding sterilized water. Twenty one days after inoculation, 1 g from Th-14 colonized air-dried samples was estimated for population count and elemental analysis.

Population count of *Trichoderma* spp.:

Trichoderma colonized and un-colonized and sterilized and unsterilized FYM was taken and a dilution of 1: 1000 employing serial dilution technique was prepared. One ml of this suspension was poured on the Petri plates seeded with TSM medium. The plates were incubated at 28^{0}C for 5 days. Observation on the appearance of colonies was recorded from 3rd to the 5th day.

Total elemental analysis:

Total N content in sample was determined by Kjeldahl method as per procedure outlined by Page (1982). The total content of P, K, S and micronutrients in manure samples was determined in di-acid digest (HNO_3:$HClO_4$, 3:1, v/v) by ascorbic acid reduced ammonium molybdate procedure at 880 nm, flame photometry, turbidimetry and atomic absorption spectrometry (Avanta. M, GBC), respectively following the procedures detailed by Page (1982). The final contents were calculated on oven dry weight basis.

Water soluble fraction of elements:

Twenty grams fresh manure was extracted in 100 ml water for 2 h at 120 rpm. The content were filtered. The contents of soluble humic matter was calculated by recording absorbance of extracts at 650 nm and comparing it with those of humic acid standards. Twenty ml filtrate was digested in 10 ml di-acid (HNO_3: $HClO_4$, 3:1, v/v) and final volume was made to 20 ml using distilled water. The contents of P, K, S and micronutrients in the filtrate were determined following the procedures mentioned in the preceding section. The results were expressed on oven-dry weight basis.

RESULTS AND DISCUSSION

The various characteristics of *Trichoderma* colonized and non-colonized and sterilized and unsterilized FYM were investigated. As shown in Tables 1 and 2, colonization of FYM by *Trichoderma harzianum* improved the quality of FYM. The results presented in Table 3 indicated that the population of *Trichoderma harzianum* in colonized compost was several folds higher (48.60 X 10^{12} cfu/g) than non-colonized compost (3.00 x10^4 cfu/g). Similar results were obtained by Singh *et al*. (2004) who reported that FYM serve as substrate for better colonization of *Trichoderma.* Overall, addition of bioagent did not significantly influence the general properties of FYM. There was not much difference in per cent moisture, per cent ignition loss, per cent ash, per cent organic matter, humic matter, pH and EC of non-colonized and bioagents' colonized FYM as shown in Table 4. There was no significant change in the general properties even after autoclaving of the colonized FYM.

Both total and water soluble content of a number of macro and micronutrients like P, K, S, Zn, Cu and Fe were significantly higher in *Trichoderma harzianum* (Th-14) colonized compost as compared to non-colonized FYM (Tables 1 and 2). While comparing the total nutrient content of

Table 1 : Total nutrient content of bioagents colonized or non-colonized sterilized/unsterilized FYM

Treatments	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
FYM alone (Unsterilized)	2.75	1.60	0.075	0.28	6.66	1.22	213.49	31.04	2457.42	402.38
Th-14 colonized FYM (Unsterilized)	3.50	2.97	0.149	0.31	9.67	1.66	292.09	47.77	3142.14	531.68
FYM alone (Sterilized)	2.87	1.84	0.055	0.33	7.60	1.21	236.49	29.67	2686.43	475.50
Th-14 colonized FYM (Sterilized)	3.44	1.90	0.140	0.48	10.64	2.32	422.12	49.75	3183.10	580.35
C.D. (P=0.05)	0.09	0.51	0.005	0.01	0.26	0.38	51.65	4.31	12.80	11.38

FYM= Farm yard manure

Table 2 : Water soluble nutrient content of colonized or non-colonized sterilized/unsterilized FYM

Treatments	H. M (g/100 g)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)
FYM alone (Unsterilized)	0.020	0.08	0.0098	0.0156	0.407	0.10	0.57	0.04	2.73
Th-14 colonized FYM (Unsterilized)	0.124	0.21	0.0133	0.0019	0.304	0.20	0.63	0.08	3.33
FYM alone (Sterilized)	0.045	0.09	0.0115	0.0185	0.493	0.08	0.57	0.06	2.39
Th-14 colonized FYM (Sterilized)	0.133	0.07	0.0151	0.0179	0.445	0.14	0.86	0.05	5.34
C.D. (P=0.05)	0.020	0.07	0.0005	0.0004	0.014	0.05	0.02	0.01	0.38

Table 3: Population of bioagents in colonized FYM 21 days after inoculation just before nutrients analysis

Treatments	Cfu /g air dried FYM
Th-14 colonized FYM (Unsterilized)	$48.60x10^{12}$
Th-14 in non colonized FYM (Unsterilized)	$3.00x10^{4}$
Th-14 colonized FYM (Sterilized)	0.00
Th-14 in non colonized FYM (Sterilized)	0.00
C.D. (P= 0.05)	$30.48x10^{7}$

Table 4 : General properties of bioagents colonized or non colonized sterilized/unsterilized FYM

Treatments	Moisture (%)	Ignition loss %	Ash (%)	O. M (%)	H. M (g/100g)	pH (1:2)	E. C ($dS.m^{-1}$)
FYM alone (Unsterilized)	0.119	55.88	44.00	15.59	4.03	6.16	0.86
Th-14 colonized FYM (Unsterilized)	0.975	53.43	45.60	14.90	4.44	5.80	0.75
FYM alone (Sterilized)	0.232	53.67	46.10	14.97	4.03	5.14	0.56
Th-14 colonized FYM (sterilized)	2.507	53.29	44 .20	14.86	4.49	5.71	1.76
C.D. (P=0.05)	-	-	-	-	0.22	0.09	0.03

colonized and non-colonized FYM, it was observed that, the per cent nitrogen, phosphorus and potassium content was higher in unsterilized *Trichoderma harzianum* (Th-14) colonized FYM (Table 1). There was no significant difference in nutrient increase between sterilized and unsterilized *Trichoderma* colonized compost. However, sterilized Th-14 colonized FYM was found better in most of the studied macro and micronutrient enhancement in FYM. In present study, it was found that colonization of FYM by *Trichoderma harzianum* significantly improved the quality of FYM. The results are in agreement with previous reports (Bandyopadhyay *et al*., 1988), who reported increased N content and other plant nutrients in compost colonized by *Trichoderma*. It is concluded from the above experiments that the nutritive status of bioagent colonized compost was increased manifold in comparison to uncolonized composts.

It was interesting to observe that the total humic matter was higher in bioagents colonized FYM as compared to non-colonized FYM. Maximum content was in *Trichoderma harzianum* (Th-14) colonized FYM. The humic matter content was more in colonized sterilized FYM as compared to colonized unsterilized FYM. Water-soluble humic matter content was much higher (³ 6 times) in FYM colonized with bioagents as compared to non-colonized FYM. Nutritive value of composts colonized by bioagents was retained even after such composts were made free from microbes by autoclaving. Similar findings were reported by Zaidi (2006), who reported higher total humic matter content in *Trichoderma* colonized sterilized and unsterilized FYM in comparision to uncolonized FYM. These nutrients probably got released during the process of decomposition. This activity may explain, at least partially, the ability of bioagent colonized compost to improve plant health even in nutrient deficient soils. Since humic matter is reported to have got growth promoting effect, so in addition to better availability of macro and micro nutrients, high humic matter content would be responsible for the better plant growth by using *Trichoderma harzianum* colonized cowdung/ FYM under field conditions..

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