INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

ISOLATION AND IDENTIFICATION OF *PSEUDOMONAS PUTIDA* FROM BACTERIAL LEAF BLIGHT DISEASE OF *CHRYSANTHEMUM* AND ITS MANAGEMENT USING BACTERICIDES AND BIO-AGENTS *IN VITRO* AND *IN VIVO* IN KARNATAKA (INDIA)

G. Shamala and GR. Janardhana*

Phytopathology Laboratory, Department of Studies in Botany
University of Mysore, Manasagangotri, Mysuru- 570 006,
Karnataka, India.

ABSTRACT

Bacterial blight of *Chrysanthemum* was observed during 2015 at the flowering stages of crop in Mandya district of Karnataka state (India). Bacterial blight symptoms includes, dark brown spots and blotches on leaves extending beyond plant leaves with water-soaked lesions on stems, darkening and death of buds and stem terminals. Affected plants showed brown to black decay at the base. The leaf spots measured 1-2mm in diameter, and as the number of spots progressed the leaves were completely dried up. The disease incidence (72.8%) and severity (71.39%) were very high in Banangadi region of Mandya district compared to other surveyed regions. The pathogen responsible for the disease was isolated and identified as *Pseudomonas putida* based biochemical and molecular methods. Pathogenecity was proved by Koch's postulates. Three different bactericides were evaluated *in vitro* against *Pseudomonas putida*. Among the three, Bactinash at 0.1% was most effective in inhibiting the bacterial growth followed by Anucin at 0.2%, Cristocyclene at 0.5% respectively. Field experiments also revealed that, Anucin at 0.2% was most effective in reducing the bacterial blight incidence with least disease severity of 7.19% and per cent disease index (4.88%). The control plants showed maximum disease severity. Bio-agents such as *Hypocrea rufa (Trichoderma viride)* and *Pseudomonas fluorescens* were also found effective in inhibiting the bacterial pathogen both *in vitro* and *in vivo*. To the best of our knowledge, this is the first report of the occurrence of bacterial blight disease of *Chrysanthemum* caused by *Pseudomonas putida* in Karnataka, India.

Keywords: Pseudomonas putida, bactericides, bio-agents, disease management.

INTRODUCTION

Chrysanthemum (Asteraceae), one of the most important floricultural crops grown in Karnataka state for its beautiful cut flowers and potted plants. In India, Karnataka state ranks second on an area of about 12, 100 hectares with a production of 2,11,000 tons and productivity of 12.05 tons per hectare. In Karnataka, Tumkur district ranks first with the production of 9,612 metric tons in an area of 801 ha followed by Chitradurga with 7,824 m. tons (530 ha), Haveri with 7,140 m. tons (493 ha), Mandya with 5,951 m. tons (472 ha) and Belgaum with 4,639 m. tons (331 ha) as loose flowers. *Chrysanthemum* is vegetative propagated flowering plant and is affected by many diseases caused by fungi and bacteria. Major bacterial diseases severely reducing the crop yields were stem necrosis (*Pseudomonas cichorii)*, bud blight (*Pseudomonas syringae)* and bacterial wilt or blight (*Erwinia chrysanthemi*) [1,12,16,22]. Bacterial blight caused by *Pseudomonas* species affects a broad range of ornamentals and horticultural crops including Gerbera, Tulip, Geranium, Cabbage, Coriander etc. [2,9,13,23]. A sudden appearance of bacterial blight disease in

Karnataka is of serious concern for *Chrysanthemum* growers as it directly affects the quality of cut flowers. The present study was taken up to understand the disease incidence, severity, identification of the causative agent and management of the disease using bactericides and bioagents *in-vitro* and *in-vivo*.

MATERIALS AND METHODS

Field Survey

Field survey was conducted during November 2014 to January 2015 at major *Chrysanthemum* growing regions namely Mudugere, Banangadi, Dinca and Ballenahalli of Mandya district (Karnataka state, India), revealed the appearance of bacterial blight disease incidence. The disease incidence was determined by dividing the crop fields into 5 plots (8m x 8m) having 25 *Chrysanthemum* plants each. Ten plants in each plot were randomly selected and the disease severity was calculated with total number of leaves infected against total number of leaves in each plant. Per cent disease index was calculated by rating the randomly selected plants on a scale of 0-4 (where 0 = no disease, 1 = 1-10 diseased spots, 2 =11-30 diseased spots, 3 = >30 diseased spots on the leaves, and 4 = blighted leaves)[2,5] using the formula;

$$\% \text{ Disease index} = \frac{\text{Sum of Numerical Values}}{\text{Total number of leaves observed X Maximum grading}} \times 100$$

Isolation and identification of the pathogen

Ten (10) *Chrysanthemum* plants showing typical symptoms of leaf blight were brought to the laboratory for further isolation and identification of the pathogen. Leaf blight affected samples were subjected to pus exudation test in sterile clean water. Infected leaves were washed with sterile water and fresh cuts were made on the petiole, immersed in water and observed under light microscope for the presence of ooze containing bacterial cells[8]. Further, leaves showing typical symptoms of blight were also washed with running tap water; air dried and cut into small pieces (5 mm diameter), disinfected with 1% Sodium hypochlorite (NaOCl) solution for 2min. and rinsed thrice with sterilized water. Leaf samples were placed aseptically on nutrient agar (NA) medium and incubated in an inverted position at 37°C for 24hr. Expressed bacterial colonies were sub-cultured and subjected for identification[2].

The bacterial pathogen associated with leaf blight disease was identified as per the methods described by Bergy's Manual of Determinative Bacteriology[10]. A smear of bacterial growth on NA was tested for Gram's reaction. The isolate was streaked on King's medium B (KB) to test the fluorescence. Further, biochemical tests such as oxidation reaction, fermentation of glucose, growth at 41°C, KOH test, gas from glucose, reducing substances from sucrose, acid production from lactose, catalase activity, nitrate test, indole test, MR/VP test, gelatin liquefaction were performed in triplicates and repeated three times[2].

Molecular identification of the bacterial pathogen associated with the leaf blight disease of *Chrysanthemum* was done based on partial sequencing of 16S rRNA[8]. Total genomic DNA from 3ml of overnight bacterial culture was isolated and directly used for PCR. 16S rDNA of the culture was amplified using conventionally purified genomic DNA using the primers [forward primer (5' -AGAGTTTGATCATGGCTCAG- 3') and the reverse primer (5'-GGTTACCTTGTTACGACTT- 3')] that amplifies approximately 1500 bp length of 16S rDNA gene of the bacterium. The PCR mixture include 2 µl of genomic DNA, 1X PCR buffer, 2 mM of $MgCl_2$, 50mM dNTPs 0.6 pmol of forward and reverse primer and 1.25units of Taq DNA polymerase. The final reaction volume was made up to 20 µl using nuclease free water. PCR conditions include an initial denaturation for 4 min at 94 °C, 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 58 °C, extension for 45 s at 72 °C and final extension for 8 min at 72 °C. The PCR was performed using Primus 25 advanced thermal cycler (Peq Lab Germany). Approximately 10 µl of PCR product was visualized on 1% agarose gel stained with 1% ethidium bromide. Nucleotide sequence of the PCR product was obtained by sequencing using Sanger's method. Nucleotide sequence was deposited in GenBank (KY750243) and compared with the sequences of other closely related bacteria using the Gen Blast program (NCBI). Species with greatest similarity were considered for phylogenetic analysis[8]. Sequences were aligned with CLUSTAL W Omega program and phylogenetic analysis was carried out using Mega 6 and a weighted neighbor-joining tree was constructed.

Pathogenicity test
Two day old bacterial culture was used for testing the pathogenicity on 30 days old healthy *Chrysanthemum* plants[2,4]. *Chrysanthemum* plants (10) were selected and small pricks were made with the help of tooth pick and the bacterial inoculum (1×10^8cfu/ml) was applied as foliar spray until it run off. Plants sprayed with only sterilized water served as control. After foliar application, plants were covered with polythene bags and kept in green house conditions for 48 hrs and observed for the appearance of leaf blight symptoms.

***In vitro* evaluation of bactericides against the pathogen**
Three bactericides (Bactinash [2 bromo-2 nitropropane-1 (95%)], Anucin [Streptomycin sulphate + Tetracycline hydrochloride (90:10)] and Cristocycline [Streptomycin sulphate + Tetracycline hydrochloride (90:10)] were tested *in vitro* against the pathogenic bacterium *Pseudomonas putida* using disc diffusion method[20]. The bactericides with different concentrations (0.05%, 0.1%, 0.15%, 0.2%, 0.25%, 0.5%, 0.75% and 1.0%) were prepared using distilled water and evaluated against the pathogen. Each experiment was conducted in triplicates. The test plates were incubated at 37°C for 24 hrs and the zone of inhibition was measured in millimeter scale and the data was tabulated.

***In vitro* evaluation of bio-agents against the bacterial pathogen**
Hypocrea rufa and *Pseudomonas fluorescens* were tested *in vitro* for antagonistic effect on bacterial pathogen using dual culture method [15,17]. Briefly, 5mm-mycelial disc of *Hypocrea rufa* was placed on one side of potato dextrose agar (PDA) medium and the pathogenic bacterium was streaked on the other side of the medium in a Petri dish. Plates were incubated at 37°C for 6 - 8 days. The experiment was performed in triplicates. The antagonistic effect of *Hypocrea rufa* on the bacterial pathogen was measured by zone of inhibition.
To test the antagonistic activity of *Pseudomonas fluorescens* 24 hr old bacterial culture was inoculated as a line on the surface of a nutrient agar (NA) medium. After 72 hr of incubation at 37°C the pathogenic bacterium *Pseudomonas putida* was inoculated perpendicular to the *Pseudomonas fluorescens*. The inoculated Petri plates were incubated for 24 hr at 37°C and the antagonistic activity was measured as a zone of inhibition in a millimeter scale.

***In vivo* evaluation bactericides and bio-agents against pathogen**
A field experiment was conducted in the Botanic Garden of the Department of Studies in Botany, University of Mysore, to evaluate the effectiveness of selected bactericides and bio-agents to reduce the incidence of bacterial leaf blight disease caused by *Pseudomonas putida*. One month old healthy *Chrysanthemum* plants grown in earthen pots filled with 1:1:2 ratio of soil: sand: and compost was used for the study. The experiment was conducted in 12 different treatments (Table. 4). The concentrations of bactericides and bio-agents which gave the best inhibition against the pathogen *in vitro* were selected for *in vivo* experiments. Bacterial suspension of *Pseudomonas putida* (1×10^8cfu/ml) was sprayed as foliar application. After the initiation of blight symptoms, all the treatments were sprayed as foliar applications. The bio-agents *Pseudomonas fluorescens* and *Hypocrea rufa* were prepared as per manufacturer's instructions and sprayed. All the inoculums were sprayed twice at regular intervals of 15 days as foliar application to check the disease controlling ability. The experiments were conducted during June to July 2015. All the plants were maintained in a green house conditions and observed for the appearance of leaf blight symptoms. The disease incidence, severity and disease index were calculated after 15 days of post inoculation.

Statistical Analysis
One way ANOVA and Duncan Multiple Range Test (DMRT) were performed using the statistical package SPSS 14.00 to validate the signification of the results. The data are presented as means (± SE) of at least three replicates; the p-values < 0.05 were considered as significant.

RESULTS
Bacterial blight disease was noticed for the first time in *Chrysanthemum* growing regions of Karnataka (India) during November 2014 to January 2015. The disease was prevalent in Banangadi, Mudugere, Dinca and Ballenahalli regions of Mandya district. The White variety of *Chrysanthemum* was more susceptible to bacterial blight disease when compared to Chandini and Karnel varieties. The mean bacterial blight incidence of 60.60% was recorded from Mandya district. The maximum disease incidence, severity and disease index were 72.8%, 61.6% and 19.99% in Banangadi region followed by 61.6%, 43.88% and 15.09% in Mudugere, 56.00%, 57.11% and 18.57% in Ballenahalli regions respectively. In Dinca

region the low disease incidence (52%), severity (47.8%) and disease index (14.63%) were recorded (Tab. 1).

The bacterial blight symptoms were characterized by tan to dark brown spots and blotches on leaves extending beyond plant leaves with water-soaked lesions on stems, darkening and death of buds and stems, blackening of terminals, wilt and collapse of upper portions of the plants (Fig. 1). The spots measured 1-2mm in diameter, and as the disease progressed the leaves were completely dried up. Wilting and death of severely affected plants was also observed. Infected plants showed brown to black decay at the base.

The bacterial pathogen was isolated from the infected leaf, stem and flowers of affected plant consistently from the samples collected from different *Chrysanthemum* growing regions of Mandya district. The white dense bacterial colonies were observed around the margins of the inoculated tissues (Fig. 2). The bacterium was aerobic, Gram negative rod, oxidase positive. Hydrolysis of starch and gelatin were negative. Bacteria did not grow at 42°C and 4°C. Colonies produced diffusible fluorescent pigment and did not produce levan from sucrose (Table.2). The bacterium responsible for bacterial blight was identified as *Pseudomonas putida* based on biochemical. Further , the identity of the pathogen was also confirmed with the partial sequencing of 16S rRNA. The n-BLAST results revealed that sequence showed 98% homology with *Pseudomonas putida*. The sequence was deposited in GenBank (Accession Number KY750243). Phylogenetic analysis of the sequence revealed that, the *Pseudomonas putida* associated with leaf blight disease was also closely related to other strains of *Pseudomonas putida* with their accession numbers GQ303714, KX369582.1, KJ735915.1, KT380609, KC422702.1 etc. and showed the genetic distance of 0.0002 (Fig. 3). The bacterium responsible for bacterial blight was identified as *Pseudomonas putida* based on biochemical and molecular methods.

The pathogenicity test conducted on 10 healthy *Chrysanthemum* plants showed the occurrence of bacterial blight disease with circular or irregular, brown, sunken leaf spots on leaves after 15 days of post-inoculation. The spots were well defined, rarely coalesced. Necrosis and wilting of leaves were also observed. Infected leaves either fall off or droop down and found hanged on the plant for a week (Fig. 4). All the ten inoculated plants showed leaf blight symptoms with varying degrees of disease severity. The pathogen was re-isolated from all the post-inoculated plants and identity of the pathogen was confirmed. No such symptoms were observed on control plants.

Among the three different bactericides were tested *in vitro*, the bacterial pathogen *Pseudomonas putida* was more sensitive to Bactinash followed by Anucin and Cristocyclene respectively (Table.3). Bactinash at 0.1% significantly inhibited the bacterial growth (13.33mm) followed by Anucin at 0.2% (11.66 mm) and Cristocyclene at 0.5% (14.66mm) respectively. Further both the bio-agents significantly inhibited the growth of *Pseudomonas putida* at 0.3% concentrations.

Data on *in vivo* management of bacterial blight disease revealed that, among all the treatments, Anucin at 0.2% was most effective in disease protection with lowest disease severity (7.19%) followed by *Trichoderma viride* at 0.3% treatment with 7.50% disease severity. Minimum disease protection was recorded by the plants treated with Bacteria+Cristocylcine (0.5%) with 40.65% disease severity followed by the treatment Bacteria+Bactinash (0.1%) with 28.26% severity respectively (Table.4). Anucin (Streptomycin Sulphate+Tetracycline hydrochloride) at 0.2% was more effective in reducing the leaf blight of *Chrysanthemum* and could be used for large scale application for management of the disease. Bio-control agent *Hypocrea rufa* also found effective in reducing the bacterial blight disease at 0.3%.

DISCUSSION

The occurrence of bacterial blight of *Chrysanthemum* was observed severely in regions of Banangadi, Mudugere, Dinca and Ballenahalli regions of Mandya district, Karnataka (India) during November 2014 to January 2015. The Maximum disease incidence, severity and disease index were recorded (72.8%, 61.6% and 19.99% respectively) from Banangadi region. The bacterial pathogen was isolated and identified as *Pseudomonas putida* on the basis of biochemical tests and molecular sequencing of 16S rRNA sequencing. The bacterial pathogens are known to persist in or on infected plants, crop debris, contaminated soil and their growth is favored by surface moisture, high temperatures, and high humidity. Jones et al. (1983) observed stem necrosis on *Chrysanthemum* caused by *Pseudomonas cichorii* in Florida during winter season. Carta (1993) reported leaf spot disease caused by *Pseudomonas syringae* on *Chrysanthemum morifolium*. *Pseudomonas corrugata* causing stem rot of *Chrysanthemum* was reported by Fiori (1992) and reported that, the pathogen can survive both in plant as well as in irrigation water and soil. It is well known that

Pseudomonas putida is a soil bacterium as an effective antagonist against many pathogens.
Our data on pathogenicity test provided the evidence that *Pseudomonas putida* was responsible for blight on *Chrysanthemum*. Dimaritino et al. (2011) also provided an evidence of leaf necrosis pathogenecity on tomato plants caused by *Pseudomonas putida* and *Pseudomonas fluorescens*. Polizzi et al. (2007) reported *Pseudomonas fluorescens bv. I* and *Pseudomonas putida bv. A* as vascular pathogens on tomato plants. Saygili et al. (2004) reported the occurrence of pith necrosis of tomato caused by *Pseudomonas flurescence* from Turkey. No reports are available on the occurrence of *Pseudomonas putida* on flowering and horticultural plants in India. This is the first report on occurrence of *Pseudomonas putida* causing leaf blight disease on *Chrysanthemum* in Karnataka, India.
In the present study, Bactinash at 0.1% significantly inhibited the bacterial (*Pseudomonas putida*) growth (13.33mm) followed by Anucin at 0.2% (11.66 mm) and Cristocyclene at 0.5% (14.66mm) respectively *in vitro*. Khan and Ilyas (1989) evaluated antibacterial activity of various chemotherapuents, Agromycine-100, Streptomycine sulphate and Carboxin were more effective in disease control while Pencozeb, Nemisopre, Cuperosan, Sandofan-M and Tricyclazole were comparatively less effective against *Xanthomonas axonopodis pv. malvacearum* the causative agent of bacterial blight of cotton.
In the present study, the *in vivo* evaluation of bactericides revealed that Anucin (Streptomycin Sulphate + Tetracycline hydrochloride) at 0.2% was most effective in disease protection with lowest disease severity of 7.19% followed by *Trichoderma viride* at 0.3% with 7.50% severity. Bio-control agent *Hypocrea rufa* also found effective in reducing the bacterial blight disease at 0.3%. Antibiotics such as Streptomycin sulphate and Oxytetracycline are frequently recommended for bacterial blight disease management in *Anthurium* (Nishijima and Fugjiyama, 1985). Jagtap et al. (2012) tested the efficiency of different chemicals and also biocontrol agents against bacterial blight disease incidence (PI) and disease severity (PDI). Copper Oxychloride at 0.25 % + Streptocycline at 100ppm significantly reduced the disease severity and disease incidence (11.83% and 19.36%) respectively when compared to unsprayed control (27.56%).

ACKNOWLEDGEMENT
This study was supported by a grant in aid sanctioned by University Grant Commission (Rajiv Gandhi National Fellowship) [Fellowship No.F-14-2(SC)/2010/SA-III].

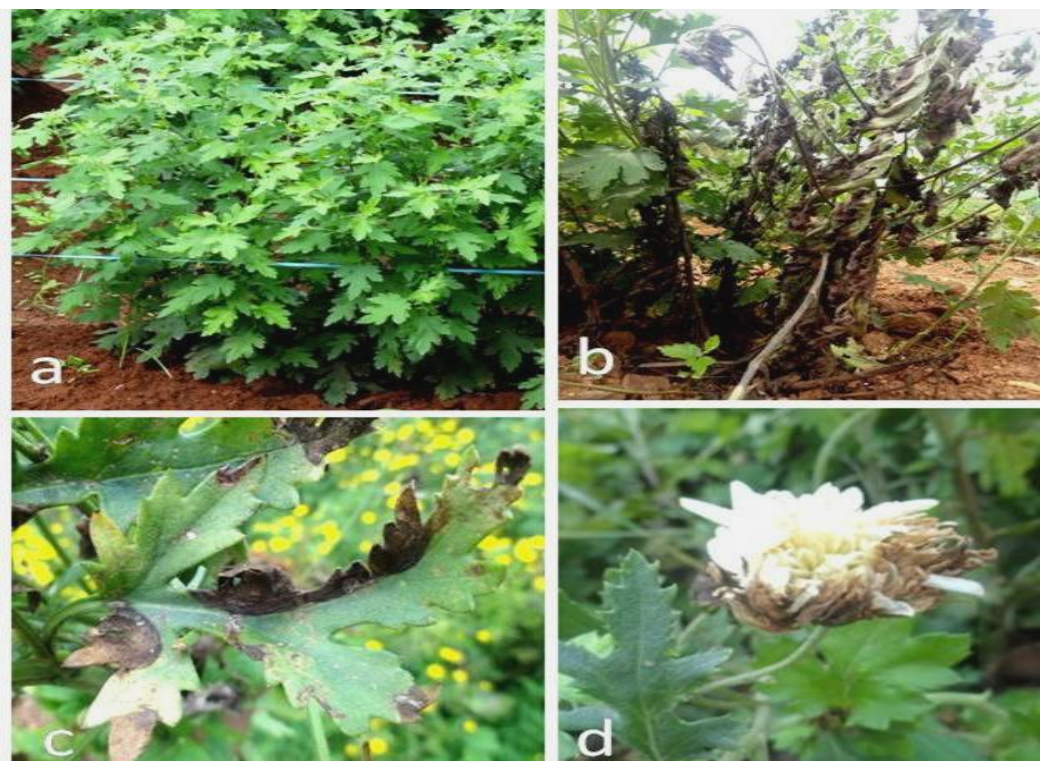

Fig. 1: a- Healthy Chrysanthemum plant; b- Blight affected plant; c- Blight affected leaf; d- Flower bud affected by blight.

Table 1: Disease incidence (DI), disease severity (DS) and per cent disease index (PDI) of bacterial blight of *Chrysanthemum* caused by *Pseudomonas putida* from regions of Karnataka

SI No	District	Locality	Variety	DI	DS	PDI
1	Mandya	Banangadi	white	72.8±3.87[b]	71.39±3.27[c]	19.99±1.30[b]
2		Mudugere	White	61.6±3.24[a]	43.88±3.01[a]	15.09±1.02[a]
3		Ballenahalli	White	56.00±4.38[a]	57.11±3.69[b]	18.57±0.26[b]
4		Dinca	White	52.00±1.26[a]	47.80±3.51[ab]	14.63±0.45[a]
Mean± SEM				**60.60±2.38**	**55.04±2.34**	**17.07±0.77**

Note: Values with superscript letters indicates the Duncans significant values at $p \leq 0.05$

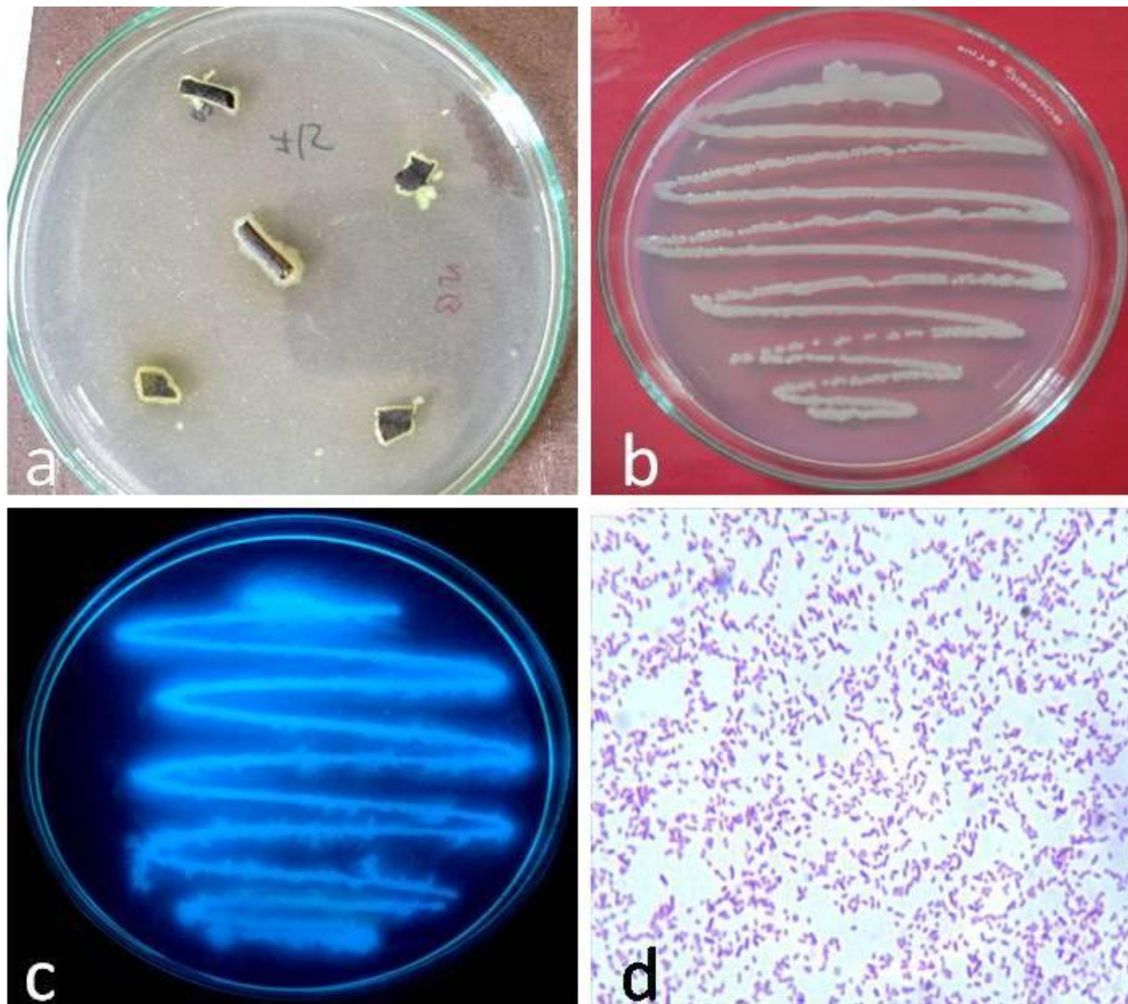

Fig. 2: *Pseudomonas putida* isolated from leaf blight affected *Chrysanthemum* plants; a- Bacterial colonies around the leaf tissue; b- Culture on NA; c- Culture showing florescence under UV light; d- Gram's reaction showing small bacterial rods.

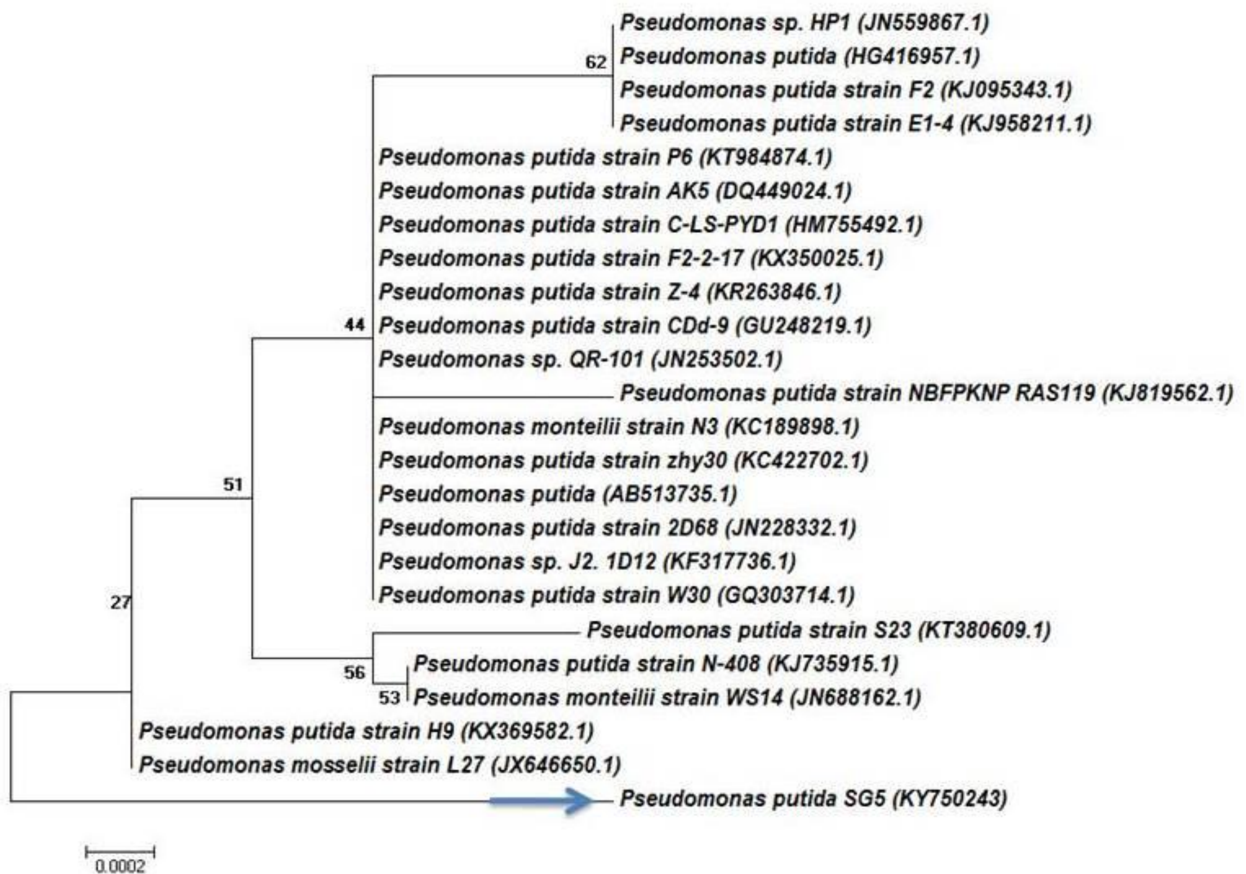

Fig. 3: Phylogenetic tree obtained by the neighbour-joining method based on the alignment of 16S rRNA sequence of *Pseudomonas putida strain SG5* and other related strains. Bootstrap analysis were performed and the scale bar indicates the distance in substitutions per nucleotide.

Fig. 4: Pathogenecity test; *Chrysanthemum* plant inoculated with bacterial pathogen showing bacterial blight after 15 days of post inoculation

Table 2: Morphological, biochemical, physiological tests and characteristics of *Pseudomonas putida*

Tests	Results
Gram reaction	-
Motility	-
MR/VP	+/+ or +/-
KOH test	+
Glucose metabolism / gas production	+/+ or +/-
Lactose	-/-
Indole	-
Fluorescence on King's B medium	+
Citrate	+
Nitrate	+
Urease	-
Gelatin liguifaction	-
Growth at 41^0C	-
Levan production	+
Oxidase activity	+
Catalase activity	+
Pathoigenicity test	+
Growth at 4^0C	-
Starch hydrolysis	-

Note:: + = positive reaction; - = negative reaction

Table 3: Effect of different concentrations of bactericides against *Pseudomonas putida in-vitro*

Sl. No.	Bactericides	Zone of Inhibition (mm)							
		0.05%	0.10%	0.15%	0.20%	0.25%	0.50%	0.75%	1.00%
		Concentrations							
1	Bactinash	11.66 ±0.33[a]	13.33 ±0.88[b]	17.00 ±1.0[c]	17.33 ±0.88[c]	20.00 ±0.57[d]	28.66 ±0.33[e]	28.66 ±0.33[f]	31.33 ±0.88
2	Cristocyclene	00.00 ±0.00[a]	6.00 ±0.00[b]	9.00 ±0.00[c]	10.00 ±0.00[c]	10.66 ±0.33[c]	14.66 ±1.45[d]	17.00 ±0.57[e]	17.66 ±0.66[e]
3	Anucin	6.00 ±0.00[a]	9.66 ±0.33[b]	11.00 ±0.57[c]	11.66 ±0.33[c]	16.00 ±0.57[d]	18.66 ±0.33[e]	21.00 ±0.00[f]	23.00 ±0.00[g]
4	Control	0.00 ±0.00	0.00 ±0.00	0 .00 ±0.00	0 .00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

Note: Values with superscript letters indicates the Duncans significant values at p≤0.05.

Table 4: Effect of bactericides and bio-agents at different concentrations on disease protection against bacterial blight of *Chrysanthemum* Caused by *Pseudomonas putida*

SI No.	Treatments	% Disease severity (DS)*	% Disease index (PDI)*	% Disease Protection
1	Bacteria ($1x10^6$ cells/ml)	41.54±4.16[d]	22.94±4.27[d]	58.46
2	Bacteria + Bactinash (0.1%)	28.26±10.71[bcd]	14.55±5.29[bcd]	71.74
3	Bactinash (0.1%)	18.43±5.87[abcd]	12.91±4.85[bcd]	81.57
4	Bacteria + Cristocylcine	40.65±8.29[cd]	19.00±3.43[cd]	59.35
5	Cristocylcine (0.5%)	16.70±9.08[abc]	8.39±4.96[abc]	83.30
6	Bacteria + Anucin (0.2%)	11.71±4.03[ab]	5.99±1.52[ab]	88.29
7	Anucin (0.2%)	7.19±3.73[ab]	4.88±2.64[ab]	92.81
8	Bacteria + Trichoderma (0.3%)	16.18±11.93[abc]	8.73±4.93[abc]	83.82
9	Trichoderma (0.3%)	7.50±5.55[ab]	3.93±2.53[ab]	92.50
10	Bacteria + Pseudomonas (0.3%)	21.86±7.83[abcd]	8.93±2.28[abc]	78.14
11	Pseudomonas (0.3%)	16.90±8.45[abc]	6.11±3.09[ab]	83.10
12	Control (water treated)	0.00±0.00	0.00±0.00	-
	Mean ± SEM	**18.91±2.70**	**9.69±1.37**	**79.37**

Note: * Values given are mean (n=3) replicates. Values with superscript letters indicates the Duncans significant values at p≤0.05.

REFERENCES

1. Bolick HJ. Bacterial bud blight of Chrysanthemum. Florida State Horticulture Society. 1960;346-351.
2. Boyraz N, Bastas KK, Maden S and Yasar A. Bacterial leaf and peduncle soft rot caused by Pectobacterium carotovorum on Tulips in Konya, Turkey. Phytoparasitica. 34(3): 272-280.
3. Carta C. Chrysanthemum morifolium Ramat: a new host for Pseudomonas syringae. Phytopathologia Mediterranea. 1993;32(2):159-161.
4. Chakravarthi G and Kalitha MC Management of bacterial wilt of brinjal by P. fluorescens based bioformulation. ARPN J Agric Bio Sci. 2011;6(3):1-11.
5. Dhanya MK and Mary CA. Management of bacterial blight of anthurium (Anthurium andreanum Linden.) using ecofriendly materials. J Trop Agric. 2006;44(1-2):74-75.
6. Dimartino M, Panebianco S, Vitale A, Castello I, Leonardi C, Cirvilleri G and Polizzi G. Occurrence and pathogenecity of Pseudomonas fluorescence and P. Putida on tomato plants in Italy. J Plant Pathol. 2011;93(1):79-87.
7. Fiori M. A new bacterial disease of Chrysanthemum: a stem rot by Pseudomonas corrugate Roberts et Scarlett. Phytopathologia Mediterranea. 1992;31:110-114.
8. Goncalves RC, Lau D, Oliveira JR, Maffia LA, Cascardo JCM and Alfenas AC. Etiology of bacterial leaf blight of eucalyptus in Brazil. Trop Plant Pathol. 2008;33(3):180-188.
9. Gupta M, Bharat N, Chauhan A and Vikram A. First report of bacterial leaf spot of Coriander caused by Pseudomonas syringae pv. coriandricola in India. Plant Dis. 2013;97(3):418.1.
10. Holt JG and Bergey DH. Bergey's Manual of Determinative Bacteriology (9th edn). Lippincott Williams and Wilkins, © 2000.
11. Jagtapa GP, Jangam AM and Deya U. Management of bacterial blight of cotton caused by Xanthomonas axonopodis pv. malvacearum. Sci J Microbiol. 2012;1(1):10-18.
12. Jones JB, Engelhard AW and Raju BC. Outbreak of a stem necrosis on Chrysanthemum incited by Pseudomonas cichorii in Florida. Plant Dis. 1983;67:431-433.
13. Kawarabayashi S and Suyama K. Bacterial blight of Gerbera caused by Pseudomonas cichorii newly found in Japan. J Agri Sci. 1990;34(4):194-201.
14. Khan MA, Ilyas MB. Antibacterial activity of various toxicants against Xanthomonas campestris pv. malvacearum for the control of bacterial blight of cotton. Pak J Agric Sci. 1989;26(3):241-246.
15. Kim WG, Weon HY, Seok SJ and Lee KH. In vitro antagonistic characteristics of bacilli isolates against Trichoderma spp. and three species of Mushrooms. Microbiol. 2008;36(4):266-269.
16. McFadden AL. Bacterial blight of Chrysanthemum. Florida State Horticulture Society. 1958;419-425.
17. Moore T, Globa L, Barbaree J, Vodyanoy V and Sorokulova I. Antagonistic Activity of Bacillus Bacteria against Food-Borne Pathogens. J Probiotic Health, 1, 110. doi: 10.4172/2329-8901.1000110. 2013.
18. Nishijima WT and Fujiyama DK. Bacterial blight of Anthurium. Commodity-Fact-Sheet, Co-operative Extension Service, Hawaii. 1985;3.
19. Polizzi G, Dimartino MA, Panebianco S and Cirvilleri G. A new emergence on soilless tomato cultures in Sicily: vascular and pith discolouration caused by Pseudomonas fluorescens and Pseudomonas putida. J Plant Pathol. 2007;89:S54-S55.
20. Sassi AB, Harzallah-skhiri F, Bourgougnon N and Aouni M. Antimicrobial activities of four Tunisian Chrysanthemum species. Indian J Med Res. 2008;127:183-192.
21. Saygili H, Aysan Y, Sahin F, Ustun N and Mirik M. Occurrence of pith necrosis caused by Pseudomonas flurescence on tomato plants in Turkey. Plant Pathol. 2004;53:803.
22. Vegh A, Nemethy ZS, Salamon P, Mandoki Z and Palkovics L. First report of bacterial wilt on Chrysanthemum caused by Dickeya chrysanthemi (syn. Erwinia chrysanthemi) in Hungary. Plant Dis. 2014;98(7): 988.
23. Wehlburg C. A bacterial spot of cabbage caused by Pseudomonas cichorii. Florida Agri Experiment Stations J Series. 1963;1724:119-122.