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In vitro exploration of bio-agents and fungicides against fruit rot of pineapple (*Ananas comosus* L.)

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Abstract
Fruit rot of pineapple caused by *Penicillium funiculosum, Fusarium guttiforme* and *Aspergillus niger* alone or in association is one of the most devastating disease which causes huge losses in the crop. In present investigation, six bio-agents *viz*., *Trichoderma harzianum, T. longibrachiatum, T. koningii, Pseudomonas fluorescens*, *Bacillus subtilis* and yeast (*Saccharomyces cerevisiae*) and eight fungicides namely Carbendazim 50% WP, Tebuconazole 25.9% EC, Azoxystrobin 23% SC, Chlorothalonil 75% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 4% +Zineb 68% (72% WP), Carbendazim 12% + Mancozeb 63% (75% WP) and Carboxin 37.5% + Thiram 37.5% (75% WP) were evaluated *in vitro* for their efficacy against pathogens causing fruit rot of pineapple. Among the bio-agents evaluated, *Trichoderma longibrachiatum* showed maximum (66.66%) inhibition of mycelial growth of *Penicillium funiculosum*. It also showed best antagonistic potential effect against *Fusarium guttiforme* with 87.77% mycelial growth inhibition followed by *T. koningii* (77.03%). Against *Aspergillus niger*, *Pseudomonas fluorescens* was found most effective in inhibiting mycelial growth by 67.77% followed by *T. longibrachiatum* (65.92%). Among the bio-agents used against all the three test pathogens, *T. longibrachiatum* showed maximum inhibition of mycelial growth with mean value of 73.45% followed by *T. koningii* with mean percent inhibition value of 64.68%. Amongst the fungicides evaluated, Tebuconazole 25.9% EC (0.1%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.2%) and Hexaconazole 4% +Zineb 68% (72% WP) (0.1%) were found most significantly effective in inhibiting mycelial growth of all the three pathogens with 100 percent inhibition over control. Chlorothalonil 75% WP was least effective against all the three pathogens of pineapple fruit rot.

Keywords: Pineapple, *Penicillium funiculosum*, *Fusarium guttiforme*, *Aspergillus niger*, bioagents, fungicides

Introduction
The pineapple (*Ananas comosus* L.) is one of the leading commercial fruit crops of the tropics belonging to family Bromeliaceae and order Poales. For its delightful flavour and wonderful taste, pineapple is considered as one of the world's most popular fruits and called as 'Prince of fruits'. Large capital expenditures on land and processing infrastructure were needed for the commercialization of pineapple, which led to monoculture over an extended period of time. Numerous crop locations have experienced severe pest and disease problems as a result of long-term monoculture, necessitating the deployment of numerous management measures. The crop is known to be infected with various fungal, bacterial and viral diseases. Among these various diseases, fruit rot of pineapple caused by *Penicillium funiculosum*, *Fusarium guttiforme* and *Aspergillus niger* in association is one of the most devastating disease which causes huge losses in the crop. The disease was responsible for reducing the market value of fruits to a great extent and also it was significant barrier to the effective production of pineapple in the *Konkan* region of Maharashtra as well as other parts of the country. It is necessary to find out the most bona fide ways of controlling the disease through integrated approach of disease management by using easily available plant extracts, fungicides and beneficial bio-agents. This study will provide the farmer with the most reliable, authentic and efficient measures required for overcoming and managing the incidence and huge losses caused by fruit rot disease on pineapple.

Materials and Methods
The present study was conducted at Department of Plant Pathology, College of Agriculture, Dapoli, and Dist. Ratnagiri. Six different bioagents *viz*., *Trichoderma harzianum*, *T. longibrachiatum*, *T. koningii*, *Pseudomonas fluorescens*, *Bacillus subtilis* and yeast

(*Saccharomyces cerevisiae*) were evaluated *in vitro* against the test pathogens separately by applying Dual culture technique (Dennis and Webster, 1971) with three replications in Factorial Completely Randomized Design (FCRD). Eight fungicides from different fungicidal groups *viz*., Carbendazim 50% WP, Tebuconazole 25.9% EC, Azoxystrobin 23% SC, Chlorothalonil 75% WP, Tebuconazole 50% + Trifloxystrobin 25% WG, Hexaconazole 4% +Zineb 68% (72% WP), Carbendazim 12% + Mancozeb 63% (75% WP) and Carboxin 37.5% + Thiram 37.5% (75% WP) were evaluated at different conc. *in vitro* against test pathogens separately by using "Poisoned Food Technique" with three replications in Factorial Completely Randomized Design. The inoculated plates were incubated at room temperature for seven days and radial colony growth was measured. The efficacy of bioagents and fungicides against the test fungi was expressed as inhibition of mycelial growth over control percent calculated by using the formula given by Vincent (1947) [10].

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

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

Results and Discussion

Results (Table 1, Plate 1 a, b & c and Fig.1) revealed that against *Penicillium funiculosum*, *Trichoderma longibrachiatum* was found most effective with least colony growth of the pathogen (30.00 mm) and highest inhibition (66.66%) followed by yeast [*Saccharomyces cerevisiae*] (35.00 mm and 61.11%), *Bacillus subtilis* (39.33 mm and 56.30%), *T. harzianum* (40.67 mm and 54.81%), *T. konigii* (40.67 mm and 54.81%) and *Pseudomonas fluorescens* (41.00 mm and 54.44%), respectively of the pathogen's colony growth and its inhibition.

The study also revealed that, *T. longibrachiatum* was found most effective against *Fusarium guttiforme* with least colony growth (11.00 mm) and highest inhibition (87.77%) followed by, *T. koningii* (20.67 mm and 77.03%), yeast [*S. cerevisiae*] (26.00 mm and 71.11%), *T. harzianum* (34.00 mm and 62.22 %), *P. fluorescens* (38.00 mm and 57.77 %) and *B. subtilis* (40.00 mm and 55.55 %), respectively.

Against *Aspergillus niger*, *P. fluorescens* was found most effective with least colony growth of the pathogen (29.00 mm) and its highest inhibition (67.77%) followed by *T. longibrachiatum* (30.67 mm and 65.92%), *T. koningii* (34.00 mm and 62.22%), *T. harzianum* (36.00 mm and 60.00%), *B. subtilis* (36.00 mm and 60.00%) and yeast [*S. cerevisiae*] (43.00 mm and 52.22%), respectively.

Among the bio-agents evaluated against all the three test pathogens, *T. longibrachiatum* showed maximum inhibition of mycelial growth with mean value of 73.45% followed by *T. koningii* with mean percent inhibition value of 64.68%. Yeast [*S. cerevisiae*], *P. fluorescens* and *T. harzianum* were also effective with 61.47%, 60.00% and 59.01% inhibition of mycelial growth, respectively. Least inhibition of test pathogens was observed due to *B. subtilis* with mean percent inhibition value of 57.28%.

The results of present investigation are in accordance with the results obtained by Lone *et al*. (2012) [5] who reported that *Trichoderma harzianum* caused the maximum growth inhibition of *Aspergillus niger* (75%) followed by *Cladosporium spherospermum* (72.2%) and *Fusarium oxysporum* (25%). The results were also in close proximity to Matos *et al*. (2014) [6] who tested *Trichoderma* spp. against *Fusarium guttiforme* and observed *T. viride* and *T. harzianum* isolates most promising. The results are also comparative with Pudake *et al*. (2018) [7] who tested seven bio-control agents against *Aspergillus niger* and reported *T. harzianum* as most effective with 99.93% inhibition followed by *T. koningii* with 88.44% and *P. fluorescence* was 50.59%. Hussain (2018) [4] who also studied antagonistic potential of *Trichoderma* isolates against *Penicillium digitatum* and reported inhibition range of 64.1% to 79.9% of mycelial growth of *P. digitatum* by *T. harzianum*.

Table 1: *In vitro* efficacy of bio-agents against pineapple fruit rot pathogens.

Tr. No.	Bio-agents	S_1- *Penicillium funiculosum*		S_2- *Fusarium guttiforme*		S_3- *Aspergillus niger*		Mean	
		Colony dia. (mm)*	Percent inhibition	Colony dia. (mm)*	Percent inhibition	Colony dia. (mm)*	Percent inhibition	Colony dia. (mm)*	Percent inhibition
T_1	*Trichoderma harzianum*	40.67	54.81	34.00	62.22	36.00	60	36.89	59.01
T_2	*T. longibrachiatum*	30.00	66.66	11.00	87.77	30.67	65.92	23.89	73.45
T_3	*T. koningii*	40.67	54.81	20.67	77.03	34.00	62.22	31.78	64.68
T_4	*Pseudomonas fluorescens*	41.00	54.44	38.00	57.77	29.00	67.77	36.00	60.00
T_5	Yeast (*Saccharomyces cerevisiae*)	35.00	61.11	26.00	71.11	43.00	52.22	34.67	61.47
T_6	*Bacillus subtilis*	39.33	56.30	40.00	55.55	36.00	60	38.44	57.28
T_7	Control	90.00	-	90.00	-	90.00	-	90.00	-
Mean		45.24		37.10		42.67		41.67	
		F test	S.E.m ±	C.D at 1%					
	T	Sig.	0.298	1.136					
	S	Sig.	0.195	0.744					
	T x S	Sig.	0.516	1.968					

(* = Mean of three replications.)

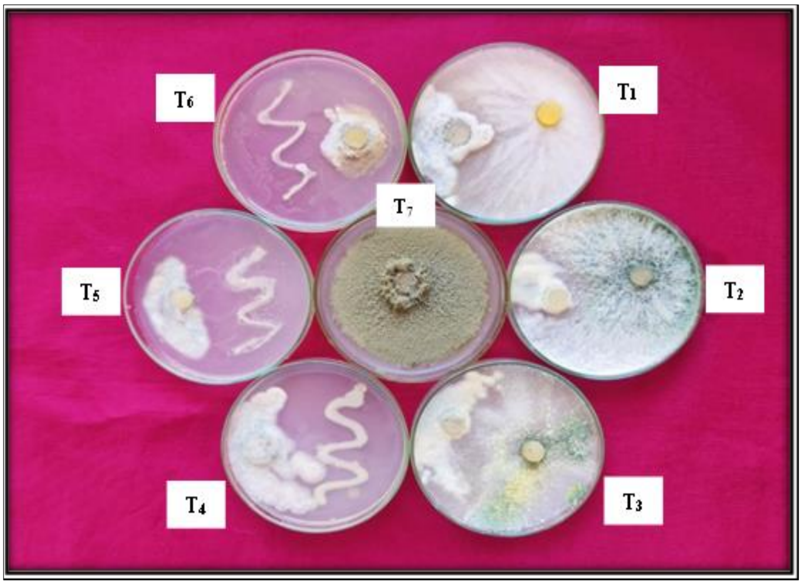

Plate I (a): *In vitro* efficacy of bio-agents against *P. funiculosum*.

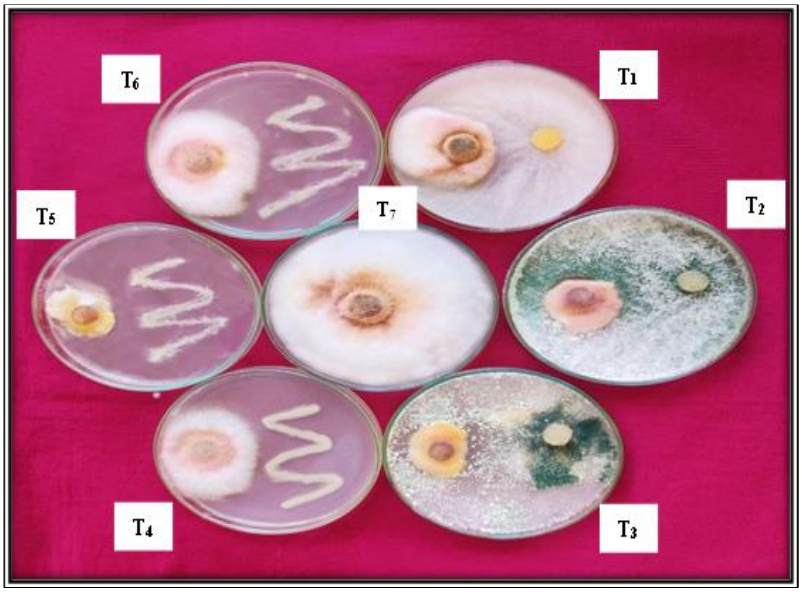

Plate I (b): *In vitro* efficacy of bio-agents against *F. guttiforme*.

Plate I (c): *In vitro* efficacy of bio-agents against *A. niger*.

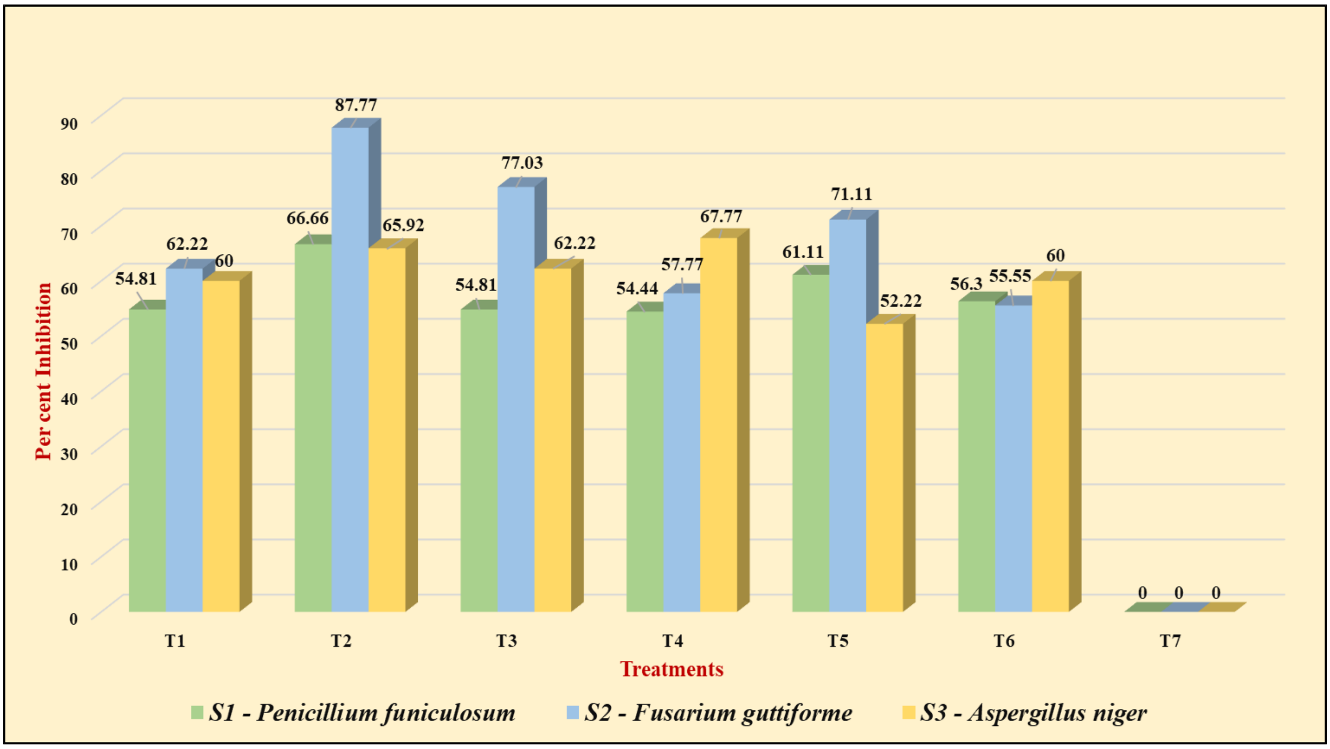

Fig 1: Percent inhibition of different bio-agents against *Penicillium funiculosum*, *Fusarium guttiforme and Aspergillus niger*.

Results from Table 2, Plate 2 a, b & c and Fig.2 revealed that all the fungicides evaluated were significantly effective against all the three fruit rot pathogens *viz*., *Penicillium funiculosum, Fusarium guttiforme* and *Aspergillus niger*.

Carbendazim 50% WP (0.1%), Tebuconazole 25.9% EC (0.1%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.2%), Hexaconazole 4% +Zineb 68% (72% WP) (0.1%) and Carbendazim 12% + Mancozeb 63% (75% WP) (0.1%) were the most significantly effective fungicides in inhibiting the mycelial growth of *P. funiculosum* with 100 percent inhibition over control. Carboxin 37.5% + Thiram 37.5% (75% WP) (0.1%) and Chlorothalonil 75% WP (0.2%) were the next best treatments in order of merits with percent mycelial growth inhibition by 88.25%, 78.52%. Azoxystrobin 23% SC (0.1%) was least effective which inhibited the mycelial growth of the *P. funiculosum* to the tune of 33.33%.

Amongst the various fungicides tested Carbendazim 50% WP (0.1%), Tebuconazole 25.9% EC (0.1%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.2%), Hexaconazole 4% +Zineb 68% (72% WP) (0.1%) and Carbendazim 12% + Mancozeb 63% (75% WP) (0.2%) were found most significantly effective in inhibiting mycelial growth of *F. guttiforme* with complete inhibition over control. The next best treatment was of Chlorothalonil 75% WP (0.2%) and Carboxin 37.5% + Thiram 37.5% (75% WP) (0.1%) which inhibited mycelial growth of test fungus by 88.25% and 83.33%, respectively. The least effective fungicide against *F. guttiforme* was Azoxystrobin 23% SC (0.1%) which showed 80.00% inhibition of mycelial growth over control.

Against *Aspergillus niger*, Tebuconazole 25.9% EC (0.1%), Azoxystrobin 23% SC (0.1%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.2%), Hexaconazole 4% +Zineb 68% (72% WP) (0.1%) and Carboxin 37.5% + Thiram 37.5% (75% WP) (0.1%) resulted with cent percent (100%) mycelial growth inhibition. Carbendazim 12% + Mancozeb 63% (75% WP) (0.2%) and Chlorothalonil 75% WP (0.1%) were also effective against *A. niger* with 55.55 and 51.11 percent inhibition of mycelial growth. Least inhibition of the test fungus was recorded in case of Carbendazim 50% WP (0.1%) with just 19.25% as against control.

Results (Table 2, Plate 2 a, b &c and Fig. 2) also revealed that all the fungicide tested were effective in inhibiting the mycelial growth of fruit rot causing fungi *viz*., *P. funiculosum*, *F. guttiforme* and *A. niger*. Tebuconazole 25.9% EC (0.1%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.2%) and Hexaconazole 4% +Zineb 68% (72% WP) (0.1%) were the most significantly effective fungicide against all the three fungi *viz*., *P. funiculosum*, *F. guttiforme* and *A. niger* with mean value of 100 percent inhibition over control. Carboxin 37.5% + Thiram 37.5% (75% WP) (0.1%) and Carbendazim 12% + Mancozeb 63% (75% WP) (0.2%) were the next best treatments in order of merits with mean percent mycelial inhibition of 90.62% and 85.18%, respectively. Carbendazim 50% WP (0.1%), Chlorothalonil 75% WP (0.2%) and Azoxystrobin 23% SC (0.1%) were also found effective against test fungi which inhibited the mycelial growth of *P. funiculosum*, *F. guttiforme* and *A. niger* to the tune of 73.08%, 72.71% and 71.11%, respectively.

The results of the present study are in conformity with several earlier workers, Attrassi *et al*. (2016) [1] reported Azoxystrobin highly effective in inhibiting mycelial growth of *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, *Penicillium digitatum*, *Rhizopus stolonifera* and *Penicillium italicum* responsible for the fruit rot of oranges. Dahal *et al*. (2018) [2] reported cent percent mean mycelial growth inhibition (100%) of who investigated efficacy of *Fusarium oxysporum* with Carbendazim (50% WP) followed by Chlorothalonil (75% WP) with 71.49% inhibition. Similarly, Sharma *et al*. (2019) [9] observed Tebuconazole 25.9% EC and Azoxystrobin 25% EC inhibited mycelial growth of *Penicillium digitaum* causing green mould of Kinnow by 92.57% and 62.57%, respectively. The results are also in close proximity with the results of

Reddypalli (2020) [8] tested six fungicides against *Aspergillus niger* and reported complete inhibition (100%) of mycelial growth of *A. niger* by Tebuconazole at concentrations of 25, 50, 100 and 200 ppm and Azoxystrobin at concentrations of 100 and 200 ppm.

Table 2: *In vitro* efficacy of fungicides against pineapple fruit rot pathogens.

\Tr. No.	Name of fungicides	Conc. (%)	S_1- *Penicillium funiculosum*		S_2- *Fusarium guttiforme*		S_3- *Aspergillus niger*		Mean	
			Colony dia. (mm)*	Percent inhibition	Colony dia. (mm)*	Percent inhibition	Colony dia. (mm)*	Percent inhibition	Colony dia. (mm)*	Percent inhibition
T_1	Carbendazim 50% WP	0.1	0.00	100	0.00	100	72.67	19.25	24.22	73.08
T_2	Tebuconazole 25.9% EC	0.1	0.00	100	0.00	100	0.00	100	0.00	100
T_3	Azoxystrobin 23% SC	0.1	60.00	33.33	18.00	80.00	0.00	100	26.00	71.11
T_4	Chlorothalonil 75% WP	0.2	19.33	78.52	10.33	88.25	44.00	51.11	24.56	72.71
T_5	Tebuconazole 50% + Trifloxystrobin 25% WG	0.2	0.00	100	0.00	100	0.00	100	0.00	100
T_6	Hexaconazole 4% +Zineb 68% (72% WP)	0.1	0.00	100	0.00	100	0.00	100	0.00	100
T_7	Carbendazim 12% + Mancozeb 63% (75% WP)	0.2	0.00	100	0.00	100	40.00	55.55	13.33	85.18
T_8	Carboxin 37.5% + Thiram 37.5% (75% WP)	0.1	10.33	88.25	15.00	83.33	0.00	100	8.44	90.62
T_9	Control	-	90.00	-	90.00	-	90.00	-	90.00	-
Mean			19.96		14.18		27.41			
		F test	S.E.m ±		C.D at 1%					
	T	Sig.	0.205		0.773					
	S	Sig.	0.118		0.446					
	T x S	Sig.	0.354		1.338					

* = Mean of three replications.)

Plate II (a): *In vitro* efficacy of fungicides against *P. funiculosum*.

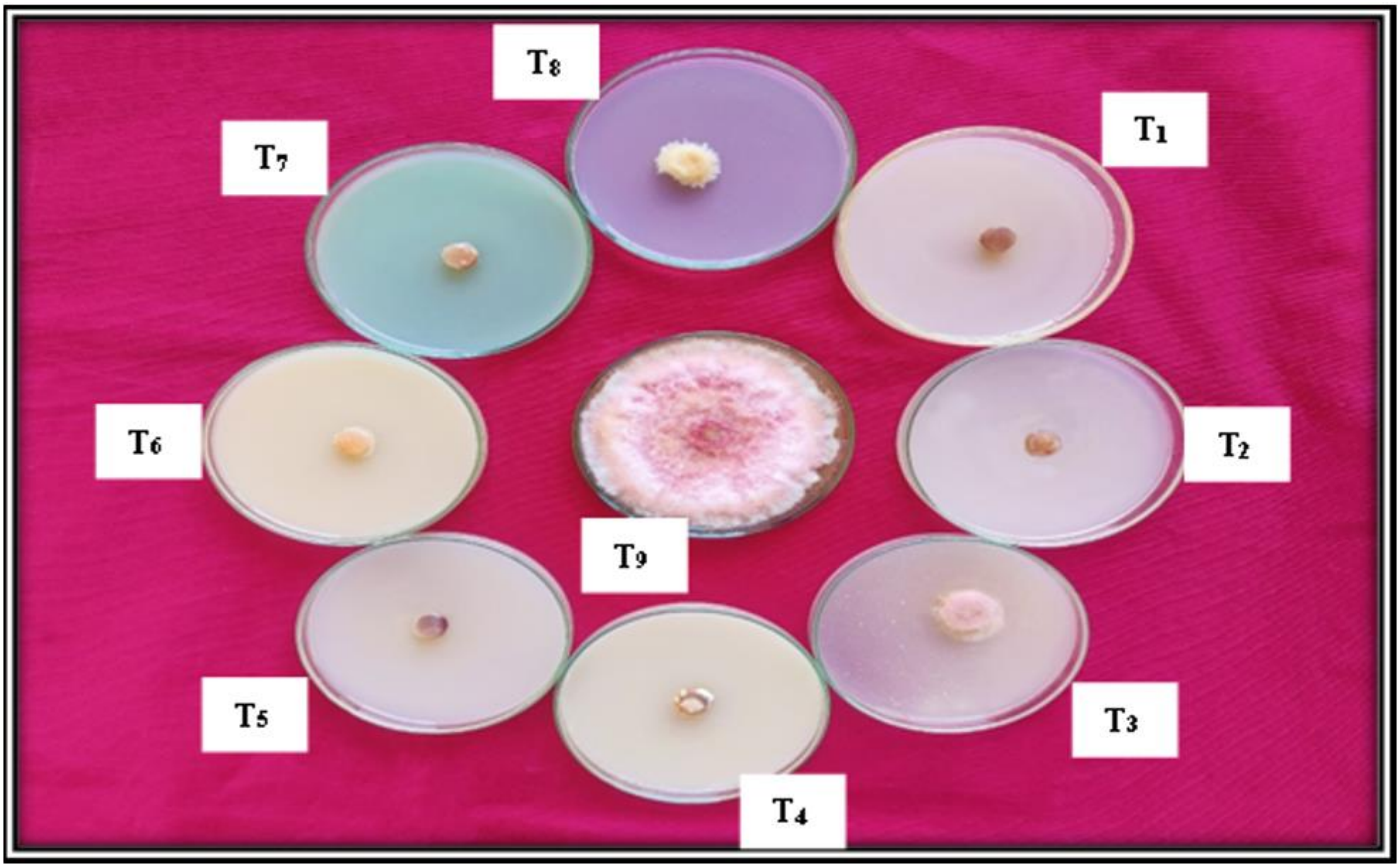

Plate II (b): *In vitro* efficacy of fungicides against *F. guttiforme*

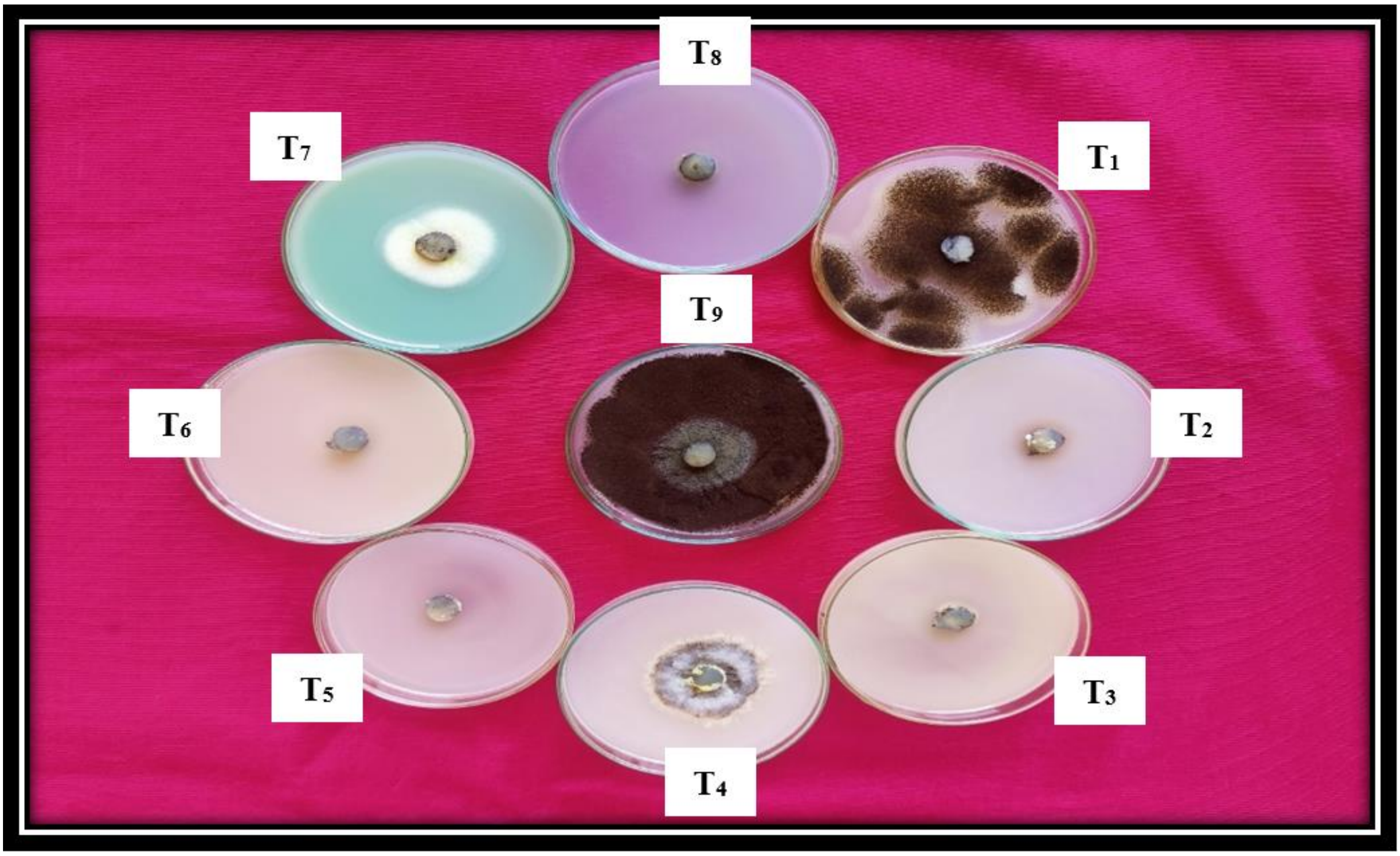

Plate II (c): *In vitro* efficacy of fungicides against *A. niger*

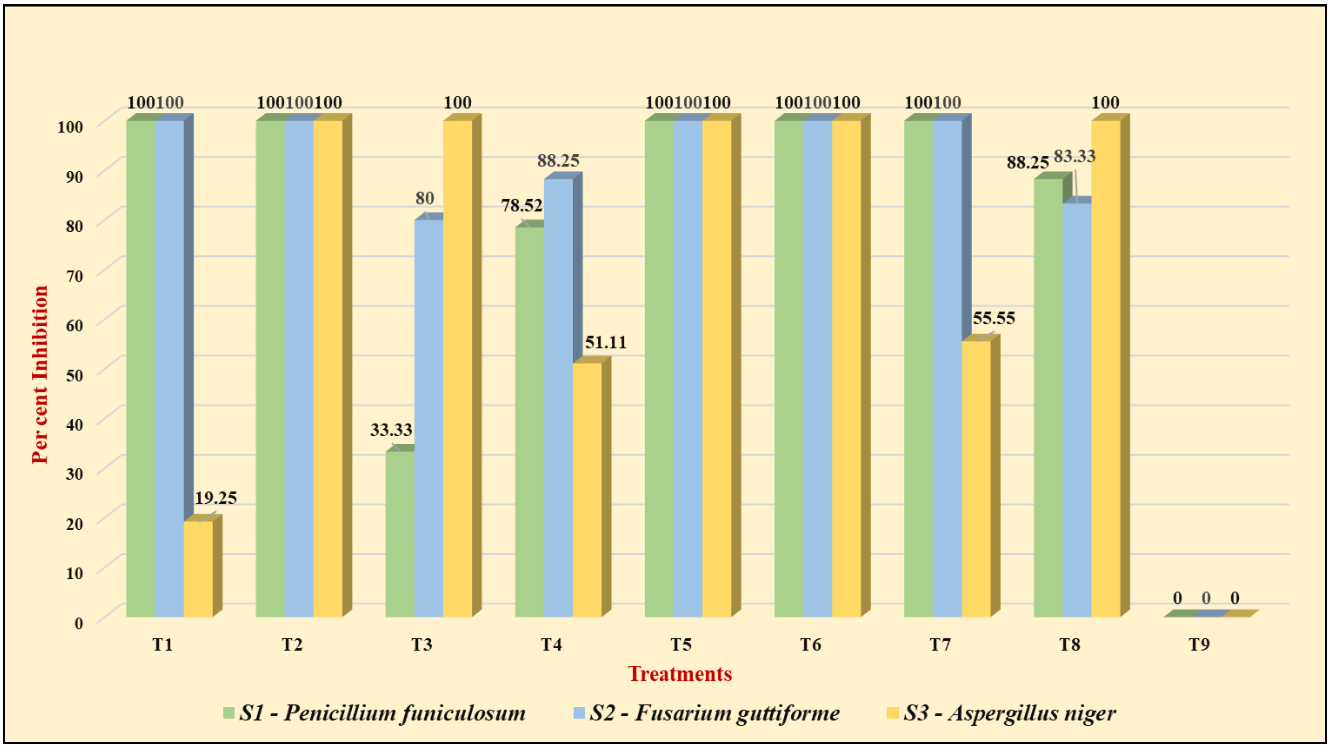

Fig 2: Percent inhibition of different fungicides against *Penicillium funiculosum*, *Fusarium guttiforme and Aspergillus niger*.

Conclusions

From the results of present experiment, it is concluded that fruit rot of pineapple incited by *Penicillium funiculosum* or *Fusarium guttiforme* or *Aspergillus niger* can be effectively controlled by bioagents namely *T. longibrachiatum* and *T. koningii* and of the fungicides by Tebuconazole 25.9% EC (0.1%) or Tebuconazole 50% + Trifloxystrobin 25% WG (0.2%) or Hexaconazole 4% +Zineb 68% [72% WP] (0.1%).

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