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In vitro compatibility of *Pseudomonas fluorescens* with different systemic fungicides

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Abstract
Bioagents are beneficial for plant and soil health and are proved to be compatible with different fungicides till the threshold level is achieved. The effect of compatibility of P. fluorescens (strain 28) with four fungicides *viz*., Hexaconazole, Nativo (Tebuconazole50%+ Trifloxystobin25%), 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 found that the systemic fungicides i.e. Hexaconazole and Tebuconazole were found to be comparatively more toxic than other fungicides. However 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. It was therefore concluded that the possibilities of compatible fungicide could be incorporated along with bioagents for effective and sustainable disease management causing less disturbance to agro-ecosystem. Thus, the study was undertaken to determine the threshold level of different fungicides at suitable concentrations for effective growth of bioagents.

Keywords: *Pseudomonas fluorescens*, Systemic fungicides, 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) [7]. 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) [8] 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) [14] 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 fungicides Hexaconazole, Nativo, Propionazole and Tubuconazole 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) [6]. 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

Results and Discussion

Fungicides *viz.* Hexaconazole, Nativo, Propiconazole and Tebuconazole at four concentration *viz.*10ppm, 15ppm, 20ppm, and 25ppm were evaluated for their compatibility with *Pseudomonas fluorescens in vitro* (Table 1 and Figure 1, 2). It is evident that all the four concentrations of Nativo were highly compatible with low toxic effect against *P. fluorescens in vitro.* The percent inhibition of population dynamics of *P. fluorescens* due to Nativo at 10ppm, 15ppm, 20ppm and 25ppm concentrations was recorded to be 67.65, 75.00, 91.18 and 92.65 respectively, even after a prolong exposure *i.e.* up to 96 hours. Vimi *et al* (2006) [19] also reported the compatibility of copper fungicides, copper oxychloride and copper hydroxide at 0.05% whereas it was found to be incompatible at other concentrations. Similar results were also reported by using *P. fluorescens* (KAU strain) with eleven fungicides with poisoned food technique. The fungicides were tested at four concentrations *viz*., 0.05%, 0.1%, 0.2% and 0.3%. The fungicides *viz*., propiconazole, hexaconazole, tebuconazole, difenconazole, azoxystrobin, carbendazim and famoxadone + cymoxanil were observed to be compatible at all concentrations whereas Kresoxim methyl was found to be less compatible and mancozeb, copper oxychloride and copper hydroxide were found to be at par with *P. fluorescens*.

The compatibility of *P. fluorescens* with azoxystrobin was also reported to be compatible at different concentrations up to 300 ppm (Devi 2013) [5]. Valarmathi *et al* (2013) [16] reported the compatibility effect of *P. fluorescens* strains with higher concentration of fungicides. Prasanna *et al*. (2002) [12] studied *in vitro* compatibility of Thiomethoxam 70 WS and its effect on the growth and sporulation of *T. harzianum*. It was concluded that there was no inhibition of its mycelial growth at all concentrations, except @ 1.25 percent. Thus, *P. fluorescens* was found to be incompatible at the higher concentrations of fungicides.

Propiconazole and Nativo also showed their toxicity behavior against *P. fluorescens*. There was complete inhibition of *P. fluorescens* at 10, 15, 20 and 25 ppm during the initial period of incubation (24 hours). However, the toxic effect of chemical was found to show a decreasing trend with the increase in days of incubation. This led to advancement in the bacterial population at 10 ppm concentration of Propiconazole, when kept for 48 hours. Similarly, the inhibitory effect of Propiconazole at 15, 20 and 25 ppm concentration up to 48 hours was also observed, but a negative effect in growth of *P. fluorescens* was noticed at 72 hours. However, the toxicity level was found to be reduced at 96 hours. The percent inhibition of Propiconazole was recorded to be 76.47, 80.88, 89.71 and 92.65 at 10, 15, 20 and 25ppm concentration respectively. Similar results were reported by Archana *et al.,* (2012) [ ]. The compatibility of Azoxystrobin 23 SC with bacterial and fungal biocontrol agent's *viz., P. fluorescens* and *Bacillus subtilis* was found to be compatible at 300 ppm as compared to fungal biocontrol agent *Trichoderma viride* above 15 ppm. Naik *et al.,* (2013) [10] also 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. Deepthi (2013) [4] found the compatibility of *Trichoderma* isolate GRHF-4 with Mancozeb @ 0.2% and Copper oxychloride @ 0.1%, Carbendazim @ 0.2%, Thiophanate methyl @ 0.1%, Hexaconazole @ 0.1% and Propiconazole @ 0.1%.

Hexaconazole was found to show a 100 per cent inhibitory effect on the bacterial population during the initial period of incubation. However, there was 89.66 per cent inhibition at 15, 20 and 25 ppm concentrations at 48 hours. The toxicity level of Hexaconazole was found to be reduced and was recorded to be 83.33, 88.10, 90.48 and 95.24 per cent when kept for an incubation period of 72 hours at 10, 15, 20 and 25 ppm. It was found to be further decreasing to 80.88, 82.35, 88.24, and 94.12 percent *at* 10, 15, 20 and 25 ppm concentration of Hexaconazole, respectively at an incubation period of 96 hours. Similar results were observed by Nandeesha *et al.,* (2013) [11]. *In vitro* compatibility of bioagent, Mancozeb was carried with *Trichoderma harzianum*. Vijayaraghavan and Abraham (2004) [18] reported that *T. viride* and *T. harzianum* were incompatible with Bordeaux mixture, Copper, Captan and Kavach, while they were found to be compatible with Indofil M-45, Ridomil MZ, Akomin and Antracol. Fytolan was found to be partially compatible and *T. longibrachiatum* was found to be incompatible. Madhusudhan *et al*. (2010) [9] evaluated the compatibility behavior of two *T. viride* isolates (T2 and T4) with six fungicides *viz*; Carbendazim 50% WP, Propiconazole 25% EC, Hexaconazole 5% EC, Tridemorph 80% EC, Chlorothalonil 75% WP and Mancozeb 75% WP (each @ 50, 100, 250, 500 and 1000 ppm). It was reported that Mancozeb was highly compatible with Chlorothalonil, Carbendazim, Hexaconazole and Propiconazole. They showed 100% inhibition at 50 to 100 ppm. Sarkar *et al*. (2008) observed the effect of systemic fungicides, Hexaconazole and it was reported to be most toxic, followed by Propiconazole and Triflumizole. Bhattiprolu (2008) [3] reported the compatibility of Mancozeb, Copper oxychloride and Thiram, at recommended doses. However, the isolate could not tolerate the fungicides like, Carbendazim, Hexaconazole, Benomyl and Thiophanate-methyl, hence found to be incompatible.

The toxicity level of Tebuconazole was found to show an inhibitory effect at 10, 15, 20 and 25 ppm concentrations during the initial period of incubation *i.e.* at 24 hours. However, the percent inhibition was recorded to be inhibitory at all concentrations while it was recorded to be 75.86 and 78.57 per cent at 48 and 72 hours at 10 ppm. The level of toxicity of Tebuconazole was found to be further reduced to 79.41, 86.76, 91.18 and 95.59 per cent at 10, 15, 20 and 25 ppm concentration respectively. Rai *et al*. (2016) [13] studied compatibility of *T. harzianum* (Th 14) with six systemic fungicides *viz*., Hexaconazole, Tebuconazole, Difenoconazole, Propiconazole, Carbendazim and two contact fungicides *viz*., Mancozeb and Captan (each @ 25, 50 and 100 ppm). It was reported that all systemic fungicides caused 100% mycelial growth inhibition at the test concentrations, except Metalaxyl. Sreeja and Girija (2015) [15] studied the compatibility of 12 fungicides with *T. viride*, *P. fluorescens* and *Rhizobium* spp. They reported that Propiconazole, Flusilazole, Tebuconazole, and Carbendazim (each @ 0.1%) were incompatible and led to 100 per cent mycelial growth inhibition of *T. viride*. Veena *et al*. (2014) [17] tested compatibility of *Trichoderma* isolate-7 (CT7) with commonly used fungicides *viz*., Copper oxychloride (0.25%), Captan (0.25%), Hexaconazole (0.2%), Tebuconazole (0.1%) and Validamycin (0.1%) and reported that the test bioagent was more compatible with Validamycin (72.22%), followed by Copper oxychloride (66.66%); whereas, it was incompatible with Hexaconazole and Tebuconazole and Captan showed 22.22 per cent compatibility.

Conclusion

Overall, it was found that the systemic fungicides *i.e.* Hexaconazole and Tebuconazole during the course of investigation were found to be comparatively more toxic than other concentrations against *P. fluorescens* than two other fungicides *i.e.* Nativo and Propiconazole. However, Tebuconazole was comparatively more toxic than Hexaconazole, with respect to the percent inhibition of *P. fluorescens* at 10ppm, 15ppm, 20ppm and 25ppm concentration respectively after 96 hours. They were also found to be unsafe against *P. fluorescens* while Nativo and Propiconazole were found to be comparatively safer for *Pseudomonas fluorescens*. Thus, it can be concluded that the use of fungicides with *P. fluorescens* for achieving sustainable plant diseases and agroecosystem management is highly recommended.

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

S.No.	Fungicide/ Treatmens	Concentration	CFUs after 24 hrs	Percent Inhibition	CFUs after 48 hrs	Percent Inhibition	CFUs after 72 hrs	Percent Inhibition	CFUs after 96 hrs	Percent Inhibition
1.	Hexaconaz ole	10ppm.	0.00	100	3.00	89.66	7.00	83.33	13.00	80.88
	Nativo	10ppm.	0.00	100	8.00	72.41	18.00	57.14	22.00	67.65
	Propiconazole	10ppm.	0.00	100	4.00	86.21	11.00	73.81	16.00	76.47
	Tebuconazole	10ppm.	0.00	100	7.00	75.86	9.00	78.57	14.00	79.41
2.	Hexaconaz ole	15ppm.	0.00	100	0.00	100.00	5.00	88.10	12.00	82.35
	Nativo	15ppm.	0.00	100	5.00	82.76	11.00	73.81	17.00	75.00
	Propiconazole	15ppm.	0.00	100	0.00	100.00	6.00	85.71	13.00	80.88
	Tebuconazole	15ppm.	0.00	100	0.00	100.00	0.00	100.00	9.00	86.76
3.	Hexaconaz ole	20ppm.	0.00	100	0.00	100.00	4.00	90.48	8.00	88.24
	Nativo	20ppm.	0.00	100	0.00	100.00	3.00	92.86	6.00	91.18
	Propiconazole	20ppm.	0.00	100	0.00	100.00	4.00	90.48	7.00	89.71
	Tebuconazole	20ppm.	0.00	100	0.00	100.00	0.00	100.00	6.00	91.18
4.	Hexaconaz ole	25ppm.	0.00	100	0.00	100.00	2.00	95.24	4.00	94.12
	Nativo	25ppm.	0.00	100	0.00	100.00	0.00	100.00	2.00	97.06
	Propiconazole	25ppm.	0.00	100	0.00	100.00	0.00	100.00	5.00	92.65
	Tebuconazole	25ppm.	0.00	100	0.00	100.00	0.00	100.00	3.00	95.59
	Control		15.00	0	29.00	0.00	42.00	0.00	68.00	0.00

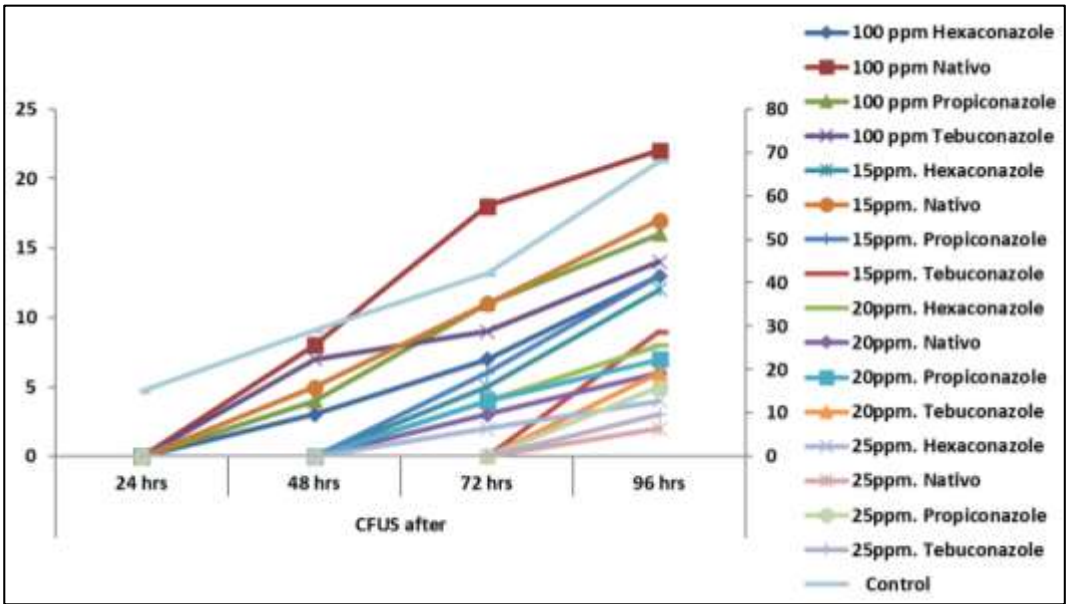

Fig 1: Effect of different concentrations of fungicides on *population* dynamics of *Pseudomonas fluorescens.*

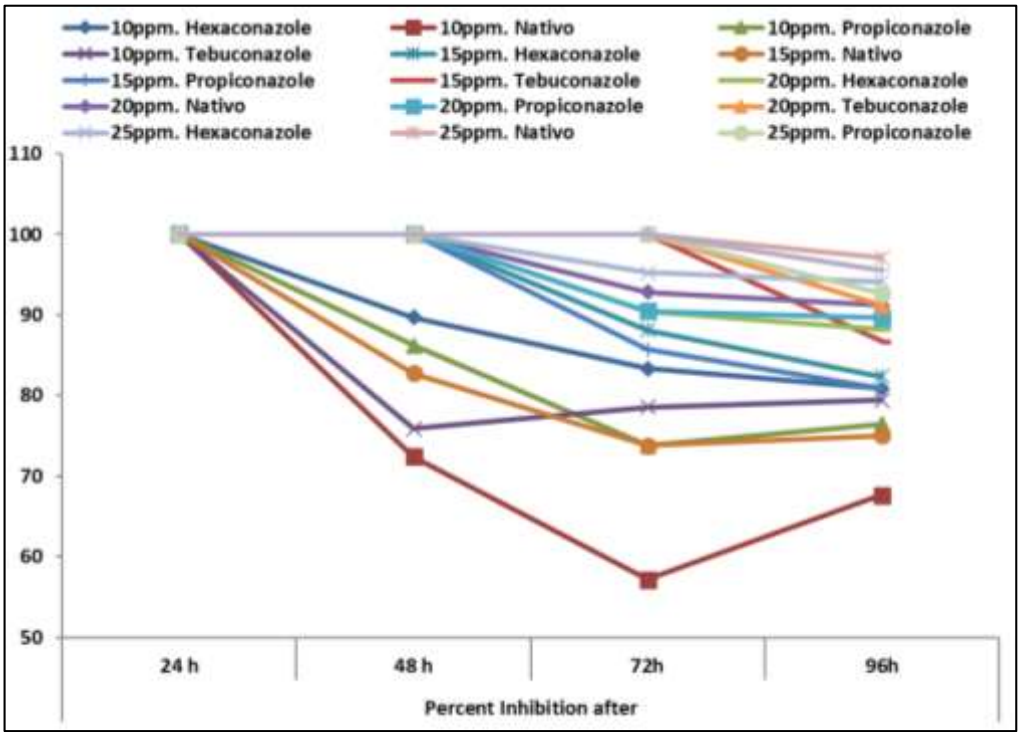

Fig 2: Effect of different concentrations of fungicides on percent inhibition at different hours after inoculation of *Pseudomonas fluorescens.*

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