Journal of Biological Control, 37(1): 06-12, 2023, DOI: 10.18311/jbc/2023/32435

Research Article

Compatibility of indigenous *Trichoderma asperellum* with chemical fungicides for the management of chickpea wilt

GURURAJ SUNKAD*, RANJANA JOSHI and MEGHANA PATIL

Department of Plant Pathology, University of Agricultural Sciences, Raichur – 584104, Karnataka, India
**Corresponding author E-mail:* sunkadgururaj@gmail.com

ABSTRACT: *Trichoderma asperellum* is an antagonistic fungus, which has the ability to inhibit the growth of pathogens in target environment. The study on compatibility of *T. asperellum* with fungicide molecules was carried out to know its compatibility with different classes of fungicide molecules during the studies on antagonistic potential of *Trichoderma* sp. against *Fusarium oxysporum* f. sp. *ciceris* causing chickpea wilt. In the present study, each of six popular systemic, non-systemic and combi-fungicide molecules were used to study the compatibility with bioagent *Trichoderma asperellum*. The fungicides were used at three different concentrations, i.e., systemic fungicides at 0.05, 0.1 and 0.15 per cent and non-systemic and combi fungicides at 0.1, 0.2 and 0.3 per cent concentrations by using poisoned food technique. Among six systemic fungicides, only azoxystrobin was highly compatible, whereas other systemic fungicides were incompatible showing 100 per cent inhibition of *T. asperellum*. Among six non-systemic fungicides, propineb, copper oxychloride and copper hydroxide were compatible at all three concentrations tested. However, mancozeb was compatible at lower concentrations but incompatible at higher (0.3%) concentrations. Further, thiram and captan were highly incompatible. Among six combi fungicides, copper oxychloride + copper hydroxide and cymoxanil + mancozeb were compatible with *T. asperellum*.

KEYWORDS: Compatibility, combi fungicides, non-systemic, per cent mycelial inhibition, systemic, *Trichoderma asperellum*

(Article chronicle: Received: 17-01-2023; Revised: 21-03-2023; Accepted: 23-03-2023)

INTRODUCTION

The human interest in safer options for plant disease management employing biological control, genetically engineered crops, and resistant sources has been sparked by a global upsurge in warnings against synthetic pesticides. In nature, antagonism serves as the balance wheel; it exists wherever there is life. This fundamental truth of nature is the basis for bio-control or bio-intense management of plant pathogens. Depending on the target species, several groups of organisms including viruses, bacteria, fungi, and nematodes are used in the bio-management to manage diseases. The compatibility of prospective bio-agents with fungicides is crucial for the development of an efficient disease management programme. In an IDM strategy, fungicides and suitable bio-agents are used to protect seeds and seedlings from inoculums that are soil-borne and seed-borne (Dubey and Patil, 2001).

Applications of fungicides at low levels were beneficial for Bio-Control Agents (BCAs) in plant disease management systems, and improved disease control was attained (Frances *et al*., 2002; Buck, 2004). Similar disease suppression would be provided by combining BCAs with fungicides as would be the case with increased fungicide use (Monte, 2001). By combining antagonists with synthetic compounds, the potential for resistance development is avoided, and the need for fungicide application is decreased. Fungicides and bio-control agents do not usually work well together. Combining biocontrol agents with widely used fungicides may produce either synergism or antagonistic interactions between the two. Therefore, it is decided to determine whether possible bio-agents are compatible with widely used fungicides in the present investigation for the benefit of farmers.

Trichoderma asperellum, a filamentous fungus that reproduces asexually and possesses a sexual teleomorph from the genus Hypocrea, has gained popularity due to its adaptability and capacity to combat a significant number of plant diseases in a variety of target conditions. The extent of resistance varies depending on the fungicide, but it has been documented that *Trichoderma* species have innate

and/or induced resistance to numerous fungicides (Omar, 2006). The aim of the current investigation was to determine whether *T. asperellum* can be used along with the fungicides in integrated disease management of chickpea wilt.

MATERIALS AND METHODS

Isolation of *Trichoderma* sp.

During the studies on antagonistic potential *Trichoderma* sp. against *Fusarium oxysporum f.* sp. *ciceris* causing chickpea wilt, the soil sample was taken from the chickpea rhizospheric soil and native *Trichoderma* sp. was isolated on *Trichoderma* Specific Medium (TSM) ($MgSO_4$. $7H_2O$: 0.20 g; K_2HPO_4: 0.90 g; KCL: 0.15 g; NH_4NO_3: 1.00 g; Glucose: 3.00 g; Rose Bengal: 0.15 g; p-dimethyl amino benzene diazosodium sulfonate: 0.3g; Chloramphenicol: 0.25 g; Pentachloronitrobenzene: 0.20 g; Agar: 20.00 g; Distilled water: 1000 ml) by using serial dilution technique. The pure culture of *Trichoderma* sp. was obtained by the hyphal tip method and maintained in potato dextrose agar slants at 28±1°C. Later, the pure culture slant was sent to Agharkar Research Institute, Pune for identification.

Studies on compatibility of *Trichoderma* sp. with popular classes of fungicides

The studies on the compatibility of *Trichoderma* sp. with popular classes of fungicides were carried out at the Bio-input Entrepreneurship Center, Department of Plant Pathology, University of Agricultural Sciences, Raichur. Six systemic fungicides which are popular among farmers were tested for compatibility at three concentrations viz., 0.05, 0.1, and 0.15%. Apart from these, six non-systemic and six combination fungicides were assessed for compatibility at concentrations of 0.1, 0.2, and 0.3%**.**

Trichoderma sp. was tested for fungicide compatibility using the poisoned food approach (Shravelle, 1961). The *Trichoderma* sp. was first cultivated for 5-6 days on Potato Dextrose Agar (PDA) (Composition for 1 lit: Potato: 200 g; Dextrose: 20 g; Agar: 20 g; Distilled water: 1000 ml) medium. To achieve the appropriate concentration, the fungicidal suspension was added to the molten PDA medium. In 90 mm diameter Petri dishes, 20 ml of molten PDA medium with fungicide suspension was added. A 5mm disc of *Trichoderma* sp. was positioned in the middle of the plates after solidification. The control was maintained without fungicides. The plates were incubated at 28±1°C and each set of treatments was replicated three times. When the control plate's growth was at its highest, the radial growth of *Trichoderma* sp. was measured in treatments and the per cent mycelial inhibition was estimated using the following formula (Vincent, 1947).

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

Where,

I = Per cent inhibition

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

Statistical analysis

The mycelial growth in terms of diameter of *Trichoderma* sp. and per cent inhibition was subjected to statistical analysis of factorial CRD data which was analyzed using the OPSTAT software.

RESULTS

Identification of *Trichoderma* sp.

The isolated *Trichoderma* sp. was identified as *Trichoderma asperelllum* by Agharkar Research Institute, Pune. The sequence obtained was deposited in NCBI genebank, and the accession number was obtained (MW063489).

Compatibility of *Trichoderma asperelllum* with systemic fungicides

The results (Table 1) indicated that Azoxystrobin showed the least mean inhibition (2.22%) of *T. asperellum* among the six systemic fungicides tested. However, other systemic fungicides *viz*., hexaconazole, thiophanate methyl, carbendazim, tebuconazole, and benomyl were found incompatible by exhibiting 100% suppression of the bioagent at all the three concentrations. The results on interaction of fungicides versus their concentrations showed that Azoxystrobin was highly compatible with *T. asperellum* with a minimum inhibition of 6.67% at 0.15 percent concentration, while the other systemic fungicides were highly incompatible with bio-agent (Table 1 and Plate 1).

Compatibility of *Trichoderma asperellum* with non-systemic fungicides

Among non-systemic fungicides, two fungicide molecules, such as propineb (0.00%) and copper hydroxide (0.00%), showed considerably lowest mean inhibition of *T. asperellum* followed by copper oxychloride (18.52%) and mancozeb (22.22%). The inhibition of bio-agent was significant with respect to thiram (51.61%) and captan (80.62%). With an increase in concentration, the mean *T. asperellum* inhibition percentage ranged from 16.05% (0.1%) to 46.36% (0.3%). Further, copper oxychloride and

Table 1. Compatibility of *T. asperellum* with systemic fungicides

Treatment	Per cent inhibition of mycelial growth*			
	Concentration (%)			Mean
	0.05	0.1	0.15	
Azoxystrobin 23 % SC	0.00 (0.00)**	0.00 (0.00)	6.67 (14.96)	2.22 (8.57)
Hexaconazole 5 % EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Thiophanate methyl 70 % WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Carbendazim 50 % WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole 25.9 % EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Benomyl 50 % WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Mean	**83.33 (65.90)**	**83.33 (65.90)**	**84.45 (66.76)**	**83.70 (66.18)**

	S. Em ±	C. D at 1%
Fungicides (F)	0.17	0.65
Concentration (C)	0.12	0.46
F×C	0.29	1.12

*Mean of three replications, **Figures in parentheses are arc sine transformed values.

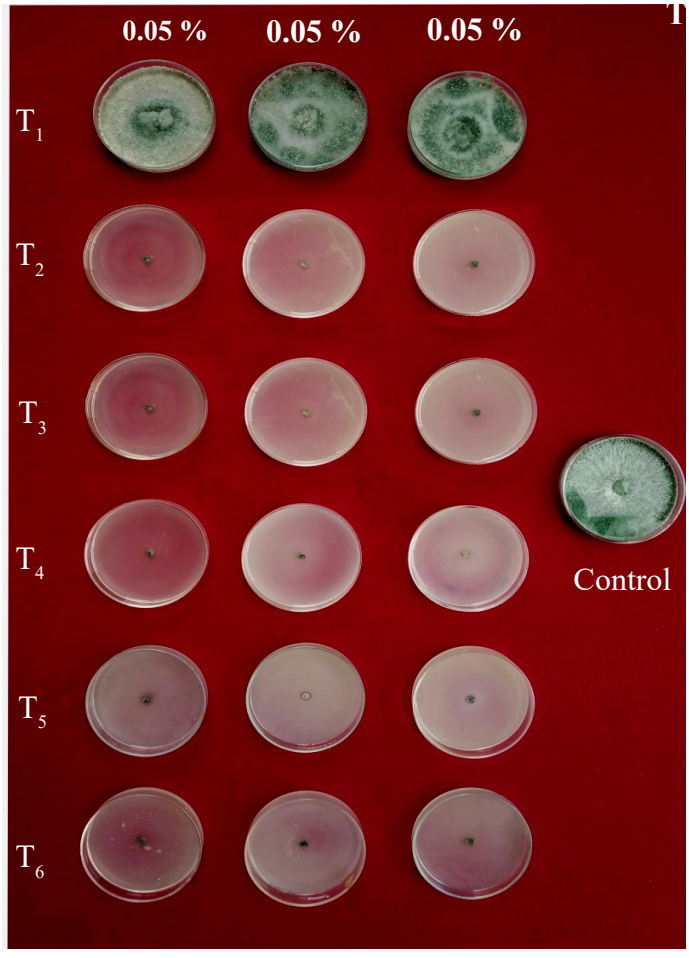

Plate 1. Compatibility of *T. asperellum* with systemic fungicides (T1: Azoxystrobin 23 % SC; T2: Hexaconazole 5 % EC; T3: Thiophanate methyl 70 % WP; T4: Carbendazim 50 % WP; T5: Tebuconazole 25.9 % EC; T6: Benomyl 50 % WP.

Table 2. Compatibility of *T. asperellum* with non-systemic fungicides

Treatment	Per cent inhibition of mycelial growth*			
	Concentration (%)			Mean
	0.1	0.2	0.3	
Captan 50% WP	75.93 (60.61)**	77.78 (61.87)	88.15 (69.85)	80.62 (63.87)
Thiram 75%WP	20.37 (26.83)	55.56 (48.18)	78.89 (62.64)	51.61 (45.91)
Mancozeb 75% WP	0.00 (0.00)	0.00 (0.00)	66.67 (54.73)	22.22 (28.12)
Propineb 70% WP	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Copper oxy chloride 50% WP	0.00 (0.00)	11.11 (19.47)	44.44 (41.80)	18.52 (25.48)
Copper hydroxide 77% WP	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	**16.05 (23.61)**	**24.08 (29.38)**	**46.36 (42.91)**	**28.83 (32.47)**
	S. Em ±	**C. D. at 1%**		
Fungicides (F)	0.27	1.04		
Concentration (C)	0.19	0.73		
F×C	0.47	1.80		

*Mean of three replications, **Figures in parentheses are arc sine transformed values.

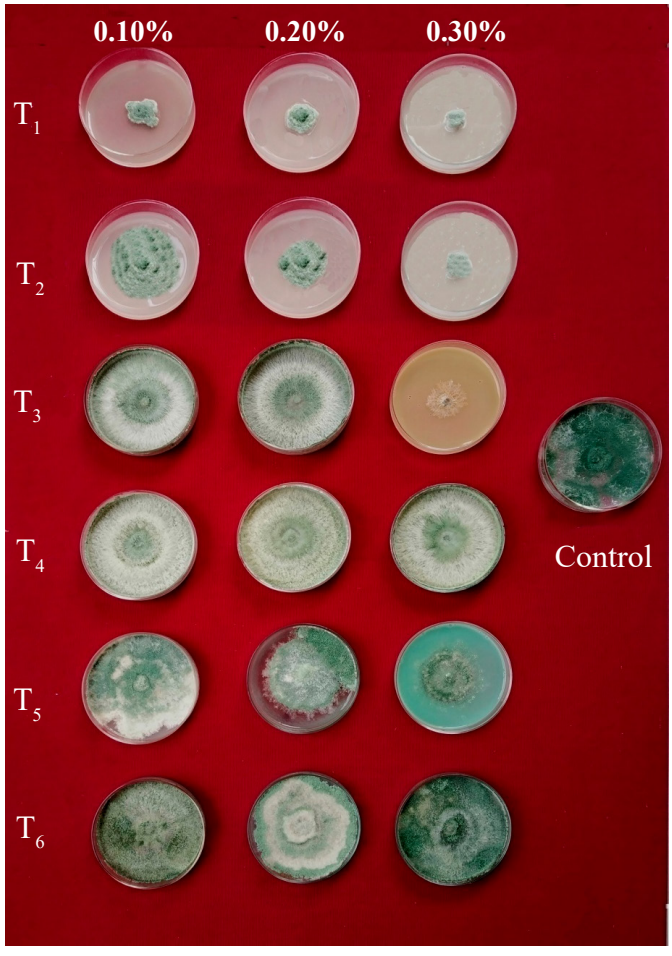

Plate 2. Compatibility of *T. asperellum* with non-systemic fungicides (T_1: Captan 50 % WP; T_2: Thiram 75 % WP; T_3: Mancozeb 75 % WP; T_4: Propineb 70 % WP; T_5: Copper oxychloride 50 % WP; T_6: Copper hydroxide 77 % WP % EC).

Table 3. Compatibility of *T. asperellum* with combi fungicides

Treatment	Per cent inhibition of mycelial growth*			
	Concentration (%)			Mean
	0.1	0.2	0.3	
Thiophanate methyl 450 g/l + Pyraclostrobin 50g/l (w/v) FS	57.41 (49.25)**	83.33 (65.90)	94.44 (76.36)	78.39 (62.29)
Carbendazim 12 % + Mancozeb 63 % WP	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Tebuconazole 50 %+ Trifloxystrobin 25 % WG	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
Carboxin 37.5 %+ Thiram 37.5 %WS	93.33 (75.03)	97.78 (81.42)	100.00 (90.00)	97.04 (80.08)
Copper oxychloride + Copper hydroxide 14 % WG	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Cymoxanil 8 % + Mancozeb 64 % WP	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	**58.46 (49.86)**	**63.52 (52.84)**	**95.74 (54.17)**	**62.57 (52.27)**

	S. Em ±	C. D at 1%
Fungicides (F)	0.53	2.03
Concentration (C)	0.37	1.43
F×C	0.91	3.51

*Mean of three replications, **Figures in parentheses are arc sine transformed values.

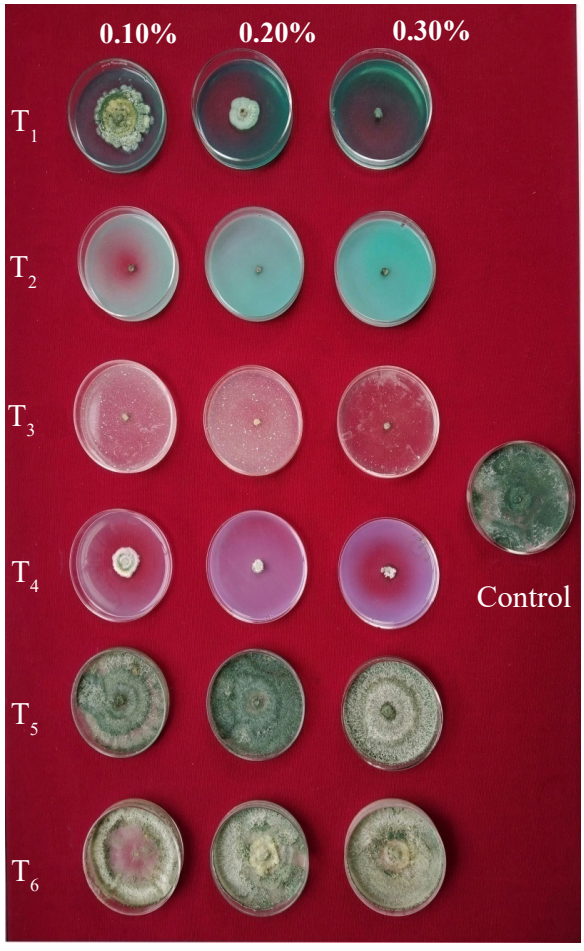

Plate 3. Compatibility of *T. asperellum* with combi fungicides (T_1: Thiophanate methyl 450g/l + Pyraclostrobin 50g/l (w/v) FS; T_2: Carbendazim 12 % + Mancozeb 63 % WP; T_3: Tebuconazole 50 % + Trifloxystrobin 25 % WG; T_4: Carboxin 37.5 % + Thiram 37.5 % WS; T_5: Copper oxychloride + Copper hydroxide 14 % WG; T_6: Cymoxanil 8 % + Mancozeb 64 % WP).

mancozeb permitted the growth of *T. asperellum* at lower concentrations but inhibited it by 11.11% and 66.67% at 0.2 and 0.3 per cent concentrations, respectively. However, propineb and copper hydroxide were found compatible with zero inhibition per cent at all three concentrations tested (Table 2 and Plate 2).

Compatibility *T. asperellum* with combi fungicides

The results presented in Table 3 and Plate 3 indicated that the combi fungicide molecules such as copper oxychloride + copper hydroxide and cymoxanil + mancozeb demonstrated considerably lowest inhibition of *T. asperellum*. The mean maximum inhibition (100%) of *T. asperellum* was recorded with carbendazim + mancozeb and tebuconazole + trifloxystrobin at all three concentarations. With an increase in concentration, the mean *T. asperellum* inhibition percentage ranged from 58.46% (0.1%) to 95.74% (0.3%). With respect to interaction effect of fungicides and their concentrations. Copper oxychloride + copper hydroxide and cymoxanil + mancozeb were found compatible by recording zero inhibition per cent at all three concentrations tested. Further, results also shown that at 0.1% concentration thiophanate methyl + pyraclostrobin and carboxin + thiram inhibited *T. asperellum* by 57.41% and 93.33 %, respectively. However, carbendazim + mancozeb and tebuconazole + trifloxystrobin were completely incompatible with *T. asperellum* (Table 3 and Plate 3).

DISCUSSION

The results of present investigation indicated that azoxystrobin was compatible with *T. asperellum,* which showed the least mean inhibition of *T. asperellum* among the six systemic fungicides tested. Further, other systemic fungicides such as hexaconazole, thiophanate methyl, carbendazim, tebuconazole, and benomyl were found incompatible by exhibiting 100% suppression of the bio-agent. The prior research has demonstrated that *Trichoderma* sp. were incompatible with carbendazim, benomyl, carboxin, propiconazole, hexaconazole, tricyclozole, tridemorph, and chlorothalonil with 100% radial growth suppression (Ranganathswamy *et al*., 2012). Further, *T. viride* was completely incompatible with systemic fungicides, including carbendazim, hexaconazole, tebuconazole and propiconazole as reported by Bindu *et al*. (2011). *T. viride* isolates were not safe for these fungicides to use, according to Madhusudhan *et al.* (2010) in screening of carbendazim (50% WP), propiconazole (25% EC), tridemorph (50% EC), and hexaconazole (5% EC) against *Trichoderma viride*. Ashok (2005) evaluated *in vitro* compatibility of *T. viride* and *T. harzianum* and found that carbendazim and hexaconazole were extremely incompatible with at all concentrations examined. Propiconazole was harmful and incompatible with *T. harzianum*, according to Ajay *et al*. (2018). Malathi *et al*. (2002) reported that *Trichoderma* could not develop at even 1 ppm of carbendazim and 10 ppm of thiophanate methyl.

With respect to non-systemic fungicides, the present investigation results indicated that propineb and copper hydroxide were found highly compatible whereas, thiram and captan were incompatible with *T. asperellum.* Ashok (2005) reported that copper oxychloride and mancozeb were found compatible with commercial and native isolates of *T. viride* and *T. harzianum*. According to Madhusudhan *et al*. (2010), isolated *T. viride* was shown to be unaffected by mancozeb. According to Saxena *et al*. (2014), mancozeb up to 0.25 percent and thiram up to 0.1 percent were compatible with the test antagonist *T. viride* growth and did not negatively affect it. The findings of Valarmathi *et al*. (2013) showed that copper hydroxide inhibited *T. viride* at concentrations greater than 0.25 percent. However, in the current study, *T. asperellum* still being compatible with copper hydroxide at concentrations as low as 0.3%, which may be due to the effect of different species of our investigation.

With regard to combi- fungicides, the results indicated that copper oxychloride + copper hydroxide and cymoxanil + mancozeb were found compatible with *T. asperellum*. Whereas, thiophanate methyl + pyraclostrobin, carboxin + thiram carbendazim + mancozeb and tebuconazole + trifloxystrobin were completely incompatible with *T. asperellum.* The findings of the current investigation are consistent with those of Amaresh *et al*. (2019), who reported that *T. viride* and *T. hamatum* were incompatible with carboxin + thiram. Theertha *et al*. (2017) observed that even at the lowest concentration tested (0.01%), the fungicides containing carbendazim were strongly suppressive to the mycelial growth of *Trichoderma*. According to Lakshmi *et al*. (2018), carbendazim + mancozeb was also incompatible and showed a 100% inhibition on the growth of the fungal antagonist, and cymoxanil + mancozeb at a concentration of 0.1% recorded the lowest inhibition of 13.58% on the growth of the fungal bio-agent. In the present investigation, tebuconazole + trifloxystrobin were found incompatible with *T. asperellum* and this study was not previously reported by others.

CONCLUSION

The fungicide molecules such as azoxystrobin, propineb, copper hydroxide, copper oxychloride, copper hydroxide + copper oxychloride and cymoxanil + mancozeb were found highly compatible with *T. asperellum*. The other fungicide molecules viz., carbendazim, tebuconazole, hexaconazole, benomyl, captan, thiram, thiophanate methyl + pyraclostrobin, carbendazim + mancozeb, tebuconazole + trifloxystrobin and carboxin + thiram were highly incompatible with the test bio-agent.

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