The Pharma Innovation Journal 2023; 12(12): 2889-2892

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(12): 2889-2892

www.thepharmajournal.com
Received: 06-09-2023
Accepted: 08-10-2023

P Mahalakshmi
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

M Ayyandurai
Department of Plant Pathology, Agricultural College and Research Institute, Madurai, Tamil Nadu, India

P Mareeswari
Department of Plant Pathology, Agricultural College and Research Institute, Madurai, Tamil Nadu, India

Corresponding Author:
P Mahalakshmi
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Suppression of fusarium wilt (*F. oxysporum* f. sp. *dianthi*.) by liquid formulation of *Pseudomonas fluorescens* and enhancement of growth of in carnation (*Dianthus caryophyllus* L.)

P Mahalakshmi, M Ayyandurai and P Mareeswari

Abstract
Studies were conducted to test the efficacy of liquid formulation of *Pseudomonas fluorescens* against fusarial wilt of carnation under *in vitro* and *in vivo* conditions. Thirty four isolates of *P. fluorescens* were screened against the mycelial growth of *F. oxysporum* f. sp. *dianthi*. Among the isolate tested, the isolate of Pf12 significantly inhibited the pathogen mycelial growth (2.70 cm) which accounted 70.0 percent reduction over control with 6.30 mm inhibition zone followed by Pf7 recorded 64.78 percent reduction of mycelial growth with 5.83mm of inhibition zone. The effective strain of Pf12 in different age old culture were tested their effect on the mycelial growth of the pathogen. The result showed that the cultures from 7 to 15 days recorded the least mycelial growth of 2.51 to 2.56 cm followed by the cultures from second and 30 days with the mycelial growth of 2.64 and 2.88 cm which was on par with each other. The poly house experiment revealed that among the treatments, Application of liquid formulation of Pf12 by soil drenching + seedling dip combined with basal application of Th2 recorded lowest mean percent disease index of 5.44 which was on par with chemical check which was recorded 5.34%. and also significantly increased the growth characters of plants which showed higher stalk length (66.35 cm), stalk girth (3.94 cm), number of branches (4.24/plant), number flowers per plant (3.56/plant) and flower diameter (4.57 cm).

Keywords: Carnation, wilt, *F. oxysporum* f. sp. *dianthi*, *Pseudomonas fluorescens*, liquid formulations

Introduction
Carnation (*Dianthus caryophyllus* L.) is one among the most popular commercial cut flowers of the world, ranking second in commercial importance next only to rose. Carnation is preferred to roses and chrysanthemums by several exporting countries, on account of its excellent keeping quality, wide range of forms and colours and ability to withstand long distance transportation. Cut carnations, roses and chrysanthemums contribute close to 50 percent of the world cut flower trade (Jawaharlal *et al.*, 2009) [6]. In India, carnation is grown in Nasik, Pune, Kodaikanal, Nilgiris, Kalimpong, Darjeeling, Bangalore, Solan, Palampur, Shimla, Srinagar, Nainital and Chaubattia. The most suitable climate for commercial carnation flower production in India prevails in the Nilgiris and Kodaikanal of Tamil Nadu and parts of Himachal Pradesh (Bhatt, 1993) [1]. This is being grown in all these cities in highly controlled conditions in polyhouses / greenhouses. In Tamil Nadu, Ooty had occupied first place, within area of 2,86,700 sq.mt and production of 363.12 lakh cut flowers per annum. There are several diseases reported in carnation including rust caused by *Uromyces dianthi*, leaf blight by *Alternaria dianthi*, grey mold by *Botrytis cinerea*, fusarium wilt caused by *Fusarium oxysporum* f. sp. *dianthi*, leaf spot by *Cercospora* and *Cladosporium*, and root and stem rots caused by *Rhizoctonia solani* or *F. roseum*. Among them, fusarium wilt is an important soil borne disease occurring prevalently in carnation fields (Kyounge *et al.*, 2001) [8]. An important area of biological control is the development of formulations that would cause for viable microbial activity for long period of time. Many of the studies are reported for the use of powder or talc based carrier of PGPR, having the shelf life of three months. However only few reports are available on the development of liquid formulation. Liquid formulation has the advantages of high cell count, zero contamination, longer shelf life, greater protection against environmental stresses, increased field efficacy and convenience of handling (Vendan and Thangaraju, 2006) [12].

Materials and Methods

Screening of different age old stock cultures of *P. fluorescens against F. oxysoporum* f. sp. *Dianthi in vitro*

A nine mm mycelial disc of carnation wilt pathogen *F. oxysoporum* f. sp. *dianthi* were placed in the centre of the Petri plate. Sterile whatman No.40 filter paper discs with six mm dia were placed 1 cm away from the edge at four sides centering on the fungal disc. Twenty five micro liters of broth cultures of different age old *P. fluorescens* (Pf12) were dropped over the filter paper discs. Observations were taken after five days for the presence of inhibition zone over the pathogen and nearer to the bacterial spot. Control was maintained with the sterile distilled water instead of bacterial inoculum.

Results and Discussion

In vitro screening of different isolates of *P. fluorescens* against *F. oxysporum* f. sp. *dianthi*

The efficacy of isolates of *P. fluorescens* against *F. oxysporum* f. sp. *dianthi* was evaluated and the results are furnished in Table. 1. The result revealed that all the isolates of *P. fluorescens* showed inhibitory against the growth of the fungus. Among the isolate tested, the isolate of Pf12 significantly inhibited the pathogen mycelial growth (2.70 cm) which accounted 70.0 percent reduction over control with 6.30 mm inhibition zone followed by Pf7 recorded 64.78 percent reduction of mycelial growth with 5.83mm of inhibition zone. The minimum per
cent reduction of 6.44 was recorded in Pf31 with 0.58 mm of inhibition zone. Thangavelu *et al*. (2004) [14] screened Pf10 from 11 isolates of *P. fluorescens* and this was the most effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *cubense.* Saravanan *et al.* (2004) [11] reported that the strains of *P. fluorescens* isolated from the banana rhizosphere had significant inhibitory action against *F. oxysporum* f. sp. *cubense.* Kaur *et al.* (2007) [7] observed that the antagonist activity of *P. fluorescens* against growth of *F. oxysporum f. sp. ciceri* in chickpea under *in vitro* condition. The present study revealed that the liquid culture of Pf12 in different age has the ability to inhibit the mycelial growth of the pathogen. The result indicated that the cultures from 7 to 15 days recorded the least mycelial growth of 2.51 to 2.56 cm but the ability was slightly decreased towards 180 days (5.12 cm) (Table 2). This might be due to slight decrease in the cell population of Pf12 lead to slight decrease in the production of secondary metabolites. The similar results were reported by Manikandan (2008) [9] who explained that the liquid product of Pf1 reduced the mycelial growth of wilt pathogen.*F. oxysporum* f. sp. *lycopercici* in tomato plants. The role of secondary metabolites of fluorescent pseudomonads in the induction of resistance was reported by several workers (Haas *et al.*, 2000) [3]. Secretion of lytic enzymes *viz*., β-1,3 glucanase and chitinase by fluorescent pseudomonads play an important role in the biological control of pathogens (Viswanathan and Samiyappan, 2001; Harish, 2005) [13, 4].

Effect of liquid formulation of bio inoculants on wilt incidence and growth parameters of carnation plants under poly house conditions

An experiment was conducted to assess the efficacy of liquid formulation of antagonists and organic amendments against wilt disease in carnation and the results were depicted in Table 2. The result showed that all the treatments remarkably reduced the intensity of wilt. Among the treatments, basal application of Th2 combined with soil drenching + seeding dip of Pf12 liquid formulation recorded lowest mean percent disease index of 5.44%, this was on par with chemical check of carbendazim (0.1%) which recorded of 5.34 % disease incidence followed by talc based formulation of above treatments with mean disease index of 6.67 percent disease incidence. In addition, the growth parameters of the carnation plants were also improved by the application of liquid formulation of Pf12 by soil drenching + seedling dip combined with basal application of Th2 significantly increased the growth characters of plants which showed higher stalk length (66.35 cm), stalk girth (3.94 cm), number of branches (4.24/plant), number flowers per plant (3.56/plant) and flower diameter (4.57cm) Our results supported by the earlier observations that a combination of biocontrol agents with different mechanisms of disease control will have an additive effect and results in enhanced disease control compared to their individual application (Guetsky *et al*., 2002) [2]. Manikandan (2008) [9] also indicated that the liquid formulations of Pf1 of *P. fluroscens* strains effectively reduced the leaf blight and wilt incidence in tomato and also induce growth parameters.

Table 1: Efficacy of different isolates of *P. fluorescens* against growth of *F. oxysporum* f. sp. *dianthi in vitro*

S. No	Isolates	Mycelial growth (cm)*	Percent reduction over control	Inhibition zone (mm)
1	Pf1	7.57	15.88	1.43
2	Pf2	5.12	43.11	3.88
3	Pf3	4.82	46.44	4.18
4	Pf4	5.55	38.33	3.45
5	Pf5	7.71	14.33	1.29
6	Pf6	8.23	8.56	0.77
7	Pf7	3.17	64.78	5.83
8	Pf8	7.32	18.66	1.68
9	Pf9	5.75	36.12	3.25
10	Pf10	4.17	53.66	4.83
11	Pf11	6.81	24.33	2.19
12	Pf12	2.70	70.00	6.30
13	Pf13	4.67	48.11	4.33
14	Pf14	3.41	62.11	5.59
15	Pf15	5.84	35.11	3.16
16	Pf16	6.58	26.89	2.42
17	Pf17	4.52	49.77	4.48
18	Pf18	6.46	28.22	2.54

19	Pf19	8.01	11.00	0.99
20	Pf20	5.87	34.77	3.13
21	Pf21	8.34	7.33	0.66
22	Pf22	7.32	18.67	1.68
23	Pf23	6.15	31.65	2.85
24	Pf24	4.91	45.44	4.09
25	Pf25	3.62	59.77	5.38
26	Pf26	7.62	15.33	1.38
27	Pf27	7.60	15.55	1.40
28	Pf28	5.39	40.11	3.61
29	Pf29	4.55	49.44	4.45
30	Pf30	8.18	9.11	0.82
31	Pf31	8.42	6.44	0.58
32	Pf32	6.33	29.66	2.67
33	Pf33	4.45	50.55	4.55
34	Pf34	5.02	44.22	3.98
35	Pf35	7.26	19.33	1.74
36	Pf36	7.90	12.22	1.10
37	Pf37	4.74	47.33	4.26
38	Control	9.0	0.0	0.0
	CD (P=0.05)	0.20	2.13	0.11

* Mean of three replications

Table 2: Effect of bio inoculants on disease incidence and growth parameters of carnation plants under field condition

Treatments	Mean Disease incidence (%)	Stalk length (cm)*	Stalk girth(cm)*	*No of branches/ plant	*No of flowers/plant	Flower diameter(cm)*
T_1 -BA of (Th2)	9.76 (18.20)	88.45	4.00	4.01	3.31	4.48
T_2 -SD of Pf12 (liquid)	8.28 (16.72)	92.14	4.11	4.45	3.74	4.56
T_3 - SD of Pf12 (talc)	9.50 (17.95)	88.68	4.08	4.31	3.40	4.51
T_4 - Seedling dip of Pf12 (liquid)	12.47 (20.67)	85.25	3.88	3.85	3.11	4.32
T_5 - Seedling dip of Pf12(talc)	14.45 (22.34)	84.12	3.80	3.57	2.87	4.27
T_6 - BA of neem cake	16.04 (23.61)	80.16	3.63	3.42	2.64	3.88
T_7 - BA of vermicompost	18.96 (25.81)	81.14	3.51	3.31	2.52	3.73
T_8 - BA of coirpith	19.46 (26.17)	78.75	3.38	3.01	2.38	3.57
T_9 - T_1 +T_2+ T_4	5.44 (13.48)	98.67	4.89	6.54	5.83	5.13
T_{10} - T_1 +T_3 +T_5	6.67 (14.96)	95.28	4.51	5.61	5.32	5.08
T_{11} - SD of carbendazim (0.1%)	5.34 (13.36)	95.16	4.20	5.57	5.12	5.01
T_{12} - Control	44.46 (41.82)	47.81	2.32	2.34	2.01	1.60
CD=(0.05)	0.22	2.83	0.13	0.12	0.10	0.19

*Mean of three replications BA - Basal Application; SD - Soil Drenching

References

1. Bhatt NR. Carnation in commercial flowers, Eds. Bose, T.K. and Yadaw, L.P., Naya Prakash, Calcutta, India, 1993, p. 355-377.
2. Guetsky R, Stienberg D, Elad Y, Fischer E, Dinoor A, Manjula K. Improving biological control by combining biocontrol agents each with several mechanisms of disease suppression. Phytopathol. 2002;92:976-985.
3. Haas DC, Blumer C, Keel C. Biocontrol ability of fluorescent pseudomonads genetically directed dissected: importance of positive feedback regulation. Curr. Optin. Biotechnol. 2000;11:290-297.
4. Harish S. Molecular biology and diagnosis of Banana bunchy top virus and its management through induced systemic resistance. Ph.D. Thesis, TamilNadu Agricultural Univercity, Coimbatore. 2005, p. 126.
5. Hegde SV. Liquid biofertilizers in Indian Agriculture. Biofert. News Lett. 2002;12:17-22.
6. Jawaharlal M, Ganga M, Padmadevi R, Jegadeeswar V, Rarthikeyan S. A technical guide on carnation. Tamil Nadu Agricultural University, Coimbatore, 2009.
7. Kaur R, Singh RS, Alabouvette C. Antagonistic Activity of Selected Isolates of Fluorescent Pseudomonas against *Fusarium oxysporum* f.sp. *ciceri*, Asian J Plant Sci.

2007;6(3):446-454.
8. Kyounge Suk Han, Jong Han Park, Yong Mun Choi. Stub Dieback of Carnation Caused by *Fusarium graminearm.* J Plant Pathol. 2001;17(2):101-105.
9. Manikandan R. Development and evaluation of aqueous formulation of *Pseudomonas fluorescens* (Pf1) for the management of early blight and *Fusarium* wilt of tomato. M.Sc. (Agri.) Thesis. Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, 2008.
10. Saravanakumar D. Rhizobacteria induced systemic resistance against Biotroph (*Exobasidium vexans*) and Necrotroph (*Macrophomina phaseolina*) pathogens in tea and green gram. M.Sc. (Agri.) Thesis, Tamil Nadu Agricultural University, Coimbatore. 2002, p. 120.
11. Saravanan T, Bhaskaran R, Muthusamy M. *Pseudomonas fluorescens* induced enzymological changes in banana roots (cv. Rasthali) against *Fusarium* wilt disease. Plant Pathol. 2004;3:72-80.
12. Vendan RT, Thangaraju. Development and standardization of liquid formulation for *Azospirillum* bioinoculant. Indian J microbiol. 2006;46(4):379-387.
13. Viswanathan R, Samiyappan R. Role of chitinases in *Pseudomonas* spp. induced systemic resistance against *Colletotrichum falcatum* Went in sugarcane. Indian Phytopathol. 2001;54:418-423.
14. Thangavelu R, Palaniswami A, Velazhahan R. Mass production of *Trichoderma harzianum* for managing fusarium wilt of banana. Agric Ecosyst Environ. 2004;103:259-263.