

Fungal root endophyte *Piriformospora indica* mitigates the residues of systemic fungicide in vegetable cowpea

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Abstract

Anthraxnose, caused by *Colletotrichum gloeosporioides*, is a major fungal disease of vegetable cowpea that affects the leaves, stems, petioles, flowers, and pods, leading to considerable yield loss. Modern fungicides, including strobilurins and triazoles, are frequently used to control this disease. Additionally, *Piriformospora indica*, a beneficial root endophytic fungus, is commonly utilized in plants of varied genera for its disease-suppressing effects and its capacity to enhance plant growth. The present study investigated the application of *P. indica*-compatible fungicides (trifloxystrobin + tebuconazole and carbendazim) on colonized plants to assess their combined effect on anthracnose management under pot culture conditions. Additionally, fungicide residue on the leaves was analyzed using the QuEChERS-LC-MS/MS method, and degradation percentages were calculated to determine whether *P. indica* contributes to the reduction of residue buildup. *P. indica*-colonization, along with spraying of trifloxystrobin 25 % + tebuconazole 18.3 % SC and carbendazim 50 % WP brought down the anthracnose infection by 86.67 and 84.56 per cent in vegetable cowpea seedlings. Residue analysis in *P. indica* colonised plants showed a higher degradation of 84.83%, 88.25%, and 89.32% with respect to tebuconazole, trifloxystrobin, and carbendazim, and 68.35%, 75.14%, and 76.64% in controls, respectively. The initial deposition of fungicides was lesser in colonized plants. *P. indica* colonisation also reduced the half-life to 7.57, 7.12, and 7.19 days in response to 12.35, 10.05, and 9.63 days in control plants. Therefore, it was inferred that *P. indica* reduces the residue build-up in vegetable cowpea by enhancing the degradation percentage.

Key words: *P. indica*, vegetable cowpea, fungicide residue, dissipation, half-life

Introduction

Vegetable cowpea (*Vigna unguiculata* subsp. *sesquipedalis* (L) Verdcort), is a highly preferred vegetable crop in Kerala for its diverse cuisine and favourable climatic conditions. The crop is infected by many fungal and viral diseases, of which anthracnose caused by *Colletotrichum gloeosporioides* is highly infective, leading to a severity of 55 per cent (Sreeja, 2014). Dark brown to black spots appear on the leaves, and elongated and sunken lesions encircling stems, pods, peduncles, and petioles are major symptoms. Presently the disease is managed with the application of triazole fungicides, a mixture of carbendazim and mancozeb and the use of biocontrol agents like *Trichoderma* spp. (Sreeja, 2014).

Much likely, indiscriminate use of these chemicals during the fruiting stage can develop residue on the produce as well as in the soil and growers are most concerned about residue accumulation at the harvest stage, which exceeds the maximum residue limits (MRLs). There is a growing demand for organically grown crops as consumers are more aware of health issues. Central Insecticides Board and Registration Committee (CIBRC) have recommended the consumption of agricultural products applied with chemicals after checking the half-life

period of the pesticides. To safeguard the safety of consumers, the European Union (EU) has released laws that establish the MRLs for certain fungicides in various fruits and vegetables (EFSA, 2021). The residue degradation following foliar pesticide application to a plant surface is dependent on multiple degrading factors, including photoperiod rate of volatilization in combination with rainwater washing off.

According to Qin *et al.* (2010), residue greater than 70 per cent is reported in cowpeas, peaches, grapes and celery. MRLs of myclobutanil, tetraconazole and fluzilazole used against powdery mildew of grapes are above the specified limit and require a minimum waiting period of 30 to 60 days (Salunkhe *et al.*, 2015). Tripathy *et al.* (2022) observed residue of 56 fungicides like tebuconazole, metalaxyl, asoxystrobin, trifloxystrobin and difenoconazole from brinjal, capsicum, tomato and cucurbits. In order to meet the MRLs, harvesting is frequently postponed past the point of physiological maturity until the fruit is deemed safe, costing financial loss. Triazole fungicide tends to persist in crops and the environment for a long time. The residue level of propiconazole in apple was above the MRLs (Machowska *et al.*, 2008), and in strawberries the levels increased after each application (Heleno *et al.*, 2014).

Although the chemical stability and physical properties of a pesticide ultimately influence its persistence in the environment, environmental factors also play a role and can be used to accelerate the pesticide's breakdown. Of them, the biodegradation of pesticides by microorganisms is a significant, secure and effective method for eliminating residues. Bacterial and fungal strains which are naturally isolated are proven to detoxify and metabolize organic substances which are environmentally safe. An investigation by Eizuka *et al.* (2003) has successfully demonstrated the capability of *Kitasatospora* sp. and *Streptomyces* sp. in degrading ipconazole, a triazole fungicide used in treating paddy seeds. Similarly, *Enterobacter sakazakii* and *Serratia* sp. have effectively degraded 51 per cent concentration of tebuconazole (Sehnm *et al.*, 2009) and selected strains of *Pseudomonas putida* from rhizosphere of tea plantations have biologically degraded propiconazole. Salunkhe *et al.* (2014) also shared the possibility of detoxifying the action of *Bacillus subtilis* against carbendazim in grapes. According to Zhang *et al.* (2019), the endophytic *Paenibacillus polymyxa* that was isolated from ginseng destroyed fluazinam, chlorpyrifos, DDT, 46.24, and 21.05 percent in the roots and 75, 58, 46, 55.61, and 73.65 percent in the leaves. After being inoculated with the endophytic *Trichoderma atroviride*, the levels of chlorothalonil, cyproconazole, trifloxystrobin, and azoxystrobin were reduced by 89.0, 46.4, 33.2, and 82.20 per cent, respectively. These fungicides were degraded with the aid of glutathione s-transferase (GST) production (Escudero-Leyva *et al.*, 2022). Therefore, use of biocontrol agents is highly influential against plant diseases and can reduce chemical fungicide usage, residue and its impending health hazards.

A combined application of fungicide and fungicide-tolerant biocontrol agents has been effective in managing a variety of fungal diseases, including sugarcane red rot (Malathi *et al.*, 2002), botrytis blight in ornamental plants (Buck, 2004), chilli anthracnose (Anand *et al.*, 2010), botrytis grey mold of grapes (Stefan *et al.*, 2015), botrytis crown and root rot in tomatoes (Omar *et al.*, 2006), and rhizoctonia aerial blight on rosemary (Conway *et al.*, 1997). According to Roylawar *et al.* (2021), the combined use of fungicides and the periodic release of *P. indica* could reduce the frequency and amount of chemical spraying to halt the progression of stemphylium blight of onion. Moreover, this combination can also be effective in producing safer foods, reducing the dependency of chemicals in managing plant diseases and serving as a natural bioremediation tool to reduce environmental contamination.

No work has been carried out till now that explains the use of compatible fungicide with *P. indica* and its role in residue management. It is our first effort to investigate the compatibility of new-generation fungicides with *P. indica* under pot culture conditions. Henceforth, we attempted to find the compatible new-generation fungicides with *P. indica* under *in vitro* conditions and the effect of their combination in bringing down anthracnose infection under *in vivo* (pot culture) conditions. The ability of *P. indica* in controlling various plant diseases is already known and its capability in degrading fungicide residues offers exciting promises with dual advantage of protecting vegetable cowpea from anthracnose and for breaking down the fungicide residue on cowpea pods. Hence, the present investigation was done to

study the residue behavior, dissipation and half life of fungicides in vegetable cowpea colonized with *P. indica*.

Materials and methods

Maintenance and multiplication of *P. indica*: *Piriformospora indica* (Accession No. INBA3202001787) was cultivated in Potato Dextrose Agar (PDA, pH 6.5) by placing 5mm discs at the centre. After that, the dishes were kept in a room temperature (27±1°C) and 80 per cent humidity for 10 days, with 12-hour cycles of light and dark. To preserve its viability, the culture was then periodically sub-cultured every fifteen days (Johnson *et al.*, 2013).

Multiplication of *C. gloeosporioides* and preparation of spore suspension: *C. gloeosporioides* was isolated from the infected vegetable cowpea leaves following standard procedure onto PDA medium, sub-cultured and re-isolated periodically to maintain its virulence. The pathogen was multiplied by transferring 5 mm disc in PDB for 21 days until pink spore formation. Spore mass was filtered by passing through a muslin cloth and concentration was made to 10⁻⁶ spores mL⁻¹ using haemocytometer.

Compatibility of *P. indica*-colonized seedlings with fungicides against *C. gloeosporioides*: Pot culture experiments were conducted to analyze the efficacy of *P. indica*-colonization with the fungicides against anthracnose. Vegetable cowpea seedlings of variety Vellayani Jyothika were colonized with *P. indica* by following the procedure described by Jojy (2020). Similarly control plants were also maintained separately for the experiment. The endophyte-colonized seedlings were re-potted on 15th day and raised as per the Package of Practices of KAU (2016). Thirty-five day-old seedlings were spray-inoculated with a conidial suspension of *C. gloeosporioides* @ 10⁻⁶ spores mL⁻¹ and covered with polypropylene covers to maintain humidity. 10 days after symptom expression, two best fungicides from the *in vitro* experiment were sprayed at the recommended doses. The disease severity was calculated before and after the application of fungicides. The experiment was laid out as a completely randomized design (CRD) with seven treatments and five replications. The treatments were vegetable cowpea seedlings colonized with *P. indica*; the seedlings inoculated with spore suspension of *C. gloeosporioides*; the seedlings pre-colonized with *P. indica*, subsequently inoculated with *C. gloeosporioides* and then sprayed with best fungicide (trifloxystrobin 25 % + tebuconazole 18.3 % SC); the seedlings pre-colonized with *P. indica*, subsequently inoculated with *C. gloeosporioides* and then sprayed with best compatible fungicide (carbendazim 50 % WP); the seedlings inoculated with *C. gloeosporioides* and sprayed with best compatible fungicide (trifloxystrobin 25 % + tebuconazole 18.3 % SC); the seedlings inoculated with *C. gloeosporioides* and sprayed with best compatible fungicide (carbendazim 50 % WP); and the seedlings without the endophyte and fungicides (control).

Fungicide residue analysis of *P. indica*-colonized vegetable cowpea plants: The persistence and degradation of fungicide residues in vegetable cowpea leaves was carried out at Pesticide Residue Research and Analytical Laboratory (PRRAL) of the All India Network Research Project on Pesticide Residues (AINRPPR), College of Agriculture, Vellayani, Thiruvananthapuram. Vegetable cowpea leaves were collected from each treatment at 2 h, 1, 3, 5, 7, 15 and 21 days after the

application of trifloxystrobin + tebuconazole and carbendazim. Three replications of each sample from the treatments involving *P. indica* and *C. gloeosporioides* with and without the best 2 fungicides were collected for the residue analysis.

Standard stock solutions of tebuconazole, carbendazim, and trifloxystrobin (400 µg/ml) were prepared in methanol. The working standard mixture of 10 µg/mL was prepared by serial dilution. The standard required for plotting the calibration curve (1.00, 0.50, 0.25, 0.10, and 0.05, 0.01, 0.005 µg/ml) were prepared by serially diluting the working standard with methanol and were used for spiking samples and studying the linear dynamic range in the liquid chromatographic analysis. All standard solutions were stored in darkness at 4°C.

Ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS/MS) parameters were set and 10 µL of each of the standards were injected into it. The instrument's detection threshold was calculated depending on the lowest effective concentration of each fungicide and each standard was injected three times, which could be identified under UPLC-MS/MS conditions. The LOD (Limit of Detection) was defined as the lowest concentration at which a signal-to-noise ratio (S/N) was any number more than three and it was identified as 0.01 mg kg⁻¹.

For residue extraction and cleanup of vegetable cowpea leaves, multi-residue estimation method for vegetables and fruits using QuEChERS technique 10 was used. 25 g of leaves was grinded for 3 min using a high-speed blender. The extract was collected in 50 mL centrifuge tube and to it 50 mL high-performance liquid chromatography (HPLC) grade acetonitrile was added and homogenized (Heidolph Silent Crusher-M) for 3 min at 14000 rpm. To separate the acetonitrile level, 10 g of activated sodium chloride (NaCl) was added and the mixture was shaken for 2 min. The samples were then centrifuged for four minutes at 2500 rpm, followed by the transfer of 16 mL of the clear upper layer into 50 mL centrifuge tubes with 6 g of sodium pre-activated sulphate for 2 min while vortexing. The extracts from acetonitrile were cleaned up using the solid phase extraction method (DSPE). For this, 12 mL of the top layer was moved into centrifugation tubes (15 ml) having 1.2 g of anhydrous magnesium with 0.2 g primary secondary amine (PSA) sulphate and vortexed for 30 seconds and centrifuged once more for 3 min at 2500 rpm. The 5 mL of each supernatant liquid was added to a Turbovap tube and evaporated to dryness while submerged in gentle nitrogen at Table 1. Effect of *P. indica*-root colonization and foliar spraying of systemic fungicides on anthracnose symptom appearance and lesion size in vegetable cowpea var. Vellayani Jyothika

Treatments	DTSA	Lesion size (cm)*				
		3 rd DAI	5 th DAI	7 th DAI	10 th DAI	15 th DAI
<i>P. indica</i> alone	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
<i>C. gloeosporioides</i> alone	1	1.30 ± 0.11 ^a	3.05 ± 0.15 ^a	5.40 ± 0.19 ^a	7.80 ± 0.05 ^a	8.90 ± 0.12 ^a
<i>P. indica</i> -colonized seedlings + <i>C. gloeosporioides</i> + Trifloxystrobin 25 % + Tebuconazole 18.3 % SC	5	0.00 ± 0.00	0.72 ± 0.27 ^d	1.98 ± 0.06 ^c	2.70 ± 0.10 ^c	3.40 ± 0.19 ^c
<i>P. indica</i> -colonized seedlings + <i>C. gloeosporioides</i> + Carbendazim 50% WP	5	0.00 ± 0.00	0.76 ± 0.09 ^d	1.94 ± 0.51 ^c	2.58 ± 0.78 ^c	3.30 ± 0.21 ^c
<i>C. gloeosporioides</i> + Trifloxystrobin 25 % + Tebuconazole 18.3 % SC	3	0.82 ± 0.18 ^b	2.31 ± 0.17 ^c	3.98 ± 0.19 ^b	5.19 ± 1.21 ^b	6.40 ± 0.31 ^b
<i>C. gloeosporioides</i> + Carbendazim 50 WP	3	0.75 ± 0.26 ^b	2.48 ± 0.15 ^b	4.04 ± 0.22 ^b	5.28 ± 1.29 ^b	6.25 ± 0.12 ^b
Absolute control	0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SE(m)		0.03	0.06	0.07	0.07	0.09
CD (0.05)		0.08	0.16	0.21	0.20	0.25

*Mean of four replications ± standard deviation; DTSA - Days taken for symptom appearance; Superscripts with same alphabets indicate on par values and those in different alphabets indicate significant difference at 5 per cent level of significance

45°C on Turbovap 7.5 psi nitrogen flow. 2 mL of methanol was used to reconstitute the residues and before using to LC-MS/MS, filtered through a 0.2-micron filter.

The residues of tebuconazole, carbendazim, and trifloxystrobin were estimated using a reversed phase Atlantis d c-18 (100 x 2.1 mm, 5 µm particle size) column. The mobile phase system received eluent chemicals as: "(A) 5 millimolar Ammonium Formate + 0.1% Formic acid in Water and (B) 5 millimolar Ammonium Formate + 0.1% Formic acid in Methanol". The flow rate stays at 0.3 ml/min, and the injection volume was 2 µL. A 40°C temperature was maintained in the column. With retention times of 3.57, 3.96 and 0.90 min, respectively, the residues of tebuconazole, trifloxystrobin and carbendazim were verified by GC- MS (Shimadzu GC- MS QP 2010 Plus). The limit of quantification (LOQ) and limit of detection (LOD) values were 0.01 mg kg⁻¹ respectively. The half-life period of each fungicide was also calculated.

The residues were estimated using the formula:

$$\text{Residues } (\mu\text{g g}^{-1}) = \frac{\text{Sample area} \times \text{Concentration of standard } (\mu\text{g g}^{-1}) \times \text{Final volume of extract (mL)}}{\text{Standard Area} \times \text{Weight of sample (g)}}$$

Statistical analysis: All the experiments performed in pot culture were designed in CRD. The data were analyzed statistically using Grape 2.0 software (Gopinath *et al.*, 2020). Data were subjected to ANOVA and treatment means were compared with t-test.

Results

Compatibility of *P. indica* and systemic fungicides under pot culture: Based on the findings of the *in vitro* experiment, trifloxystrobin 25 % + tebuconazole 18.3 % SC and carbendazim 50WP were selected for pot culture studies. Untreated plants did not show any symptoms of anthracnose and *P. indica*-colonization significantly reduced the disease incidence and severity with improved growth of the plant. At various time intervals, the size and severity of the anthracnose lesion were substantially reduced by all of the treatments. The combination of *P. indica* colonization and systemic fungicide spray considerably reduced the disease severity when compared to untreated plants sprayed with fungicides following artificial inoculation of *C. gloeosporioides*. Fungicide-sprayed plants colonized by *P. indica*, control plants sprayed with fungicides, and plants containing *C. gloeosporioides*

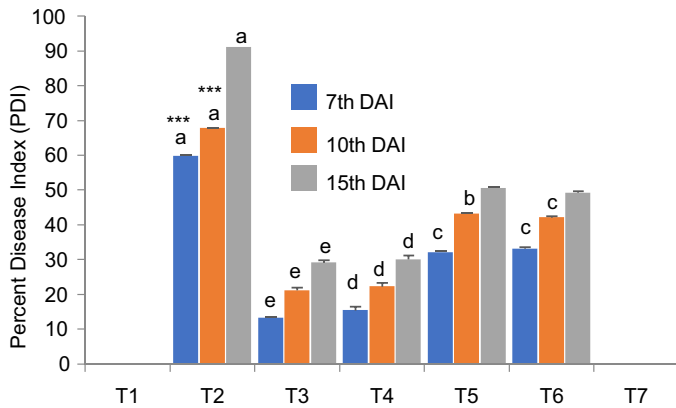


Fig 1. Percent disease severity of anthracnose incited by *C. gloeosporioides* in *P. indica*-primed vegetable cowpea seedlings var. Vellayani Jyothika along with the application of compatible systemic fungicides at 7, 10 and 15 DAI T₁: *P. indica* alone; T₂: *C. gloeosporioides* alone, T₃: *P. indica*-primed seedlings + *C. gloeosporioides* + Trifloxystrobin 25 % + Tebuconazole 18.3 % SC; T₄: *P. indica*-colonized plants + *C. gloeosporioides* + Carbendazim 50WG; T₅: *C. gloeosporioides* + Trifloxystrobin 25 % + Tebuconazole 18.3 % SC; T₆: *C. gloeosporioides* + Carbendazim 50WG; T₇: Absolute control

alone showed lesion sizes ranging from 2.58 to 7.8 cm on 10 DAI (Table. 1) and PDIs ranging from 21.23 to 67.81.

Ten days following the *C. gloeosporioides* inoculation, vegetable cowpea plants not treated with fungicides and *P. indica* exhibited infection rates as high as 67.81 percent. When *P. indica*-colonized plants were treated with the suitable fungicide trifloxystrobin 25% + tebuconazole 18.3% SC and carbendazim 50WP, the severity values were 21.23 and 22.29 at 10 DAI. In contrast, plants that *P. indica* did not colonize showed comparatively greater severity of 43.21 and 42.19 following the administration of carbendazim 50WP and trifloxystrobin 25% + tebuconazole 18.3% SC on 10 DAI (Plate 1). When compared to plants without *P. indica* colonization, the application of trifloxystrobin 25% + tebuconazole 18.3% SC and carbendazim 50WP decreased the severity of anthracnose by 50.86 and 47.17 percent (Fig. 1).

Pesticide residue: In light of the above study, dissipation patterns of the field-tested fungicides were investigated. An initial deposit of 2.64, 2.77 and 1.03 mg kg⁻¹ on leaves was observed 2 h after spraying of plants with carbendazim (0.1%), tebuconazole (0.1%) and trifloxystrobin (0.1%) in plants colonized with *P. indica* and sprayed with *C. gloeosporioides*. In contrast, a higher residue of 3.46, 3.95 and 1.67 mg kg⁻¹ of the above fungicides were observed on leaves collected from plants without colonization of *P. indica* and also inoculated with the pathogen (Fig. 2). *P. indica* has increased the degradation of fungicides. With respect to the initial deposit, residues had been separated to 88.25 per cent by 21 days in carbendazim and 84.83 and 89.32 per cent in the case of tebuconazole (0.1%) and trifloxystrobin (0.1%). In plants without *P. indica*-colonization, dissipation occurred very slowly, i.e., 68.35, 75.14 and 76.66 per cent. The dissipation of fungicides showed a non-linear over time. Fig. 2 shows the correlation coefficient and regression equations for three fungicides over two treatments.

The calculated half-life for carbendazim, tebuconazole and trifloxystrobin was 9.71, 12.35 and 10.05 days in control and it was considerably reduced by *P. indica* treatment to 7.19, 7.42 and 7.12 days. Degradation of fungicides was slower in plants

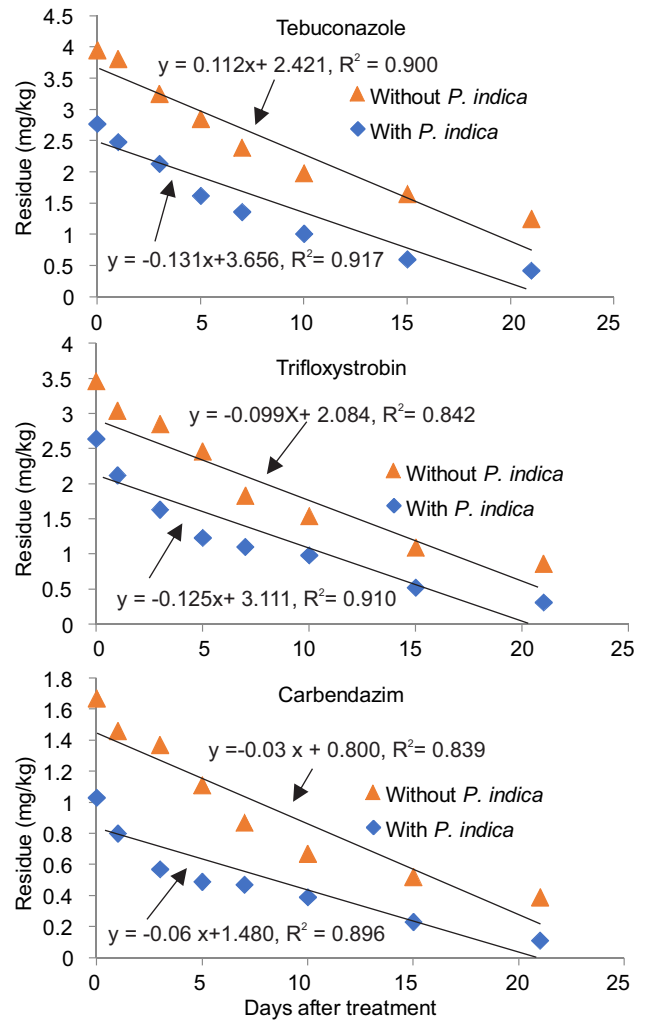


Fig. 2. Dissipation kinetics of active substances of a) tebuconazole b) trifloxystrobin and c) carbendazim over time in different treatments in vegetable cowpea variety Vellayani Jyothika

which were not colonized with *P. indica*. *P. indica*-colonization significantly increased the dissipation by 27.23 per cent in case of carbendazim, 28.81 per cent in tebuconazole and 20.29 per cent in the case of trifloxystrobin. *P. indica*-colonization reduced fungicide residues of trifloxystrobin 25 % +tebuconazole 18.3 % SC by 17 per cent and in carbendazim 50WG by 16.6 per cent in the treated vegetable cowpea plants.

Discussion

Results from the present experiment confirm that priming *P. indica* onto vegetable cowpea plants can significantly reduce anthracnose infection. Severity was brought down to 13.33 and 15.44 per cent with the combined application of *P. indica* and fungicides. Sugarcane setts with *P. fluorescens* and carbendazim treatment reduced the red rot infection by 66.67 per cent (Malathi *et al.*, 2002) and 99.39 per cent of chilli anthracnose with *P. fluorescens* and azoxystrobin (Anand *et al.*, 2010). In an integrated strategy, the survival rate of the beneficial microbe is not significantly impacted by the activity of the targeted fungicide. Use of compatible fungicides was found to be significantly effective against rosemary Rhizoctonia aerial blight (Conway *et al.*, 1997), Botrytis blight in ornamental plants (Buck, 2004), Fusarium crown and root rot in tomato (Omar *et al.*, 2006), Botrytis grey mold of grapes (Stefan *et al.*, 2015) and Fusarium

wilt disease in dry beans (Abd-El-Khair *et al.*, 2019). Roylawar *et al.* (2021) opined the use of fungicides like azoxystrobin 20% + difenoconazole 12.5%, metiram 55% + pyraclostrobin 5% WG with the periodic application of *P. indica* could minimize the frequency and volume of chemical sprays to slow down the progression of the disease. In light of the above study, dissipation patterns of the field-tested fungicides were investigated.

The present study on the effect of endophyte against fungicide residue showed a positive result as the colonization reduced the degradation and residue of three fungicides (carbendazim, tebuconazole and trifloxystrobin) upto 18 per cent. According to Patel *et al.* (2016) initial deposit trend of 2.77 mg kg⁻¹ in vegetable cowpea plants by tebuconazole was similar to what was observed in spring onion and 1.95 mg kg⁻¹ in grapes (Mohapatra *et al.*, 2014). Dubey *et al.* (2020) conducted a study in tomato, chilli and onion, where the initial residues of tebuconazole degraded to 97 per cent within 7 days. *P. indica* also increased fungicide degradation, with residues separated to 87.25 per cent by 21 days. Dissipation of tebuconazole + trifloxystrobin to 50 per cent of the initial deposit (0.95 and 1.88 mg kg⁻¹) was observed by Sahoo *et al.* (2012) within 2 days. In gherkins, residue deposited by tebuconazole + trifloxystrobin was less than 1 mg/kg⁻¹ (Mohapatra, 2015). As detailed by Ramadan *et al.* (2020), residue of carbendazim was detected in chilli, brinjal, cucumber and cabbage, wherein the value exceeded MRLs only in cabbage. Results from Cui *et al.* (2023) recorded higher residues of trifloxystrobin in cowpeas (1.25 mg/kg⁻¹) than in cucumber (0.277 mg/kg⁻¹), which increased after subsequent spraying. Moreover, the degradation of trifloxystrobin was slower in cowpea than in cucumber. It is challenging to generalize about the likely causes of variation in dissipation of trifloxystrobin, tebuconazole and carbendazim in different crops because the main factors influencing the final state of residues are; crop growth features like distance among canopy variable climatic parameters, physiochemical and biological properties of the soil (Seiber and Kleinschmidt, 2010).

The half-life of carbendazim, tebuconazole and trifloxystrobin was significantly reduced to 7 days by *P. indica* treatment, while as fungicide degradation was slower in plants not colonized with *P. indica*, half life varied from 9 to 12 days. The residues were degraded very slowly as the leaves were used for the study. Dubey *et al.* (2020) calculated the half-life of tebuconazole to be 2.58 days in apple, 2.1 days in onion and chilli. Half-life values were much less for tebuconazole in onion and mango (6), chilli (1), and tomato (0.9), as observed by Mohapatra (2014); Sahoo *et al.* (2012) and Saha *et al.* (2016).

The degradation of ipconazole, a triazole fungicide used to treat rice seedlings, has been effectively reduced by *Kitasatospora* sp. and *Streptomyces* sp., as demonstrated by Eizuka *et al.* (2003). Comparably, Propionazole has been physiologically decomposed by certain strains of *Pseudomonas putida* from the rhizosphere of tea plantations. At the same time, *Enterobacter sakazakii* and *Serratia* sp. have successfully broken down a 51 per cent concentration of tebuconazole (Sehnem *et al.*, 2009). *Bacillus subtilis* may have a detoxifying effect on carbendazim in grapes, according to Salunkhe *et al.* (2014). Similarly, a combination of bioagents with fungicides significantly reduced the residue of fungicides in many studies *viz.*, residues of azoxystrobin and penconazole by *Ampelomyce squisqualis*, *Bacillus subtilis* and

Trichoderma harzianum against powdery mildew of strawberry (Pertot *et al.*, 2008); fludioxonil and fenhexamid by *Bacillus subtilis* strain, *Bacillus amyloliquefaciens* and *Aureobasidium pullulans* against grey mold of grapes (Rotolo *et al.*, 2018), boscalid, cyprodinil, and fenexamide by *Papilio trematerrestris* and *Bacillus subtilis* against brown rot of pear (Palmieri *et al.*, 2022). Similarly, De Curtis *et al.* (2019) also observed a reduced residue with increased dissipation of cyprodinil or boscalid in stone fruits coated with yeast *Rhodotorula kratochvilovae* against monilia brown spot.

From the present investigation it was found that fungicides can be used in *P. indica*-colonized vegetable cowpea plants to protect the plants from anthracnose infection. This method can also reduce the concentration of chemicals used and also reduces the buildup of residue on the plant surface, thereby reducing health and environmental risks. The residue persisted in the leaves of vegetable cowpea for more than 21 days in all the treatments. But *P. indica* treatment could considerably increase the degradation by 17 to 19 per cent. *P. indica* treatment also reduced the half-life of the fungicides by 7.19, 7.42 and 7.12 days instead of 9.71, 12.35 and 10.05 days in control plants for carbendazim, tebuconazole and trifloxystrobin.

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