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Compatibility studies of *Bacillus* spp. with commonly used agrochemicals

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Abstract
The compatibility of bacterial biocontrol agent (*Bacillus* spp.) was assessed with seven seed dressers (Thiophanate Methyl + Pyraclostrobin, Mancozeb + Carbendazim, Carboxin + Thiram, Captan, Captan + Hexaconazole, Thiamethoxam and Imidacloprid + Hexaconazole), nine fungicides (Hexaconazole, Propiconazole, Tebuconazole, Thiophanate Methyl, Chlorothalonil, Propineb, Copper Oxychloride, Carbendazim + Mancozeb and Azoxystrobin + Difenoconazole) and five weedicides (2,4-D Amine Salt, Pendimethalin, Oxyfluorfen, Ammonium Salt of Glyphosate and Glyphosate), commonly used by farmers in India for the control of soil borne plant pathogens. Bacillus spp. (BRSN-B2) was found compatible with seed dressing chemicals (Thiophanate Methyl 45% + Pyraclostrobin 5% FS and Thiamethoxam 30% FS), fungicides (Thiophanate Methyl 70% WP and Copper Oxychloride 50% WP) and weedicides (2,4-D Amine Salt 58% SL, Oxyfluorfen 23.5% EC and Ammonium Salt of Glyphosate 71% SG). The usage of these bio-pesticides was made more widespread by their pesticide compatibility since they can be used in integrated disease management to control soil-borne plant diseases.

Keywords: Compatibility, *Bacillus*, weedicides, fungicides & seed dressers

Introduction

Soil-borne plant pathogens are highly destructive pathogens and cause tremendous yield losses to all kinds of crops. By using antagonistic bacteria, plant diseases can be effectively controlled (Cook and Baker, 1983) [3]. The application of bio control agents to protect some commercially significant crops shows promise after substantial research on the interactions between bio control agents and plant diseases (Vesseur *et al*., 1990) [10]. A competitive advantage for the establishment of the introduced bio control agents and an improvement in the bio control may result from the addition of particular chemicals. When combined with bio control agents, the use of fungicide at decreased rates has dramatically improved disease control in comparison to treatments using bio control agents alone in a number of disease management techniques (Frances *et al*., 2002; Buck, 2004) [4, 2]. The objectives of the present study is to test the growth of different bio control agents with commonly used pesticides at different concentrations under *in vitro* conditions for the control of soil borne plant pathogens.

Materials and Methods

The compatibility of bacterial bio control agents with commonly used Agrochemicals (seed treatment chemicals, fungicides and weedicides) were evaluated by the Disk diffusion method using a completely randomized design (CRD). Sterile filter paper discs (Whatman No. 42) of 5 mm diameter will be soaked in different concentrations of agrochemicals. The discs were placed at the center of Petri dishes containing the NA medium seeded with 48 hr the old culture of the bacterial antagonists. The inoculated plates were kept in the refrigerator at 4°C for 4 h to allow diffusion of the chemical into medium. Control was consisting of filter paper disc soaked in sterile distilled water. Three replications were maintained. The plates were incubated at 27±2 °C for 48 h and observed for the production of inhibition zone around filter paper discs.

Table 1: *In vitro* evaluation of compatibility of seed treatment chemicals with bacterial antagonists

Sl. No.	Treatments	Trade name	Dose
1	Thiophanate Methyl 45% + Pyraclostrobin 5% FS	Xelora	0.30%
2	Mancozeb 50% + Carbendazim 25% WS	Sprint	0.30%
3	Carboxin 37.5% + Thiram 37.5% DS	Vitavax power	0.30%
4	Captan 50%WP	Captaf	0.30%
5	Captan 70% WP+ Hexaconazole 5%	Taqat	0.15%
6	Thiamethoxam 30% FS	Anant	0.50%
7	Imidacloprid 18.5% + Hexaconazole 1.5% FS	Trailer	0.20%

Table 2: *In vitro* evaluation of compatibility of fungicides with bacterial antagonists

Sl. No.	Treatments	Trade name	Dose
1	Hexaconazole 5% EC	Contaf Plus	0.20%
2	Propiconazole 25% EC	Propizole	0.10%
3	Tebuconazole 25.9% EC	Folicur	0.15%
4	Thiophanate Methyl 70% WP	Roko	0.20%
5	Chlorothalonil 75% WP	Kavach	0.25%
6	Propineb 70% WP	Antracol	0.20%
7	Copper Oxychloride 50% WP	Blitox	0.30%
8	Carbendazim 12% + Mancozeb 63% WP	Kuber	0.20%
9	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	Amistar Top	0.10%

Table 3: *In vitro* evaluation of compatibility of weedicides with bacterial antagonists

Sl. No.	Treatments	Trade name	Dose
1	2,4-D Amine Salt 58% SL	Kay-D	0.22%
2	Pendimethalin 30% EC	Stomp Xtra	0.62%
3	Oxyfluorfen 23.5% EC	Goal	0.12%
4	Ammonium Salt of Glyphosate 71% SG	Excel Mera 71	0.62%
5	Glyphosate 41% SL	All Kill	12%

Results and Discussion

Seed treatment chemicals

Data presented in Table 4. showed that the isolates of *Bacillus* spp. were found safer with Thiophanate Methyl 45% + Pyraclostrobin 5% FS, Carboxin 37.5% +Thiram 37.5% DS and Thiamethoxam 30% FS as 85 mm dia. radial growth was noted by these seed dressers with no inhibition. Other seed treatments pesticides *viz.*, Captan 70% + Hexaconazole 5% WP, Imidacloprid 18.5%+ Hexaconazole 1.5% FS, Captan 50% WP, and Mancozeb 50% + Carbendazim 25% WS showed 73.73, 72.90, 72.57and 74.77 mm dia. radial growth with 14.63, 14.24, 13.26 and 12.04 per cent inhibition of *Bacillus* spp. isolate respectively.

Similar *in vitro* bacteriostatic inhibition of the bacterial antagonists was reported by many workers around the world. Mohiddin and Khan (2013) [7] observed that, maximum tolerance concentration for *B. subtilis* were 3200 µg captan/ml, 600 µg mancozeb/ml. Whereas, in case of carbendazim, the bacteria showed tolerance even for a concentration of 50,000 µg/ml and Suneeta *et al.*, (2016) [9] reported that *Bacillus* spp. strains showed growth tolerance against carbendazim, and hexaconazole. According to, Aswathi *et al.*, (2016) [1] Maximum tolerance concentration of *B. subtilis* was found to be at lower concentration i.e., imidacloprid @ 0.01%

Fungicides

Data is presented in Table 5. showed that the isolates of *Bacillus* spp. was found safer with Thiophanate Methyl 70% WP and Copper Oxychloride 50% WP suggesting that this isolate can be used with Thiophanate Methyl 70% WP and Copper Oxychloride 50% WP as no percent inhibition was observed. Other chemicals i.e., Carbendazim 12% + Mancozeb 63% WP, Propineb 70% WP, Tebuconazole 25.9% EC, Azoxystrobin 18.2% + Difenoconazole 11.4% SC, Propiconazole 25% EC, Chlorothalonil 75% WP and Hexaconazole 5% EC were found compatible safer to this isolate exhibited 54.57, 61.23, 69.50, 72.50, 73.10, 74.97 and 77.43 mm dia. growth with 33.81, 26.41, 17.22, 13.89, 13.22, 11.15 and 8.41 per cent growth inhibition respectively.

Similar bacteriostatic as well as bactericidal effects of the fungicides with bacterial antagonists were reported earlier by several workers. Suneeta *et al.*, (2016) [9] reported that, Tebuconazole 50, propineb 70 WP and propiconazole 25% EC, Difenoconazole 25% EC, Azoxystrobin 25% SC, Carbendazim, and then followed by hexaconazole were incompatible with *B. subtilis* at higher concentration. Similarly, Mohiddin and Khan (2013) [7] observed that Mancozeb was incompatible above 600 µg mancozeb/ml and Harshita *et al.*, (2019) [5] reported that Topsin M (Thiophanate Methyl 70% WP) was compatible with *B. subtilis*

Weedicides

Data presented in Table 6. showed that the isolates of *Bacillus* spp. were compatible with 2,4-D Amine Salt 58% SL, Oxyfluorfen 23.5% EC, Ammonium Salt of Glyphosate 71% SG and Glyphosate 41% SL suggesting that this isolate can be used with these weedicides as no percent inhibition were observed. Pendimethalin 30% EC was found compatible with *Bacillus* spp. to the extent of 13.03 per cent inhibition.

Results of the present study on the compatibility of weedicides with *Bacillus* spp. were in consonance with those reported earlier by several workers, Aswathi *et al.*, (2016) [1] tested the compatibility of imidacloprid, carbendazim and pendimethalin with *B. subtilis* and found that maximum tolerance concentration of *B. subtilis* was found to be at lower concentration i.e., imidacloprid @ 0.01%, carbendazim @ 0.05% and moderately sensitive to pendimethalin @ 0.2%

Table 4: *In vitro* evaluation of compatibility of seed treatment chemicals with *Bacillus* spp.

T	Treatments	Dose	Dia. of bacterial colony (mm)	Inhibition (%)
T_1	Thiophanate Methyl 45% + Pyraclostrobin 5% FS	0.30%	85.00	0.00 (0.00)*
T_2	Mancozeb50% + Carbendazim 25% WS	0.30%	74.77	12.04 (20.30)
T_3	Carboxin 37.5% + Thiram 37.5% DS	0.30%	85.00	0.00 (0.00)
T_4	Captan 50%WP	0.30%	72.57	14.63 (22.48)
T_5	Captan70%WP + Hexaconazole 5%	0.15%	73.73	13.25 (21.35)
T_6	Thiamethoxam 30% FS	0.50%	85.00	0.00 (0.00)
T_7	Imidacloprid 18.5% + Hexaconazole 1.5% FS	0.20%	72.90	14.24 (22.16)
T_8	Control	-	85.00	0.00 (0.00)
SE±			0.13	0.13
CD @ 1%			0.55	0.53

(*Figures in parenthesis are arc sine transformed value)

Table 5: *In vitro* evaluation of compatibility of fungicides with *Bacillus* spp.

T	Treatments	Dose	Dia. bacterial colony (mm)	Inhibition (%)
T_1	Hexaconazole 5% EC	0.20%	77.43	8.90 (17.36)*
T_2	Propiconazole 25% EC	0.10%	73.10	14.00 (21.97)
T_3	Tebuconazole 25.9% EC	0.15%	69.50	18.24 (25.28)
T_4	Thiophanate Methyl 70% WP	0.20%	85.00	0.00 (0.00)
T_5	Chlorothalonil 75% WP	0.25%	74.97	11.80 (20.09)
T_6	Propineb 70% WP	0.20%	61.23	27.96 (31.92)
T_7	Copper Oxychloride 50% WP	0.30%	85.00	0.00 (0.00)
T_8	Carbendazim 12% + Mancozeb 63% WP	0.20%	54.57	35.80 (36.75)
T_9	Azoxystrobin 18.2% + Difenoconazole 11.4% SC	0.10%	72.50	14.71 (22.55)
T_{10}	Control	-	85.00	0.00 (0.00)
SE±			0.25	0.21
CD @ 1%			0.99	0.86

(*Figures in parenthesis are arc sine transformed value)

Table 6: *In vitro* evaluation of compatibility of weedicides with *Bacillus* spp.

T	Treatments	Dose	Dia. bacterial colony (mm)	Inhibition%
T_1	2,4-D Amine Salt 58% SL	0.22%	85.00	0.00 (0.00)*
T_2	Pendimethalin 30% EC	0.62%	73.93	13.03 (21.16)
T_3	Oxyfluorfen 23.5% EC	0.12%	85.00	0.00 (0.00)
T_4	Ammonium Salt of Glyphosate 71% SG	0.62%	85.00	0.00 (0.00)
T_5	Glyphosate 41% SL	12%	85.00	0.00 (0.00)
T_6	Control		85.00	0.00 (0.00)
SE±			0.04	0.04
CD @ 1%			0.17	0.17

(*Figures in parenthesis are arc sine transformed value)

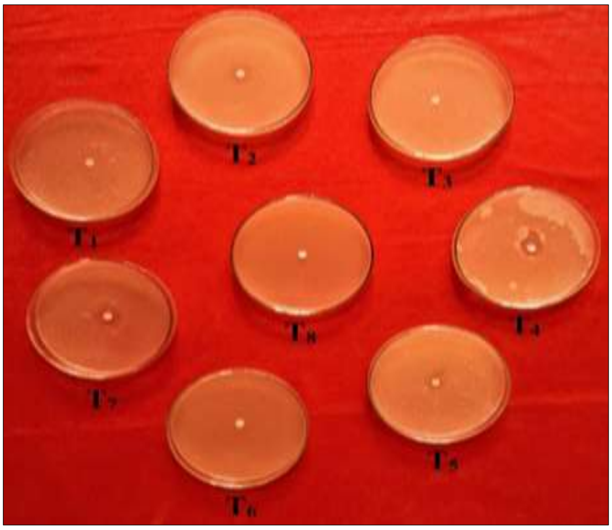

Fig 1: Seed treatment

Fig 2: Fungicides

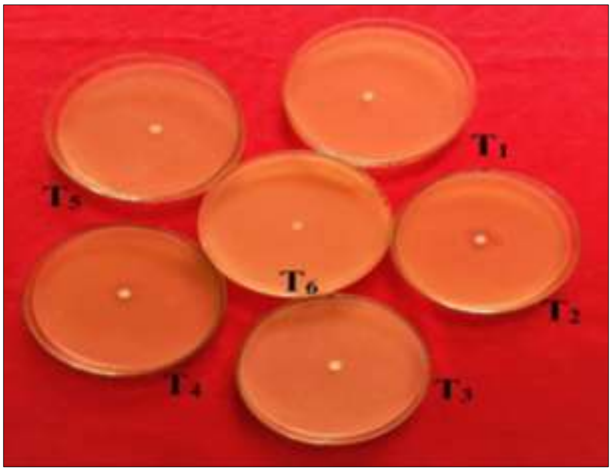

Fig 3: Weedicides

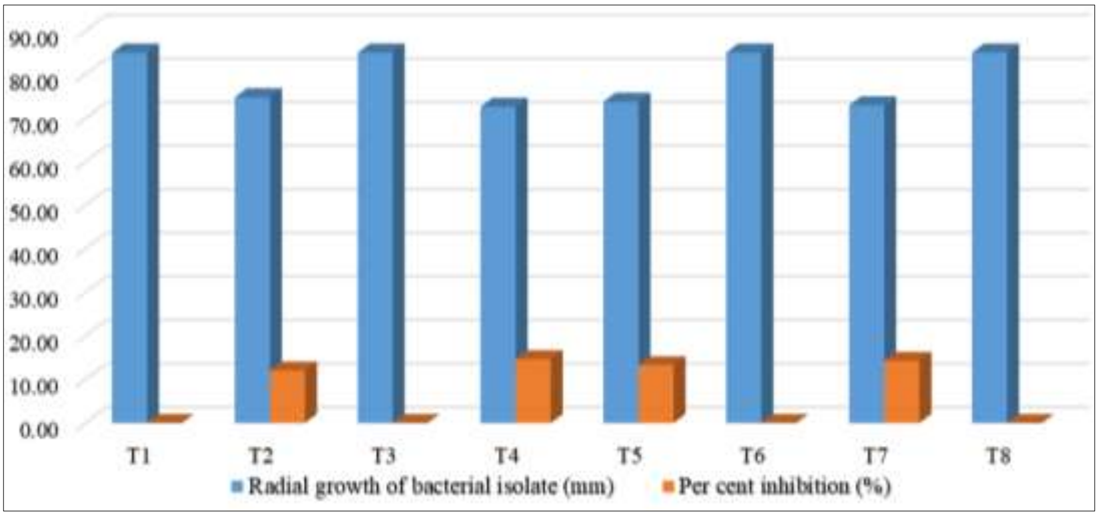

Fig 4: *In vitro* evaluation of compatibility of seed treatment chemicals with *Bacillus* spp.

T_1	:	Thiophanate Methyl 45% + Pyraclostrobin 5% FS
T_2	:	Mancozeb50% + Carbendazim 25% WS
T_3	:	Carboxin 37.5% + Thiram 37.5% DS
T_4	:	Captan 50%WP
T_5	:	Captan70%WP + Hexaconazole 5%
T_6	:	Thiamethoxam 30% FS
T_7	:	Imidacloprid 18.5% + Hexaconazole 1.5% FS
T_8	:	Control

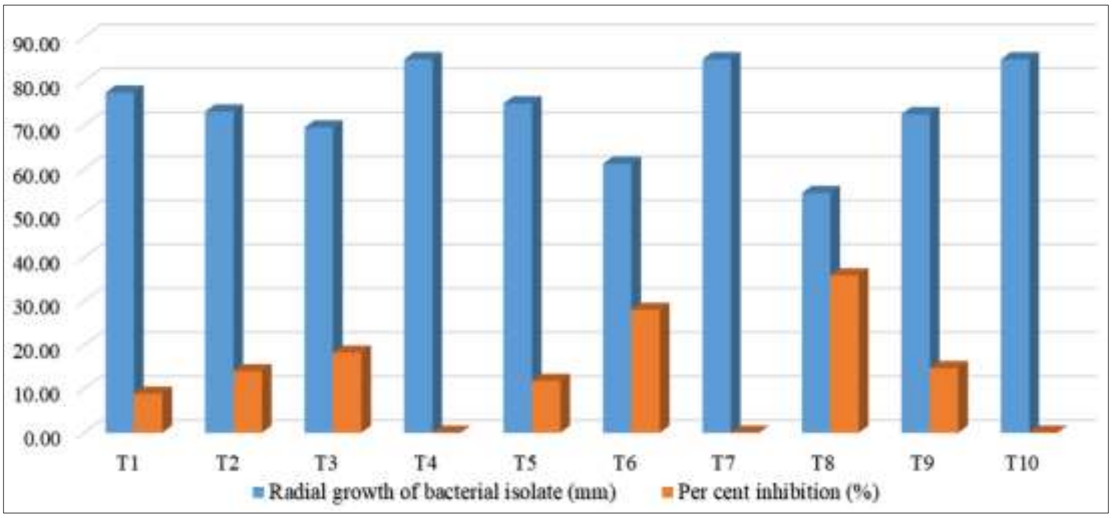

Fig 5: *In vitro* evaluation of compatibility of fungicides with *Bacillus* spp.

T_1	:	Hexaconazole 5% EC
T_2	:	Propiconazole 25% EC
T_3	:	Tebuconazole 25.9% EC
T_4	:	Thiophanate Methyl 70% WP
T_5	:	Chlorothalonil 75% WP
T_6	:	Propineb 70% WP
T_7	:	Copper Oxychloride 50% WP
T_8	:	Carbendazim 12% + Mancozeb 63% WP
T_9	:	Azoxystrobin 18.2% + Difenoconazole 11.4% SC
T_{10}	:	Control

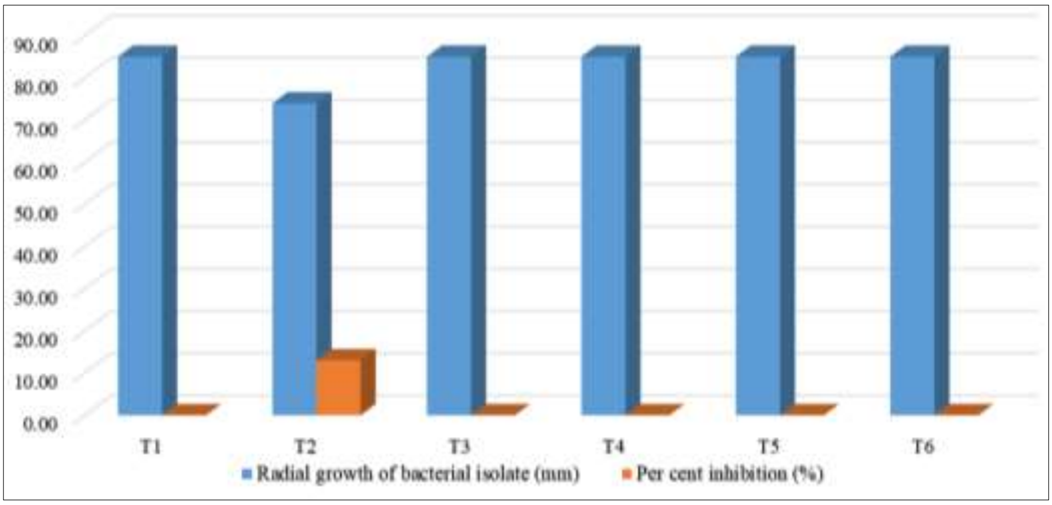

Fig 6: *In vitro* evaluation of compatibility of weedicides with *Bacillus* spp.

T_1	:	2,4-D Amine Salt 58% SL
T_2	:	Pendimethalin 30% EC
T_3	:	Oxyfluorfen 23.5% EC
T_4	:	Ammonium Salt of Glyphosate 71% SG
T_5	:	Glyphosate 41% SL
T_6	:	Control

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