In vitro compatibility of *Trichoderma* and *Bacillus* biocontrol agents with different fungicides

ABSTRACT

Biocontrol agents are beneficial for plant and soil health and are effective in controlling many plant diseases. These biocontrol agents in combination with fungicides at reduced doses can be more effective than using alone. Keeping this in view, an *in vitro* study was carried out to test the compatibility of commonly used fungicides *viz.*, captan 50 WP, thiram 75% DS, tebuconazole 5.36% FS, carboxin 37.5% + thiram 37.5% DS, prochloraz 24.4% + tebuconazole 12.1% w/w EW and thiophanate methyl 450 g/l + pyraclostrobin 50 g/l with two fungal (*Trichoderma asperellum* and *T.viride*) and three bacterial biocontrol agents (*Bacillus subtilis* S4KB5, *B. subtilis* S8KB2 and *B. subtilis* B3). All the five fungicides were found to be compatible with all the biocontrol agents at 100ppm and 250ppm. All the fungicides except thiram, showed complete inhibition of *T. asperellum* at 1500ppm and 2000ppm and with *T. viride,* 100 per cent inhibition is shown by all the fungicides at 1500ppm and 2000ppm except carboxin + thiram. With biocontrol isolates, *B. subtilis* S4KB5, *B. subtilis* S8KB2 and *B. subtilis* B3, highest inhibition zone were recorded by prochloraz + tebuconazole (2000ppm), tebuconazole (2000ppm) and thiophanate methyl + pyraclostrobin (2000ppm). Present findings suggest that compatible fungicides can be used with biocontrol agents in an integrated disease management practices for the control of seed and soil borne pathogens.

Key words: Biocontrol agents, Sustainable Agriculture, Compatibility, Fungicides, *Trichoderma* and *Bacillus.*

INTRODUCTION

The productivity of crops is limited by various biotic and abiotic constraints such as incidence of pests and diseases, high/low temperatures, drought, erratic rainfall, and unfavourable soil factors etc. Among various biotic stresses, disease caused by both fungal and bacterial phytopathogens are causing major yield losses. Seed and soil borne diseases caused by the different fungal genera such as *Alternaria, Colletotrichum, Aspergillus, Pencillium, Helminthosporium, Fusarium, Verticillium, Sclerotium* and *Macrophomina,* etc., are considered as a major limitation causing 50 – 75 per cent yield losses in field and horticultural crops (Baysal-Gurel and Kabir, 2018) [3]. To control these seed and soil borne pathogens,

conventional synthetic chemical fungicides and fumigants need to be applied at regular intervals throughout the growing season of the crop. Though the use of fungicides against these pathogens can manage some of these diseases, frequent and indiscriminate use can adversely affect the environment and health and can also lead to development of fungicide resistance (Panth *et al.,* 2020) [18].

In the recent years, use of biocontrol agents for the management of seed and soil borne diseases has been advocated widely. Control of plant diseases using antagonistic microorganisms can be an effective means. Microorganisms, such as *Trichoderma* spp. and *Bacillus* spp. have emerged as most powerful biocontrol agents against plant diseases. *Trichoderma* spp. have long been recognized for their biocontrol properties and have become an important tool in agricultural disease management. Similarly, *Bacillus* spp. found in soil are considered as safe alternatives to harmful chemicals, as they exhibit antagonistic activities against various fungal and bacterial phytopathogens (Pandey *et al.,* 2015 [16]; Zhao *et al.,* 2015 [23]; Kumar *et al.,* 2018 [9]). Since the biocontrol agents are applied either to seed or soil or both, there is a possibility of interaction and interference that would arise if fungicides were also applied to the crops. Combined application of biocontrol agents with commonly used fungicides may result either in synergism/antagonism between them. In several disease management practices, the addition of fungicide at a reduced rate in combination with biocontrol agents has significantly inhibited the pathogen compared to biocontrol agents alone (Buck, 2004) [4]. Combining antagonists with fungicides eliminates the chance of resistance development and reduces the fungicides application (Kumar *et al.,* 2018) [9].

By finding compatible combinations of biocontrol agents and fungicides, sustainable and environmentally friendly strategies can be developed to mitigate the impact of these diseases on crop productivity. The ultimate goal of the research mentioned is to develop effective combination of biocontrol agents and fungicides for controlling seed and soil borne plant diseases. In view of this, laboratory experiments were conducted to test the possibility of compatibility of fungal and bacterial bioagents with fungicides.

MATERIAL AND METHODS

The current investigation was carried out at Department of Plant Pathology, College of Agriculture, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, India. Two fungal (*Trichoderma asperellum* and *T. viride*) and three bacterial biocontrol agents (*Bacillus subtilis* S4KB5, *B. subtilis* S8KB2 and *B. subtilis*

B3) were collected from the Department of Plant Pathology. They were tested for their compatibility with six fungicides at different concentrations (100ppm, 250ppm, 500ppm, 1000ppm, 1500ppm and 2000ppm) under *in vitro* conditions.

Table 1 List of the fungicides used to test compatibility with biocontrol agents used in the present study

S. No	Name of the fungicide	Trade name	Mode of action	Manufacturing company
1.	Captan 50 WP	Captaf	Non systemic	Rallis India Ltd.
2.	Thiram 75% DS	Seedcap	Non systemic	Jaivik Crop Care Ltd.
3.	Tebuconazole 5.36% FS	Raxil	Systemic	Bayer Crop Science Ltd.
4.	Carboxin 37.5% + thiram 37.5% DS	Vitavax power	Systemic & contact	Dhanuka Agritech Ltd.
5.	Prochloraz 24.4% + Tebuconazole 12.1% EW	Zamir	Systemic	Adama Ind. Pvt. Ltd.
6.	Thiophanate methyl 450 g/l + Pyraclostrobin 50 g/l	Xelora	Systemic	BASF Ind. Ltd.

***In vitro* evaluation of compatibility of fungicides with fungal biocontrol agents**

The compatibility of six fungicides at six concentrations *i.e.*, 100ppm, 250ppm, 500ppm, 1000ppm, 1500ppm and 2000ppm with fungal biocontrol agents *viz.*, *Trichoderma asperellum* and *T.viride* was tested using poisoned food technique (Nene and Thapliyal., 1993) [15]. Stock solution of 1,00,000 ppm concentration was prepared using 10ml of sterilized distilled water. Desired concentration of fungicide was obtained by diluting the stock solution using the following formula.

$$C_1V_1 = C_2V_2$$

Where, C_1 = concentration of the stock solution (ppm), V_1 = volume of the stock solution to be added (ml), C_2 = desired concentration (ppm) and V_2 = volume of Potato Dextrose Agar (PDA) in which fungicide is to be amended (ml).

Desired concentration of fungicide was added to media using micropipette. Poisoned medium (20ml) containing fungicide was poured into sterilized Petri plate under aseptic

conditions in laminar air flow and were allowed to solidify. Each plate was inoculated in the centre with a five mm diameter of five-day old fungal culture disc cut from the periphery of actively growing culture under aseptic conditions and incubated at 28±1^{o}C in a BOD incubator. Fungal cultured on Potato Dextrose Agar plates with non-poisoned medium served as control. Radial growth of the fungus was recorded daily in the control plate starting from the initiation of the fungal growth in correspondence to treatment plates till the fungal growth was full in control. Per cent inhibition of the biocontrol agent growth over control was calculated using the formula given by Vincent (1947) [22].

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

Where, I = Per cent growth inhibition,

C = Growth of biocontrol agent in control plate and

T = Growth of biocontrol agent in treatment plate

***In vitro* evaluation of compatibility of fungicides with bacterial biocontrol agents**

The compatibility of six fungicides at six concentrations *i.e.*, 100ppm, 250ppm, 500ppm, 1000ppm, 1500ppm and 2000ppm with bacterial biocontrol isolates *viz., Bacillus subtilis* S4KB5, *B. subtilis* S8KB2 and *B. subtilis* B3 was tested using well diffusion technique (Magaldi, 2004 [10]; Valgas *et al.*, 2007 [21]). The sterilized Petri plates is poured with nutrient agar medium and allowed to solidify. Overnight culture of bacteria was evenly spread over the media surface by means of sterilized spreader. Thereafter, 5mm diameter well was made in each agar plate by using sterilized cork borer. The required concentration of fungicide was loaded into the each well (50µl/well) in Petri plates separately with the help of micropipette. The petri plates without fungicidal suspension served as control. The plates were then incubated at 28±1^{0}C for 48hours and observed for the inhibition zone. Experiment was replicated thrice. The fungicidal suspension diffuses in the agar medium creating a zone of inhibition in case of incompatibility of bacterial bioagents with the fungicide. The inhibition zone was measured in mm with the help of a scale after 48hr of incubation. Absence of inhibition zone indicated the compatibility with respective bacterial strains.

STATISTICAL ANALYSIS

Data obtained from the experiment were statistically analysed by a completely randomized design (CRD). The data pertaining to percentages were square root transformed

wherever necessary. Duncan Multiple Range Test (DMRT) was used to analyse the data. Statistical analysis was carried out as per the procedures outlined by Gomez and Gomez (1984) [5] and Panse and Sukhatme (1985) [17].

RESULTS AND DISCUSSION

The present experiment was aimed to find the compatibility of biocontrol agents *viz., T. asperellum, T. viride, Bacillus subtilis* S4KB5, *B. subtilis* S8KB2 and *B. subtilis* B3 with different fungicides.

In vitro evaluation of compatibility of fungicides with fungal biocontrol agents

Compatibility of six fungicides at different concentrations with fungal biocontrol agent *T. asperellum* are indicated in Table 2 and Plate 1. Among the six fungicides evaluated with *T. asperellum*, captan and thiram showed significantly lowest inhibition on mycelial growth of 88.29 and 90.00mm respectively and is on par with control. On the contrary, tebuconazole showed significantly highest inhibition of 46.35 per cent with a radial mycelial growth of 48.28 mm at 100ppm. Fungicide thiram was found to be significantly compatible with *T. asperellum* with minimum per cent inhibition ranging from 0 per cent to 92.15 per cent at 100ppm to 2000ppm concentrations respectively, followed by captan with minimum per cent inhibition ranging from 1.9 per cent to 87.98 per cent at all concentrations except at 1500ppm and 2000ppm and it was on par with carboxin + thiram at all tested concentrations. Complete inhibition on radial mycelial growth of *T. asperellum* was shown by all the fungicides at 1500ppm and 2000ppm except carboxin + thiram. Fungicides, prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin showed 100 per cent inhibition from 500ppm. Overall, fungicides captan and thiram were more compatible with *T. asperellum* from 100 to 2000ppm and prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin were least compatible.

At 100ppm and 250ppm, both captan and thiram were highly compatible with *T. viride* and showed significantly lowest inhibition with highest mycelial growth of 90mm and is on par with control. While tebuconazole showed significantly highest inhibition with lowest mycelial growth of 27.44mm and 24.19mm at 100pm and 250ppm, respectively followed by prochloraz + tebuconazole (39.24mm and 27.42mm). The fungicide carboxin + thiram was significantly compatible with *T. viride* with minimum per cent inhibition ranging from 0 per cent to 93.75 per cent at 100 to 2000ppm concentrations, respectively. All the remaining fungicides *viz.,* captan, thiram, tebuconazole, prochloraz + tebuconazole and

thiophanate methyl + pyraclostrobin showed 100 per cent inhibition at 1500ppm and 2000ppm (Table 3 and Plate 2). Overall, fungicides captan and thiram were more compatible with *T. viride* from 100 to 2000ppm and tebuconazole and prochloraz + tebuconazole were least compatible.

Earlier various studies have been conducted on compatibility of biocontrol agents with fungicides. Similar results have been obtained by other workers**.** Mclean *et al.* (2001) [11] reported that *T. harzianum* was least sensitive to procymidone and captan and most sensitive to mancozeb, tebuconazole and thiram. Bagwan (2010) [2] reported that thiram (0.2%) copper oxychloride (0.2%) and mancozeb (0.2%) were found comparatively safer against *T. harzianum* and *T. viride.* Kay and Stewart (1994) [7] reported that four fungal antagonists (*Chaetomium globosum, Trichoderma harzianum, T. viride, Trichoderma* spp.) were found insensitive to captan, mancozeb and thiram but were sensitive to benomyl and procymidone. More or less similar results have been found by other workers also (Nallathambi *et al.,* 2009) [14]. Different workers have reported chlorothalonil and captan as tolerant for *T. harzianum* even at higher concentrations up to 2000mg/ml in spore germination tests (Abdel-Moity *et al.,* 1982 [1]; Papavizas *et al.,* 1982 [19]; Mishra *et al.,* 2004 [12]).

***In vitro* evaluation of compatibility of fungicides with bacterial biocontrol agents**

Six fungicides *viz.,* captan, thiram, tebuconazole, carboxin + thiam, prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin at six concentrations *viz.,* 100ppm, 250ppm, 500ppm, 1000ppm, 1500ppm and 2000ppm were evaluated for their compatibility with three *Bacillus subtilis* isolates (S4KB5, S8KB2 and B3). The results revealed an absence of a distinct inhibition zone around the wells indicating compatibility of the three bacterial biocontrol agents with different concentrations of fungicides. The inhibition zone values significantly differed in all the fungicides evaluated at different concentrations.

At 100ppm, all the fungicides were significantly compatible with all the *B. subtilis* isolates (S4KB5, S8KB2 and B3) showing zero inhibition except prochloraz + tebuconazole (8.95mm) and on par with control. Fungicides, captan and thiram showed zero inhibition and were significantly compatible with all three isolates and on par with control. With *B. subtilis* isolate, S4KB5 prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin were recorded significantly highest inhibition zone of 39.18mm and 36.20mm respectively at 2000ppm as indicated in Table 4 and Plate 3. Overall, fungicides captan and thiram were

more compatible with *B. subtilis* isolate, S4KB5 from 100 to 2000ppm and tebuconazole and prochloraz + tebuconazole were least compatible.

Tebuconazole and thiophanate methyl + pyraclostrobin were recorded significantly highest inhibition zone of 35.74mm and 35.11mm respectively and were highly incompatible with *B. subtilis* isolate, S8KB2 at 2000ppm. Overall, fungicides thiram and carboxin + thiram were more compatible with *B. subtilis* isolate, S8KB2 from 100 to 2000ppm and prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin were least compatible (Table 5 and Plate 4).

With *B. subtilis* isolate, B3 prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin were recorded significantly highest inhibition zone of 33.33mm and 31.71mm respectively. Overall, fungicides captan and thiram were more compatible with *B. subtilis* isolate, B3 from 100 to 2000ppm and prochloraz + tebuconazole and carboxin + thiram were least compatible (Table 6 and Plate 5).

Similar bacteriostatic as well as bactericidal effects of the fungicides with bacterial antagonists were reported earlier by several workers. Mohiddin and Khan (2013) [13] reported that fungal and bacterial bioagents are tolerant to fungicides *viz.*, carbendazim, mancozeb, metalaxyl, captan, thiram, and nemacur at lower concentrations and as the concentration increases the bioagents become more sensitive. Singh and Pandey (2020) [20] observed that maximum tolerance concentration of mancozeb was 15µg/ml, vitavax was 0.1µg/ml, metalaxyl was 1000µg/ml for *Bacillus* spp. Harsha *et al.* (2023) [6] reported that *Bacillus* spp. was found safer with thiophanate methyl + pyraclostrobin, carboxin +thiram and thiamethoxam. It also found that bacterial biocontrol agents were found more tolerant to fungicides than fungi. Kishore and Jacob (1987) [8], Mohiddin and Khan (2013) [13] made similar kind of observations and concluded that it may be due to the reason that, some bacteria can use chemicals as nutrients and hence can tolerate higher concentrations of chemicals.

CONCLUSION

The present study on fungicide compatibility clearly indicates that all the six fungicides *viz.*, captan, thiram, tebuconazole, carboxin + thiam, prochloraz + tebuconazole and thiophanate methyl + pyraclostrobin during the course of investigation were found to be

comparatively more compatible at 100ppm and 250ppm against fungal (*T. asperellum* and *T. viride)* and bacterial (*Bacillus subtilis* S4KB5, *B. subtilis* S8KB2 and *B. subtilis* B3) biocontrol agents. It also came to known that lesser concentration of the fungicides had lesser inhibitory effect as compared to higher concentration. However, all the fungicides were comparatively more toxic against all the five biocontrol agents at 1500ppm and 2000ppm. It also clearly indicates the selective response of biocontrol agents to fungicides. The variation in the sensitivity of biocontrol agents to fungicides might be due to their inherent ability to degrade them. Further the data on fungicide tolerance helps to select suitable selective fungicides that are compatible with biocontrol agents. Thus, it can be concluded that the use of fungicides at lower concentrations with biocontrol agents for achieving sustainable plant diseases and agroecosystem management is highly recommended.

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Table 2 Compatibility of fungicides with *T. asperellum* under *in vitro* conditions

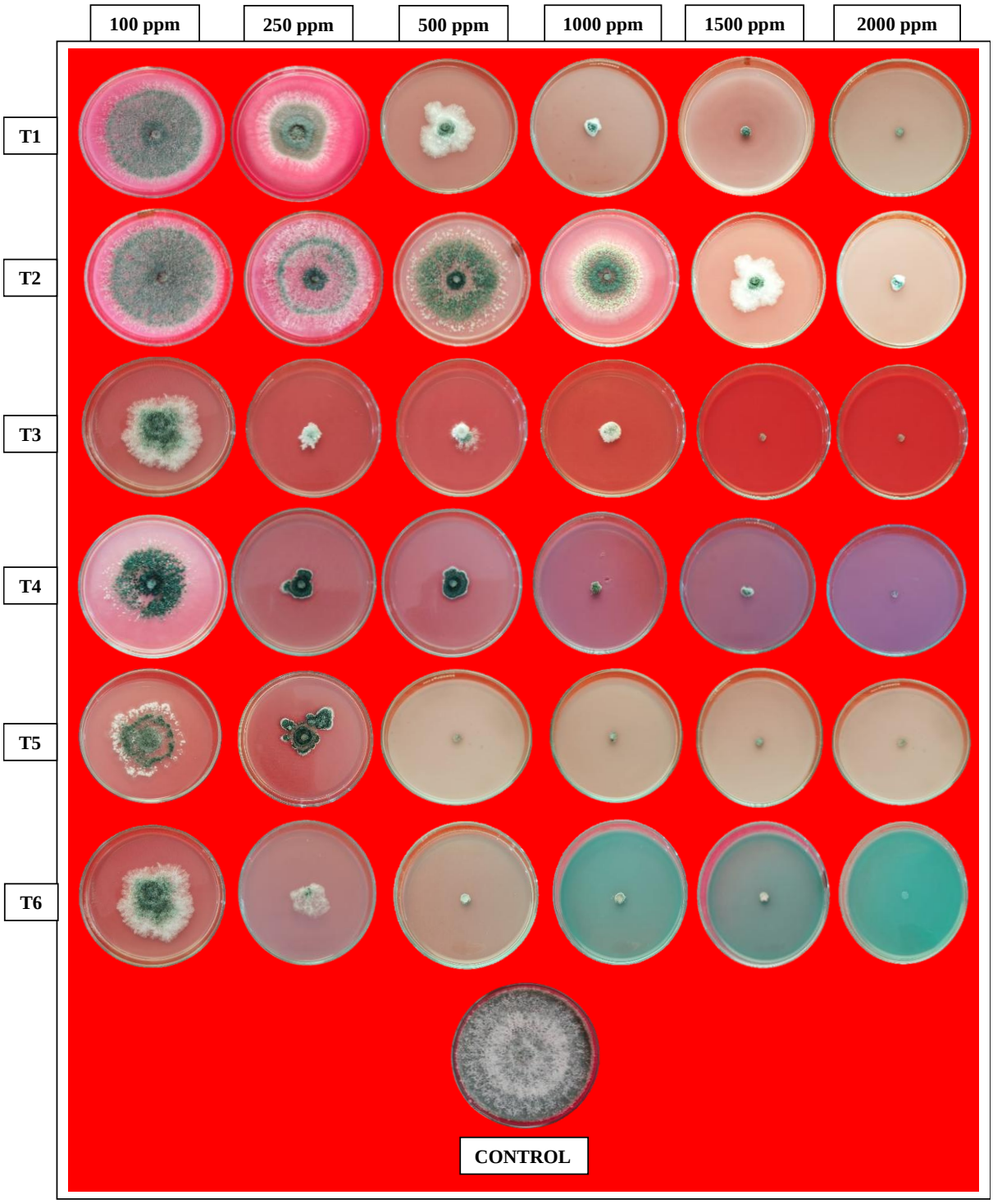
Fungicide/ Concentration (ppm)	Radial growth (mm)							Per cent inhibition						
	100	250	500	1000	1500	2000	Mean	100	250	500	1000	1500	2000	Mean
Captan	88.29^{a}	60.33^{c}	30.56^{c}	10.82^{c}	0.00^{c}	0.00^{c}	31.67^{c}	1.90^{c} (1.62)	32.97^{b} (5.83)	66.04^{c} (8.19)	87.98^{c} (9.43)	100.00^{a} (10.05)	100.00^{a} (10.05)	64.82^{c} (7.53)
Thiram	90.00^{a}	86.55^{b}	79.61^{b}	66.55^{b}	34.26^{b}	7.07^{b}	60.67^{b}	0.00^{c} (1.00)	3.83^{d} (2.14)	11.55^{d} (3.47)	26.06^{d} (5.19)	61.93^{b} (7.93)	92.15^{b} (9.65)	32.59^{d} (4.90)
Tebuconazole	48.28^{d}	19.54^{f}	17.46^{d}	8.94^{c}	0.00^{c}	0.00^{c}	15.70^{de}	46.35^{a} (6.88)	78.29^{a} (8.90)	80.60^{b} (9.03)	90.06^{b} (9.54)	100.00^{a} (10.05)	100.00^{a} (10.05)	82.55^{b} (9.08)
Carboxin + Thiram	54.62^{b}	24.19^{e}	21.19^{d}	6.53^{c}	0.00^{c}	0.00^{c}	17.75^{d}	39.31^{b} (6.35)	73.13^{b} (8.61)	76.46^{bc} (8.80)	92.74^{b} (9.68)	100.00^{a} (10.05)	100.00^{a} (10.05)	80.27^{b} (8.92)
Prochloraz + Tebuconazole	52.38^{bc}	33.88^{d}	0.00^{e}	0.00^{d}	0.00^{c}	0.00^{c}	14.38^{e}	41.80^{b} (6.54)	62.36^{c} (7.96)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	84.03^{a} (9.12)
Thiophanate methyl + Pyraclostrobin	49.54^{cd}	23.85^{e}	0.00^{e}	0.00^{d}	0.00^{c}	0.00^{c}	12.23^{e}	44.96^{bc} (6.78)	73.50^{b} (8.63)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	86.41^{a} (9.27)
Control	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	0.00^{c} (1.00)	0.00^{d} (1.00)	0.00^{e} (1.00)	0.00^{e} (1.00)	0.00^{c} (1.00)	0.00^{c} (1.00)	0.00^{e} (1.00)

Values expressed are mean of three replications;

Figures in the parenthesis are square root transformed values;

Note: Means with same letter are not significantly different at 5% level.

Plate 1 Compatibility of fungicides with *T. asperellum* under *in vitro* conditions



Where, T1 – Captan; T2 – Thiram; T3 – Tebuconazole; T4 – Carboxin + Thiram; T5 – Prochloraz + Tebuconazole and T6 – Thiophanate methyl + Pyraclostrobin.

Table 3 Compatibility of fungicides with *T. viride* under *in vitro* conditions

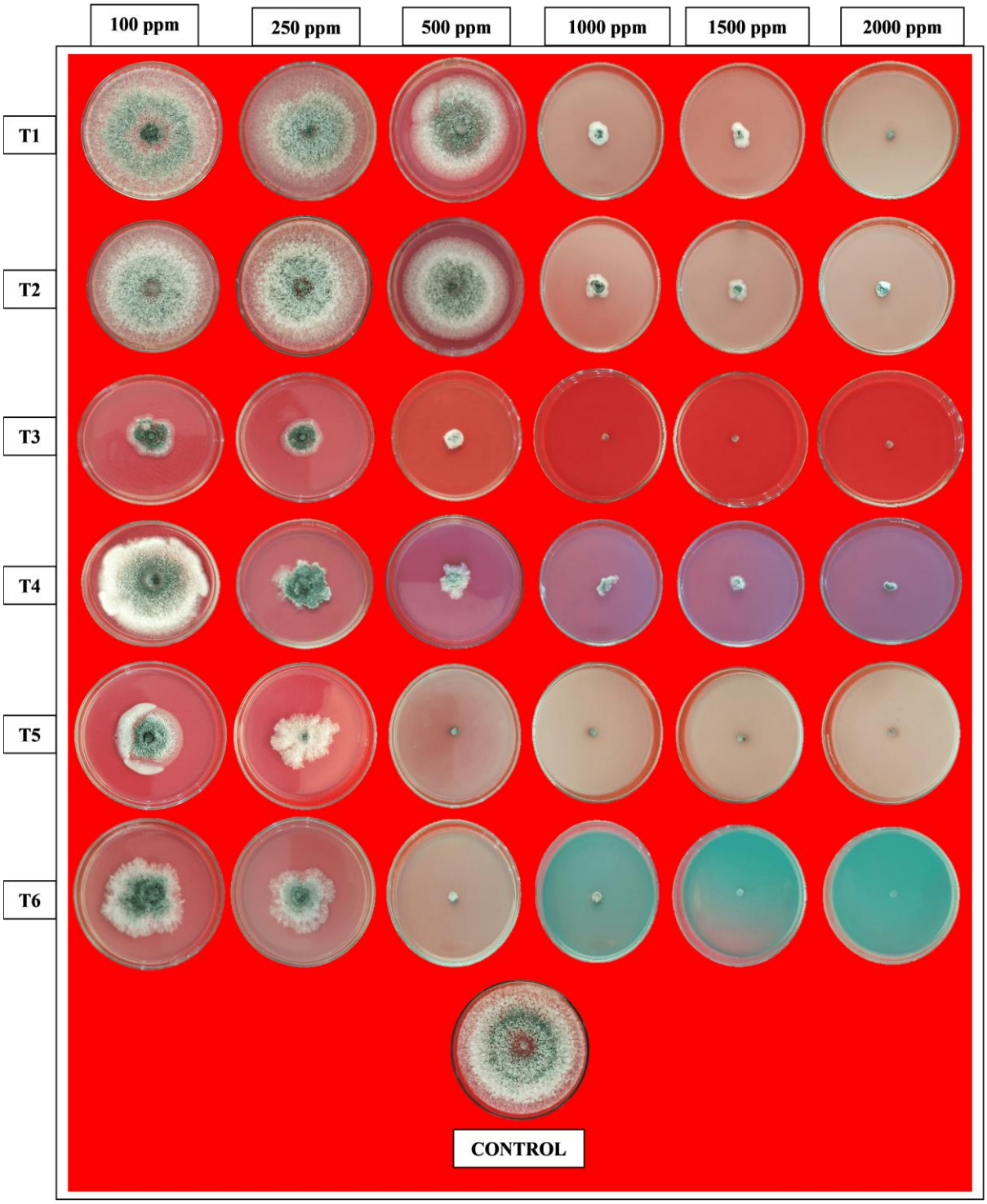
Fungicide/ Concentration (ppm)	Radial growth (mm)							Per cent inhibition						
	100	250	500	1000	1500	2000	Mean	100	250	500	1000	1500	2000	Mean
Captan	90.00^{a}	90.00^{a}	79.34^{b}	17.64^{b}	0.00^{c}	0.00^{c}	46.16^{b}	0.00^{e} (1.00)	0.00^{e} (1.00)	11.84^{d} (3.55)	80.40^{b} (9.02)	100.00^{a} (10.05)	100.00^{a} (10.05)	48.71^{c} (5.78)
Thiram	90.00^{a}	90.00^{a}	81.07^{b}	18.99^{b}	0.00^{c}	0.00^{c}	46.68^{b}	0.00^{e} (1.00)	0.00^{e} (1.00)	9.92^{d} (3.26)	78.90^{b} (8.94)	100.00^{a} (10.05)	100.00^{a} (10.05)	48.14^{c} (5.72)
Tebuconazole	27.44^{e}	24.19^{d}	15.71^{d}	0.00^{c}	0.00^{c}	0.00^{c}	11.22^{d}	69.51^{a} (8.40)	73.12^{a} (8.61)	82.55^{b} (9.14)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	87.53^{a} (9.38^{a})
Carboxin + Thiram	84.35^{b}	35.66^{b}	22.72^{c}	16.25^{b}	8.43^{b}	5.63^{b}	28.84^{c}	6.28^{d} (2.67)	60.37^{d} (7.83)	74.76^{c} (8.70)	81.94^{b} (9.11)	90.63^{b} (9.57)	93.75^{b} (9.73)	67.96^{b} (7.94)
Prochloraz + Tebuconazole	39.24^{d}	27.42^{cd}	0.00^{e}	0.00^{c}	0.00^{c}	0.00^{c}	11.11^{d}	56.40^{b} (7.58)	69.54^{bc} (8.40)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	87.66^{a} (9.36)
Thiophanate methyl + Pyraclostrobin	48.24^{c}	31.73^{bc}	0.00^{e}	0.00^{c}	0.00^{c}	0.00^{c}	13.33^{d}	46.40^{c} (6.88)	64.75^{b} (8.11)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	100.00^{a} (10.05)	85.19^{a} (9.20)
Control	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	90.00^{a}	0.00^{e} (1.00)	0.00^{e} (1.00)	0.00^{e} (1.00)	0.00^{c} (1.00)	0.00^{c} (1.00)	0.00^{c} (1.00)	0.00^{d} (1.00)

Values expressed are mean of three replications;

Figures in the parenthesis are square root transformed values;

Note: Means with same letter are not significantly different at 5% level.

Plate 2 Compatibility of fungicides with *T. viride* under *in vitro* conditions



Where, T1 – Captan; T2 – Thiram; T3 – Tebuconazole; T4 – Carboxin + Thiram; T5 – Prochloraz + Tebuconazole and T6 – Thiophanate methyl + Pyraclostrobin.

Table 4 Compatibility of fungicides with *B. subtilis* isolate S4KB5 under *in vitro* conditions

Fungicide/ Concentration (ppm)	Inhibition zone (mm)						
	100	250	500	1000	1500	2000	Mean
Captan	0.00^{b}	0.00^{b}	13.61^{b}	18.62^{b}	23.40^{d}	25.18^{e}	13.47^{b}
Thiram	0.00^{b}	0.00^{b}	11.98^{b}	13.31^{c}	18.27^{e}	28.38^{d}	11.99^{b}
Tebuconazole	0.00^{b}	14.26^{a}	26.43^{a}	28.40^{a}	30.35^{b}	33.19^{c}	22.11^{a}
Carboxin + Thiram	0.00^{b}	16.10^{a}	22.40^{a}	24.49^{a}	26.59^{c}	35.50^{b}	20.85^{a}
Prochloraz + Tebuconazole	8.95^{a}	13.49^{a}	20.34^{a}	25.35^{a}	32.42^{a}	39.18^{a}	23.29^{a}
Thiophanate methyl + Pyraclostrobin	0.00^{b}	12.02^{a}	22.80^{a}	27.46^{a}	29.88^{b}	36.20^{b}	21.40^{a}
Control	0.00^{b}	0.00^{b}	0.00^{c}	0.00^{d}	0.00^{f}	0.00^{f}	0.00^{c}

*Values expressed are mean of three replications;
Note: Means with same letter are not significantly different at 5% level.

Table 5 Compatibility of fungicides with *B. subtilis* isolate S8KB2 under *in vitro* conditions

Fungicide/ Concentration (ppm)	Inhibition zone (mm)						
	100	250	500	1000	1500	2000	Mean
Captan	0.00^{b}	0.00^{c}	0.00^{e}	7.76^{e}	18.55^{f}	21.44^{b}	7.96^{c}
Thiram	0.00^{b}	10.79^{b}	19.06^{b}	24.51^{b}	33.14^{a}	35.74^{a}	20.54^{a}
Tebuconazole	0.00^{b}	0.00^{c}	11.94^{d}	17.42^{d}	25.64^{d}	29.43^{ab}	14.07^{b}
Carboxin + Thiram	11.40^{a}	13.58^{a}	21.71^{a}	27.33^{a}	31.24^{b}	31.26^{ab}	22.75^{a}
Prochloraz + Tebuconazole	0.00^{b}	15.44^{a}	21.18^{a}	27.08^{a}	29.33^{c}	35.11^{a}	21.35^{a}
Thiophanate methyl + Pyraclostrobin	0.00^{b}	0.00^{c}	0.00^{e}	0.00^{f}	0.00^{g}	0.00^{c}	0.00^{d}
Control	0.00^{b}	0.00^{c}	14.00^{c}	20.33^{c}	23.71^{e}	26.52^{ab}	14.09^{b}

*Values expressed are mean of three replications;
Note: Means with same letter are not significantly different at 5% level.

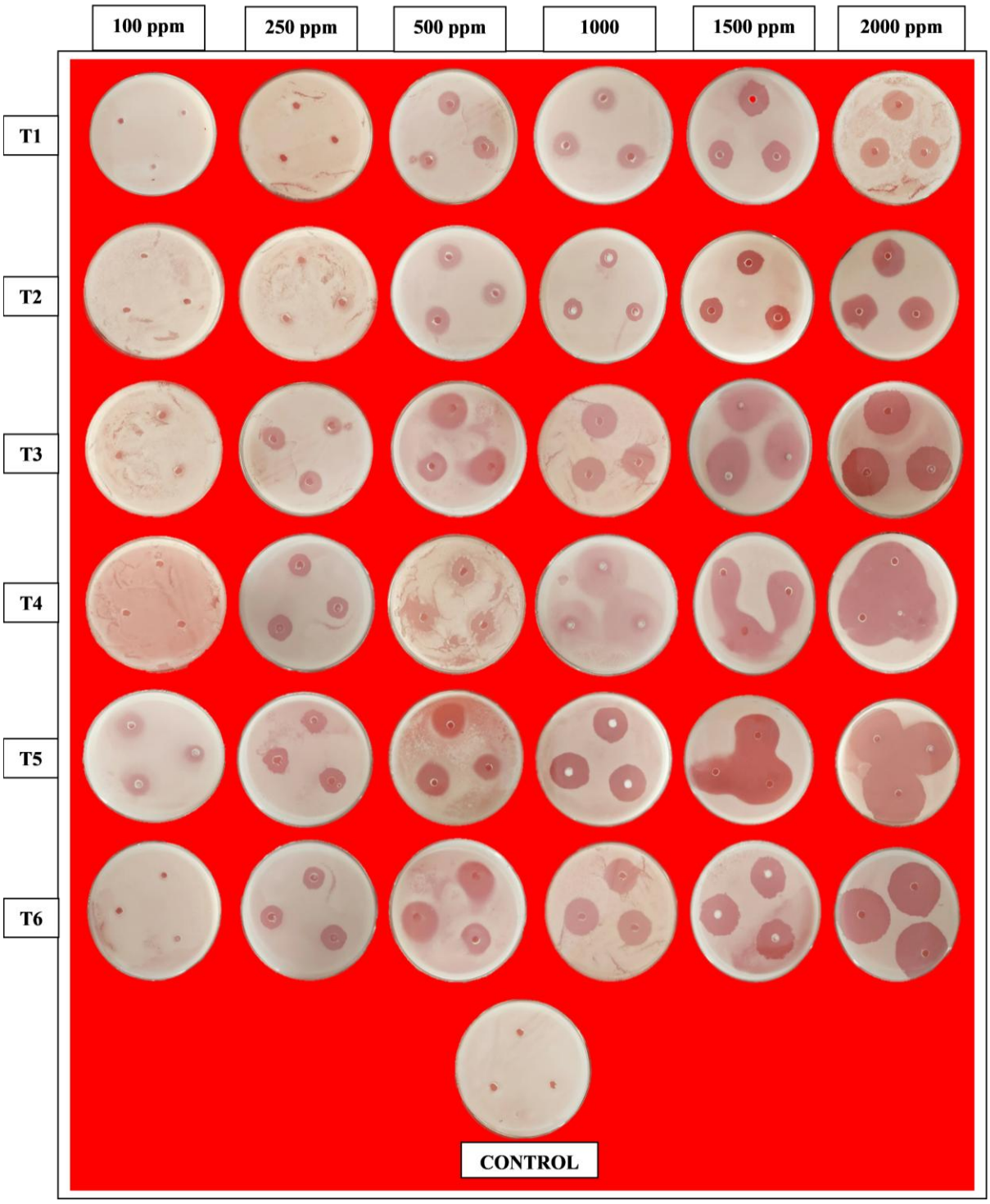
Table 6 Compatibility of fungicides with *B. subtilis* isolate B3 under *in vitro* conditions

Fungicide/ Concentration (ppm)	Inhibition zone (mm)						
	100	**250**	**500**	**1000**	**1500**	**2000**	**Mean**
Captan	0.00^{b}	0.00^{c}	0.00^{c}	17.23^{b}	21.39^{e}	27.71^{c}	11.06^{c}
Thiram	0.00^{b}	0.00^{c}	0.00^{c}	12.41^{c}	23.04^{cd}	28.35^{c}	10.63^{c}
Tebuconazole	0.00^{b}	0.00^{c}	14.27^{b}	21.22^{ab}	24.01^{bc}	31.25^{b}	15.12^{b}
Carboxin + Thiram	0.00^{b}	9.53^{b}	13.05^{b}	20.07^{ab}	22.23^{de}	28.56^{c}	15.57^{b}
Prochloraz + Tebuconazole	12.35^{a}	15.34^{a}	19.58^{a}	23.31^{a}	27.88^{a}	33.33^{a}	21.96^{a}
Thiophanate methyl + Pyraclostrobin	0.00^{b}	0.00^{c}	11.25^{b}	18.44^{b}	24.95^{b}	31.71^{b}	14.39^{b}
Control	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{f}	0.00^{d}	0.00^{d}

*Values expressed are mean of three replications;

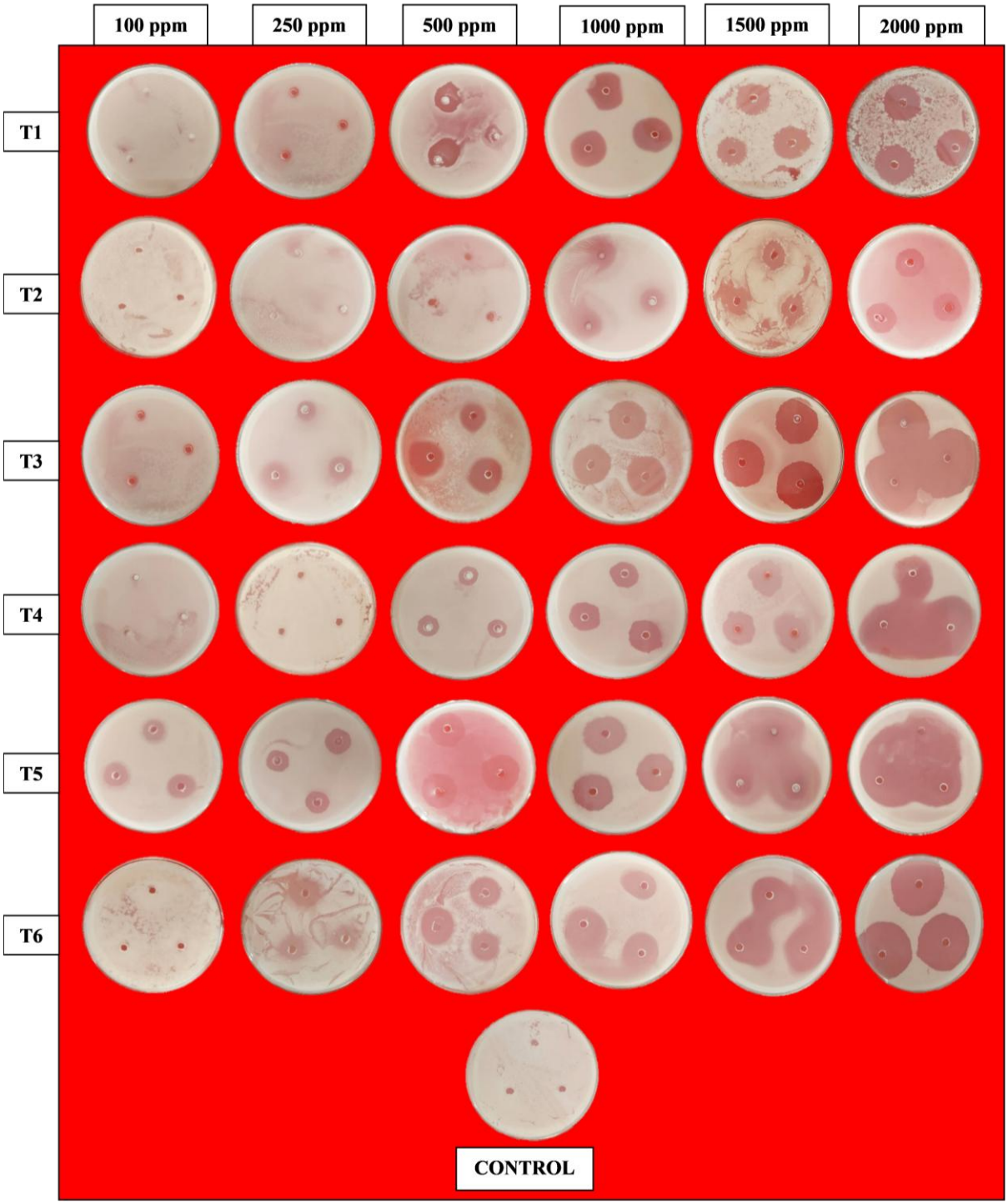
Note: Means with same letter are not significantly different at 5% level.

Plate 3 Compatibility of fungicides with *B. subtilis* isolate S4KB5 under *in vitro* conditions



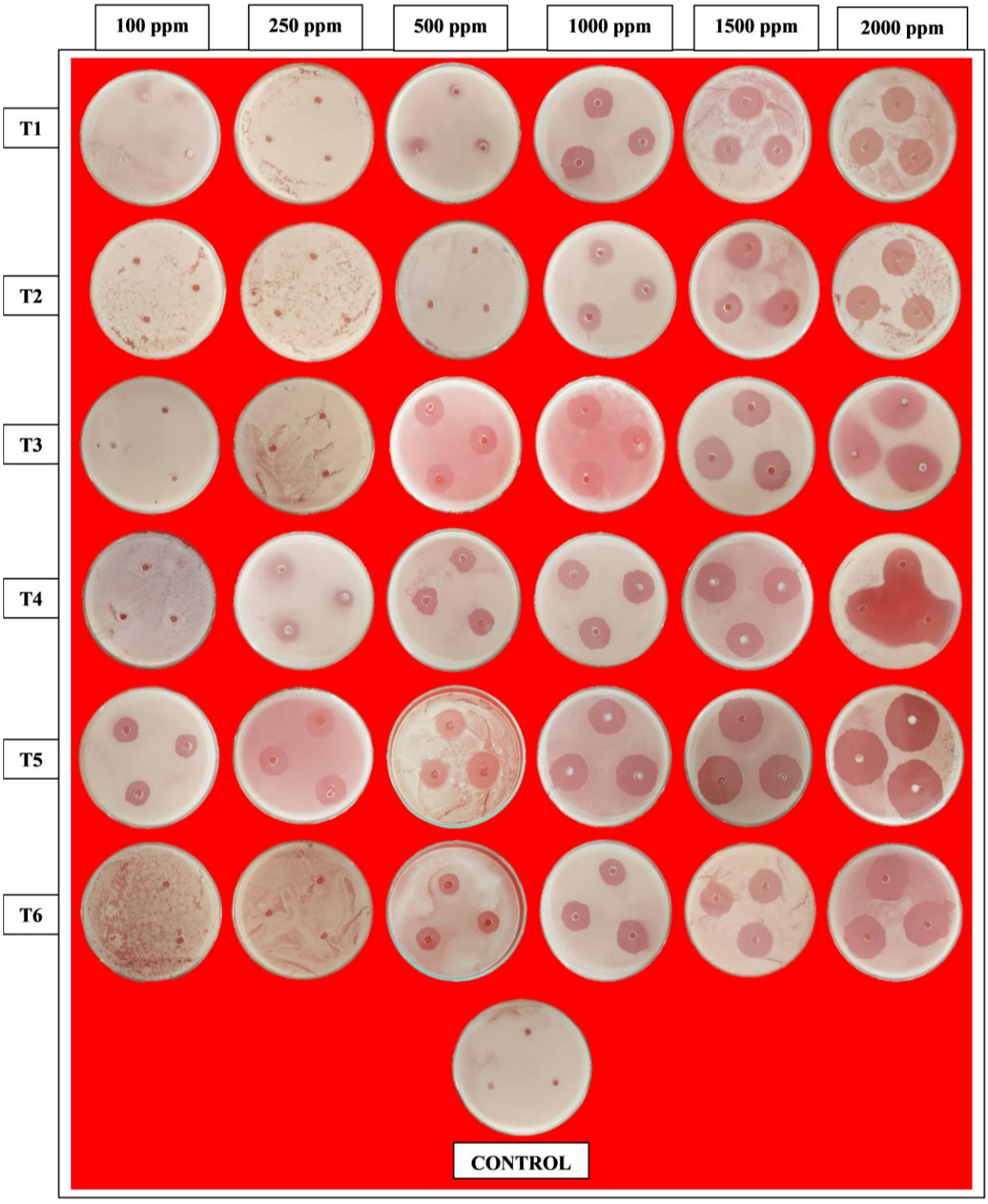
Where, T1 – Captan; T2 – Thiram; T3 – Tebuconazole; T4 – Carboxin + Thiram; T5 – Prochloraz + Tebuconazole and T6 – Thiophanate methyl + Pyraclostrobin.

Plate 4 Compatibility of fungicides with *B. subtilis* isolate S8KB2 under *in vitro* conditions



Where, T1 – Captan; T2 – Thiram; T3 – Tebuconazole; T4 – Carboxin + Thiram; T5 – Prochloraz + Tebuconazole and T6 – Thiophanate methyl + Pyraclostrobin.

Plate 5 Compatibility of fungicides with *B. subtilis* isolate B3 under *in vitro* conditions



Where, T1 – Captan; T2 – Thiram; T3 – Tebuconazole; T4 – Carboxin + Thiram; T5 – Prochloraz + Tebuconazole and T6 – Thiophanate methyl + Pyraclostrobin.