International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2024: 7.101

Effect of *Trichoderma* Seed Dressing on Rhizosphere Mycoflora of *Brassica juncea*

Walay Tagade

Department of Botany, C. J. Patel College Tirora, Dist - Gondia (M. S.), India
Email: *walaytagade[at]gmail.com*

Abstract: ***Rhizosphere mycoflora were isolated by serial dilution technique from two varieties of Brassica juncea (L.) czern. and coss. cv. Varuna (containing high amount of glucosinolate) and selection EH - 3 (containing very low amount of glucosinolate) were selected for rhizosohere study. The seeds of Brassica juncea cv. EH - 3 and Varuna were treated with Trichoderma - talk powder. At different stages of plant growth (Seedling, flowering and maturity) the rhizosphere mycoflora were isolated and recorded. The highest number of rhizosphere mycoflora was isolated during flowering stage. It was observed that the number of mycoflora was more in rhizosphere soil than non - rhizosphere soil. Aspergillus species was the most dominant species in the rhizosphere of Varuna and EH - 3. Other common fungi isolated from the rhizosphere soil are Curvularia sp., Alternaria sp, Fusarium sp., etc.***

Keywords: Brassica juncea, Rhizosphere, mycoflora, Aspergillus, Trichoderma - talk powder

1. Introduction

Interactions between soil microorganisms and plant roots satisfy important nutrient requirements for both plant and associated microorganisms. This interaction of plant roots and rhizosphere microorganisms is largely based on interactive modification of soil environment by processes such as the release of organic chemicals of the soil by roots, water uptake by plants, microbial production of plant growth factors and microbially mediated availability of mineral nutrients (Hinsinger, 1998). The rhizosphere is a zone of predominantly commensal and mutualistic interaction between the plant and microbes. Apart from these types of associations, antagonism also prevails amongst the biotic community of rhizosphere. Chemically and physically, the rhizosphere is the most complex and changeable to environment (Curl and Truelove, 1986). Plant roots are known to influence aggregation of soil particles in the rhizosphere. The pattern of exudation and the composition of root exudates play a key role in the success or failure of infection by soil - borne pathogens (Schroth and Hildebrand, 1964). Attempts have been made in recent years to influence the rhizosphere microflora through various extraneous means like soil amendments with organic matter and fertilizers, foliar applications of various nutrients, antibiotics, growth regulators and fungicides (Annapurna and Rao, 1982). Such changes are readily reflected in the plant rhizosphere (Dublish, 1986).

India is the major mustard oil producing country and cultivated in about 6.8 million hectares. Many states like Rajasthan, Punjab, Gujarat, Haryana, and Madhya Pradesh are major mustard oil producing states of India. White rust (*Albugo candida* (Lev.) Kuntze) of mustard is a serious widespread disease causing leaf phase infection up to 27.4 % (Saharan and Lakra, 1988). Whereas, *Alternaria* blight is caused by *Alternaria brassicae* (Berk.) Sacc. is the most important disease and it occurs every year in all the rapeseed – mustard growing states of India. This disease causes an average yield loss of 46 - 47 % in yellow sarson and 35 – 38% in mustard (Kolte et al., 1987). In addition to direct yield losses, the disease adversely affects the seed quality by reducing seed size, seed discolouration and reduction in oil contents (Kaushik et al., 1984). Other soil born disease is *Sclerotinia* rot of mustard caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is potentially a serious threat, generally it infect all above ground parts of the plants but the most destructive symptoms is on stem. With regard to all these above mentioned diseases, it is important to know how the rhizosphere of *Brassica* plant behaves in different stages of plant growth.

With respect to economical importance of mustard in India and worldwide the present study is based on two varieties of *Brassica juncea* (L.) czern. and coss. cv. Varuna (containing high amount of glucosinolate) and selection EH - 3 (containing very low amount of glucosinolate).

2. Material and Methods

1) **Description of the study area:** To investigate the rhizosphere mycoflora of *Brassica juncea* var. Varuna and cultivar line EH - 3 were selected for experimental study. The seeds were procured from the Dhara Vegetable Oil and Foods Company Ltd. (DOFCO) sponsored project, Department of Botany, R. T. M. Nagpur University, Nagpur, Maharashtra, India. The seeds were grown in the field and foliar applications were made to alter the rhizosphere mycoflora. (Plate I).
2) **Seed Dressing:** Antagonistic effect of *Trichoderma* was checked by seed dressing technique. For this *Trichoderma* powder was made as per the procedure of *Trichoderma* - Talc - powder formulation (Sinha, 2001). *T. viride* isolate II was grown on PDA plate for 8 days. Peptone dextrose broth was prepared and dispensed at the rate of 100 ml in each 250 ml Erlenmeyer flask. Discs were cut with sterilized cork borer from the plate cultures of *T. viride* II and inoculated at 28 ± 10C for 7 days.
3) **Collection of rhizosphere soil samples:** Soil samples were collected from the Experimental field of Post Graduate Teaching Department of Botany; RTM Nagpur University Campus Nagpur, Maharashtra, India. Roots with adhering soils of healthy plants and NRS

(Non - rhizosphere soil) were collected and transferred to the sterile plastic bags and brought to the Laboratory for experimental purposes. (Plate II)

4) **Isolation of Rhizosphere Mycoflora:** Rhizosphere mycoflora were isolated by serial dilution plate technique (Johnson and Curl, 1972) on Czepak's dox agar medium. After incubation the number of colonies appeared on plates were counted and percent occurrence of particular fungi was calculated. (Plate III)
5) **Identification of Mycoflora:** The fungi isolated from rhizosphere and rhizoplane were identified with the help of standard literature available in the Department. (Nagmani et. al.2006, Booth, 1977, Raper and Fennell, 1965 and Raper and Thom, 1949.).

Plate I: *Brassica juncea* cv EH-3 and Varuna field at Department of botany, R. T. M. Nagpur University, Nagpur

Plate II: A-Rhizosphere soil with roots of EH-3, B-Rhizosphere soil with roots Varuna

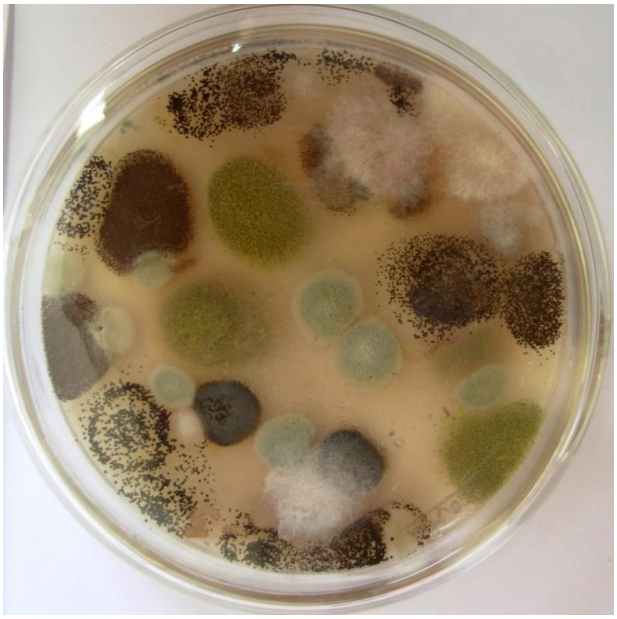

Plate III: Rhizosphere mycoflora of Brassica juncea by serial dilution technique

3. Results and Discussion

The seed dressing technique was used for biological control of seed borne plant pathogens. During the investigation *Trichoderma* isolate II was used as biological control agent while doing this its effect on rhizosphere mycoflora was also observed. Due to *Trichoderma* seed dressing (TSD), the rhizosphere mycoflora was significantly altered as compared to control plants of EH - 3 and Varuna. The percent occurrence of *Aspergillus* sp. was significantly increased in rhizosphere of EH - 3 and Varuna. Apart from this percent occurrence of *Curvularia* sp., *Fusarium* sp. and *Rhizopus* sp. also increased. However, occurrence of *Penicillium* sp. and *Alternaria* sp. was decreased in rhizosphere of EH - 3 and Varuna, respectively. The occurrence of *Cladosporium* sp. and *Helminthosporium* sp was increased in rhizosphere of EH - 3. It was also observed that *Chaetomium globosum* and Mycelia sterilia were not isolated from rhizosphere of EH - 3 and Varuna (Fig.1).

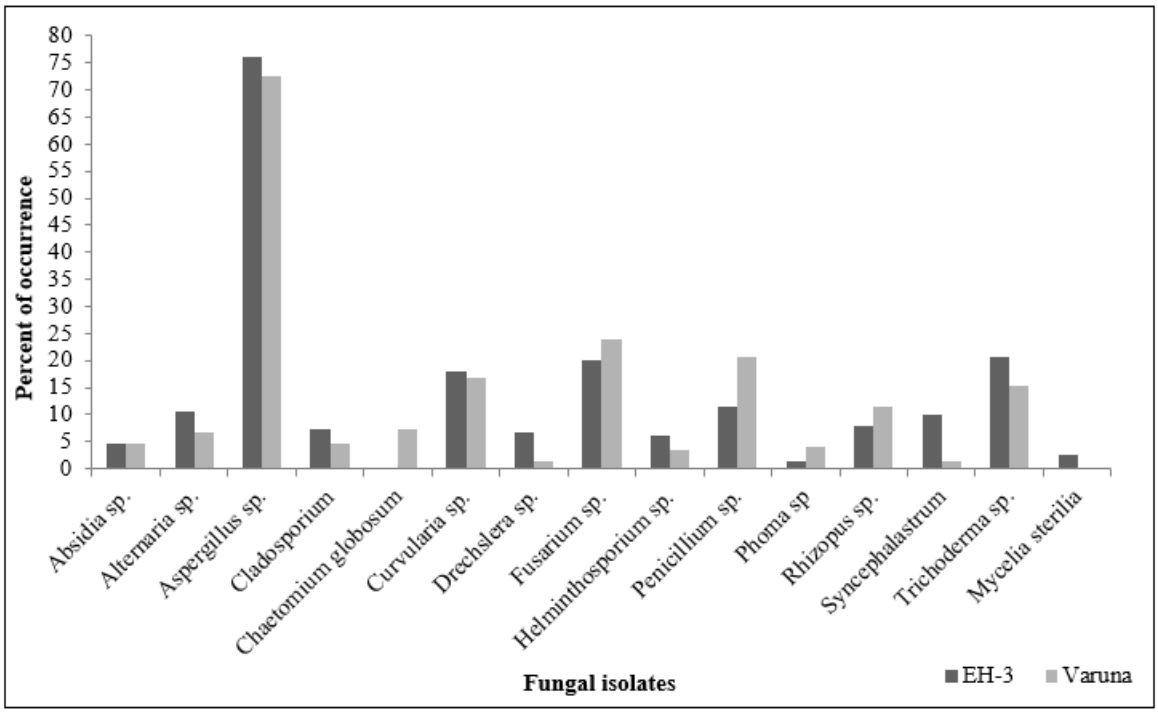

Figure 1: Percent occurrence of fungi in TSD treated plants

During the study 32 and 33 fungi were recorded from TSD plants of EH - 3 and Varuna respectively. *Aspergillus niger*, *A. fumigatus*, *A. nidulans*, *A. terreus*, *A. ochraceus*, *Curvularia lunata*, *Penicillium multicolor*, *Trichoderma* isolate I and II and *Syncephalastrum* was isolated throughout from TSD plants of EH - 3 whereas, *A. niger*, *A. nidulans*, *Cladosporium*, *Curvularia lunata*, *Fusarium graminearum*, *Fusarium oxysporum*, *Chaetomium globosum*, *Penicillium oxalicum*, *Trichoderma* isolate II and *Rhizopus* sp. were isolated throughout from the TSD plants of Varuna (Table 1).

Table 1: Percent occurrence of non - rhizosphere, rhizosphere soil and effect of *Trichoderma* on *Brassica juncea* (EH - 3 and Varuna) during various growth stages (cfug $^{-1}$X 10^3)

S. No.	Fungal isolates	NRS			Control						EH - 3			Varuna		
					EH - 3			Varuna			TSD			TSD		
		S	F	M	S	F	M	S	F	M	S	F	M	S	F	M
1	*Absidia* sp.	-	5.26	6.90	3.23	-	1.37	4.84	-	5.19	-	-	7.07	3.26	3.70	-
2	*Alternaria alternata*	-	5.26	-	-	7.32	9.59	6.45	7.41	10.39	-	2.65	7.07	-	4.63	5.49
3	*Alternaria* sp.	-	-	5.17	4.84	-	-	-	3.70	3.90	-	2.65	3.03	-	-	-
4	*Aspergillus clavatus*	-	-	-	-	2.44	-	-	-	-	-	-	-	-	-	5.49
5	*Aspergillus fischeri*	-	-	-	-	-	-	-	-	6.49	-	6.19	-	-	5.56	4.40

6	*Aspergillus flavipes*	-	-	-	-	-	-	-	3.70	-	-	-	-	-	-	-
7	*Aspergillus flavus*	9.43	7.02	12.07	6.45	4.88	4.11	8.06	-	3.90	4.30	2.65	-	-	-	5.49
8	*Aspergillus fumigatus*	13.21	12.28	8.62	3.23	7.32	5.48	8.06	8.64	7.79	5.38	5.31	8.08	5.43	6.48	4.40
9	*Aspergillus nidulans*	-	-	-	4.84	3.66	8.22	6.45	3.70	2.60	7.53	4.42	4.04	8.70	4.63	5.49
10	*Aspergillus niger*	7.55	5.26	15.52	9.68	6.10	8.22	11.29	7.41	5.19	11.83	7.08	6.06	9.78	6.48	7.69
11	*Aspergillus ochraceus*	-	5.26	-	-	4.88	-	-	4.94	-	-	4.42	7.07	-	-	8.79
12	*Aspergillus sulphureus*	-	-	10.34	4.84	-	-	6.45	-	-	-	-	7.07	4.35	-	-
13	*Aspergillus sydowii*	5.66	-	-	8.06	-	-	-	-	-	-	-	3.03	-	-	-
14	*Aspergillus terreus*	-	5.26	6.90	6.45	8.54	5.48	-	-	-	7.53	5.31	5.05	5.43	-	5.49
15	*Aspergillus wentii*	9.43	-	-	-	-	-	-	-	-	-	-	-	6.52	3.70	-
16	*Cladosporium* sp.	5.66	7.02	6.90	-	3.66	-	6.45	6.17	-	6.45	4.42	-	-	4.63	2.20
17	*Chaetomium globosum*	-	-	-	-	-	8.22	-	6.17	6.49	-	-	-	2.17	6.48	2.20
18	*Curvularia lunata*	5.66	5.26	3.45	11.29	7.32	4.11	9.68	8.64	5.19	4.30	5.31	7.07	8.70	9.26	5.49
19	*Curvularia* sp.	-	-	-	4.84	4.88	8.22	-	2.47	9.09	-	5.31	4.04	2.17	-	-
20	*Drechslera* sp.	-	-	-	-	4.88	6.85	-	3.70	-	4.30	-	6.06	-	-	2.20
21	*Fusarium graminearum*	5.66	-	-	-	-	-	-	3.70	2.60	-	-	6.06	6.52	4.63	5.49
22	*Fusarium oxysporum*	11.32	7.02	6.90	9.68	7.32	6.85	8.06	4.94	6.49	7.53	7.08	-	6.52	5.56	6.59
23	*Fusarium* sp.	-	-	-	4.84	-	-	6.45	2.47	-	3.23	5.31	-	-	1.85	-
24	*Helminthosporium* sp.	-	-	-	-	3.66	-	-	-	5.19	3.23	5.31	-	-	3.70	1.10
25	*Penicillium citrinum*	-	8.77	-	-	7.32	8.22	3.23	-	-	4.30	-	-	-	3.70	5.49
26	*Penicillium liliacinum*	-	8.77	-	-	3.66	-	-	2.47	6.49	-	-	2.02	-	-	-
27	*Penicillium multicolor*	7.55	-	-	9.68	-	-	-	3.70	-	-	0.88	-	5.43	-	-
28	*Penicillium oxalicum*	5.66	-	-	-	-	4.11	4.84	1.23	-	-	2.65	-	3.26	0.93	-
29	*Penicillium purpurogenum*	-	-	-	-	-	-	4.84	-	-	7.53	-	-	3.26	-	-
30	*Penicillium* sp.1	-	-	6.90	-	-	-	-	-	-	-	-	-	-	2.78	-
31	*Penicillium* sp.2	-	-	-	-	1.22	5.48	-	-	-	-	-	-	-	6.48	-
32	*Phoma* sp.	-	-	-	-	4.88	-	-	4.94	3.90	-	-	2.02	6.52	-	-
33	*Rhizopus stolonifer*	9.43	8.77	5.17	8.06	6.10	5.48	4.84	6.17	9.09	6.45	5.31	-	6.52	7.41	3.30
34	*Syncephalastrum* sp.	3.77	8.77	5.17	-	-	-	-	3.70	-	4.30	3.54	7.07	-	1.85	-
35	*Trichoderma isolate I*	-	-	-	-	-	-	-	-	-	6.45	6.19	3.03	-	2.78	7.69
36	*Trichoderma isolate II*	-	-	-	-	-	-	-	-	-	5.38	4.42	5.05	5.43	2.78	5.49
37	*Mycelia sterilia*	-	-	-	-	-	-	-	-	-	-	3.54	-	-	-	-

NRS= Non - rhizosphere soil, RS = Rhizosphere soil, S = Seedling (20 days), F - Flowering (45 days), M - Maturity (95 days), TSD - Trichoderma seed dressing.

Occurrence of fungi varied with the age of plant growth. *Aspergillus niger* was observed as the most dominant fungus during seedling and flowering stages. In addition to this *Fusarium oxysporum* was also dominant fungus during flowering stage. Whereas, *Aspergillus fumigatus* was observed as the most dominant during maturity stage in TSD treated plants of EH - 3. Simultaneously, in TSD plants of Varuna rhizosphere mycoflora was significantly altered at various growth stages. *Aspergillus niger* was occurred as the most dominant fungus at seedling stage. While *Curvularia lunata* and *Aspergillus ochraceus* were reported to be the dominant fungi (Table 1).

Seed treatment with biocontrol agents for disease management particularly against soil - borne pathogens was attempted by many workers like Papavizas (1985), Kehri and Chandra (1991) Harman and Hadar (1983) reported that seed treatment with *Trichoderma* spp. prevents emergence of damping off and Suriachardraselvan et al., (2004) also used the seed treatment technique with the help of *Trichoderma* spp. for the control of Charcoal rot in sunflower caused by *Macrophomina phaseolina*.

The result showed that *Trichoderma viride* I and II occurred throughout the growth of plants. Similar results were obtained by Roy and Pan (2004). They showed that all the isolates (wild and mutants) of *Trichoderma harzianum* and *T. virens* multiplied in groundnut rhizosphere soil at different stages of plant growth. Shankar and Jeyarajan (1996) stated that proliferation of *Trichoderma* population in the rhizosphere takes place due to seed treatment with *Trichoderma* sp.

Apart from *Trichoderma* species other fungi like *Aspergillus fischeri*, *Fusarium graminearum*, *Penicillium purpurogenum*, *Syncephalastrum* and Mycelia sterilia were isolated from the rhizosphere of TSD plant of EH - 3 while *Aspergillus clavatus*, *A. flavipes*, *A. sydowii*, *A. terreus*, *A. wentii*, *Penicillium citrinum*, *Penicillium* sp 1 and 2 were isolated from the rhizosphere of TSD of Varuna plants. However, few fungi were inhibited due to seed dressing of *Trichoderma viride*. Howell *et al.*, (1997) stated that the combination of *Trichoderma virens* and fungicide (Metalaxyl) were most effective against the seedling diseases of cotton.

Alternaria sp. was not isolated from the rhizosphere of treated plats of EH - 3 and Varuna. Similarly, *Aspergillus clavatus*, *Chaetomium globosum* and *Penicillium* sp.2 were not isolated from the TSD treated plants of EH - 3 while *P. lilacinum* was not isolated from the rhizosphere of TSD treated plants of Varuna (Table 7 and 8). The *Alternaria alternata* occurred only at maturity stage in TSD treated plants of EH - 3 and Varuna while in control plants of EH - 3 it was observed at flowering and maturity stage whereas in control plants of Varuna it was present through out the growth of Plants. Kaur and Mukhopadhyay (1992) and Mukhopadhyay and Mukherjee (1991) have also demonstrated effectiveness of *Trichoderma* and *Gliocladium*

against the chickpea wilt complex in glass house and in field trials.

References

[1] Annapurna, J., Rao, R. P., 1988. Foliar spray of fungicides and rhizosphere microflora of maize. Indian Phytopath.41 (4), 558 - 561.
[2] Booth C.1977. Fusarium, Laboratory guide. CMI, Kew, Surrey, England, 1 - 237
[3] Curl, E. A., Truelove, B., 1986. The Rhizosphere. Springer Verlag, Berlin Heidelberg, New York.
[4] Dublish, P. K., 1986. Foliar application of antibiotics, growth harmones and urea in relation to rhizosphere mycoflora of A. esculentus and Lagenaria vulgaris. Indian Phytopath.39 (2), 264 - 268.
[5] Hinsinger, P., 1998. How do plant roots acquire mineral nutrients? Chemical processes involved in the rhizosphere. Adv. Agron.64, 225 - 265.
[6] Howell, C. R., DeVay, J. E., Garber, R. H., Batson W. E., 1997. Field control of cotton seedling diseases with Trichoderma virens in combination with fungicides seed treatments. The Journal of Cotton Science 1, 15 - 20.
[7] Johnson, L. F., Curl, E. A., 1972. Methods for research on the ecology of soil - borne pathogens. Burgess Publishing Company. pp.7 - 15.
[8] Kaur, N. P., Mukhopadhyay, 1992. Integrated control of chickpea wilt complex by Trichoderma and chemical methods in India. Trop. Pest management.38, 20 - 23.
[9] Kaushik, C. D., Saharan, G. S., Kaushik, J. C., 1984. Magnitude of loss in yield and management of Alternaria blight in rapeseed mustard. Indian Phytopath.37, 398.
[10] Kehri, H. K., Chandra, S.1991. Antagonism of Trichoderma viride to Macrophomina phaseolina and its application in the control of dry root rot of mungbean. Indian Phytopath.44, 60 - 63.
[11] Kolte, S. J., Awasthi, R. P., Vishwanath, 1987. Assessment of yield losses due to Alternaria blight in rapeseed and mustard. Indian Phytopath.40, 209 - 211.
[12] Mukhopadhyay, A. N., Mukharjee, P. K., 1991. Innovative approaches in biological control of soil borne disease in chickpea. Fourth Int. Trichoderma and Gliocladium workshop. Belgrade, Italy, July 17 - 20. Petria 1, 146.
[13] Nagmani A., Kunwar I. K., and Manoharachary C.2006. Hand book of Soil fungi, I. K., International Private limited.1 - 496.
[14] Papavizas, G. C., 1985. Trichoderma and Gliocladium: biology, ecology and potential for biocontrol. Ann. Rev. Phytopathol.23: 23 - 54.
[15] Raper K. B. and Fennell D. I.1965. The genus Aspergillus. The Williams and Wilkins Company, Baltimore 1 - 686.
[16] Raper K. B. and Thom C.1949. A manual of the Penicillia. Williams & Wilkins Company, Baltimore 1 - 875.
[17] Roy, A., Pan, S., 2004. Comparative study on rhizosphere colonization by some mutant and wild isolates of Trichoderma species. Indian Phytopath.57 (3), 255 - 259.
[18] Saharan, Lakra, 1988. Correlation of leaf and stag - head infection intensities of white rust with yield components of mustard. Indian J. Mycol. Plant Pathol.18, 81.
[19] Schroth, M. N., Hildebrand, D. C., 1964. Influence of plant exudates on root infecting fungi. Ann. Rev. Phytopathology 2, 101 - 132.
[20] Shankar, P., Jeyarajan, R., 1996. Seed treatment formulation of Trichoderma and Gliocladium for biological control of Macrophomina phaseolina in Sesamum. Indian Phytopath.49, 148 - 151.
[21] Suriachardraselvan, M., Salalrajan F., Aiyanathan, K. E. A., Seetharaman, K., 2004. Seed treatment with Trichoderma spp. for the control of Charcoal Rot in sunflower caused by Macrophomina phaseolina. J. Mycol. Pl. Patnol., 34 (2), 366 - 368.