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Mayekar AN
M.Sc. Agri., Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Bhagwat RG
Associate Professor, Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India.

Kadam JJ
Associate Professor, Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Arai MFN
M.Sc. Agri., Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Arote RR
M.Sc. Agri., Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Dhole GS
M.Sc. Agri., Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Divkar PM
M.Sc. Agri., Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Phondekar UR
Ph.D Scholar, Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Corresponding Author:
Mayekar AN
M.Sc. Agri., Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

In vitro exploration of bio-agents and fungicides against collar rot of groundnut (*Arachis hypogaea* L.)

Mayekar AN, Bhagwat RG, Kadam JJ, Arai MFN, Arote RR, Dhole GS, Divkar PM and Phondekar UR

Abstract
Collar rot of groundnut caused by *Aspergillus niger* is an important seed and soil borne disease. Collar rot is reported to cause losses in yield up to 40% in India. Utilization of biocontrol agents along with fungicides is best suited for integrated disease management. In this study, total six bioagents were evaluated *in vitro* against *A. niger*, out of which *Pseudomonas fluorescens* recorded significantly highest mycelial growth inhibition (61.66%) of the test pathogen, followed by *T. longibrachiatum* (58.33%), *T. harzianum* (57.41%), *T. koningii* (53.52%), *Bacillus subtilis* (53.15%) and *Penicillium funiculosum* (50.00%). Among eight fungicides evaluated *in vitro* were found effective against *A. niger*. However, Copper oxychloride 50% WP @ 0.25%, Carbendazim 12% + Mancozeb 63% WP @ 0.2%, Tebuconazole 25.9% EC, Carbendazim 50% WP and Carboxin 37.5% + Thiram 37.5% WP each @ 0.1% resulted with cent per cent (100%) mycelial growth inhibition. These were followed by Azoxystrobin 23% SC @ 0.1% (57.51%), Mancozeb 75% WP @ 0.2% (52.04%) and Thiram 75% WP @ 0.2% (50.74%).

Keywords: Groundnut, *Aspergillus niger*, bioagents, fungicides

Introduction
Groundnut (*Arachis hypogaea* L.) is called as the 'King' of Oilseeds' or "Wonder nut" and "Poor men's Cashew nut", which belongs to family Fabaceae and sub-family Papilionaceae. Worldwide, over 100 countries grow groundnut, which includes the developing countries mostly concentrated in Asia and Africa, contributing 56% and 40%, respectively the global area and 68% and 25% production, respectively. Major groundnut producing countries in the world are: India, China, Nigeria, Senegal, Sudan, Burma and USA. India occupies first position, both in respect of the area and production of groundnut in the world. Among the various diseases of groundnut, collar rot (*Aspergillus niger*) is an important seed and soil borne disease. This disease appears in two phases *viz*, pre-emergence and post-emergence phase. Collar rot is devastating on stem tissues near ground surface causing rotting, wilting and plant death (Pande and Rao, 2000) [9]. Collar rot disease on groundnut seedlings was first reported by Jochem (1926) [4] and in India it was first reported by Jain and Nema (1952) [3] as *Aspergillus* blight. It is a toxic fungus which causes yellowing of the leaves, blighting effect on the shoot part and at last finally leading to death of crown portion of the plant (Suzui and Makino, 1980) [11]. Collar rot disease of groundnut is one of the most important factors contributing to low yield.

Materials and Methods
The present study was conducted at Dept. of Plant Pathology, College of Agriculture, Dapoli, and Dist. Ratnagiri. A total of six most potential bioagents *viz*., *Trichoderma harzianum, T. koningii, T. longibrachiatum, Pseudomonas fluorescens, Bacillus subtilis* & *Penicillium funiculosum* were evaluated *in vitro* against *A. niger*, by applying 'Dual Culture Technique' (Dennis and Webster, 1971) [1] and using PDA as basal culture medium with three replications in Completely Randomized Design (CRD). Efficacy of eight fungicides *viz*., Copper oxychloride 50% WP, Carbendazim 12% + Mancozeb 63% WP, Tebuconazole 25.9% EC, Carbendazim 50% WP, Carboxin 37.5% + Thiram 37.5% WP, Azoxystrobin 23% SC, Mancozeb 75% WP and Thiram 75% WP were evaluated *in vitro* at various concentrations against *Aspergillus niger*, by applying Poisoned food technique (Nene and Thapliyal, 1993) [8] and using Potato dextrose agar (PDA) as basal culture medium with three replications in Completely Randomized Design (CRD).

The efficacy of bioagents and fungicides against the test fungus was expressed as inhibition of mycelial growth over control per cent calculated by using the formula given by Vincent (1947) [12].

$$\text{Percent Inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,
C = Growth (mm) of test fungus in untreated control plate.
T = Growth (mm) of test fungus in treated plate.

Results and Discussion

The results obtained on mycelial growth and inhibition of *Aspergillus niger* with four fungal and two bacterial antagonists are presented in Table 1.

Results (Table 1, Plate I and Fig. 1) revealed that all of the bioagents evaluated exhibited fungistatic action against *A. niger* and significantly inhibited its growth, over untreated control. However, *Pseudomonas fluorescens* was found most effective with least colony growth of the pathogen (34.50 mm) and its highest inhibition (61.66%), followed by *T. longibrachiatum* (37.50 mm and 58.33%), *T. harzianum* (38.33 mm and 57.41%), *T. koningii* (41.83 mm and 53.52%), *Bacillus subtilis* (42.16 mm and 53.15%) and *Penicillicum funiculosum* (45.00 mm and 50.00%), respectively of the pathogens colony growth and its inhibition.

Table 3: *In vitro* bio-efficacy of the bioagents against *Aspergillus niger*, causing groundnut collar rot

Tr. No.	Treatments	Colony Diam.* of Pathogen (mm)	% Inhibition
T_1	*Trichoderma harzianum*	38.33	57.41
T_2	*T. koningii*	41.83	53.52
T_3	*T. longibrachiatum*	37.50	58.33
T_4	*Pseudomonas fluorescens*	34.50	61.66
T_5	*Bacillus subtilis*	42.16	53.15
T_6	*Penicillium funiculosum*	45.00	50.00
T_7	Control (untreated)	90.00	-
	S.E. ±	0.52	0.57
	C.D. (P=0.01)	1.59	1.76.

*Mean of three replications. Diam.: Diameter

Thus, the bioagents *viz*., *Pseudomonas fluorescens*, *Trichoderma longibrachiatum*, *T. harzianum*, *T. koningii* and *Bacillus subtilis* were found most potential antagonists against *Aspergillus niger*.

These results of the present study are in consonance with the reports of several earlier workers. Gajera *et al.* (2011) [2] reported *T. viride* (Isolate-60) as most effective with highest mycelial growth inhibition (86.20%) of *A. niger* (groundnut collar rot), followed by *T. harzianum* (Isolate-2J) (80.40%). Nathawat and Pratap (2014) reported maximum mycelial growth inhibition of *A. niger* (groundnut collar rot) with *T. harzianum* (Isolate- Navsari) (81.40). Kumari and Singh (2017) [5] reported significantly highest mycelial growth inhibition of *A. niger* (groundnut collar rot) with *T. harzianum* (81.66%), followed by *T. viride* (66.94%) and *P. fluorescens* (43.14%).

In vitro bioefficacy of the bioagents against *Aspergillus niger*, causing groundnut collar rot

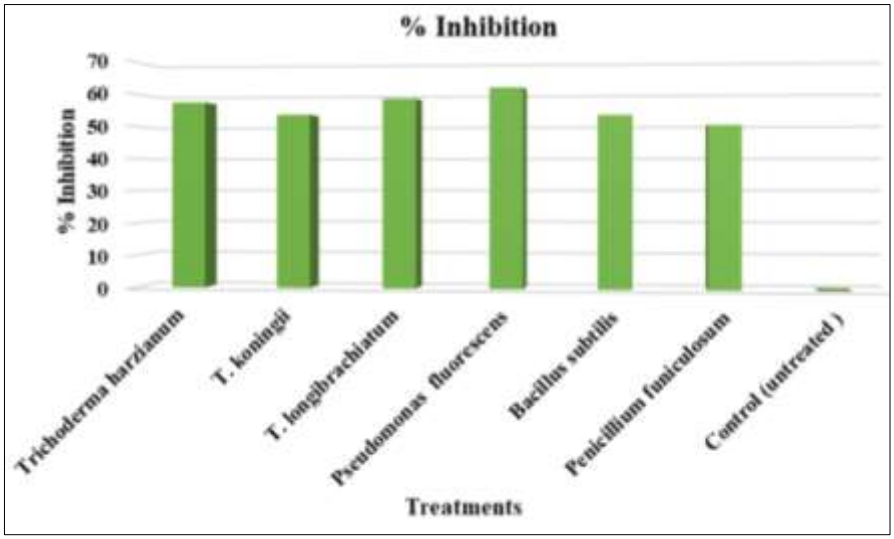

Fig 1: *In vitro* bioefficacy of the bioagents against *Aspergillus niger*, causing groundnut collar rot

A total of eight fungicides were evaluated *in vitro*, against *A. niger*, causing groundnut collar rot by applying poisoned food technique and using PDA as basic culture medium.

Table 2: *In vitro* efficacy of fungicides against *Aspergillus niger*, causing groundnut collar rot

Tr. No.	Treatments	Conc. (%)	Colony Diameter* (mm)	(%) Inhibition
T_1	Mancozeb 75% WP	0.2	43.16	52.04
T_2	Copper oxychloride 50% WP	0.25	00.00	100.00
T_3	Tebuconazole 25.9% EC	0.1	00.00	100.00
T_4	Azoxystrobin 23% SC	0.1	38.00	57.51
T_5	Thiram 75% WP	0.2	44.33	50.74
T_6	Carbendazim 50% WP	0.1	00.00	100.00
T_7	Carbendazim 12% + Mancozeb 63% (75% WP)	0.2	00.00	100.00
T_8	Carboxin 37.5% + Thiram 37.5% (75% WP)	0.1	00.00	100.00
T_9	Control	-	90.00	-
	S.E. ±		0.47	0.49
	C.D. (P=0.01)		1.42	1.48

*Mean of three replications

The results obtained thereof are presented in the Table 2, Plate II and Fig. 2 and narrated here under.

Mycelial growth

Results (Table 2, Plate II and Fig. 2) revealed all of the test fungicides tested were found effective against the test pathogen. The mycelial growth with the test fungicides ranged from 00.00 mm to 44.33 mm, as against 90.00 mm in untreated control. However, Copper oxychloride 50% WP @ 0.25%, Carbendazim 12% + Mancozeb 63% WP @ 0.2%, Tebuconazole 25.9% EC, Carbendazim 50% WP and Carboxin 37.5% + Thiram 37.5% WP each @ 0.1% resulted with none of the mycelial growth (00.00 mm), These were followed by Azoxystrobin 23% SC @ 0.1%, with next least mycelial growth (38.00 mm), Mancozeb 75% WP @ 0.2% (43.16 mm) and Thiram 75% WP @ 0.2% (44.33 mm).

Mycelial inhibition

Results (Table 2, Plate II and Fig. 2) revealed all of the test fungicides tested were found effective against the test pathogen. The mycelial growth inhibition with the test fungicides ranged from 44.33 to 100 per cent. However, Copper oxychloride 50% WP @ 0.25%, Carbendazim 12% + Mancozeb 63% WP @ 0.2%, Tebuconazole 25.9% EC, Carbendazim 50% WP and Carboxin 37.5% + Thiram 37.5% WP each @ 0.1% resulted with 100 per cent mycelial growth inhibition. These were followed by Azoxystrobin 23% SC @ 0.1% (57.51%), Mancozeb 75% WP @ 0.2% (52.04%) and Thiram 75% WP @ 0.2% (50.74%).

Thus, all of the eight fungicides were found fungistatic against *Aspergillus niger* and significantly inhibited its mycelial growth, over untreated control. However, the fungicides found most effective in the order of merit were Tebuconazole 25.9% EC, Carbendazim 50% WP, Carboxin 37.5% + Thiram 37.5% WP, Carbendazim 12% + Mancozeb 63% WP, Copper oxychloride 50% WP, Azoxystrobin 23% SC, Mancozeb 75% WP and Thiram 75% WP.

These results of the present study are in conformity with findings of several earlier workers. Nathawat and Pratap (2014) reported that Tebuconazole 2% DS @ 100,250,500,750 and 1000 ppm, Carbendazim 12% + Mancozeb 63% WP @ 250,500,1000 and 1500, Captan @ 2000 and Carboxin 37.5 + Thiram 37.5% WP @ 1000 and 1500 ppm resulted with cent per cent (100%) mycelial growth inhibition of *A. niger* (groundnut collar rot). Kumari *et al.* (2016) [6] based on *in vitro* evaluation, reported highest mycelial growth inhibition of *A. niger* (groundnut collar rot), with the fungicides *viz*., Carbendazim 12% + Mancozeb 63% WP (95.67%), followed by Carboxin 37.5 + Thiram 37.5% DS (95.10%), Carbendazim 50% WP (92.44%). Rohtas *et al.* (2016) reported that highest mean mycelial growth inhibition of *A. niger* (groundnut collar rot), with the fungicides *viz*., Propiconazole 25% EC (94.16%), followed by Carbendazim 50% WP (88.00%), Captan 50% WP (58.56), Thiram 50% WP (52.00%).

In vitro efficacy of fungicides against *Aspergillus niger*, causing groundnut collar rot

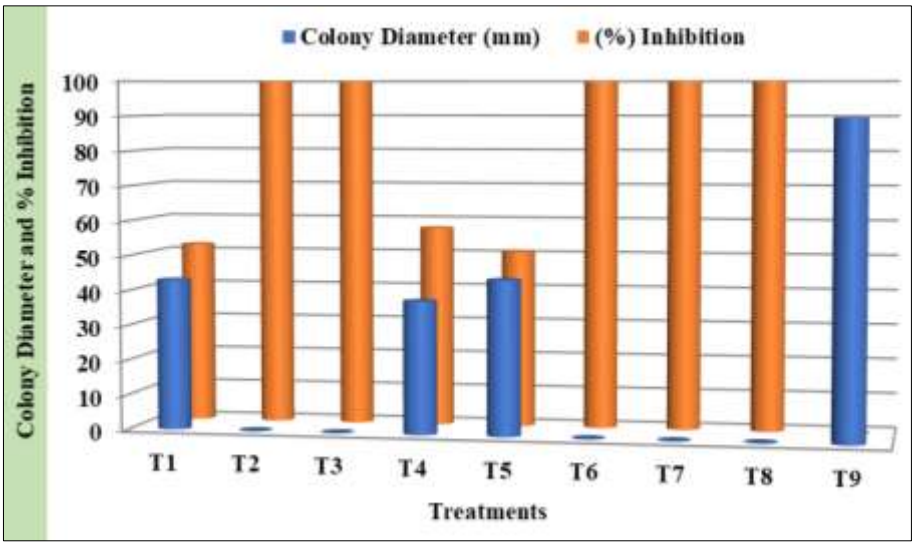

Fig 2: *In vitro* efficacy of fungicides against *Aspergillus niger*, causing groundnut collar rot

Conclusion

From the result of current study, it is concluded that Collar rot of groundnut caused by *Aspergillus niger* can be successfully controlled by bioagents namely *Pseudomonas fluorescens* & *T. longibrachiatum* and the fungicides by Copper oxychloride 50% WP @ 0.25%, Carbendazim 12% + Mancozeb 63% WP @ 0.2%, Tebuconazole 25.9% EC, Carbendazim 50% WP and Carboxin 37.5% + Thiram 37.5% WP each @ 0.1%.

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