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RESEARCH ARTICLE Agricultural Science Digest

Bio-efficacy of Antagonists and its Culture Filtrates against Black Mould Rot [*Aspergillus niger* Van Tieghem] of Garlic (*Allium sativum* L.)

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10.18805/ag.D-5307

ABSTRACT

Background: Black mould rot of garlic caused by *Aspergillus niger,* is the most destructive disease in storage as well as in field conditions. The present investigation was carried out to evaluate bio-efficacy of antagonists and culture filtrates of *Trichoderma* spp., against black mould rot of garlic.

Methods: For eco-friendly and effective management of black mold rot of garlic, eight antagonist and culture filtrates of six *Trichoderma* spp. were selected and screened by dual culture and poisoned food technique.

Result: Among all the antagonistic fungus and bacteria, *Trichoderma asperellum* and its cultural filtrate were found effective.

Key words: Antagonist, *Aspergillus niger*, Black mould, Culture filtrates, Garlic.

INTRODUCTION

Garlic (*Allium sativum* L.) is the most important bulbous crop after onion, belonging to *Amaryllidaceae* family (Mishra *et al*., 2014). Garlic is cultivated in India as the second major bulbous crops next to onion, covering an area of 227.39 thousand hectares with a total production of 1181.37 thousand MT having productivity of 5195 kg per hectare (Anon., 2015a). Garlic crop suffers from many diseases post-harvest diseases *viz*., white rot (*Sclerotium cepivorum* Berk), blue mold rot (*Penicillium* spp.), black mold (*A. niger*), pink rot (*Pyrenochaeta terrestis* Hansen), internal bulb rot (*Macrophomina phaseolina* (Tassi) Goid, basal rot (*Fusarium oxysporum* f. sp. *cepae*) and neck rot (*Botrytis allii* Munn) (Anonymous., 2015b), which cause considerable losses, apart from reduction in crop yields. The diseases also pose harmful effects during harvesting, post harvesting, processing and marketing stages, which lower the quality and export potentiality of the crop that significantly cause the economic loss (Mishra *et al*., 2014).

Black mould rot is caused by *Aspergillus niger,* is the most common fungi causing food spoilage and bio deterioration of other materials (Samson *et al*. 2004). Black mold is responsible for incurring losses to the extent of 12-25 percent due to various bulb rots and total losses during storage of 5-6 months as high as 30-40 per cent (Maini and Chakravarti, 2000). Nandeesha *et al*. (2013) reported that *Trichoderma* spp. inhibited 81.36 per cent growth of *A. niger* by dual culture method. *Trichoderma* spp. is well documented as effective biological control agent of plant diseases (Harman *et al*. 1980; Sivan *et al*. 1984 and Coley-Smith *et al*. 1991). Therefore, the present investigation was carried out to evaluate bio-efficacy of antagonists and culture filtrates of *Trichoderma* spp., against black mold rot of garlic.

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How to cite this article: Chavda, H.C. and Rathwa, M.G. (2023). Bio-efficacy of Antagonists and its Culture Filtrates against Black Mould Rot [*Aspergillus niger* Van Tieghem] of Garlic (*Allium sativum* L.). Agricultural Science Digest. doi: 10.18805/ag.D-5307.

Submitted: 05-02-2021 **Accepted:** 13-01-2023 **Online:** 01-02-2023

MATERIALS AND METHODS

The present study was carried out in the Department of Plant Pathology, B.A. Collage of Agriculture, Anand Agricultural University, Anand, Gujarat during 2015-16. The Completely Randomized Design (CRD) was used for the experimentation.

Collection and isolation of pathogen

Garlic bulb showing typical symptoms of black mold rot were brought to the laboratory and pathogen isolated by tissue isolation method under aseptic conditions. The pure culture of pathogen was obtained by single spore isolation and maintained on Potato Dextrose Agar (PDA) medium. The pathogen was identified by morphological characteristics and molecular studies. The molecular identification was carried out by DNA barcoding using ITS region sequencing. Various antagonists *viz*., *Trichoderma viride, T. harzianum, T. virens, T. atroviride, T. asperellum, T. fasciculatum, Pseudomonas fluorescens* and *Bacillus subtilis* were available in the Department of Plant Pathology, B.A. College of Agriculture, Anand Agricultural

University, Anand, Gujarat screened to test their efficacy against *A. niger*.

Dual culture method

The dual culture method (Dennis and Webster, 1971) was used to test the antagonists against *A. niger.* The antagonists and pathogen were grown on PDA medium. Sterilized PDA medium (20 mL) was poured aseptically in 90 mm diameter sterilized Petri plate. Mycelial disc of 5 mm diameter cut from the periphery of both antagonist and test pathogen and were placed at 50 mm apart from each other in Petri plates. The test pathogen was grown alone in petri plate for comparison. Each treatment was repeated three times. The Petri plates were incubated at 25±1°C in BOD incubator. The observations on mycelial growth (mm) and per cent growth inhibition of test pathogen were recorded after 7 days of incubation. The per cent growth inhibition (PGI) of pathogen in each treatment was calculated by formula given by Asalmol *et al.* (1990).

Bulb dip method

Pre- inoculation and post-inoculation treatment was employed by using bulb dip method (Chastagner and DeBauw, 2011). In pre-inoculation, healthy, mature and uniform sized garlic bulbs were first randomly pin-pricked and then dipped in the spores suspension (10^6 spores/mL) and LCF of *Trichoderma* spp. for 5 min and allowed to air dried overnight and then inoculated with the test pathogen (10^6 spores/mL). The interval between spore suspension treatment and inoculation was kept 12 hours and were incubated at room temperature. Control was maintained separately with pathogen. Each treatment was replicated three times. The per cent rot severity was recorded on 7th and 14th day after inoculation with the help of formula given by Siyom and Yusuf (1990). In post-inoculation, the procedure detailed in pre-inoculation was followed except that the bulbs were first inoculated with pathogen and then with antagonists.

Effect of liquid culture filtrate (LCF) of *Trichoderma* spp. against *A. niger*

Bioefficacy of liquid culture filtrate of different *Trichoderma* spp. were evaluated by "Poisoned food technique" suggested by Nene and Thapliyal (1979) against *A. niger in-vitro*. Conical flask containing 100 mL of sterilized Potato Dextrose Broth (PDB) medium was inoculated with 5 mm diameter mycelial disk cut from margin of seven days old culture of six *Trichoderma* spp. The flasks containing individual bio-agents were incubated in an orbital shaker cum BOD incubator at 27±1°C for 21 days. After 21 days of incubation the culture filtrates were filtered separately by what man filter paper No. 1 to remove mycelial mat and the spores were removed by vacuum filter. The LCF of *Trichoderma* spp. (300 µL/ mL of PDA) were added to PDA medium separately and after solidification; 5 mm discs of *A. niger* were inoculated in the centre of each Petri plates separately. Petri plates inoculated with *A. niger,* without amendment of filtrate served as a control. The inoculated Petri plates were incubated in BOD incubator at 27±2ºC. Each treatment was repeated three times and the per cent mycelial growth inhibition of the pathogen was recorded after 7 days of incubation. Further, pre and post inoculation treatment was given to bulbs through bulb dip method as described earlier in antagonist study.

RESULTS AND DISCUSSION

Dual culture method

The lowest mycelial growth *i.e.* 24.33 mm with highest percent mycelial growth inhibition *i.e.* 72.96 was recorded in *T. asperellum,* which was at par with *T. harzianum*. While mediocre effect on per cent mycelial growth inhibition of *A. niger* was noticed in *T. atroviride* and *T. virens i.e.* 64.07 and 53.96. Meanwhile, Per cent lowest mycelial growth inhibition was observed in *T. fasciculatum* which were at par with *P. fluorescens.* The results are presented in Table 1 and Plate 1.

Result similar to the present investigation was reported by Prajapati (2016). He observed highest mycelial growth in *T. asperellum i.e.* 78.89 per cent followed by *T. harzianum* and *T. viride i.e.* 4.44 and 71.86, after seven days of incubation against *A. niger*. Nandeesha *et al.* (2013) reported that all the fourteen isolates of *Trichoderma* sp. inhibited (81.36%) growth of *A. niger* in groundnut by dual culture method. Lone *et al.* (2012) and Bhushan *et al.* (2013) reported maximum inhibition zone created by *T. harzianum* against *A. niger* (75%) followed by *Cladosporium spherospermum* (Link) (72.2%) and *F. oxysporum* (25%) in common walnut whereas *Bacillus subtilis* against *A. flavus* and *A. terreus* was 1.0 cm and against *A. niger* was 0.9 cm and the minimum zone of 0.8 cm was recorded against *A. fumigates* and *F. oxysporum* by the bacterium in bajra, respectively by using dual culture method. Bagwan (2011) reported that the *Trichoderma* spp. effectively inhibited the growth of *A. niger* up to 98 per cent in groundnut isolate by dual culture method. Wang *et al.* (2008) found four isolates *viz*., *Bacillus amyloliquefaciens* (EXWB3), *Bacillus subtillis*

Table 1: Effect of antagonists on mycelial growth inhibition of *A. niger.*

Treatments	Mycelial growth (mm) 7 DAI*	Per cent growth inhibition (PGI)
T. viride	30.00	66.67
T. harzianum	25.33	71.85
T. virens	42.33	53.96
T. atroviride	32.33	64.07
T. asperellum	24.33	72.96
T. fasciculatum	47.67	47.04
P. fluorescens	46.33	48.52
B. subtilis	27.00	70.00
Control	90.00	-
SEm±	1.07	-
C.D. at 5%	3.18	-
C.V. %	4.57	-

strains EXWB1, EXWB2 and EXWB4 effective against eight post-harvest pathogens including *B. cinerea, A. alternata, F. oxysporum, A. niger, T. roseum, Penicillium* sp. and *Cladosporium* sp. on melon fruits. Among the *Bacillus subtillis* strains EXWB1 was found to be the most effective in controlling the fungal rots. Prajapati *et al.* (2015) reported that *Trichodema asperellum* showed maximum mycelial growth inhibition against *Sclerotium rolfsii*.

Bulb dip method

All the bioagents were further tested on the bulbs following pre- and post-inoculation treatments and results are presented in Table 2 and Plate 2. In pre-inoculation treatment, *Trichoderma asperellum* found significantly superior in reducing the black mold rot severity *i.e.* 0.49 per cent both on 7th and 14th day after inoculation followed by *T. harzianum*, *T. viride* and *T. atroviride*. Whereas *T. virens* and *T. fasciculatum* gave mediocre effect while *B. subtilis* and *P. fluorescens* found least effective in reducing black mold rot severity on 7th and 14th day after inoculation over control. In Post- inoculation treatment, the trend similar to that observed in pre-inoculation was noted in post-inoculation treatment. *Trichoderma asperellum* found significantly superior in reducing the per cent black mold rot severity *i.e.* 10.13 and 15.81 followed by *T. harzianum* (13.81 and 24.82%), *T. viride* (17.85 and 24.71%) and *T. atroviride* (29.46 and 33.83%) on 7th and 14th day after inoculation. *B. subtilis* (38.11 and 48.41%) and *P. fluorescens* (49.61 and 58.90%) found least effective. It was observed that among two treatments of inoculation, pre- inoculation treatment was found superior in managing the black mold rot severity as compared to post-inoculation.

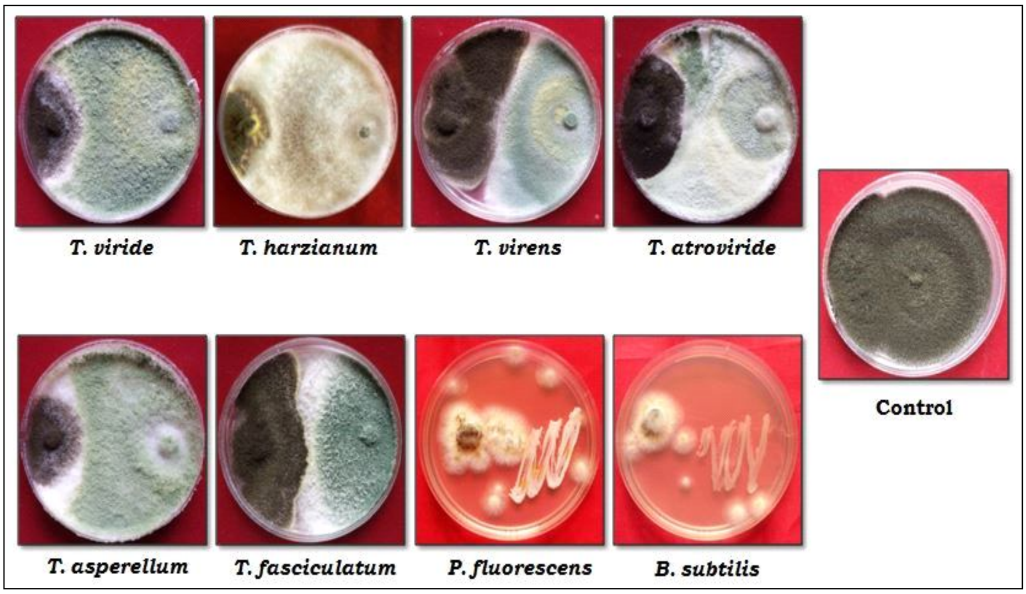

Plate 1: Bio-efficacy of antagonists against mycelial growth inhibition of *A. niger in vitro.*

Table 2: Effect of antagonists on severity of black mold rot.

Antagonist	per cent rot severity			
	Pre-Inoculation		Post-Inoculation	
	7 DAI*	14 DAI	7 DAI	14 DAI
T. viride	3.60 (0.39)**	9.96 (2.99)	17.85 (9.40)	24.71 (17.47)
T. harzianum	0.49 (0.01)	3.54 (0.38)	13.81 (5.70)	24.82 (17.62)
T. virens	16.95 (8.50)	21.07 (12.92)	37.12 (36.41)	43.74 (47.80)
T. atroviride	6.69 (1.36)	12.75 (4.87)	29.46 (24.19)	33.83 (30.99)
T. asperellum	0.49 (0.01)	0.49 (0.01)	10.13 (3.09)	15.81 (7.42)
T. fasciculatum	19.85 (11.53)	27.60 (21.46)	43.07 (46.63)	54.55 (66.36)
P. fluorescens	34.35 (31.83)	47.32 (54.04)	49.61 (58.01)	58.90 (73.31)
B. subtilis	28.46 (22.70)	37.02 (36.25)	38.11 (38.12)	48.41 (55.97)
Control	58.09 (72.05)	73.30 (91.74)	58.82 (73.19)	76.57 (94.60)
SEm±	1.53	1.26	0.64	1.24
C.D. at 5%	4.55	3.74	1.92	3.68
C.V. %	14.13	8.44	3.38	5.06

Similar trend of results was recorded by Prajapati (2016). He reported that *Trichoderma asperellum* gave cent per cent reduction in black mould rot severity after 7th and 0.33 per cent after 14th day of inoculation, followed by *T. harzianum*, *T. viride*, *T. virens*, *T. fasciculatum* and *T. atroviride* against *A. niger*. Reddy (2012) reported that *T. harzianum* was found most effective in reducing the Penicillium rot severity by 4.50 and 10.00 per cent on 4th and 8th day after inoculation. Baig *et al*. (2012) reported that *P. fluorescens* and *B. subtilis* can effectively suppress the growth of *A. niger* up to 73 and 60 per cent respectively, in groundnut seeds. Bhuvneswari and Rao (2011) reported that the *T. viride* acts as a most potent bio-agent for controlling *Aspergillus* fruit rot of mango. Meena (2006) reported that *T. viride* and *T. harzianum* at 1 $\times$ 10^{6} cfu/mL found very effective against Penicillium fruit rot of aonla. Bagwan (2003) recorded lowest Penicillium rot severity in citrus (*P. digitatum* and *P. italicum*) when the fruits were treated with *Candida oleophila, Candida* sp. and *T. viride*.

Effect of liquid culture filtrate (LCF) of *Trichoderma* spp. against *A. niger*

The lowest mycelial growth *i.e.* 28.33 mm and highest percent growth inhibition *i.e.* 68.52 was recorded in *T. asperellum* on 7th day after incubation, followed by *T. harzianum i.e.* 45 mm and 50.00, was at par with *T. atroviride* and *T. viride* 47.41 and 45.93 per cent. While, minimum mycelial growth and lowest per cent growth inhibition was recorded in *T. virens*, was at par with *T. fasciculatum*. The results are presented in Table 3 and Plate 3.

The results of present investigations corroborate with the results obtained by Prajapati (2016). He recorded highest mycelial growth inhibition by LCF of *T. harzianum* followed by *T. asperellum* and *T. viride i.e.*88.14, 87.41 and 85.56 per cent after seven days of incubation against *A. niger*. Odebode (2006) reported that the culture filtrates of *T. harzianum* revealed highest per cent mycelial growth inhibition *i.e.* 45.60 against *A. niger*. Rajendran *et al*. (2010) studied inhibitory effect of culture filtrate (50% v/v) of *T. viride* on mycelium growth inhibition of *A. niger, A. fumigatus* and *A. flavus* and reported 64, 49 and 48 per cent mycelial growth inhibition, respectively in fruits and vegetables through poisoned food technique. Terna *et al.* (2013) evaluated antagonistic potential of culture filtrates of a soil isolate of *T. viride in vitro*, against three common post-harvest rot pathogens; *A. niger*, *A. flavus* and *F. oxysporum*. They observed that the synergistic combination of all three enzyme-induced culture filtrates (chitinase, protease and cellulase) of *T. viride* gave the highest radial growth inhibitions by *A. niger* (77.69%), *A. flavus* (67.89%) and *F. oxysporum* (46.95%), respectively. Kataty and Emam (2012) found that, culture filtrate of *T. harzianum* (T3 and T24) greatly inhibited spore germination of the test post-

Table 3: Effect of culture filtrates of *Trichoderma* spp. on growth inhibition of *A. niger.*

Trichoderma spp.	Mycelial growth (mm) 8 DAI	Percent growth Inhibition (PGI)
T. viride	48.67	45.93
T. harzianum	45.00	50.00
T. virens	64.33	28.52
T. asperellum	28.33	68.52
T. fasciculatum	64.33	28.52
T. atroviride	47.33	47.41
Control	90.00	0.00
SEm±	1.89	-
C.D. at (5%)	5.72	-
C.V. %	5.89	-

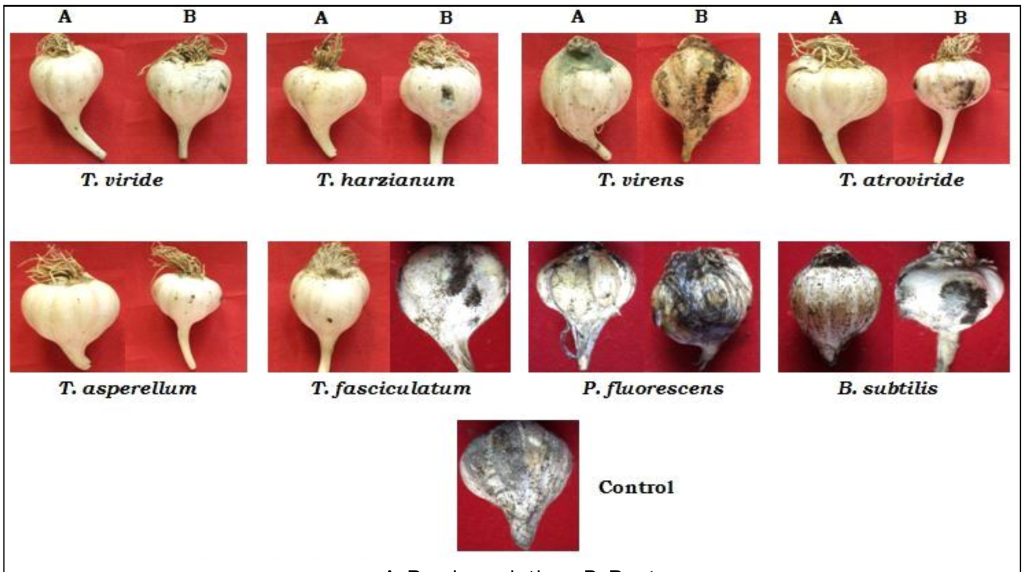

Plate 2: Bio-efficacy of of different antagonists against black mold rot of garlic *in vivo* after 14 days of inoculation.

harvest pathogenic fungi (*Geotricum candidum*, *Penicillium steckii, Rhizopus* sp., *Fusarium* sp. and *Aspergillus* sp.). Nirupama and Singh (2011) screened culture filtrates of ten isolates of *Trichoderma* spp. and evaluated the production of volatile and non-volatile inhibitors against the *F. oxysporum* f. sp. *lycopersici,* causing wilt in tomato. Culture filtrate of *T. viride* (TV19) at 15 percent concentration gave highest inhibition of pathogen growth followed by *T. harzianum* (TH7). The similar trend of results in growth inhibition of the pathogen was observed in volatile metabolites of 15-days old cultures of TV19 and TH7. Prajapati *et al*. (2015) showed that the LCF of *T. harzianum* and *T. viride* revealed complete inhibition of mycelial growth of *Sclerotium rolfsii*.

In pre-inoculation treatment, the LCF obtained from *Trichoderma asperellum* found superior in reducing the black mold rot severity *i.e.* 20.05 and 21.32 after 7th and 14th day after inoculation followed by *T. harzianum* and *T. viride i.e.* 20.96, 25.49 and 23.29, 30.14, respectively. *T. virens* and *T. atroviride* showed mediocre effect. While, the culture filtrate of *T. fasciculatum* found least effective in managing black mold rot severity *i.e.* 33.21 and 44.46 per cent as compared to control after 7th and 14th days after inoculation, respectively. Similar trend of results was observed in post-inoculation treatments. The culture filtrate of *T. asperellum* was found effective in reducing the per cent black mold rot severity *i.e.* 27.43 and 28.60 after 7th and 14th days of inoculation followed by *T. harzianum*, *T. viride*. *T. virens* and *T. atroviride* gave mediocre effect. *T. fasciculatum* found least effective. The results obtained are presented in Table 4 and Plate 4.

The results of present investigations corroborate with the results obtained by Prajapati (2016) he reported that the liquid culture filtrates of *Trichoderma asperellum*, *T. harzianum* and *T. viride* found significantly superior showing

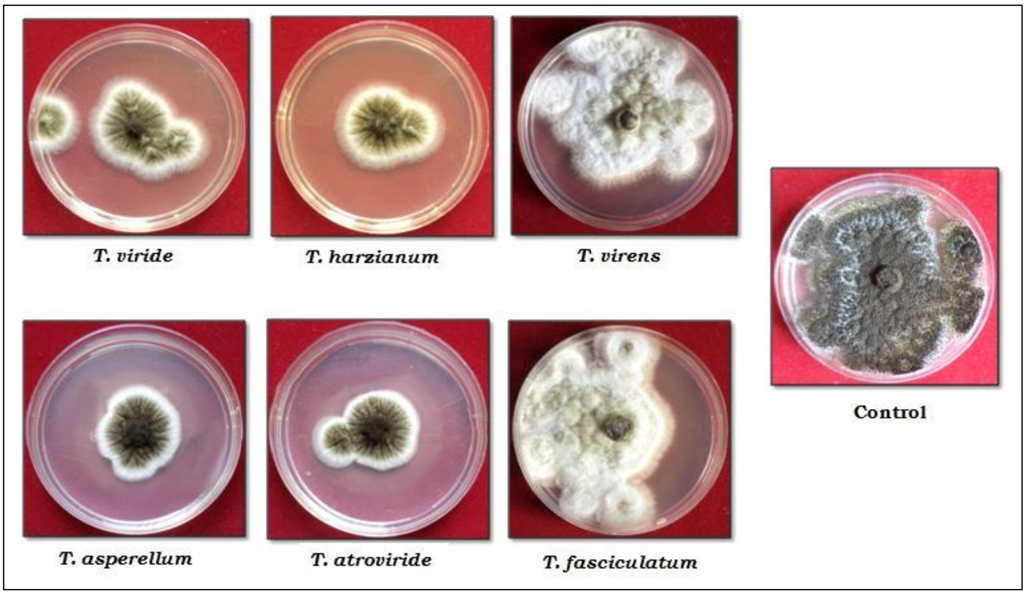

Plate 3: Effect of liquid culture filtrates of *Trichoderma* spp. on mycelial growth of *A. niger in vitro*.

Table 4: Effect of culture filtrates of *Trichoderma* spp. on severity of black mold rot.

Trichoderma spp.	Per cent rot severity			
	Pre-Inoculation		Post-Inoculation	
	7 DAI*	14 DAI	7 DAI	14 DAI
T. viride	25.49 (18.52)**	30.14 (25.21)	29.30 (23.95)	38.30 (38.41)
T. harzianum	20.96 (12.80)	23.29 (15.63)	28.73 (23.11)	31.42 (27.18)
T. virens	30.14 (25.21)	38.68 (39.06)	36.40 (35.21)	49.01 (56.98)
T. asperellum	20.05 (11.75)	21.32 (13.22)	27.43 (21.22)	28.60 (22.91)
T. fasciculatum	33.21 (30.00)	44.46 (49.06)	45.18 (50.31)	59.79 (74.68)
T. atroviride	30.41 (25.62)	39.66 (40.73)	40.80 (42.70)	48.78 (56.58)
Control	60.07 (75.11)	64.63 (81.64)	60.78 (76.17)	69.19 (87.38)
SEm±	0.55	0.63	0.84	1.14
C.D. at 5%	1.68	1.91	2.57	3.46
C.V. %	3.05	2.92	3.83	4.25

*DAI= Days after inoculation.

**Figures in the parenthesis are retransformed values of arc sine transformation.

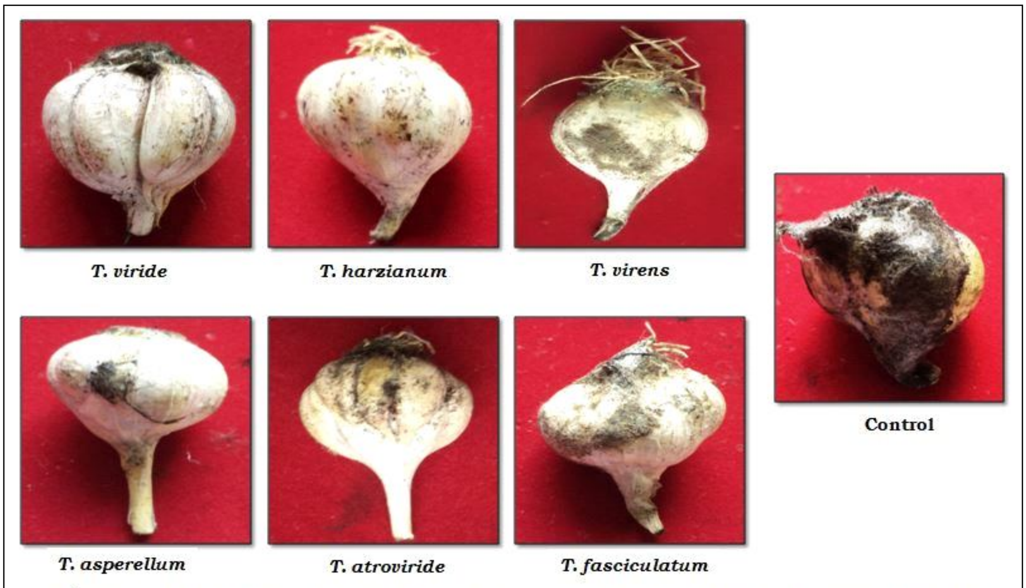

Plate 4: Effect of liquid culture filtrates of *Trichoderma* spp. on black mold rot of garlic after 14 days of inoculation *in vivo*.

no black mould rot severity followed by *T. fasciculatum i.e.* 5.66 and 9.67 per cent on 7th and 14th day after inoculation. Adebesin *et al.* (2009) showed that the culture filtrate of *T. asperellum* (NG-T161) inhibited the mycelial growth of *F. oxysporum* and *Colletotrichum musae* by 49.7 and 60.3 per cent, respectively at 50 per cent (v/v) concentrations. Patel (2009) reported that culture filtrate of *T. harzianum* at 1250 µl/ml gave complete mycelial growth inhibition of *A. niger* of groundnut while in dual culture it was 71.11 per cent. Mortuza and Ilag (1999) reported that conidia and culture filtrates of *Trichoderma* spp. effectively reduced the rotting in banana fruits when artificially inoculated with *Lasiodiplodia theobromae.*

CONCLUSION

Among all the antagonists significantly lowest mycelial growth with highest mycelial growth inhibition was recorded in *T. asperellum* followed by *T. harzianum*. Pre-inoculation treatment with, *T. asperellum* found significantly superior in reducing the garlic black mould rot severity on 7th and 14th day after inoculation followed by *T. harzianum*, *T. viride*, *T. atroviride* over control. Similar trend of results was observed in post-inoculation treatment. Culture filtrate of *T. asperellum T. harzianum* found effective in *in vitro* and *in vivo*.

The future of food production and environmental safety are in jeopardy due to issues in agriculture. The emergence of plant pathogenic microorganisms in a crop plantation caused a pandemic plant diseases phenomenon. The best solution to overcome this problem is the application of biological control by using *Trichoderma* spp. in agricultural products. *Trichoderma* spp. significantly suppress the growth of plant pathogenic microorganisms and regulate the rate of plant growth.

Conflict of interest: None.

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