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Research Article

Compatibility of *Bacillus thuringiensis* var. *kurstaki* and *Pseudomonas fluorescens* with pesticides recommended for rice pest management at drone and Taiwan spraying concentrations

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ABSTRACT: *In vitro* studies were carried out at Institute of Rice Research, PJTSAU, Rajendranagar, Hyderabad, in 2023 to assess the compatibility of biopesticides with chemical pesticides applied at drone and Taiwan sprayer concentrations. The possibility of utilizing both biopesticides and chemical pesticides together with drone for managing major rice insect-pests and diseases was explored. The compatibility of *Bacillus thuringiensis* var. *kurstaki* with four insecticides (flubendiamide 39.35% SC, chlorantraniliprole 18.50% SC, cartap hydrochloride 50% SP and tetraniliprole 18.18% SC) and *Pseudomonas fluorescens* with three fungicides (propiconazole 10.7% + tricyclazole 34.2% SE, tebuconazole 50% + trifloxystrobin 25% WG and propiconazole 25% EC) was determined by following the inhibition zone technique on suitable media with sterile filter paper discs. *Bacillus thuringiensis* var. *kurstaki* combinations tested with flubendiamide 39.35% SC, chlorantraniliprole 18.50% SC, cartap hydrochloride 50% SP exhibited slight inhibition over control while, tetraniliprole 18.18% SC exhibited high compatibility with *B. thuringiensis* var. *kurstaki* without any growth inhibition at both drone and Taiwan sprayer concentrations. Similarly, all the tested fungicides were found compatible with *P. fluorescens*, displaying negligible growth inhibition compared to the control. Notably, tebuconazole 50% + trifloxystrobin 25% WG showed cent per cent compatibility with no growth inhibition at both drone and Taiwan sprayer concentrations. These findings underscore the potential for seamlessly integrating biopesticides with chemical pesticides, paving the way for effective management of pests and diseases without compromising efficacy, particularly as a pesticide resistance management strategy.

KEYWORDS: *Bacillus thuringiensis,* biopesticides, compatibility, *Pseudomonas fluorescens,* rice

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INTRODUCTION

Biopesticides, derived from natural sources such as plants, microbes and certain minerals, have emerged as sustainable alternatives to conventional chemical pesticides in integrated pest management (IPM) programs (Koul *et al.,* 2008). These eco-friendly agents offer targeted control against pests while minimizing adverse effects on non-target organisms and simultaneously safeguarding the environment. Despite these advantages, their standalone efficacy may be limited under high pest pressure conditions (Chandler *et al.,* 2011). Consequently, the integration of biopesticides with chemical pesticides has gained traction as a strategy to enhance overall pest management efficacy. Yet, the interactions between biopesticides and chemical pesticides can be intricate and multifaceted, necessitating comprehensive investigation on compatibility of biopesticides with agrochemicals. Previous research has underscored the complexity of interactions between microbial insecticides and chemical pesticides, highlighting the need for systematic evaluation under varying conditions (Ruiu, 2015).

Inhibition studies focusing on the combination of biopesticides with chemical pesticides aim to elucidate the potential synergistic or antagonistic effects on pest control efficacy. Understanding these interactions is vital for optimizing IPM strategies and devising sustainable pest management practices (Gupta *et al.,* 2019). Synergistic interactions may enhance pest control efficacy, reducing the reliance on chemical inputs and mitigating resistance development (Chandler *et al.,* 2011). Conversely, antagonistic interactions may compromise the effectiveness

of biopesticides, necessitating careful selection and timing of the pesticide applications.

The choice of application method (knapsack/Taiwan/ power sprayer/drone) further adds to the complexity of application of pesticides. With the advent of drone technology, precision agriculture has witnessed remarkable advancements, offering efficient and targeted delivery of pesticides across vast agricultural landscapes. Conversely, traditional manual spraying techniques continue to be prevalent, particularly in small-scale farming operations. Particularly as the concentration of chemicals increases by about 10 folds, as a result of reduced water volume investigating compatibility under both drone and manual spraying concentrations (Taiwan sprayer) provides valuable insights into the practical feasibility of integrating biopesticides and chemical pesticides for pest management across diverse agricultural scenarios.

In light of these considerations, this study aims to systematically assess the compatibility of biopesticides and chemical pesticides at two spraying concentrations, with a focus on both drone and Taiwan spraying applications. Understanding these interactions is crucial for evolving pest management strategies that combine the strengths of biopesticides and mitigate the drawbacks of chemical pesticides**.**

MATERIAL AND METHODS

Compatibility studies of *B. thuringiensis* var. *kurstaki* with insecticides under *in vitro* conditions

Studies were conducted under laboratory conditions at Institute of Rice Research, ARI, Rajendranagar, in 2023 with *B. thuringiensis* var. *kurstaki* (BTK) and insecticides, to identify compatible combinations. The experiment comprising nine treatments, replicated thrice was laid out in Completely Randomized Design (CRD). The study followed Central Insecticide Board and Registration Committee (CIB&RC) guidelines for pesticide dosages (g/ml a.i. ha^{-1}) in both drone and Taiwan sprayer applications. Pesticide combinations, including biopesticides and insecticides, were recalculated per litre of water based on recommended acre doses, adjusting for spray volumes of 375 L ha^{-1} for Taiwan sprayer and 36.75 L ha^{-1} for drone spraying. The details of insecticides used in the experiment are presented in Table 1.

The compatibility of bacteria with insecticides was determined by following inhibition zone technique (Nene and Thapliyal, 1993). The biopesticide was carefully cultured from commercial formulation *i.e., B. thuringiensis* var. *kurstaki* 0.5% WP (Lipel) of M/s. Agrilife (India) Pvt. Ltd., Hyderabad. Sterile filter paper discs (Whatman No. 42) with a diameter of 15 mm were immersed at drone and Taiwan sprayer concentrations of insecticides as mentioned in Table 1. These treated discs were then positioned at the centre of Petri dishes containing nutrient agar (NA) medium, that were pre-inoculated with 0.5 ml of *B. thuringiensis* var. *kurstaki* spore suspension @ 10^8 CFUs/ml. The experimental assessment incorporated three control conditions wherein nutrient agar (NA) plates with filter paper discs soaked in sterile distilled water and NA plates with filter paper discs soaked in the chemical insecticide served as negative controls, which aided to verify the absence of bacterial contamination in the culture medium and assessing the integrity of the insecticide solutions. Additionally, NA plates inoculated solely with *B. thuringiensis* var. *kurstaki* were utilized as the positive control. Data analysis exclusively focused on the results derived from the positive controls to ensure the accuracy and reliability of the experimental findings. The plates were incubated at 27±2°C and 70±10% RH for 48 hours and subsequently examined for the presence of inhibition zones around the filter paper discs. The zone of growth inhibition was measured at 2, 4 and 5 days after inoculation (DAI) which was compared with standard check. Per cent growth inhibition of bacteria over control was calculated using the following formula,

Table 1. Insecticide details

Trt. no.	Treatment Particulars	Dose (g or ml/l)	Spraying Concentration
T1	BTK + Flubendiamide 39.35% SC @ 24 g a.i./ha	10^8 CFUs/ml + 1.36 ml/l	Drone
T2	BTK + Chlorantraniliprole 18 .50% SC @ 30 g a.i./ha	10^8 CFUs/ml + 4.08 ml/l	Drone
T3	BTK + Cartap hydrochloride 50% SP @ 500 g a.i./ha	10^8 CFUs/ml + 27.21 g/l	Drone
T4	BTK + Tetraniliprole 18.18% SC @ 60 g a.i./ha	10^8 CFUs/ml + 8.16 ml/l	Drone
T5	BTK + Flubendiamide 39.35% SC @ 24 g a.i./ha	10^8 CFUs/ml + 0.13 ml/l	Taiwan sprayer
T6	BTK + Chlorantraniliprole 18 .50% SC @ 30 g a.i./ha	10^8 CFUs/ml + 0.40 ml/l	Taiwan sprayer
T7	BTK + Cartap hydrochloride 50% SP @ 500 g a.i./ha	10^8 CFUs/ml + 2.66 g/l	Taiwan sprayer
T8	BTK + Tetraniliprole 18.18% SC @ 60 g a.i./ha	10^8 CFUs/ml + 0.80 ml/l	Taiwan sprayer
T9	*B. thuringiensis* var. *kurstaki* (BTK)	10^8 CFUs/ml	-

$$\text{Percentage growth inhibition} = \frac{\text{Growth (mm) in control} - \text{Growth (mm) in treatment}}{\text{Growth (mm) in control}} \times 100$$

Compatibility studies of *P. fluorescens* with fungicides under *in vitro* conditions

The inhibition studies of *P. fluorescens* (PF) with chemical fungicides were conducted at Institute of Rice Research, ARI, Rajendranagar, in 2023 under laboratory conditions, to identify best compatible combinations to control rice diseases or to utilize in combined application of biopesticides and fungicides. The experiment with seven treatments, each with three replications was laid out in Completely Randomized Design (CRD). The details of fungicides used in the experiment are given in the Table 2.

The effect of different fungicides on growth inhibition of the *P. fluorescens* were evaluated by following inhibition zone technique (Nene and Thapliyal, 1993). The bacterial culture was carefully grown from commercial formulation *i.e., P. fluorescens* 1.75% WP (Sheathguard – PF, M/s Agrilife (India) Pvt. Ltd., Hyderabad). Three fungicides of different concentrations based on field application rate (as per CIB&RC recommendations), were prepared at drone or Taiwan sprayer concentrations and assessed for their compatibility with *P. fluorescens* under *in vitro* conditions. Sterile filter paper discs (Whatman No. 42) of 15 mm diameter were immersed in the respective treatments. These treated discs were then placed at the centre of petri dishes containing kings (B) media, which had been inoculated with the bacterial suspensions, at the concentration of 10^8 CFUs/ml. The experiment utilized three controls including kings (B) media with sterile distilled water-soaked discs and kings (B) media with the fungicide-soaked discs which served as negative controls, confirming media and solution sterility. The kings (B) medium with *P. fluorescens* alone acted as the positive control, with data analysis exclusively based on these controls for accuracy. Observations on growth of the bacterial colony were recorded at 2, 4, 6, 8 and 10 days after inoculation and per cent inhibition of bacterial growth over control was calculated.

Statistical analysis

The data were analysed statistically using analysis of variance (ANOVA) with Completely Randomized Design (CRD) (Gomez and Gomez, 1984) and Duncan's test at a significance level of 95% using OPSTAT software.

RESULTS AND DISCUSSION

Compatibility of *B. thuringiensis* var. *kurstaki* with insecticides under *in vitro* conditions

The results on the compatibility of *B. thuringiensis* var. *kurstaki vis-a-vis* various insecticides at both drone and Taiwan sprayer concentrations was presented in Table 3, Figure 1. The results indicated that, all insecticides evaluated were compatible with very less or no growth inhibition. Among all the insecticidal treatments, tetraniliprole 18.18% SC was found highly compatible, showing no growth inhibition at both drone and Taiwan sprayer concentrations. This was followed by *B. thuringiensis* var. *kurstaki* + flubendiamide 39.35% SC, showed 2.67% inhibition at drone spraying concentration, whereas no inhibition of growth was observed at Taiwan sprayer dosage. *B. thuringiensis* var. *kurstaki* + cartap hydrochloride 50% SP showed 3.11% inhibition at drone spraying concentration, whereas 1.33% of growth inhibition was observed at Taiwan sprayer dosage. Significantly highest growth inhibition was observed in *B. thuringiensis* var. *kurstaki* + chlorantraniliprole 18.50% SC, which has shown 3.56 % and 2.22% inhibition at drone and Taiwan sprayer concentrations, respectively. According to Amizadeh *et al*. (2015), the increase in colonization was statistically significant solely in the chlorantraniliprole treatment. Bt might have utilized the pesticides as supplementary nutrient sources by degrading them, a capability also demonstrated by other researchers (Mandal *et al*., 2013). The application of imidacloprid insecticide at a concentration of 20% (600 g/l) did not hinder the effectiveness of *Bacillus* sp. strains

Table 2. Fungicide details

Trt. no.	Treatment Particulars	Dose (g or ml/l)	Spraying Concentration
T1	PF + Propiconazole 10.7% + Tricyclazole 34.2% SE @ 393.75 ml a.i./ha	10^8 CFUs/ml + 17.01 ml/l	Drone
T2	PF + Propiconazole 25% EC @ 125 g a.i./ha	10^8 CFUs/ml + 13.60 ml/l	Drone
T3	PF + Tebuconazole 50% + Trifloxystrobin 25% WG @ 100 + 50 g a.i./ha	10^8 CFUs/ml + 5.44 g/l	Drone
T4	PF + Propiconazole 10.7% + Tricyclazole 34.2% SE @ 393.75 ml a.i./ha	10^8 CFUs/ml + 1.66 ml/l	Taiwan sprayer
T5	PF + Propiconazole 25% EC @ 125 g a.i./ha	10^8 CFUs/ml + 1.33 ml/l	Taiwan sprayer
T6	PF + Tebuconazole 50% + Trifloxystrobin 25% WG @ 100 + 50 g a.i./ha	10^8 CFUs/ml + 0.53 g/l	Taiwan sprayer
T7	*P. fluorescens* (PF)	10^8 CFUs/ml	-

Table 3. Compatibility of *B. thuringiensis* var. *kurstaki* with insecticides

Trt. no.	Treatment Particulars	Dose (g or ml/l)	Bacterial growth (mm)	%Growth Inhibition (GI)
T1	BTK + Flubendiamide 39.35% SC @ 24 g a.i./ha	10^8 CFUs/ml + 1.36 ml/l	73.00	2.67 (9.40)[d]
T2	BTK + Chlorantraniliprole 18 .50% SC @ 30 g a.i./ha	10^8 CFUs/ml + 4.08 ml/l	72.33	3.56 (10.85)[f]
T3	BTK + Cartap hydrochloride 50% SP @ 500 g a.i./ha	10^8 CFUs/ml + 27.21 g/l	72.67	3.11 (10.14)[e]
T4	BTK + Tetraniliprole 18.18% SC @ 60 g a.i./ha	10^8 CFUs/ml + 8.16 ml/l	75.00	0.00 (0.00)[a]
T5	BTK + Flubendiamide 39.35% SC @ 24 g a.i/ha	10^8 CFUs/ml + 0.13 ml/l	75.00	0.00 (0.00)[a]
T6	BTK + Chlorantraniliprole 18 .50% SC @ 30 g a.i./ha	10^8 CFUs/ml + 0.40 ml/l	73.33	2.22 (8.55)[c]
T7	BTK + Cartap hydrochloride 50% SP @ 500 g a.i./ha	10^8 CFUs/ml + 2.66 g/l	74.00	1.33 (6.62)[b]
T8	BTK + Tetraniliprole 18.18% SC @ 60 g a.i./ha	10^8 CFUs/ml + 0.80 ml/l	75.00	0.00 (0.00)[a]
T9	*B. thuringiensis* var. *kurstaki* (BTK)	10^8 CFUs/ml	75.00	0.00 (0.00)[a]
	SEM±			0.22
	CD at 5% significance			0.66
	CV (%)			7.51

Values in the parentheses are angular transformed values; Alphabets represent the DMRT analysis.

Table 4. Compatibility of *P. fluorescens* with fungicides

Trt. no.	Treatment Particulars	Dose (g or ml/l)	Bacterial growth (mm)	%Growth Inhibition (GI)
T1	PF + Propiconazole 10.7% + Tricyclazole 34.2% SE @ 393.75 ml a.i.	10^8 CFUs/ml + 17.01 ml/l	73.33	2.22 (8.57)[d]
T2	PF + Propiconazole 25% EC @ 125 g a.i.	10^8 CFUs/ml + 13.60 ml/l	73.67	1.78 (7.62)[c]
T3	PF + Tebuconazole 50% + Trifloxystrobin 25% WG @ 100 + 50 g a.i.	10^8 CFUs/ml + 5.44 g/l	75.00	0.00 (0.00)[a]
T4	PF + Propiconazole 10.7% + Tricyclazole 34.2% SE @ 393.75 ml a.i.	10^8 CFUs/ml + 1.66 ml/l	74.00	1.33 (6.62)[b]
T5	PF + Propiconazole 25% EC @ 125 g a.i.	10^8 CFUs/ml + 1.33 ml/l	75.00	0.00 (0.00)[a]
T6	PF + Tebuconazole 50% + Trifloxystrobin 25% WG @ 100 + 50 g a.i.	10^8 CFUs/ml + 0.53 g/l	75.00	0.00 (0.00)[a]
T7	*Pseudomonas fluorescens* (PF)	10^8 CFUs/ml	75.00	0.00 (0.00)[a]
	SEM±			0.19
	CD at 5% Significance			0.58
	CV (%)			10.09

Values in the parentheses are angular transformed values; Alphabets represent the DMRT analysis.

100 ppm, while at 500 ppm they were moderately compatible. However, at 1000 ppm, the strain showed poor growth with propiconazole, tebuconazole, trifloxystrobin + tebuconazole, and carbendazim, although it maintained good compatibility with azoxystrobin (Hanuman and Bindu Madhavi, 2018). Pandian *et al.* (2013) demonstrated compatibility of *P. fluorescens* with lower concentrations of copper hydroxide (0.5 g/l). However, in contrast, Dhanya *et al*. (2016) reported high incompatibility with copper hydroxide at higher concentrations (2 g/l). Keshgond and Naik (2013) observed minimal inhibition of *P. fluorescens* by carbendazim, while mancozeb and captan caused complete inhibition. According to Gokil (2013), fungicides like tricyclazole and carbendazim were compatible with *P. fluorescens* up to concentrations

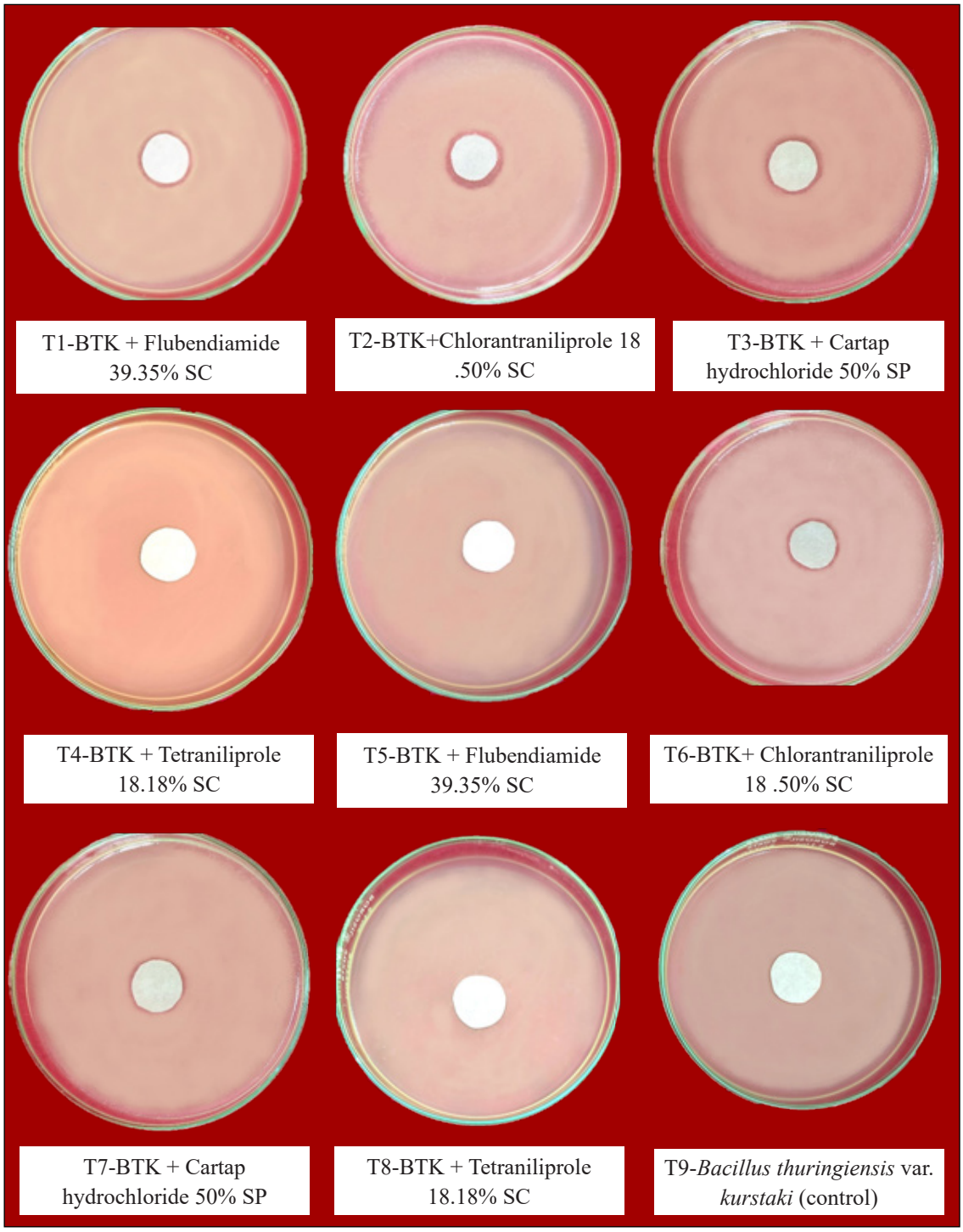


Figure 1. Effect of insecticides on the growth of *B. thuringiensis* var. *kurstaki*.

of 2000 ppm. Maheshwari (2013) also reported good compatibility of *P. fluorescens* with carbendazim, thiram, and mancozeb. *P. fluorescens* demonstrated greater compatibility with fungicides compared to *B. subtilis*, with the highest tolerance concentrations recorded at 1600 µg/ml for mancozeb and 50,000 µg/ml for both captan and carbendazim (Mohiddin and Khan, 2013). *P. fluorescens* and *B. subtilis* showed higher fungicide tolerance may be due to their ability to utilize pesticides as nutrients, enabling them to withstand higher chemical concentrations (Kishore and Jacob, 1987; Aislabie and Jones, 1995). These findings are consistent with the compatibility studies by Ahila Devi and Prakasam (2013) and Shahida *et al.* (2010) found that *P. fluorescens* was compatible with potassium phosphonate but incompatible with *Bacillus*. Additionally, Kataria *et al.* (2002) reported that lower azoxystrobin rates combined with

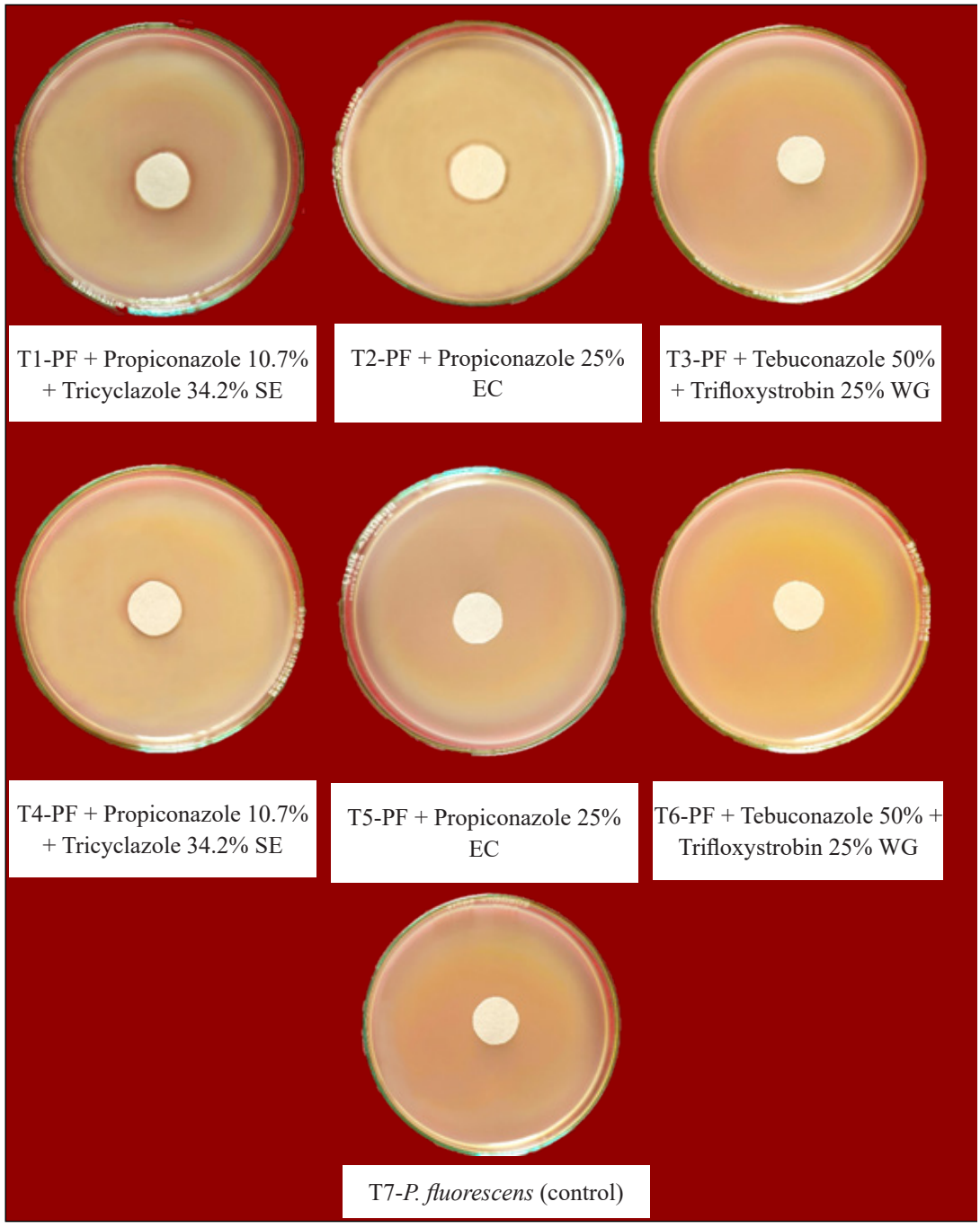


Figure 2. Effect of fungicides on the growth of *P. fluorescens in vitro*.

utilized (Constantinescu *et al.,* 2014). The present findings are in agreement with the above findings.

Compatibility of *P. fluorescens* with fungicides under *in vitro* conditions

The results pertaining to the compatibility of *P. fluorescens* with three fungicides are presented in Table 4 and Figure 2. The study showed that all three fungicides were compatible with *P. fluorescens,* as they were showing less or negligible percentage of growth inhibition compared to control. The growth inhibition of *P. fluorescens* in propiconazole 10.7% + tricyclazole 34.2% SE was 2.22% at drone concentration and was significantly higher compared to propiconazole 25% EC (1.78%). Similarly, propiconazole 10.7% + tricyclazole 34.2% SE exhibited 1.33 % growth inhibition of *P. fluorescens* at Taiwan sprayer concentrations compared to no inhibition with propiconazole 25% EC. Whereas, tebuconazole 50% + trifloxystrobin 25% WG was found fully compatible, showing no growth inhibition at both drone and Taiwan sprayer concentrations.

The *P. fluorescens* has exhibited higher compatibility with propiconazole, tebuconazole, trifloxystrobin + tebuconazole, azoxystrobin, carbendazim, and carbendazim + mancozeb at

P. fluorescens strain 36 enhanced antagonistic effects against *Rhizoctonia solani* Kuhn. Sendhil Vel *et al.* (2004) also found that the growth of Pf1 and *B. subtilis* was unaffected by high azoxystrobin concentrations. Mathew (2003) investigated the compatibility of *P. fluorescens* with nine pesticides, finding that mancozeb, carbendazim, chlorpyrifos, and imidacloprid were highly compatible with the *P. fluorescens* strain P11 at their recommended field use doses.

CONCLUSION

The study demonstrates the compatibility of *B. thuringiensis* var. *kurstaki* with various insecticides and *P. fluorescens* with fungicides at concentrations applicable for drone and Taiwan sprayer. *In vitro* assessments revealed that, minimal growth inhibition, indicating a high degree of compatibility between these biopesticides and chemical pesticides. These results underscore the potential for integrating biopesticides with conventional pesticides in pest management strategies, offering a synergistic approach to enhance overall efficacy. The demonstrated compatibility supports the integration of biopesticides into plant protection regimes, particularly when applied *via* drones, facilitating rapid and sustainable pest and disease control. This approach aligns with modern agricultural practices, aiming for efficient and environmentally sound pest management solutions.

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