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In vitro Evaluation of Fungicides and Bio-rationals against *Pestalotiopsis psidii* Causing Guava Fruit Canker

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Guava (*Psidium guajava* L.), originating from Tropical America and part of the Myrtaceae family, is a highly adaptable and nutritionally valuable fruit. Known as the "Apple of the Tropics," it ranks as the fourth most important fruit crop globally. India leads in guava production. However, guava production faces serious threats from various diseases, particularly canker caused by *Pestalotiopsis psidii*. The disease, varying in severity across guava varieties, manifests as brown necrotic spots on fruits that progress to crater-like lesions covered with white mycelium. *In vitro* evaluation of fungicides and bio-rationals revealed that carbendazim 12% + mancozeb 63% WP

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and azoxystrobin 11% + tebuconazole 18.3% SC at all the tested concentration *i.e.* 500, 1000 and 1500 ppm demonstrated complete inhibition of the pathogen, followed by mancozeb 75% WP showed 97.41 per cent inhibition rate at highest concentration *i.e.* 2000 ppm. Among the bio-rationals, panchgavya completely inhibited mycelial growth at both 5% and 10% concentration, followed closely by buttermilk, which showed growth inhibition of 45.93 and 17.78 per cent at 10% and 5% concentration, respectively.

Keywords: Guava fruit canker; Pestalotiopsis psidii (Kwee and Chong); fungicides; botanicals.

1. INTRODUCTION

Guava (*Psidium guajava* L.), originating from Tropical America and part of the Myrtaceae family, is a vital fruit in tropical and subtropical regions due to its adaptability and nutritional value. Known as the "Apple of the Tropics," it grows in diverse soil conditions and climates, even tolerating high rainfall and drought [1]. Globally, guava is the fourth most important fruit crop, celebrated for its rich vitamin C content (50-300 mg per 100 g) and other nutrients like niacin, riboflavin, and vitamin A [2].

India leads global guava production, with significant cultivation in states like Bihar, Uttar Pradesh, Karnataka, Madhya Pradesh, Maharashtra, and Gujarat. In Gujarat, guava cultivation covers 14,708 hectares, producing 179,510 metric tons annually (Department of Horticulture, Government of Gujarat, 2022-23).

However, guava production faces substantial losses from diseases such as, wilt (*Fusarium oxysporum* (Fr.) Shcl. f. sp. *psidii*), dieback (*Botrydiplodia theobromae* Pat.), leaf spot [*Cercospora psidii* (Yamamoto)], anthracnose (*Gloeosporium psidii* Delacr.), red rust (*Cephaleuros virescens* Kunze), damping off (*Rhizoctonia solani* Kuhn.) and particularly canker caused by *Pestalotiopsis psidii* Pat., prevalent at temperatures between 25-30°C with high humidity, severely impacts fruit quality and yield, with potential losses up to 100 per cent [3]. Canker disease symptoms mainly appear on green guava fruits, starting as small brown or rust-colored necrotic spots. These expand, breaking the epidermis and forming shallow, crater-like lesions. The severity varies across guava varieties [4]. In severe cases, numerous raised cankerous spots form on guava fruits, breaking open to expose the seeds. These fruits become hard, malformed, and mummified, often dropping prematurely [5].

As the disease is becoming severe day by day and negligible information available on the management of the disease by use of fungicides and botanicals. Therefore, the present study has been carried out to investigate the efficacy of various fungicides and botanicals against *Pestalotiopsis psidii* under *in vitro* conditions.

2. MATERIALS AND METHODS

The research on *Pestalotiopsis psidii* causing guava fruit canker under *in vitro* conditions, conducted during the 2022-2023 academic year at the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari which is situated in South Gujarat Heavy Rainfall Agro-climatic Zone of Gujarat state. The material employed and methodology implemented throughout the investigation are presented here.

2.1 Evaluate the Efficacy of Fungicides against *Pestalotiopsis psidii In vitro*

Bio-efficacy of two contact and four combi-products fungicides *viz*., mancozeb 75% WP, zineb 78% WP at 1000, 1500 and 2000 ppm concentrations, carbendazim 12% + mancozeb 63% WP, hexaconazole 4% + zineb 68% WP, pyraclostrobin 5% + Metiram 55% WG and azoxystrobin 11% + tebuconazole 18.3% SC at 500, 1000 and 1500 ppm concentrations along with the control against guava fruit canker pathogen was studied *in vitro* by Poisoned Food Technique method (Nene and Thapliyal, 1982) using Completely Randomized Design. The calculated quantity of fungicides was added to PDA media, mixed thoroughly and poured about 20 ml of media into sterilized Petri plates and allowed to solidify. After solidification each plate was inoculated at center with a 5 mm diameter disc obtained from an actively growing margin of test fungus colony on PDA. Control plates, treated without any fungicide were maintained. There were 3 replicates of each treatment. The Petri plates were incubated at 27 ± 1°C in BOD incubator. The observations on colony diameter (mm) were recorded after 12 to 15 days of incubation. Per cent inhibition of mycelial growth

(PGI) of test fungus was calculated by using the following formula given by Vincent [6] and the results were analyzed statistically.

$$\text{PGI} = \frac{\text{DC} - \text{DT}}{\text{DC}} \times 100$$

Where,

PGI = Per cent growth inhibition

DC = Average diameter of mycelial colony from control set (mm)

DT = Average diameter of mycelial colony from treated set (mm)

2.2 Evaluate the Efficacy of Bio-Rational against *Pestalotiopsis psidii In vitro*

Six bio-rationals *viz*., cow urine, butter milk, panchgavya, garlic, neem and tulsi were filtered through Whatman No. 1 filter paper. Finally filtrate thus obtained was used as 100 per cent stock solution. Each bio-rationals was incorporated into PDA at 5 per cent and 10 per cent before sterilization. Twenty ml of sterilized and cooled PDA was poured into sterile Petri plates. All plates along with control were inoculated with 5 mm mycelial disc of *P. psidii*. Each treatment was repeated thrice and incubated at 27 ± 1°C till control plates reached the radial growth of 90 mm. Per cent inhibition of mycelial growth of test fungus was calculated as described in fungicide evaluation section and the results were analyzed statistically.

2.3 Preparation of Plant Based Products

Fresh healthy plant parts of 100 g (leaves) as indicated below were collected and washed with distilled water and air dried and crushed in 100 ml of sterile water. The crushed product was tied in muslin cloth and filtrate was collected. In case of garlic, cloves were used. The prepared solution gave 100 per cent, which was further diluted to required concentrations of 5 and 10 per cent.

Fig. 1. Symptoms of guava fruit canker

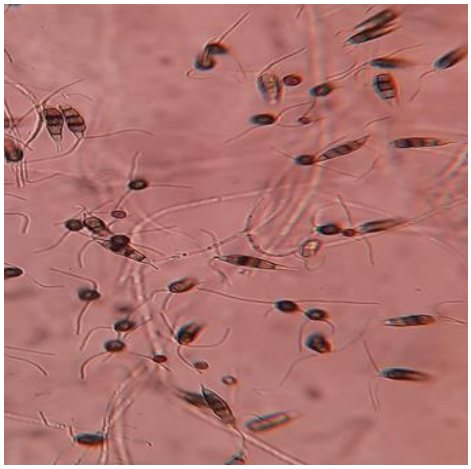

Fig. 2. Microphotograph of conidia of *Pestalotiopsis psidii*

3. RESULTS AND DISCUSSION

3.1 Efficacy of Fungicides Against *Pestalotiopsis psidii In vitro*

The study revealed that all tested fungicides significantly reduced the mycelial growth of *P. psidii* compared to the control. The data of mean colony diameter and per cent inhibition are presented in Tables 1 and 2, depicted in Figs. 3 and 4. Mancozeb 75% WP showed superior efficacy among contact fungicides, achieving 81.11, 82.59, and 97.41 per cent inhibition at concentrations of 1000, 1500, and 2000 ppm, respectively. In contrast, zineb 78% WP exhibited significantly lower inhibition rates of 50.74, 60.74, and 61.48 per cent at the same concentrations.

Among combination fungicides, both carbendazim 12% + mancozeb 63% WP and azoxystrobin 11% + tebuconazole 18.3% SC completely inhibited the pathogen (100%) at all concentrations tested (500, 1000, and 1500 ppm).

Carbendazim + mancozeb and azoxystrobin + tebuconazole show superior efficacy by targeting different stages of fungal development. Carbendazim disrupts fungal cell division, while mancozeb damages cell membranes. Azoxystrobin impairs energy production, and tebuconazole interferes with fungal membrane synthesis. This dual action effectively halts fungal growth and reproduction, leading to complete inhibition across all tested concentrations.

The findings align with earlier studies, such as Surwade [7] and Bhogal [8], which reported strong inhibition of *P. psidii* by carbendazim + mancozeb and mancozeb alone. However, our results show lower inhibition with mancozeb, consistent with Sethi et al. [9], suggesting that differences in geographic locations may influence fungicidal efficacy.

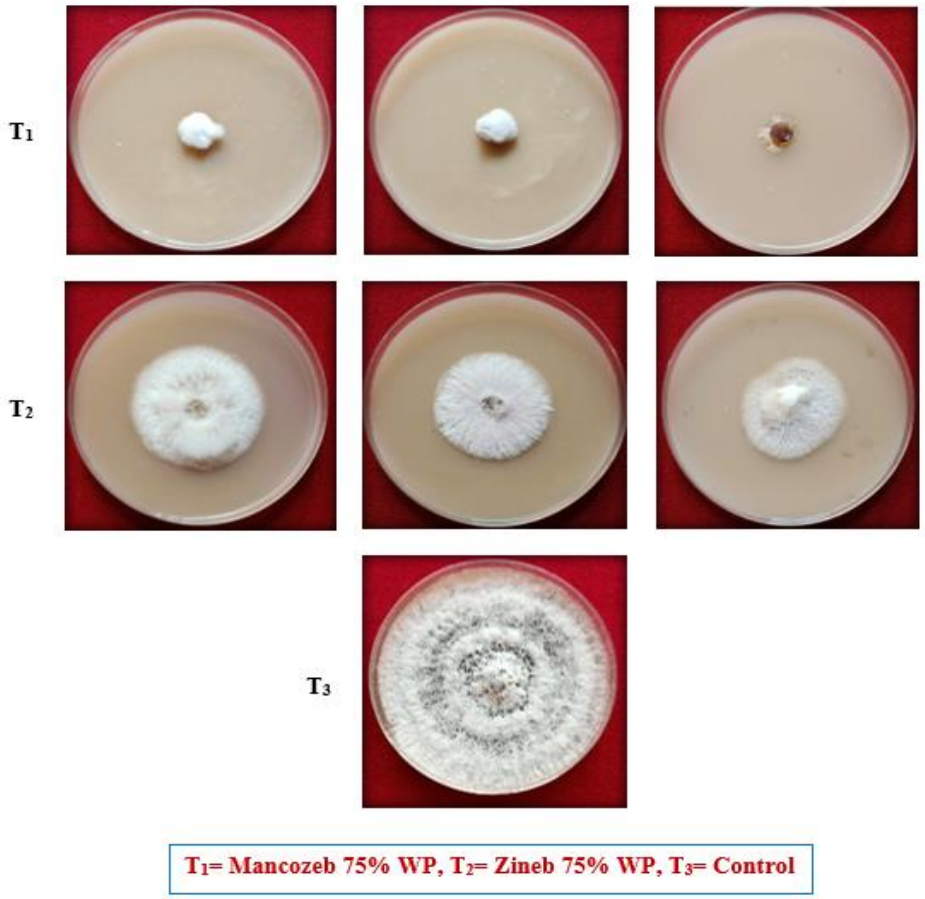

Fig. 3. Efficacy of contact fungicides on per cent growth inhibition of *Pestalotiopsis psidii*

Table 1. Efficacy of contact fungicides on per cent growth inhibition of *Pestalotiopsis psidii* *In vitro*

Sr. No.	Fungicides	PGI%			Mean colony diameter (mm)		
		Concentration (ppm)					
		1000	**1500**	**2000**	**1000**	**1500**	**2000**
1.	Mancozeb 75% WP	81.11 (64.24)*	82.59 (65.34)	97.41 (82.44)	17.00	15.67	2.33
2.	Zineb 75% WP	50.74 (45.92)	60.74 (51.20)	61.48 (51.64)	44.33	35.33	34.67
3.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	90.00	90.00	90.00
S.Em±		**1.45**			-	-	-
CD at 5%		**4.40**			-	-	-
CV%		**4.88**			-	-	-

Note: Data in parenthesis are arcsine transformed value and data outside parenthesis are original value

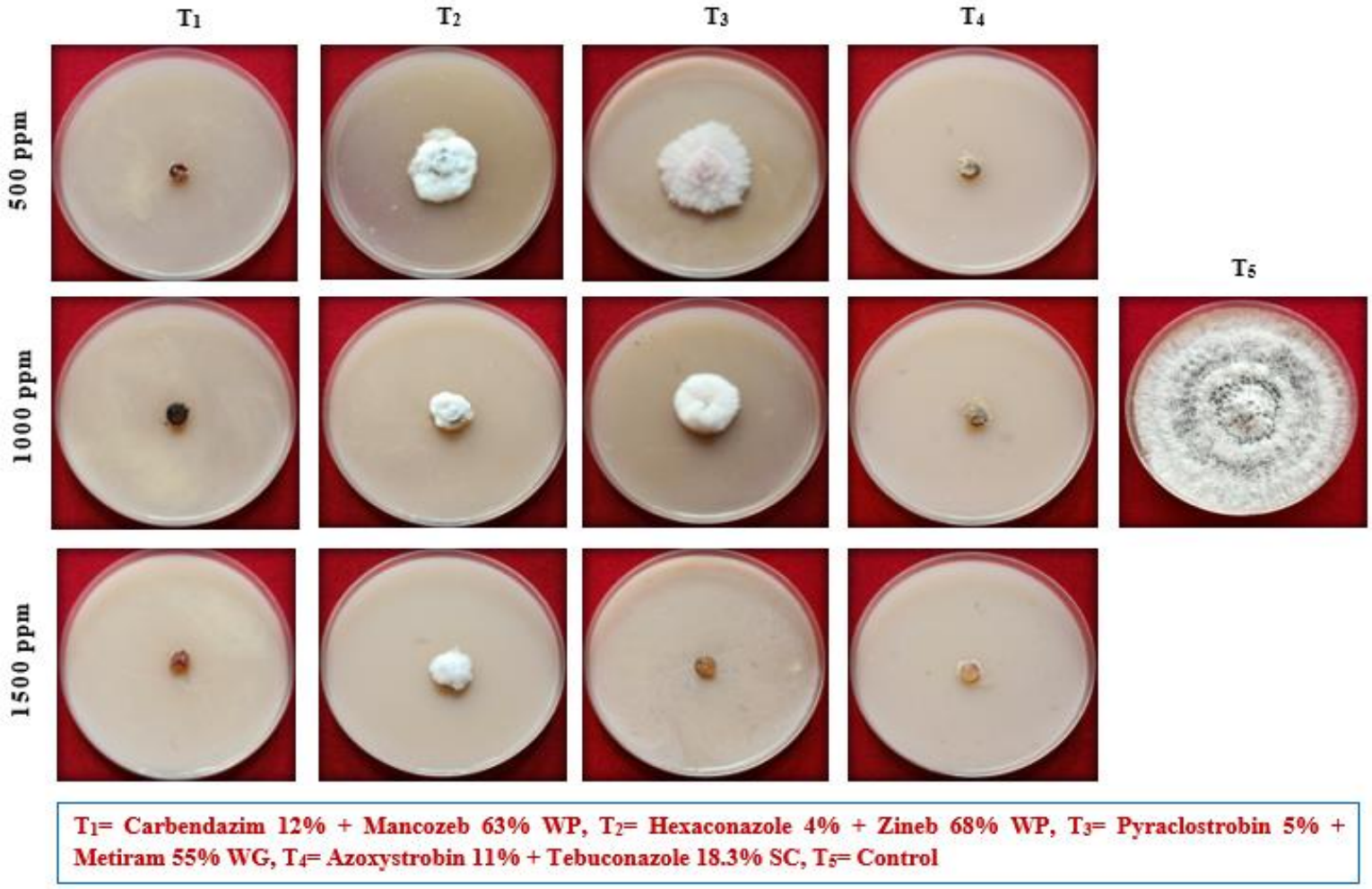

Fig. 4. Efficacy of combi-products fungicides on per cent growth inhibition of *Pestalotiopsis psidii*

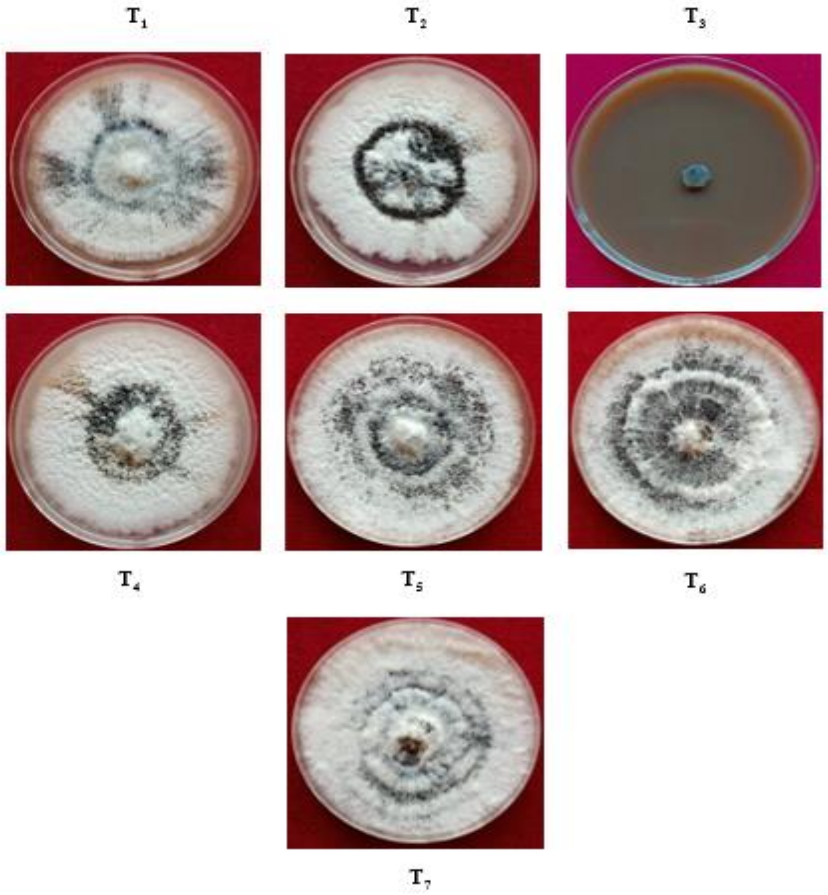

Fig. 5. Efficacy of bio-rationals on per cent growth inhibition of *Pestalotiopsis psidii* at 5 per cent

Table 2. Efficacy of combi-products fungicides on per cent growth inhibition of *Pestalotiopsis psidii In vitro*

Sr. No.	Fungicides	PGI (%) Concentration (ppm)			Mean colony diameter (mm)		
		500	1000	1500	500	1000	1500
1.	Carbendazim 12% + Mancozeb 63% WP	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)	0.00	0.00	0.00
2.	Hexaconazole 4% + Zineb 68% WP	67.04 (54.96[a])	80.74 (63.98)	81.11 (64.25)	29.67	17.33	17.00
3.	Pyraclostrobin 5% + Metiram 55% WG	65.19 (53.84[a])	72.22 (58.20)	91.48 (76.04)	31.33	25.00	7.67
4.	Azoxystrobin 11% + Tebuconazole 18.3% SC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	0.00	0.00	0.00
5.	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	90.00	90.00	90.00
S.Em±		**1.96**			**-**	**-**	**-**
CD at 5%		**5.70**			**-**	**-**	**-**
CV%		**4.84**			**-**	**-**	**-**

Note: Data in parenthesis are arcsine transformed value and data outside parenthesis are original value

Table 3. Efficacy of bio-rationals on per cent growth inhibition of *Pestalotiopsis psidii In vitro*

Sr. No.	Bio-rationals	Plant parts used for extracts	PGI (%) Concentration (%)		Mean colony diameter (mm)	
			5	10	5%	10%
1.	Cow urine	-	13.70 (21.69[a])*	23.70 (29.14)	77.67	68.67
2.	Buttermilk	-	17.78 (24.94)	45.93 (42.68)	74.00	48.67
3.	Panchgavya	-	100.00 (90.05)	100.00 (90.05)	0.00	0.00
4.	Garlic (*Allium sativum*)	Cloves	12.22 (20.46[a])	14.44 (22.31)	79.00	77.00
5.	Neem (*Azadirachta indica*)	Leaves	0.00 (0.00)	0.00 (0.00)	90.00	90.00
6.	Tulsi (*Ocimum sanctum*)	Leaves	0.00 (0.00)	0.00 (0.00)	90.00	90.00
7.	Control	-	0.00 (0.00)	0.00 (0.00)	90.00	90.00
S.Em±			**0.51**	**0.65**	**-**	**-**
CD at 5%			**1.55**	**1.95**	**-**	**-**
CV%			**3.95**	**4.27**	**-**	**-**

Note: Data in parenthesis are arcsine transformed value and data outside parenthesis are original value

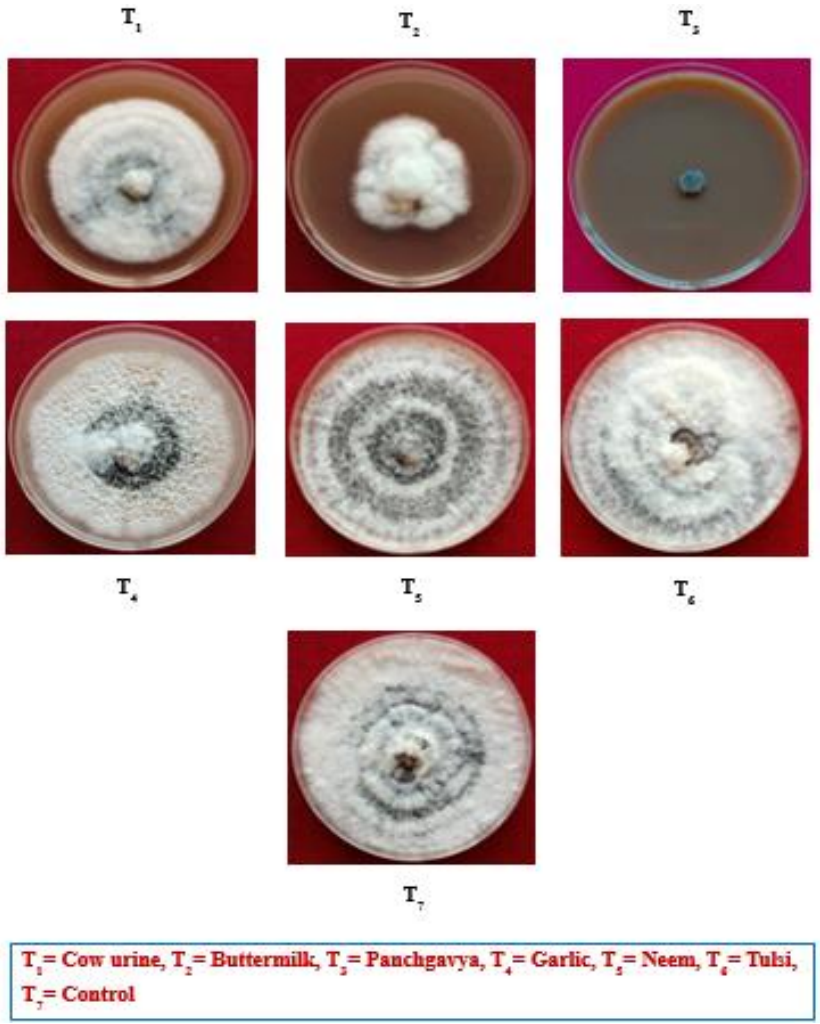

Fig. 6. Efficacy of bio-rationals on per cent growth inhibition of *Pestalotiopsis psidii* at 10 per cent

3.2 Efficacy of Bio-rationals Against Pestalotiopsis psidii *In vitro*

Table 3 and Figs. 5 and 6 demonstrate that bio-rationals significantly reduced the mycelial growth of the pathogen compared to the control. At both 5% and 10% concentrations, Panchgavya achieved complete inhibition of mycelial growth, resulting in 100 per cent suppression. Buttermilk exhibited 17.78 and 45.93 per cent reduction in mycelial growth at 5% and 10% concentrations, respectively. Conversely, neem and tulsi leaf extract showed no significant inhibitory effect (0.00%) at either concentration.

The superior effectiveness of panchgavya and buttermilk in inhibiting mycelial growth is due to their rich antimicrobial compounds, such as organic acids and peptides. Panchgavya's cow-based components and buttermilk's lactic acid create hostile environments for fungi, disrupting their metabolism and cellular processes, leading to stronger inhibition compared to neem and tulsi extracts.

Previous studies, including Surwade [7] and Kore [10], identified garlic extract as the most effective botanical against *P. psidii*, while Bhogal [8] found buttermilk and neem leaf extract to be most effective. In contrast, Tilekar [11] ranked neem seed kernel extract highest [12]. Our findings differ, likely due to geographic variations and differences in botanical properties [13].

4. CONCLUSIONS

Hence, from ongoing results and discussion, it is concluded that *in vitro* testing of fungicides and bio-rationals against *Pestalotiopsis psidii* revealed that among the fungicides, carbendazim 12% + mancozeb 63% WP and azoxystrobin 11% + tebuconazole 18.3% SC recorded complete inhibition (100%) of the pathogen at 500, 1000 and 1500 ppm concentration and among the bio-rationals, panchgavya completely inhibited (100%) mycelial growth at both 5% and 10% concentration. These fungicides and bio-rationals can further be utilized for field trials and recommendation should be provided to the farmers accordingly.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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