Int.J.Curr.Microbiol.App.Sci (2020) *9*(3): 3124-3129

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 9 Number 3 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.903.357

Survivability of Soil Bio-agents in Presence of Organic Amendment in Arid Conditions of Rajasthan, India

Nitin Chawla*, Vipen Kumar, R. K. Bagri and S. K. Jain

Rajasthan Agriculture Research Institute, (SKN Agricultural University: Jobner), Durgapura, Jaipur-302 018 (INDIA)
**Corresponding author*

Keywords

Bioagents, *Trichoderma harzianum*, *Trichoderma viride*, *pseudomonas fluorescens* and *Bacillus subtilis*, Organic amendment, Survival, Population

Article Info

Accepted:
28 February 2020
Available Online:
10 March 2020

A B S T R A C T

The effect of three organic amendment viz., farm yard manure, vermicompost and mustard cake on survival of bioagents . The population of four bioagents i.e. *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis.* significantly higher in three amended soil as compared to unamended control. The four respective bioagents were enumerated in selective media at monthly interval up to 180 days of soil application of bio-agents. The recovery of this bio-agent was found at 30 days after application as compared to 0 day i.e. immediate after soil application. The survival of the bioagent was relatively better in mustard cake amended soil as compared to farm yard manure and vermicompost amended soils. The population of *T. viride* was considerably less in unamended soil as compared to amended soils. The population of *P. fluorescens* was considerably higher in amended soils as compared to control i.e. unamended soil. The population of this bacterium in soil gradually decreased from 30 days of soil applications onwards up to 180 days. Survival of the bacterium was higher in mustard cake and vermicompost amended soil as compared to soils amended with farm yard manure. Statistical analysis of data revealed that A perusal of the data given in table 4 showed that mustard cake and vermicompost was slightly better for survival of *B. subtilis* in comparison to farm yard manure. The survival of *B. subtilis* was relatively less in unamended control in comparison to amended soils.

Introduction

Cumin (*Cuminum cyminum* L.) is one of the major seed spice crop grown in India, which is mainly grown in Rajasthan, Gujarat, Uttar Pradesh and Tamil Nadu. Cumin is valued for its typical pleasant aroma from its essential oil which ranges between 2.5 and 3.5 % in indigenous collections up to 5.5 % in exotic ones (Mahariya *et al.* 2007). The area under cumin is constantly increasing in several parts of state due to its low water requirement and lucrative market prices. But fungal diseases are serious problem in this crop and cause considerable losses in seed yield in the most of the cumin growing areas. Out of these diseases, wilt caused by *Fusarium oxysporum* (Schecht) f. sp. *cumini* is an endemic problem in most of cumin growing areas of Rajasthan and usually causes substantial yield losses (Mathur and Mathur 1965). So far, majority of cumin varieties commonly grown by the farmers are susceptible to this disease and it has become a limiting factor of cumin cultivation in most of the cumin growing region in the country. Since *fusarium* wilt of cumin is both seed and soil-borne disease and pathogen infested seed play an important role in dissemination of the disease to new areas it is urgent need to develop suitable

management strategies based on protection of the seedlings from seed borne inoculate (Lodha *et. al.,* 2000).The increased awareness with respect to deleterious effects of toxins used as agrochemicals on health of consumer and ambient environment has enthused renewed need of biological control of the plant ailments. Biological control is considered as non-hazardous, eco-friendly and sustainable approach of disease management in crop plants.

During last two decades many serious soil-borne root diseases of field and horticultural crops have been reported to be successfully managed using microbial antagonists (Anahosur *et. al.,* 1998). However, not much work has been done on management of *Fusarium* wilt of cumin under field conditions. Keeping these facts in view and seriousness of the disease the present investigation was planned using these bioagents for seed treatment as alone and in combinations to study their efficacy in controlling cumin wilt under field conditions of arid regions of Rajasthan.

Materials and Methods

Effect of three organic amendments viz., farm yard manure, vermicompost and mustard cake on survival of bioagents i.e. *T. harzianum, T. viride, P. fluorescens* and *B. subtilis* in soil under green house conditions. For this purpose talc based formulations of bioagents were applied in organic amended soils. Bareja and Lodha (2002) studied the survival of *Trichoderma harzianum* in different composts prepared from on farm wastes, farm yard manure and soil. Influence the population of microbial antagonists in presence of organic amendment in soil reported by Chattopadhaya (1999; Bora, 2000).

Effect of organic amendments on survival of bioagents in soil

Effect of three organic amendments viz., farm yard manure, vermicompost and mustard cake on survival of four test bioagents i.e. *T. harzianum, T. viride, P. fluorescens* and *B. subtilis* under green house condition. The earthen pots were filled with natural field soil and amended with farm yard manure at 20 g kg^{-1} soil, vermicompost at 10 g kg^{-1} soil and mustard cake at 2 g kg^{-1} soil. Talc based formulations of four respective bioagents were applied to amended soil at 2 g kg^{-1} soil. In case of control the test bioagents were added separately to unamended soil. Each treatment was replicated thrice. The pots were irrigated regularly.

The population of four respective bioagents present in amended and unamended soils was enumerated immediate after application of bioagent and at monthly interval up to eight months of addition to soil. The details of soil processing i.e. serial dilutions and media used for determining the population dynamics of the individual bioagent are described below.

Enumeration of *T. harzianum* and *T. viride* from soil

The experiment was conducted at Rajasthan Agricultural Research Institute Durgapura Jaipur in year 2016-17. Soil samples were collected using cork borer from each pot, mixed thoroughly and air dried in shade for 48 hours. 10 g soil was added in 90 ml sterilized water in Erlenmeyer flask and shaked gently for 4-5 minutes. Serial dilutions were made from stock soil suspension upto 10^7 add 0.2 ml soil suspension of suitable dilution (depending on stage of soil sampling) was added to the surface of *Trichoderma* selective medium (Elad and Chet, 1983) and spread uniformaly with help of glass spreader. The inoculated petridishes were incubated at 25^0C for 5 to 6 days and the *T. harzianum* and *T. viride* colonies developed were counted. Techniques for mass multiplication of *Trichoderma* spp. was reported (Kousalyagangadharan and Jeyarajan, 1988;

Papavizas, 1985; Panicker and Jeyarajan; 1993).

Enumeration *Pseudomonas fluorescens* from soil

As described above, soil samples were collected mixed thoroughly and air dried in shade for 48 hours. Stock soil solution was prepared by taking 10 g soil in 90 ml sterile distilled in Erlenmeyer flask and shaked gently for 2 to 4 minutes. Serial dilutions were prepared from the stock soil suspension upto 10^{14}.

A 0.2 ml soil suspension of suitable dilution, depending on time of soil sampling was added on surface of Pseudomonas agar fluorescens (PAF) media in petridishes and spread uniformly with the help of glass spreader. The inoculated petridishes were incubated at 27°C for 24 hours and the colonies appeared were counted.

Enumeration of *Bacillus subtilis* from soil

The method of collection of soil samples and preparation of serial dilutions were similar to that of *Pseudomonas fluorescens*. Serial dilutions were prepared up to 10^{12} using the stock soil suspension. In this case also a 0.2 ml suspension was transferred to surface of nutrient agar medium in petridishes and spread uniformly with the help of glass spreader. The inoculated petridishes were incubated 25^0C for 48 hours and the colonies appeared were counted.

Results and Discussion

Survival of bioagent in soil

The four respective bioagents were enumerated in selective media at monthly interval up to 180 days of soil application of bioagents. Population of *T. harzianum* was significantly higher in soils amended with farm yard manure, vermicompost or mustard cake. The recovery of this bioagent was higher at 30 days of applications compared to 0 days i.e. immediate after soil application. The population of the bioagent started reducing after 60 days of soil application i.e. mid January. The population was gradually reduced during the months of February, March, April and May i.e. up to 180 days of soil application in all the amended soils and control. The survival of the bioagent was relatively better in mustard cake amended soil as compared to farm yard manure and vermicompost amended soils. Whereas, survival of *T. harzianum* was relatively less as compared to amended soils all through out the study i.e. up to 180 days of soil application. Saju 2002; reported *T. harzianum* using organic matter enhance the farm production (Table 1).

The population of *T. viride* was significantly higher in three amended soil as compared to unamended control like *T. harzianum*, the population of *T. viride* in soil increased in all the three amended soils and also in control at 30 days of applications. In case of *T. viride* also, the population level started declining after 60 days of soil application. A gradual decrease in population of this bioagent was recorded up to 180 days of soil application i.e. during mid May in amended as well as in unamended soils. This bioagent survive better in mustard cake or vermicompost amended soils in comparison to farm yard manure amended soil. In this case, also survival was considerably less in unamended control soil as compared to amended soils (Table 2).

The population of *P. fluorescens* was considerably higher in amended soils as compared to control i.e. unamended soil. The population of this bacterium in soil gradually decreased from 30 days of soil applications onwards up to 180 days. The decreased in population of this bacterium was faster after 90 days i.e. March, April and May irrespective of type of amendment used.

Table.1 Effect of organic amendments on population of *Trichoderma harzianum* in soil (CFU g-1 soil) under green house condition

Soil amendments	Dose (g kg-1 soil)	Days after application						
		0 Day (x 106)	30 Day (x 106)	60 Day (x 105)	90 Day (x 105)	120 Day (x 104)	150 Day (x 104)	180 Day (x 103)
Farm yard manure	20	18.25	24.25	28.75	25.00	12.25	10.00	**22.50**
Vermicompost	10	19.50	23.75	25.50	22.50	10.50	8.25	**20.75**
Mustard cake	2	20.75	24.75	33.75	29.50	13.25	11.50	**24.00**
Control (without organic amendment)	-	19.00	17.25	15.00	11.50	5.75	3.25	**4.75**
S.Em ±		0.62	0.25	0.62	0.69	0.26	0.31	**0.42**
CD (P=0.05)		**NS**	**0.77**	**1.91**	**2.13**	**0.80**	**0.94**	**1.30**

Table.2 Effect of organic amendments on population of *Trichoderma viride* in soil (CFU g-1 soil) under green house condition

Soil amendments	Dose (g kg-1 soil)	Days after application						
		0 Day (x 106)	30 Day (x 106)	60 Day (x 105)	90 Day (x 105)	120 Day (x 104)	150 Day (x 104)	180 Day (x 103)
Farm yard manure	20	15.25	21.00	22.00	21.00	9.50	6.25	**18.50**
Vermicompost	10	20.00	28.25	35.75	27.25	13.00	10.75	**22.50**
Mustard cake	2	23.00	27.25	36.50	29.50	12.50	9.00	**25.75**
Control (without organic amendment)	-	18.00	17.25	15.50	12.25	7.00	4.00	**6.00**
S.Em ±		1.81	0.30	0.59	0.55	0.20	0.44	**0.38**
CD (P=0.05)		**NS**	**0.92**	**1.82**	**1.69**	**0.63**	**1.37**	**1.16**

Table.3 Effect of organic amendments on population of *Pseudomonas fluorescens* in soil (CFU g-1 soil) under green house condition

Soil amendments	Dose (g kg-1 soil)	Days after application						
		0 Day (x 1014)	30 Day (x 1012)	60 Day (x 1011)	90 Day (x 1011)	120 Day (x 109)	150 Day (x 107)	180 Day (x 106)
Farm yard manure	20	16.25	20.25	23.75	12.75	11.50	13.00	**14.75**
Vermicompost	10	20.75	26.00	28.00	14.25	13.25	15.25	**17.50**
Mustard cake	2	22.25	29.50	29.25	16.25	15.50	17.00	**22.00**
Control (without organic amendment)	-	18.00	16.00	15.00	13.00	8.00	5.25	**6.00**
S.Em ±		1.47	0.86	0.40	0.46	0.38	0.49	**0.76**
CD (P=0.05)		**NS**	**2.66**	**1.22**	**1.42**	**1.16**	**1.51**	**2.34**

Table.4 Effect of organic amendments on population of *Bacillus subtilis* in soil (CFU g-1 soil) under green house condition

Soil amendments	Dose (g kg-1 soil)	Days after application						
		0 Day (x 1012)	30 Day (x 1012)	60 Day (x 1011)	90 Day (x 1011)	120 Day (x 109)	150 Day (x 107)	180 Day (x 106)
Farm yard manure	20	13.75	18.50	19.25	10.75	8.75	10.25	**14.00**
Vermicompost	10	14.25	19.00	20.75	11.25	9.50	8.75	**12.75**
Mustard cake	2	14.75	19.25	21.50	11.75	10.75	11.00	**16.75**
Control (without organic amendments)	-	13.00	12.00	11.00	8.00	6.00	4.00	**5.00**
S.Em ±		0.79	0.19	0.23	0.22	0.23	0.18	**0.34**
CD (P=0.05)		**NS**	**0.59**	**0.70**	**0.67**	**0.70**	**0.55**	**1.04**

Plate 1 . Pot experiment on survival of bioagents

The survival of the bacterium was higher in mustard cake and vermicompost amended soils comparison to soils amended with farm yard manure. Further, the recovery of *P. fluorescens* was quite low in unamended soil as compared to amended soils Raj and Kapoor, 1996; Rajappan *et al.*, 2002; Srivastava and Sinha, 1971 (Table 3).

The pattern of survival of B. subtilis in amended and unamended soils was similar to that of *P. fluorescens*. In this case, also the population was enhanced at 30 days of soil applications and decline gradually till 90 days i.e. mid February. The magnitude of reduction of population was higher after 120 days i.e. mid March and continued to further decline

till mid May i.e. up to 180 days of bioagent application. A perusal of the data given in table 4 showed that mustard cake and vermicompost was slightly better for survival of *B. subtilis* in comparison to farm yard manure. The survival of *B. subtilis* was relatively less in unamended control in comparison to amended soils.

Study conducted the use of four bioagents populations in presence of three amended soil showed different results. The survivability of bioagents in arid conditions was relatively better in mustard cake amended soil as compared to farm yard manure and vermi compost amended soil.
The population of *Tricoderma viride* was less in unamended soils.

Pseudomonas fluorescens population was higher in amended soil and *Bacillus subtilis* survived better in mustard cake and vermi compost amended soil as compared to farm yard manure.

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How to cite this article:

Nitin Chawla, Vipen Kumar, R. K. Bagri and Jain, S. K. 2020. Survivability of Soil Bio-agents in Presence of Organic Amendment in Arid Conditions of Rajasthan, India. *Int.J.Curr.Microbiol.App.Sci.* 9(03): 3124-3129. doi: https://doi.org/10.20546/ijcmas.2020.903.357