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Morajdhwaj Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh, India

Ramji Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh, India

Prashant Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh, India

Madhu GS
ICAR-Central Institute of Temperate Horticulture, Rangreth, Srinagar, Jammu and Kashmir, India

Corresponding Author:
Morajdhwaj Singh
Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut, Uttar Pradesh, India

In vitro compatibility of *Pseudomonas fluorescens* with systemic weedicides

Morajdhwaj Singh, Ramji Singh, Prashant Singh and Madhu GS

Abstract
Bioagents are beneficial for plant and soil health and are proved to be compatible with different weedicides till the threshold level is achieved. The effect of compatibility of P. fluorescens (strain 28) with four weedicides *viz*., Pendimethalin, Sulfosulfuron, 2, 4-D and Isoproturon was observed. Each weedicide was evaluated at different concentrations *viz*., 10ppm, 15ppm, 20ppm and 25ppm and their inhibition on growth of Pseudomonas fluorescens was recorded. It was found that the four weedicides i.e. Pendimethalin and 2, 4-D were compatible at any concentration tested against Pseudomonas fluorescens than other weedicides i.e. Isoproturon and Sulfosulfuron. Sulfosulfuron was comparatively more toxic than Isoproturon, with respect to the level of percent inhibition of Pseudomonas fluorescens at 10ppm, 15ppm, 20ppm and 25ppm concentration respectively after 96 hours. The present study was therefore undertaken to evaluate the threshold level of different weedicides at suitable concentrations for effective growth of bioagents.

Keywords: *Pseudomonas fluorescens*, weedicides, radial growth and percent inhibition

Introduction
Pseudomonas fluorescens is used as a biocontrol agent in *in-vitro* in agriculture conditions. Pseudomonads belong to PGPR, play a major role in plant growth promotion, induced systemic resistance and biological control of pathogens. The deleterious effects of plant protection chemicals in agriculture paved the way for organic/sustainable agriculture. Application of chemical pesticides for the control of soil borne diseases causes environment and health hazards to humans and adversely affects the beneficial microorganisms in soil. The integrated use of agrochemicals and biological agents for the management of soil borne diseases is efficient and ecofriendly. The indiscriminate use of pesticides including fungicides, insecticides, herbicides and antibiotics of various chemical groups to control pest and phytopathogens are detrimental to crop productivity and microorganisms in soils. The large amount of pesticides reach in the soil which persist for long periods and destabilize the soil ecosystems and plant growth causing harm to PGPR (Ahemad *et al.* 2009) [1], (Guo *et al.* 2007) [6]. It has been reported that these concentrations could reduce the dose of fungicide (under the MRLs), the frequency of application, improve diseases control and translate the principles of IPM into practice.
Therefore, the present investigation was proposed for observing the compatibility of systemic fungicides with *Pseudomonas fluorescens.*

Materials and Methods
The experiment was conducted at the Bio-control laboratory in the Department of Plant Pathology, College of Agriculture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut (U.P.) India during 2017-18.
A previously characterized drought tolerant isolates of *P. fluorescens* strain (PfMB4) was selected on the basis of their growth performance. The isolate of *P. fluorescens* was maintained on king's (B) medium (Kings *et al*., 1954) for survival of Pseudomonas. The slants in culture tubes were sub cultured at regular intervals for its revival. *P. fluorescens* culture was maintained on king's (B) medium slants and was allowed to grow at 28±2 ^{0}C. The culture thus obtained was stored in refrigerator at 5^{0}C for further use. pH was adjusted to 7.2 ±0.2 and the final volume was made to 1000 ml using distilled water.

Poison food technique
The poison food technique (Shravelle, 1961) [12] was followed to evaluate the efficacy of

different systemic fungicides for growth inhibition of the *P. fluorescens*. Four fungicides of different concentrations *viz*., 10, 15, 20 and 25ppm, were prepared *in-vitro* for their compatibility with *P. fluorescens* using poisoned food technique. The required amount of fungicides was added in each 250 ml capacity flask, containing 100 ml sterilized kings (B) media. It was mixed thoroughly by shaking the flask prior to pouring in sterilized petri plates. The bacterial suspensions, at the concentration of 10^8 CFUs ml^{-1}, were poured into petri dishes containing King's (B) agar medium of the concentrations *viz.* 10ppm, 15ppm, 20ppm, and 25ppm of the weedicides Pendimethalin, Sulfosulfuron, 2, 4-D and Isoproturon respectively. The suspensions were spread by rotating gently on the plates. The inverted petri dishes were then incubated at 37±2°C for 48 hours. Growing bacterial colonies were counted by serial dilution method. The treatments were replicated thrice and analysed using CRD design (Gomez and Gomez, 1984) [4]. Population dynamics was recorded by counting the CFUs with the help of colony counter. The effect of individual toxicants fungicides were measured as percent inhibition with the help of following formula:

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

Where

C = Number of CFUs of bacteria in control

T = Number of CFUs of bacteria in treatments

Table 1: Effect of different concentrations of weedicides on population dynamics of *Pseudomonas fluorescens*

S. No.	Concentration	Weedicides/ Treatments	Radial growth (mm) at 24h	Percent inhibition at 24h	Radial growth (mm) at 48h	Percent inhibition at 48h	Radial growth (mm) at 72h	Percent inhibition at 72h	Radial growth (mm) at 96h	Percent inhibition at 96h
1.	10 ppm	Pendimethalin	7.00	53.33	12.00	47.83	32.00	11.11	44.00	8.33
		Sulfosulfuron	6.00	60.00	14.00	39.13	24.00	33.33	36.00	25.00
		2,4-D	4.00	73.33	16.00	30.43	29.00	19.44	40.00	16.67
		Isoproturon	3.00	80.00	18.00	21.74	24.00	33.33	37.00	22.92
2.	15 ppm	Pendimethalin	4.00	73.33	10.00	56.52	27.00	25.00	39.00	18.75
		Sulfosulfuron	4.00	73.33	12.00	47.83	21.00	41.67	32.00	33.33
		2,4-D	2.00	86.67	13.00	43.48	17.00	52.78	22.00	54.17
		Isoproturon	3.00	80.00	16.00	30.43	21.00	41.67	24.00	50.00
3.	20 ppm	Pendimethalin	3.00	80.00	8.00	65.22	20.00	44.44	36.00	25.00
		Sulfosulfuron	3.00	80.00	9.00	60.87	18.00	50.00	26.00	45.83
		2,4-D	0.00	100.00	11.00	52.17	16.00	55.56	19.00	60.42
		Isoproturon	1.00	93.33	10.00	56.52	14.00	61.11	18.00	62.50
4.	25 ppm	Pendimethalin	2.00	86.67	5.00	78.26	19.00	47.22	27.00	43.75
		Sulfosulfuron	0.00	100.00	7.00	69.57	12.00	66.67	20.00	58.33
		2,4-D	0.00	100.00	8.00	65.22	13.00	63.89	18.00	62.50
		Isoproturon	0.00	100.00	9.00	60.87	13.00	63.89	15.00	68.75
5.		Control	15.00	0.00	23.00	0.00	36.00	0.00	48.00	0.00
C.D.			0.482		1.303		2.197		3.119	
SE(m)			0.167		0.452		0.761		1.081	
SE(d)			0.236		0.639		1.076		1.528	
C.V.			8.605		6.613		6.294		6.351	

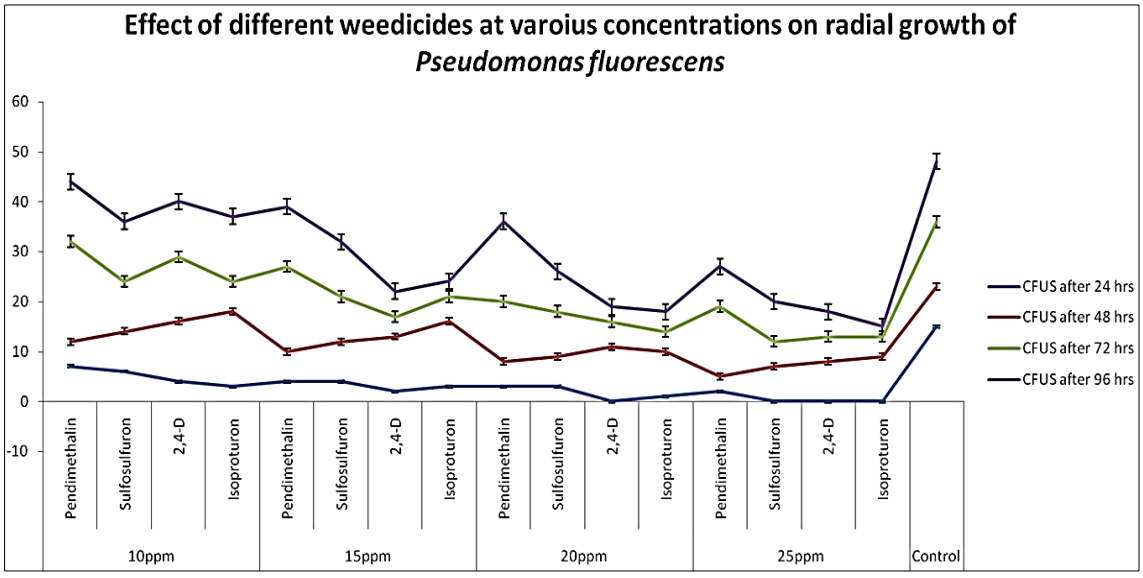

Fig 1: Effect of different concentrations of weedicides on population dynamics of *Pseudomonas fluorescens*

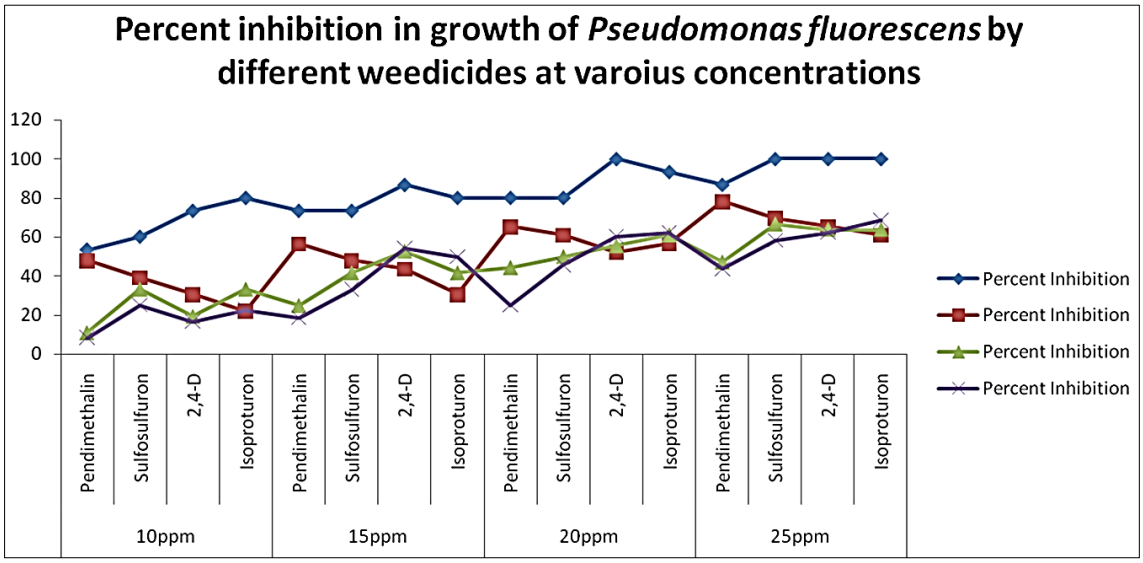

Fig 2: Effect of different concentrations of weedicides on percent inhibition at different hours after inoculation of *Pseudomonas fluorescens*

Results and Discussion

In total four weedicides *viz.* Pendimethalin, Sulfosulfuron, 2, 4-D and Isoproturon at four concentration *viz.*10ppm, 15ppm, 20ppm, and 25ppm were tested for their compatibility with *P. fluorescens in vitro* and the data recorded have been presented in Table 4.29 and Figure No 4.29. After thorough review of data table, it is evident that all the four concentrations of Pendimethalin were highly compatible with low toxic effect against *P. fluorescens in vitro.* As there was 8.33, 18.75, 25.00 and 43.75 percent inhibition in population dynamics of *P. fluorescens* due to Pendimethalin at 10ppm, 15ppm, 20ppm and 25ppm concentrations respectively, even after a prolong exposure *i.e.* up to 96 hours (4 days) incubated.

2, 4-D was little less compatible to *P. fluorescens* and next in the order of compatibility after Pendimethalin. After thorough review of data table, it is evident that all the four concentrations of 2, 4-D was quite compatible with toxic effect against *P. fluorescens in vitro.* As there was 16.67, 54.17, 60.42 and 62.50 percent inhibition of population dynamics of *P. fluorescens* due to 2, 4-D at 10ppm, 15ppm, 20ppm and 25ppm concentrations respectively, even after a prolong exposure *i.e.* up to 96 hours (4 days) incubated.

In the initial period of incubation *i.e.* at 24 hours all the concentration tested of Isoproturon were 100 per cent inhibitory of population dynamics of *P. fluorescens* at 25ppm, but as days of incubation increased it seems that toxic effect of chemical get decreased and bacterial colonies were visible at 10, 15 and 20 ppm concentration of Isoproturon which resulted in 80.00, 80.00 and 93.33 per cent inhibition. However at 48 hrs also 10, 15, 20 and 25 ppm concentration resulted in 21.74, 30.43, 56.52 and 60.87 percent inhibition. With prolonging incubation period of 72 hours it seems that level of toxicity of Isoproturon get little reduced and resulted in 33.33, 41.67, 61.11 and 63.89 per cent inhibition due to 10, 15, 20 and 25 ppm concentration of Isoproturon. The level of toxicity of Isoproturon was further decreased at 96 hours of incubation which is evidenced by per cent of inhibition *i.e.* 22.92, 50.00, 62.50 and 68.75 per cent inhibition due to 10, 15, 20 and 25ppm concentration of Isoproturon.

In the initial period of incubation *i.e.* at 24 hours all the concentration tested of Sulfosulfuron were 100 per cent inhibitory of population dynamics of *P. fluorescens* at 25ppm, but as days of incubation increased it seems that toxic effect of chemical get decreased and bacterial colonies was visualized at 10, 15 and 20 ppm concentration of Sulfosulfuron resulted in 60.00, 73.33 and 80.00 per cent inhibition. However at 48 hrs also 10, 15, 20 and 25 ppm concentration resulted in 39.13, 47.83, 60.87 and 69.57 percent inhibition. With prolonging incubation period of 72 hours it seems that level of toxicity of Sulfosulfuron get little reduce and resulted in 33.33, 41.67, 50.00 and 66.67 per cent inhibition due to 10, 15, 20 and 25 ppm concentration of Sulfosulfuron. The level of toxicity of Sulfosulfuron was further decreased at 96 hours of incubation which is evidenced by inhibition of *P. fluorescens i.e.* 25.00, 33.33, 45.83 and 58.33 per cent due to 10, 15, 20 and 25 ppm concentration of Sulfosulfuron.

Overall, it was noticed that the insecticides *i.e.* Pendimethalin and 2, 4-D were found safer, as compared to Isoproturon and Sulfosulfuron which exhibited acute toxicity for growth of *P. fluorescens.* It has been reported that the bio control agents can tolerate a certain level of weedicides when mixed with agrochemicals, resulting in eradication of diseases (De Cal *et al.*, 1994). These results are in accordance with Naik *et al.*, (2013) [9] who reported the compatibility of *P. fluorescens* and *Trichoderma viride*, with other pesticides, plant products and their utility as integral component in the sustainable management of crop diseases. Among pesticides, Carbendazim, Hexaconazole and Propiconazole showed compatibility whereas Indoxacarb and Novaluron were incompatible. Singh *et al*., (2014) reported that compatibility of *Trichoderma* spp. with pesticides by poisoned food technique and revealed that the systemic fungicide, carbendazim was the most toxic (23.3–46.6% inhibition) followed by thiophanate methyl (4.4–9.4%). A varying level of inhibition (0.0–4.4%) was observed with the weedicides. The results will enable choice of combining *Trichoderma* and agrochemicals for use in an integrated pest management approach. Archana *et al.*, (2012) [2] conducted an experiment to study the compatibility of Azoxystrobin 23 SC with bacterial and fungal biocontrol agents and insecticides *in vitro* and glass house conditions, respectively. Bacterial biocontrol agents *viz.*, *Pseudomonas fluorescens* and *Bacillus subtilis* were compatible with Azoxystrobin 23 SC even at a high concentration of 300 ppm whereas fungal biocontrol agent *Trichoderma viride* was inhibited by Azoxystrobin 23 SC at a concentration above 15 ppm. Among the four insecticides tested for compatibility, all insecticides were physically

compatible with Azoxystrobin 23 SC at 125, 250 and 500 g ai ha-1 whereas dichlorvos was biologically incompatible even at the lowest concentration tested. Ramarethinam *et al*. (2001) [11] studied *in vitro* compatibility of *T. viride* with fungicides and weedicides and reported that Mancozeb 75% WP and Copper oxychloride 88% w/w each @ 100 ppm and 500 ppm did not inhibited growth of *T. viride*. However, @ 1000 ppm, Copper oxychloride, completely inhibited its growth. Fungicides Carbendazim 50% WP, Hexaconazole 5% EC, Propiconazole 25% EC and a weedicide, Metalachlor 50% EC completely inhibited growth of *T. viride* even @ 100 ppm. Malathi *et al.* (2002) [8] studied *in vitro* compatibility of *Trichoderma* strains and *Pseudomonas fluorescens*, with systemic fungicides *viz*., Thiophanate methyl and Carbendazim. They reported that growth of *P. fluorescens* (11 strains) was not affected up to 500 ppm of both Thiophanate methyl and Carbendazim, while *Trichoderma* (6 strains) could not grew even at 1 ppm of Carbendazim and 10 ppm of Thiophanate methyl. Awasthi *et al*. (2016) studied the tolerance and sensitivity of *T. harzianum* and *T. viride* to three pesticides *viz*., Carbendazim, Imidacloprid and Pendimethalin, at their recommended lower and higher dosages. They reported that both bioagents were highly sensitive to Carbendazim, but were insensitive and compatible with Imidacloprid @ 0.02% with maximum mycelial growth of 84.66 and 79.66 mm, respectively and Pendimethalin @ 0.2% (72.66 mm and 84.66 mm respectively), in *T. harzianum* and *T. viride*, respectively. Dutta and Das (2017) [10] studied compatibility of *Trichoderma* spp. *viz., T. harzianum, T. asperellum, T. viride,* and *T. pseudokoningii* with insecticides, fungicides, herbicides and inorganic fertilizer and found compatible with insecticides like Methomyl 40% W/W (0.02% and 0.04%), Thiamethoxam 25% WG (0.125% and 0.5%), Diafenthiurom 50% WP (0.02%), fungicides like Mancozeb 75% WP (0.125% and 0.5%), herbicides like Glyphosate 41% SL (0.15% and 0.3%). Singh *et al*. (2021) [13] reported that the compatibility of P. fluorescens (strain 28) with four fungicides *viz*., Hexaconazole, Nativo (Tebuconazole 50% + Trifloxystobin 25%), Propiconazole and Tebuconazole was observed. Each fungicide was tested for four concentrations *viz*., 10ppm, 15ppm, 20ppm and 25ppm and their inhibition on growth of Pseudomonas fluorescens was recorded. It was observed that Tebuconazole was comparatively more toxic than Hexaconazole, with respect to the level of percent inhibition of Pseudomonas fluorescens at 10ppm, 15ppm, 20ppm and 25ppm concentration respectively. Hanuman and Madhavi (2018) studied the compatibility of *Pseudomonas fluorescens* with 6 fungicides, 10 insecticides and 10 weedicides was tested under laboratory condition. All insecticides and herbicides were found to be compatible with *P. fluorescens*. Surendran *et al.,* (2012) [14] reported that *P. fluorescens* (PF 43) is highly compatible with 2,4 D sodium salt, metsulfuron methyl 10% + chlorimuron ethyl 10%Wp, cyhalopop butyl 10 EC, pyrazosulfuron ethyl 10WP, pretilachlor %) EC, penoxsulam 24 SP, bispyribac sodium 10SC. Beethi and Pillai (2008) [3] reported that compatibility of *P. fluorescens* was questionable with pretilachlor while, it showed compatibility with 2, 4 D sodium salt. A combination of biocontrol agents with chemicals will have an additive effect and results in enhanced disease control compared to their individual application (Guetsky *et al.*, 2002) [5].

Thus it was concluded that Pendimethalin and 2, 4-D were found to be compatible against *Pseudomonas fluorescens* at 10 ppm concentration whereas Isoproturon and Sulfosulfuron were observed to show acute toxicity. Therefore the present study is recommended to be beneficial for the farmers.

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