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In vitro Assessment of Fluorescent Pseudomonads against Coriander (*Coriandrum sativum* L.) Powdery Mildew

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ABSTRACT

Coriander (*Coriandrum sativum* L.) is well known seed spice used in India. It is affected by powdery mildew caused by *Erysiphe polygoni* DC which is most destructive pathogen. PGPR is gaining importance in present era due to organic crop production and its concern on mankind and ecosystem. A total 25 fluorescent *Pseudomonads* (PGPRs) isolates were collected from Gokak, Khanapur and Hukkeri taluk of Belagavi district (Karnataka, India). Further, identification of fluorescent *Pseudomonads* was done by cultural, morphological and biochemical characterization. Antagonistic activity of 25 fluorescent *Pseudomonads* were assessed against powdery mildew conidial inhibition under *in vitro* condition. Among isolate, FP-11 recorded highest spore inhibition (43.28 %) followed by FP-8 (40.78 %).

Key words : *Biological control, Fluorescent pseudomonads, Induced systemic resistance, Plant Growth Promoting Rhizobacteria, Siderophore.*

Introduction

The world's largest producer, consumer, and exporter of seed spices and their products is India, which is known as the "Home of Spices."Coriander (*Coriandrum sativum* L.), native to Southern Europe and the Eastern Mediterranean region, belongs to Apiaceae family. With its delicate aerial parts, stem, leaf and fruits, this annual herbaceous plant which grows from October to February, enhances the appearance, flavour and texture of anything it cooks. d-Linalool is an essential component that gives off a pleasant aroma. The production and area in India are 866 Mt and 6,65,190 hectares, respectively. With 2,77,410 hectares and 3,91,460 tonnes of seed coriander output, respectively, Madhya Pradesh is the top state (Anon, 2019). The most destructive disease was powdery mildew (*Erysiphe polygoni* DC.), which might reduce seed quality and result in yield losses of up to 20 per cent (Yadav *et al.,* 2022).

Chemical fungicides have detrimental effects on beneficial creatures such as plants, animals, people and other plants; as a result, these organisms have turned to use other plant protection techniques. Plant Growth Promoting Rhizobacteria (PGPR), a recently developed biological control agent, colonizes plant roots to stimulate plant growth while being non-toxic and ecologically viable. They are

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extremely varied and can arise from induced systemic resistance to phytopathogens or from local antagonistic relationships. The genus *Pseudomonads* comprises gram-negative, aerobic, rod-shaped, polar flagellated bacteria, belongs to class-Gamma Proteobacteria, phylum-Proteobacteria, and *Pseudomonadaceae* family, which also includes a few non-fluorescent species. Fluorescent *Pseudomonads* such as *Pseudomonas fluorescens* and *Pseudomonas putida* were aggressive root colonizers and competes for space and nutrients, siderophores (pseudobactin and pyoverdine pigments), antibiotics such as 2,4-DAPG, pyrrolnitrin and pyoluteorin volatile compounds (HCN), defense enzymes such as phenylalanine ammonia lyase (PAL), chitinase, ß-1,3 glucanase, peroxidase, polyphenol oxidase (Lamani and Kulkarni, 2018). Hence, the present investigation was conducted to collect, isolate, identify and assess the fluorescent *Pseudomonads* against coriander powdery mildew under *in vitro* conditions.

Materials and Methods

Collection of soil samples

The current study was conducted in 2021–2022 at Department of Plant Pathology, Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka. In order to ascertain the presence of fluorescent *Pseudomonads* in the undisturbed rhizosphere of the forest areas (Zone-8) in the Belagavi district, soil samples were taken in the villages of Kadbagatti in Gokak, Asoga in Khanapur, Badakundri, and Hidkal in Hukkeri taluks, respectively.

Isolation of soil samples

Serial dilutions were prepared up to 10-7 dilutions. Pour plate technique was performed with King's B (KB) medium and incubated for 24-48 hours at 30 °C. The single, fresh colonies were picked up and zig-zag streaked on nutrient Agar (NA) using the streak plate technique. The plates were incubated at 30 ºC for 48 hours before being stored in a refrigerator (4 ºC) for future analysis and periodic subcultures were made every fortnight. For long-term storage, the purified single colonies were stored in 50 per cent glycerol at -20 ºC (King *et al.*, 1954)

Identification of fluorescent *Pseudomonads*

The fluorescent *Pseudomonads* were identified using "*Bergey's Manual of Systematic Bacteriology*". Cultural characters such as colony color, colony shape, colony surface, margin, elevation and transparency. Morphological characters such as Cell shape, fluorescence test and gram staining. Three replications were maintained (Bergey, 1994).

Fluorescence test (fluorescein pigment production

For fluorescence, the fluorescent *Pseudomonads* were streaked over fluorescent agar and cultured for 48 hours at 28 ± 2°C. Fluorescein, or colony pigmentation, was seen in ultraviolet light with wavelengths ranging from 320 to 360 nm (Lamani and Kulkarni, 2018).

Gram staining

A loopful of bacteria that grown for 24 hours was smeared on glass slide was heat-fixed, left to air dry, flooded with crystal violet for a minute and then completely cleaned before being flooded with an iodine solution for a minute, flooded with 95 % ethyl alcohol (a decolorizer), rinsed, counter stained with safranin for a minute, and then completely cleaned with distilled water and then inspected under a microscope with an oil immersion objective lens (100 X) and cedar wood oil (Lamani and Kulkarni, 2018).

Biochemical characterization of fluorescent *Pseudomonads* (Lamani and Kulkarni, 2018)

1.Oxidase test

A loop of bacterial cells that had grown for 24 hours was rubbed with 1% (w/v) aqueous N,N,N,N tetramethyl-p-phenylenediamine dihydrochloride (Kovac's reagent) on strips of Whatman's No. 1 filter paper. Intense deep blue to deep purple hue (colour) in 10 seconds is oxidase positive.

Catalase test

After applying 1 ml of 3% hydrogen peroxide to a loopful of fluorescent *Pseudomonads* of 24 hours old, immediate effervescence (gas bubbles) started to emerge right away on the slide.

Gelatine liquefaction test

The 24 hour old fluorescent *Pseudomonads* culture becomes transparent in the places where the gelatin is hydrolyzed, but precipitates gelatin when 12.50 percent mercuric chloride solution is applied.

Assessment of fluorescent *Pseudomonads* under *in vitro* condition.

Antagonistic activity of fluorescent *Pseudomonads* isolates was assessed against coriander powdery mildew by per cent conidial inhibition using the cavity slide method under *in vitro* condition and was calculated by the following formula given by Vincent (1947)

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

Where,
C = Germination of conidia in control.
T = Germination of conidia in treatment.

Statistical analysis

The data were statistically examined using Completely Randomized Design (CRD) (p = 0.01) under *in vitro* condition. Web Agri. Stat. Package 1 created by the ICAR research complex in Goa was used for the analysis.

Results and Discussion

Collection and isolation of soil samples

A total number of twenty-five fluorescent *Pseudomonads* (FP-1 to FP-25) were collected from undisturbed forest areas *viz*., Gokak, Khanapur and Hukkeri taluks of Belagavi district (Plate 1). The similar studies were also reported by Deshwal *et al.* (2013) and Sharma *et al*.(2014).

Identification of fluorescent *Pseudomonads*

Cultural studies revealed that, twenty-five fluorescent *Pseudomonads* appeared as creamy yellow colony. Colony shape varied from circular to irregular, margin was undulated to entire, elevation was flat, colony surface varied from smooth, glistening smooth and rough/dry, transparency varied from translucent to transparent. Morphological characteristics were rod cell shape, fluorescence test was positive with greenish yellow pigments and gram staining was negative. Biochemical tests such as oxidase, catalase, gelatin liquefaction was positive (Table 1). Similar characteristics of fluorescent *Pseudomonads* was also observed by Deshwal *et al.* (2013), Sharma *et al.* (2014), Lamani and Kulkarni (2018) and Das *et al*. (2021).

Assessment of fluorescent Pseudomonads under in vitro condition.

Microscopic observation revealed that, conidiophores were simple, erect, cylindrical, hyaline and they bore single or multiple conidia, each of which was barrel-shaped, unicellular, and hyaline. (Figure 1). Similar observations on morphology of powdery mildew were also made by Bandela *et al.* (2014) and Khare *et al.* (2017).

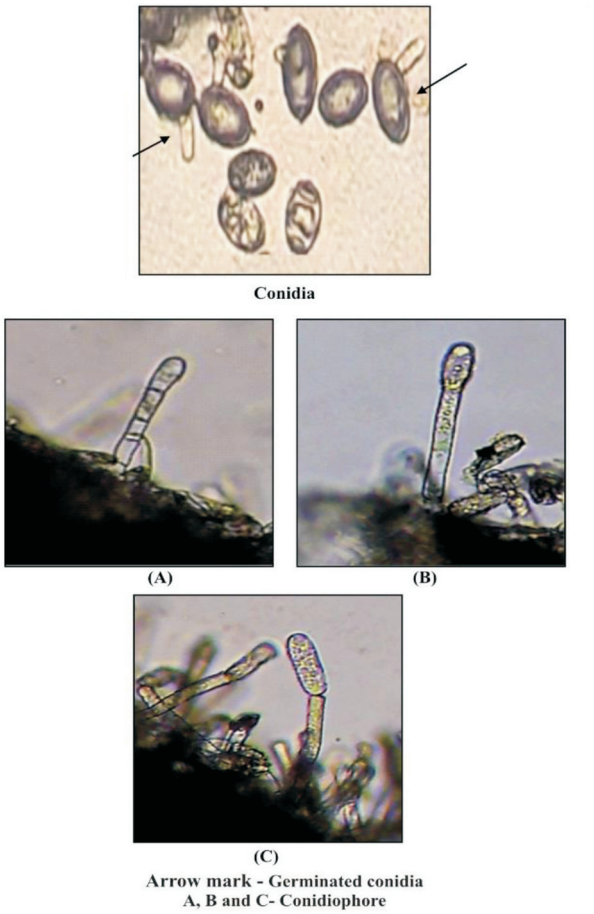

Fig. 1. Microscopic view of conidia and conidiophores of *Erysiphe polygoni* DC.

The twenty-five fluorescent *Pseudomonads* were assessed for per cent conidial inhibition against coriander powdery mildew under *in vitro* condition. Among them, FP-11 showed highest per cent conidial inhibition of 43.28 followed by FP-8 (40.78 %) and lowest per cent inhibition (26.07 %) was recorded in FP-14 (Figure 2).

The ability of fluorescent *Pseudomonads* to suppress fungal propagules depends on their ability to produce antibiotic secondary metabolites such as

Table 1. Cultural, morphological, biochemical characterization of fluorescent *Pseudomonads* on King's B medium

Isolates	Cultural						Morphological				Biochemical		
	Colony colour	Colony shape	Margin	Elevation	Colony surface	Transparency	Cell shape	Pigment (FP agar)	Fluores-cence	Gram's stain	Oxidase	Cata-lase	Gelatin liquefi-cation
FP1	Creamy yellow	Circular	Undulated	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP2	Creamy yellow	Irregular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP3	Creamy yellow	Circular	Entire	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP4	Creamy yellow	Circular	Entire	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP5	Creamy yellow	Irregular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP6	Creamy yellow	Circular	Undulated	Flat	Rough / dry	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP7	Creamy yellow	Circular	Entire	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP8	Creamy yellow	Circular	Entire	Flat	Glistening smooth	Transparent	Rod	Greenish yellow	+	-	+	+	+
FP9	Creamy yellow	Circular	Entire	Flat	Glistening smooth	Transparent	Rod	Greenish yellow	+	-	+	+	+
FP10	Creamy yellow	Circular	Entire	Flat	Rough / dry	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP11	Creamy yellow	Circular	Entire	Flat	Rough / dry	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP12	Creamy yellow	Circular	Entire	Flat	Rough / dry	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP13	Creamy yellow	Irregular	Entire	Flat	Rough / dry	Transparent	Rod	Greenish yellow	+	-	+	+	+
FP14	Creamy yellow	Circular	Entire	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP15	Creamy yellow	Circular	Undulated	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP16	Creamy yellow	Circular	Entire	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP17	Creamy yellow	Circular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP18	Creamy yellow	Irregular	Entire	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP19	Creamy yellow	Irregular	Entire	Flat	Glistening smooth	Transparent	Rod	Greenish yellow	+	-	+	+	+
FP20	Creamy yellow	Circular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP21	Creamy yellow	Circular	Entire	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP22	Creamy yellow	Irregular	Undulated	Flat	Smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP23	Creamy yellow	Irregular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP24	Creamy yellow	Circular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+
FP25	Creamy yellow	Circular	Undulated	Flat	Glistening smooth	Translucent	Rod	Greenish yellow	+	-	+	+	+

FP = Fluorescent *Pseudomonads*

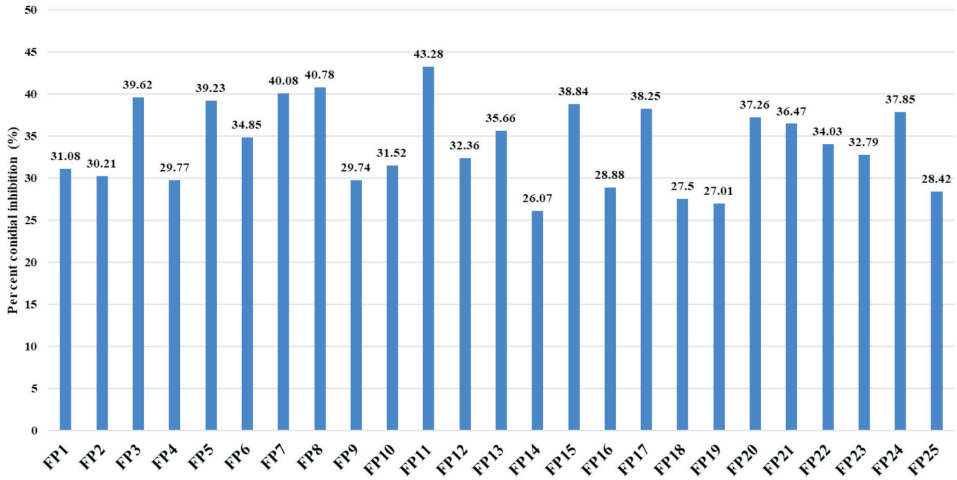

Fig. 2. Bio-efficacy of fluorescent *Pseudomonads* on per cent conidial spore inhibition of coriander powdery mildew under *in vitro* condition

pyoluteorin, pyrrolnitrin, phenazine 1- carboxylic acid, 2, 4 diacetylphloroglucinol (DAPG) hydrogen cyanide, kanosamine, pyocyanin and viscosinamide. Among these metabolites, DAPG has received a particular attention because of its broad spectrum activity. Similar findings were reported by several scientists, Sarma and Singh (2003), Baker *et al.* (2005) and Yadav *et al.* (2022).

Conclusion

Overall, the findings of this study suggest that fluorescent *Pseudomonads* (FP-11) isolated from rhizospheric soils of forest areas having strong antimicrobial properties showed highest per cent conidial inhibition against the coriander powdery mildew under *in vitro* condition.

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Conflict of interest

The author declare that in the current study, there is no conflict of interest.

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