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PB Pawar
Post Graduate Student, Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

DS Kakade
Assistant Professor, Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

KB Vavare
Post Graduate Student, Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

Corresponding Author:
PB Pawar
Post Graduate Student, Department of Plant Pathology and Agricultural Microbiology, College of Agriculture, Pune, Maharashtra, India

In vitro efficacy of bio-agents and fungicides against *Alternaria polianthi*

PB Pawar, DS Kakade and KB Vavare

Abstract
Present study revealed that all four bio-agents evaluated and exhibited antifungal activity against test pathogen (*Alternaria polianthi*) and significantly inhibited mycelial growth over untreated control. Out of four bio-agents, *Trichoderma viride* showed least mycelial growth of test pathogen (2.38 cm) and highest mycelial growth inhibition (70.31%) which was at par with *Trichoderma harzianum* with 2.78 cm mycelial growth and 65.31 per cent growth inhibition. While, *Trichoderma koningii* had maximum mycelial growth of test pathogen (3.88 cm) with minimum growth inhibition percentage (51.56%). Out of ten fungitoxicants evaluated, The fungicide Propiconazole 25% EC @ 0.1% showed maximum growth inhibition (97.92%) which was at par with Tebuconazole 25.9% EC @ 0.1%, Difenoconazole 25% EC @ 0.05% which reported 95.83 per cent and 95.00 per cent growth inhibition against test pathogen respectively.

Keywords: Tuberose, bio-control agents, *alternaria polianthi*, *trichoderma*, bio-agents, fungicides

Introduction
Tuberose (*Polianthes tuberosa* L.) is important bulbous ornamental crop cultivated in tropical and subtropical areas of world (Biswas *et al*., 2002) [2]. The propagation of tuberose is done by transplantation of daughter tubers from older plants. Many viral, bacterial, fungal and nematode diseases spread due infected tubers (Das, 1961; Rangaswamy *et al*., 1970) [3, 10] which drastically affect its production. This crop is impacted by various abiotic and biotic factors which affect the growth and leads to losses in flower yield. Tuberose is attacked by a number of fungal diseases (Vida Mahinpoo *et al*., 2013) [13]. Among fungal diseases, some air borne diseases of tuberose *viz*. blossom blight, botrytis blight, *Alternaria* leaf blight are reported (Roy, 1984) [12]. In India, leaf blight of tuberose incited by *Alternaria polianthi* was first reported from the locality of Coimbatore (Mariappan *et al*., 1977) [7] and in succeeding period once again from same state, Tamil Nadu (Muthukumar *et al*., 2007) [9]. Tuberose crop is taken well with less protection measures. However, due to change in climatic conditions, leaf blight caused by *Alternaria polianthi* accounting 15 to 20% losses in the yield and quality of tuberose and becomes the major threat in Maharashtra state.
Alternaria polianthi is one of the most important fungal pathogens of tuberose which showed characteristic symptoms on leaves. Red brown spots with faint concentric rings appear on the midrib and margins of the leaf. Dark brown spots of 10-50 mm in length appear on the peduncle. Infection leads to drying up of affected parts. The spots begin as brown specks and grow into a circular to oval form with a diameter of 4- 5 mm and a length of 10-30 mm. The number of spots on each leaf vary from one to ten and spots frequently became larger and coalesce into bigger patches. The present scrutiny was done to evaluate effective bio-agents and fungicides against fungal pathogen *Alternaria polianthi* under *in vitro* conditions. Thus, by means of these approaches, we can decrease the losses caused by leaf blight of tuberose efficiently.

Materials and Methods
Present investigations were carried out in the Department of Plant Pathology and Agricultural microbiology, College of Agriculture, Pune-05 and field experiments were carried out at Zonal Agricultural Research station, Ganeshkhind, Pune during the year 2021.

1. *In vitro* evaluation of bio-agents against *Alternaria polianthi*
1.1 Collection of antagonistic micro-organisms
The potential antagonistic activity of bio-agents *viz. Trichoderma viride*, *Trichoderma*

harzianum, Trichoderma hamatum, Trichoderma koningii were collected from Biological Nitrogen Fixation Scheme, College of Agriculture, Pune-05.

1.2 Maintenance of culture

The antagonistic fungal microorganisms were grown on PDA slants stored at 6°C in refrigerator and sub-culturing was done consequently at an interval of thirty days in order to retain virulence of the fungal bio- agents for their further study.

1.3 *In vitro* evaluation of bio-agents

The effectiveness of antagonists against the pathogen was assessed by means of dual culture technique (Dennis and Webster, 1971) on PDA medium.

1.4 Dual culture technique

About 20 milliliter of Potato dextrose agar medium was added into sterile Petri plate and allowed to solidify. The 10 days old fungal culture was taken and cut into five millimeter circular disc by means of sterile cork borer and kept nearby the periphery, on one side of PDA plate. Likewise antagonistic fungal circular disc was kept on other side. A plate with pathogen only without antagonist served as control. The incubation was done at 27±1 ºC for time period of 7 days. Each treatment was replicated four times. After the period of incubation, when the growth in the control plate reached maximum (90 mm diameter), the radial growth of the pathogen was measured and per cent inhibition over control was found out as per equation suggested by Vincent (1947) [14].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of fungal growth.

C = Growth/colony diameter of the pathogen in control plate (cm).

T = Growth /colony diameter of the pathogen in dual culture plate (cm).

2. *In vitro* evaluation of fungicides against *Alternaria polianthi*

The fungicides utilized in the present assessment along with particulars of trade name, ingredient of the chemical in formulation and source of supply were presented in Table 1. The efficacy of all following fungicides was assayed by using poisoned food technique on PDA as basal medium.

2.1 Poisoned food technique

The required quantity of specific fungicide was added separately into molten and cooled potato dextrose agar in order to get the desired concentration of fungicides. After that, 20 milliliter of the poisoned medium was added into sterile petri plates. Mycelial circular discs of 5 millimeter size from vigorously growing culture of the fungus were cut out by means of sterile cork borer and one circular disc was kept at the centre of each agar plate. Without adding any fungicides to the medium, Control plate was maintained. Each treatment was replicated thrice. The incubation was done at 27±1 oC temperature for time period of 8 days and radial fungal colony growth was determined. The effectiveness of a fungicide was assessed by calculating per cent inhibition of mycelial growth over control by utilizing the formula suggested by Vincent (1947) [14].

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition of fungal growth

C = Growth/colony diameter of the pathogen in control plate (cm)

T = Growth/colony diameter of the pathogen in treatment plate (cm)

Table 1: Particulars of fungicides used in the investigation / study

Sr. No.	Chemical name	Trade name	Active ingredient	Conc. (%)	Manufacturer
1	Mancozeb	M-45	75%WP	0.25	Indofil Industries Limited.
2	Propineb	Antracol	70% WP	0.3	Bayer Cropscience Limited.
3	Carbendazim + Mancozeb	Saaf	12%+63%WP	0.25	UPL Limited.
4	Metalaxyl-M + Mancozeb	Ridomil Gold	4%+64%WP	0.25	Syngenta India Limited.
5	Tebuconazole	Folicur	25.9% EC	0.1	Bayer Cropscience Limited.
6	Azoxystrobin	Amistar	23% SC	0.1	Syngenta India Limited.
7	Difenoconazole	Score	25% EC	0.05	Syngenta India Limited.
8	Propiconazole	Tilt	25% EC	0.1	Crystal Crop Protection Pvt. Ltd.
9	Azoxystrobin + Tebuconazole	Custodia	11%+18.3% SC	0.1	Adama India Pvt.Ltd.
10	Tebuconazole + Trifloxystrobin	Nativo	50%+25%WG	0.06	Bayer Cropscience Limited.

Results and Discussion

1. *In vitro* evaluation of bio-agents against *Alternaria polianthi*

A total four bio-agents which includes four fungal antagonistic *viz. T. viride, T. harzianum, T. koningii* and *T. hamatum* were evaluated *in-vitro* for their bio-efficacy against *Alternaria polianthi* by applying dual culture technique and using PDA as basal medium for fungal antagonism. The result obtained on mycelial growth and per cent growth inhibition of test pathogen with bio-agent are presented in Table 2, Fig 1 & 2 and PLATE 1.

1.1 Mycelial Growth Inhibition of *Alternaria polianthi*

All four bio-agents evaluated and exhibited antifungal activity against test pathogen (*Alternaria polianthi*) and significantly inhibited mycelial growth over untreated control. Out of four bio-agents, *Trichoderma viride* showed least mycelial growth of test pathogen (2.38 cm) and highest mycelial growth inhibition (70.31%) which was at par with *Trichoderma harzianum* with 2.78 cm mycelial growth and 65.31 per cent growth inhibition. *Trichoderma hamatum* showed 3.08 cm mycelial growth with 61.56 per cent growth inhibition. While, *Trichoderma koningii* had maximum mycelial growth of test

pathogen (3.88 cm) with minimum growth inhibition percentage (51.56%) (Table 2, Fig 1 & 2 and PLATE 1).

The observations of present investigation are in conformity with reports of several scientists *viz.* Morshed (1985) [8] and Babu *et al*. (2000a) [1] reported that *Trichoderma viride* and *Trichoderma harzianum* were found most effective against *Alternaria tenuis* and *Alternaria solani* in dual culture technique. Reshu and Khan (2012) [11] evaluated *Trichoderma harzianum, T. viride, T. virens* and *Aspergillus niger* against *Alternaria* spp. causing leaf blight of mustard. In dual culture test, *T. viride* reported maximum inhibition of radial growth of *A. brassicae* (74%) and *A. brassicicola* (77%).

Table 2: *In vitro* efficacy of bio-agents against mycelial growth inhibition of *Alternaria polianthi*

Tr. No.	Treatments	Colony Diameter*of bio-agent (cm)	Colony Diameter* of test pathogen (cm)	Per cent growth inhibition
T1	*Trichoderma viride*	6.63a	2.38a	70.31(56.98)
T2	*Trichoderma harzianum*	6.23a	2.78a	65.31(53.92)
T3	*Trichoderma hamatum*	5.93b	3.08b	61.56(51.69)
T4	*Trichoderma koningii*	5.13c	3.88c	51.56(45.90)
T5	Control	0.0d	8.0d	0.00(0.00)
	SE(m) ±	0.16	0.16	1.10
	CD at 1%	0.69	0.69	4.56
	CV (%)	5.31	8.40	5.25

* Mean of four replications.

Note: 1) Treatment means having common superscripts are statistically non-significant and different superscripts are statistically significant.
2) Numbers in parenthesis indicate arcsine transformed values.

Plate 1: Growth inhibition of *Alternaria polianthi* by different bio-agents

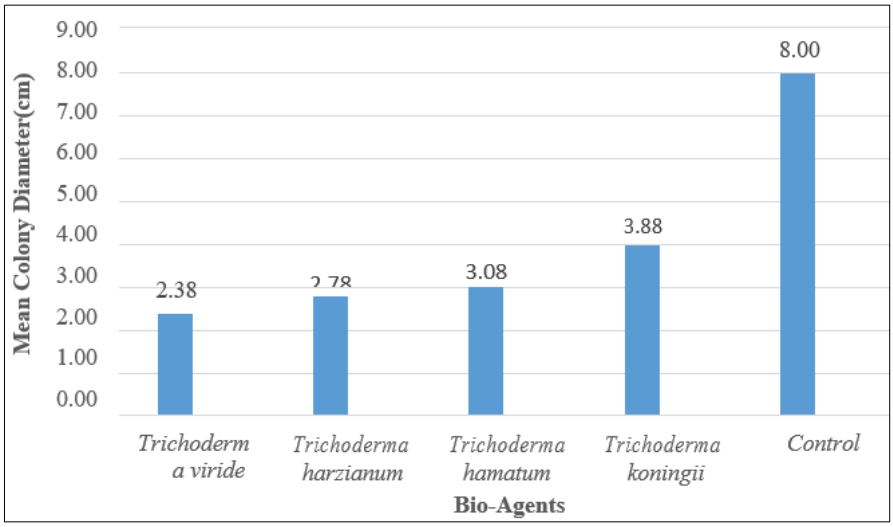

Fig 1: *In vitro* effect of different bio-agents on mycelial growth of *Alternaria polianthi*

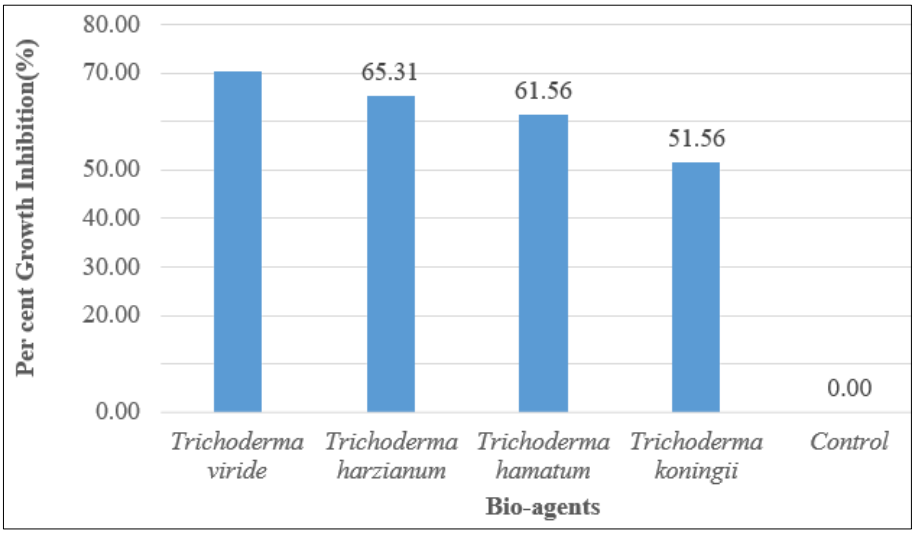

Fig 2: Per cent growth inhibition of *Alternaria polianthi* by different bio-agents

2. *In vitro* evaluation of fungicides against *Alternaria polianthi*

Ten fungitoxicants namely, contact fungicides- Mancozeb 75%WP @ 0.25%, Propineb 70% WP @ 0.3%, contact + systemic fungicides- Carbendazim 12% + Mancozeb 63%WP @ 0.25%, Metalaxyl-M 4% + Mancozeb 64% WP @ 0.25%, systemic fungicides- Tebuconazole 25.9% EC @ 0.1%, Azoxystrobin 23% SC @ 0.1%, Difenoconazole 25% EC @ 0.05%, Propiconazole 25% EC @ 0.1%, Azoxystrobin 11% + Tebuconazole 18.3% SC @ 0.1%, Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.06% were evaluated against *Alternaria polianthi* by poisoned food technique and per cent inhibition in mycelial growth was observed.

The results presented in Table 3 indicated that absolute control treatment of the fungus *Alternaria polianthi* grown profusely. On eighth day of inoculation, the mean colony diameter of fungus was 8.00 cm and growth rate is 0.40 mm hr-1. Rest of all the treatments were significantly superior over absolute control in inhibition of fungal mycelial growth. The fungicide Propiconazole 25%EC @ 0.1% showed maximum growth inhibition (97.92%) which was at par with Tebuconazole 25.9%EC @ 0.1%, Difeconazole 25%EC @ 0.05% which reported 95.83 per cent and 95.00 per cent growth inhibition respectively. This was followed by treatment of Azoxystrobin 11% + Tebuconazole 18.3%SC @ 0.1% showed 90.42 per cent growth inhibition which was at par with Tebuconazole 50% + Trifloxystrobin 25%WG @ 0.06% reported 89.17 per cent growth inhibition. While, other treatments reported growth inhibition in the range of 75 to 85 per cent with least mycelial growth inhibition (53.75%) by Azoxystrobin 23%SC @ 0.1% (Table 3, Fig 3 & 4 and PLATE 2).

Similar results of test fungicides against *A. polianthi* infecting tuberose and many other crops were reported earlier by several scientists. Lakshmi *et al.* (2017) [5] tested six systemic fungicides *viz*. Azoxystrobin, Pyraclostrobin, Difenoconazole, Hexaconazole, Propiconazole, Tebuconazole and three non-systemic fungicides *viz*. Chlorothalonil, Copper oxychloride and Mancozeb against *Alternaria polianthi* at three different concentrations using poisoned food technique. Among systemic fungicides, Hexaconazole (Contaf 5 EC), Propiconazole (Tilt 25 EC), Tebuconazole (Folicur 25 EC) and Pyraclostrobin (Cabriotop 60 WG) showed 100 per cent inhibition of test fungus at 1000 ppm, While among non-systemic fungicides, Chlorothalonil at 2000 ppm was found to be best in inhibiting fungal mycelial growth of *A. polianthi* with 52 per cent inhibition. Mallikarjun (1996) [6] assessed eight fungicides against *A. alternata* causing leaf blight of turmeric under *in vitro* condition and came to conclusion that Propiconazole (Tilt) was found more effective in inhibiting growth of test fungus.

Table 3: Effect of different fungicides with recommended concentration on mycelial growth inhibition of *Alternaria polianthi in vitro*

Tr. No.	Fungicides	Conc. %	Colony dia.* (cm) and Growth rate i.e. GR (mm hr-1) Hours after inoculation					Growth Inhibition %
			48	96	144	192	Mean GR	
T1	Mancozeb 75%WP	0.25	00.30	00.60	01.27	01.30c	0.07	83.75(66.23)
T2	Propineb 70% WP	0.3	00.77	01.00	1.67	1.90d	0.08	76.25(60.83)
T3	Carbendazim 12% + Mancozeb 63% WP	0.25	00.60	00.90	01.40	c	0.06	81.25(64.34)
T4	Metalaxyl-M 4% + Mancozeb 64% WP	0.25	00.60	00.90	01.37	01.77d	0.08	77.92(61.97)
T5	Tebuconazole 25.9% EC	0.1	00.00	00.10	00.30	00.33a	0.02	95.83(78.22)
T6	Azoxystrobin 23% SC	0.1	00.67	01.80	03.27	03.70e	0.21	53.75(47.15)
T7	Difeconazole 25% EC	0.05	00.10	00.13	00.37	00.40a	0.02	95.00(77.08)
T8	Propiconazole 25% EC	0.1	00.00	00.00	00.13	00.17a	0.01	97.92(81.70)
T9	Azoxystrobin 11% + Tebuconazole 18.3% SC	0.1	00.03	00.10	00.60	b	0.05	90.42(71.97)
T10	Tebuconazole 50% + Trifloxystrobin 25%WG	0.06	00.30	00.47	00.70	00.87b	0.04	89.17(70.68)
T11	Absolute Control		02.30	04.33	06.37	08.00f	0.40	0.00(0.00)
	SE(m) ±		0.02	0.17	0.08	0.08		1.09
	CD at 1%		0.07	0.66	0.34	0.32		4.33
	CV (%)		5.85	10.45	9.19	7.28		3.04

*Mean of three replications
Note: 1) Treatment means having common superscripts are statistically non-significant and different superscripts are statistically significant
2) Numbers in parenthesis indicate arcsine transformed values

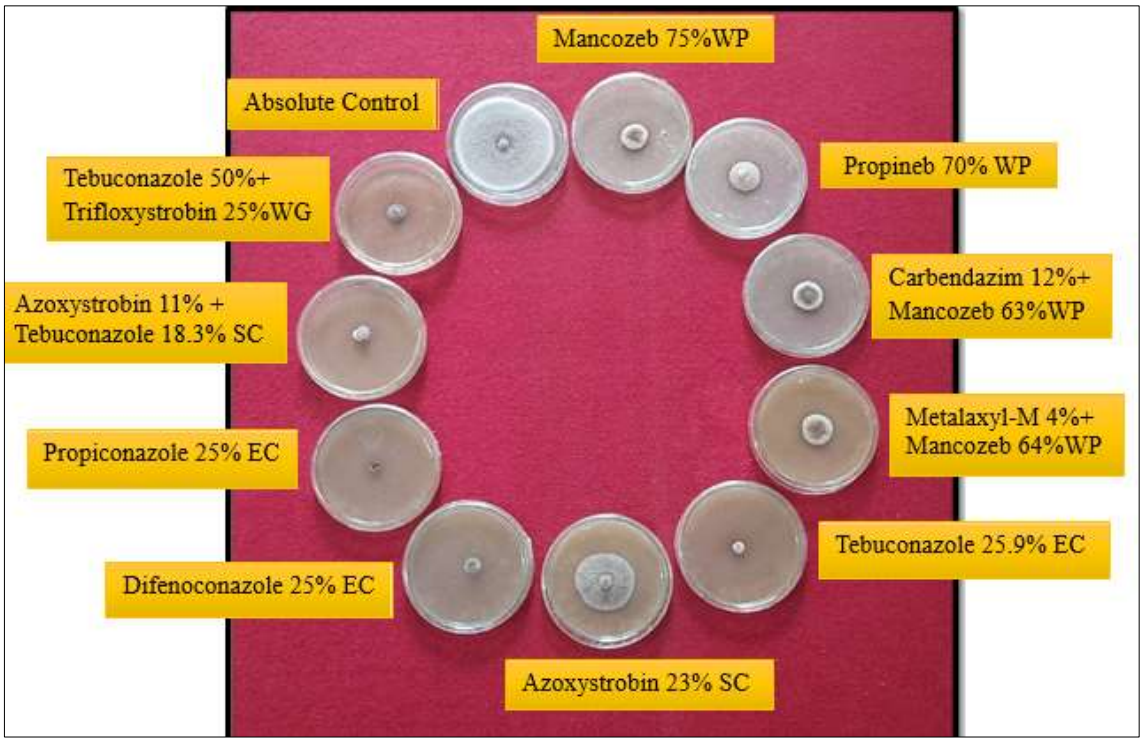

Plate 2: Effect of different fungicides with recommended concentration against *Alternaria polianthi*

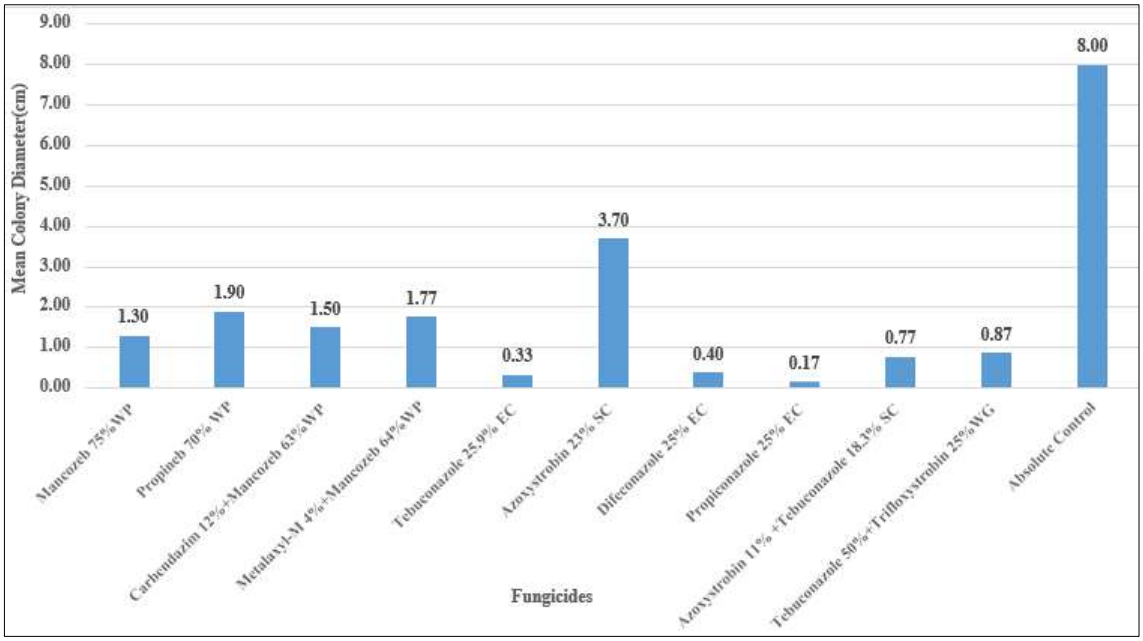

Fig 3: Effect of fungicides on mycelial growth of *Alternaria polianthi*

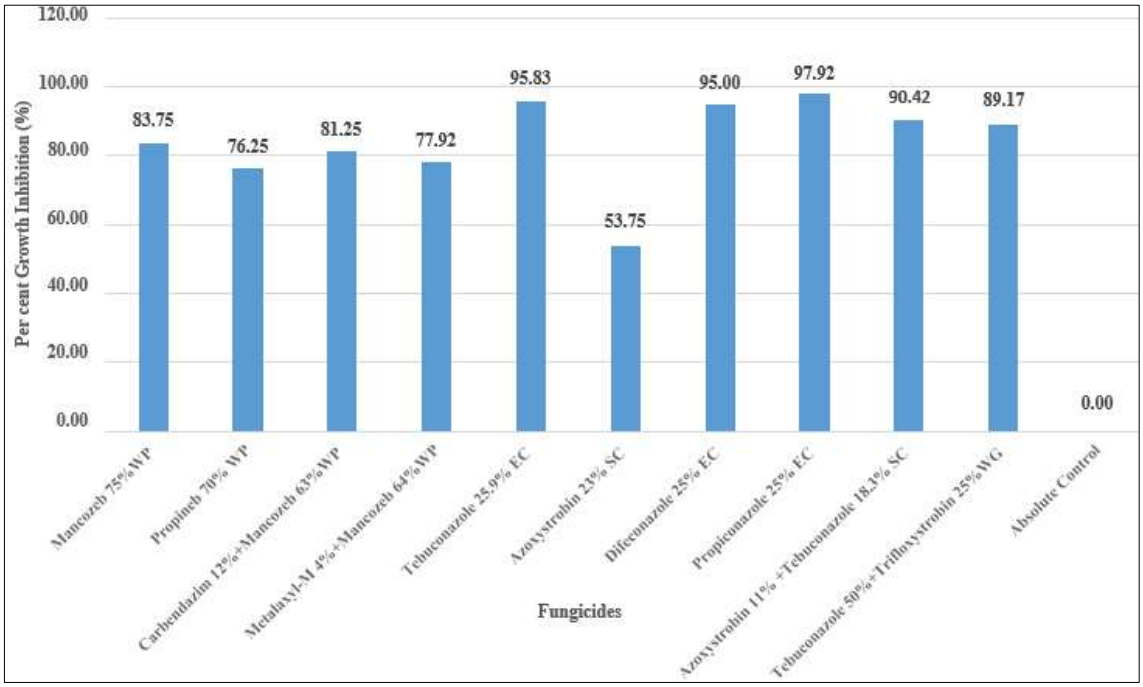

Fig 4: Per cent growth inhibition of *Alternaria polianthi* by different fungicides

Conclusion

The fungicides *viz*. Propiconazole, Tebuconazole and Difenoconazole and bio-agents *Trichoderma viride and Trichoderma harzianum* were found effective under *in-vitro* studies against *Alternaria polianthi*.

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