International Journal of Botany

ISSN: 1811-9700

science alert

ANSInet
an open access publisher
http://ansinet.com

International Journal of Botany 1 (2): 163-167, 2005
ISSN 1811-9700

Effect of Sanosil and *Pseudomonas fluorescens* on *Bean common mosaic potyvirus* Incidence in French Bean

H. Bhuvanendra Kumar, A.C. Uday Shankar, H.S. Prakash and H. Shekar Shetty
Department of Studies in Applied Botany and Seed Pathology,
University of Mysore, Manasagangotri, Mysore 570 006, India

Abstract: The effect of seed treatment with Sanosil, a commercial formulation containing hydrogen peroxide and silver and *Pseudomonas fluorescens* Trevisan (migula) on the incidence of *Bean common mosaic potyvirus* (BCMV) in French bean (*Phaseolus vulgaris* L.) was studied in screenhouse and field. The Sanosil treatment reduced the severity of disease symptoms and improved the plant stand. The treatment reduced the disease incidence from 91% in untreated plants to 1% under screenhouse condition and from 52 to 17% in field condition. *Pseudomonas fluorescens* reduced the symptoms from 53 to 10% in screenhouse. However, some of the apparently healthy seedlings in both the treatments were ELISA positive, indicating that the treatments masked the BCMV symptoms. Seedling treatment with Sanosil or *Pseudomonas fluorescens* also reduced the severity of symptoms.

Key words: Biocontrol, *Pseudomonas fluorescens, Bean common mosaic virus*, management, BCMV, sanosil, hydrogen peroxide, silver

INTRODUCTION

Bean common mosaic potyvirus (BCMV) is an economically important pathogen of French bean (*Phaseolus vulgaris* L.). It is found in all parts of the world[1] including India[2,3]. Yield loss of up to 84% has been recorded[4].

Strategies used to manage viral diseases include elimination of the source of infection including eradication of weeds, volunteers and alternate hosts, avoidance of the source of infection, cultivation in isolated areas and use of virus-free seeds.

Pathogen-mediated protection was derived from the concept of cross-protection by a mild strain of the virus. Systemic resistance to a broad spectrum of pathogens could be induced in plants by fungi, bacteria and viruses, botanicals and certain chemicals. Resistance in cucumber against *Cucumber mosaic virus* (CMV) could be induced by inoculating plants with *Colletotrichum orbiculare, Pseudomonas syringae* pv. *lachrymans* or *Tobacco necrosis virus* (TNV)[5].

Virus infection cannot be eradicated by any viricides. However, Casein in milk was found to be a strong inhibitor of virus. Spraying skimmed milk onto plants has been shown to prevent the spread of virus[6]. Copper sulphate and copper acetate (1000 ppm) could suppress virus symptoms[7].

The plants defense mechanism could be induced by using biotic and abiotic elicitors enabling the plant to combat further invasion of the pathogens. This response is termed Induced Systemic Resistance (ISR)[8] and is effective against a broad spectrum of pathogens. Plant Growth-Promoting Rhizobacteria (PGPR) act as resistance-inducers in many crop species. Most reported strains of PGPR are from *Pseudomonas* spp., particularly *P. fluorescens* strains. These can enhance plant growth and protect the plants from various plant pathogens in several crops such as cucumber, radish, tomato, sugarcane, rice and pearl millet[9-16].

The purpose of this study was to investigate the effect of Sanosil and of *Pseudomonas fluorescens* Trevisan (migula), on the incidence of BCMV infection. The product profile of the commercial formulation Sanosil, a multi-component complex formulation supplied by Sanosil Biotech Pvt. Ltd, Bombay, India and purported to contain hydrogen peroxide and silver, claims that it controls viral, bacterial and fungal diseases of plants.

MATERIALS AND METHODS

Collection of seed sample: Seeds of French bean (Cv. Selection-9) were obtained from the seed traders.

Corresponding Author: H.S. Prakash, Professor, Department of Applied Botany and Biotechnology, Manasagangotri, University of Mysore, Mysore 570 006, India
Tel : +91-081-2414450 Fax: +91-081-2414450

Maintenance of virus: The BCMV was maintained in the screenhouse. Leaves of French bean showing BCMV symptoms were collected from the field and extracted in 0.5 M cold potassium phosphate buffer (pH 7.0). The extract was inoculated mechanically onto the fully expanded primary and first trifoliate leaves of French bean seedlings using carborundum as the abrasive.

Sanosil: Sanosil was obtained from Sanosil Biotech Pvt. Ltd, Mumbai, India.

Preparation of *P. fluorescens* formulation: An antagonistic strain of *P. fluorescens* was isolated from the native soil[17]. The product was inoculated into King's medium[18] and incubated at 28°C for 48 h. The culture was centrifuged (10,000 rpm; 10 min), pellet was isolated, resuspended in distilled water and centrifuged, this process was repeated thrice, after which the washed pellets were suspended in sterile distilled water to give a turbid suspension from which inoculum was prepared. The Optical Density (OD) of the solution was adjusted to 0.45 (A_{610} nm) in a UV-spectrophotometer (Hitachi U-2000, Japan) to obtain a bacterial concentration of $1X10^8$ cfu mL^{-1}. OD was adjusted to 0.225 (A_{610} nm) to get $1X10^4$ colony forming units mL^{-1}. The formulation was prepared by mixing 400 mL of bacterial suspension containing $9X10^8$ cfu mL^{-1} to 1 kg of purified talcum powder under sterile conditions and stored in polyethylene bags at 5°C.

Seed treatment: French bean seeds were immersed in Sanosil solution (0.5, 1.0 or 1.5 % by volume) for 1h or in *P. fluorescens* suspension ($1X10^8$ cfu mL^{-1}) for 12 h. Untreated seeds served as control. Four replicates of 25 seeds were sown in a mixture of soil, sand and farmyard manure (1:2:1) contained in clay pots in a screenhouse. Ten-day-old seedlings were inoculated with BCMV at primary leaf stage and the plants were monitored periodically for expression of virus symptoms. Sanosil treated seeds were also tested in field.

Seedling treatment: French bean seedlings, four replicates of 10 each, were raised in the screenhouse and in the field. BCMV was inoculated onto the primary leaves of 15-day-old seedlings. Sanosil or *P. fluorescens* was sprayed onto the leaves using an atomizer, 24 h before, soon after or 24 h after application of the virus.

Indicator plant test: Symptom-bearing and apparently healthy french bean leaves were collected separately from each treatment to check for the presence of BCMV. The leaves were ground with potassium phosphate buffer (0.1 M, pH 7.0) using a pestle and mortar to produce an extract, which was inoculated onto the newly emerged primary leaves of *P. vulgaris* Cv. 'Top crop', previously dusted with carborundum. Distilled water treatment served as the control. The plants were maintained at 25±2°C with 12/12 h light and dark cycles under controlled environment room conditions. The inoculated plants were monitored for the expression of necrotic local lesion.

Enzyme Linked Immunosorbent Assay (ELISA): French bean seedlings with different levels of Sanosil and *P. fluorescens* treatments in the screenhouse and field were tested for BCMV infection by DAC-ELISA[19]. For each treatment, leaves from five seedlings were pooled into a sub-sample and 0.1 g (0.8 cm diameter) of the leaf tissue was taken by punching with a cork borer.

Leaf material (0.1 g) was extracted with antigen buffer (1 mL) consisting of a mixture of phosphate buffer saline (PBS 0.5 M, pH 7.2) to which sodium diethyl dithiocarbamate trihydrate was added. Negative (buffer) and positive controls were also prepared. Antigens (100 μL) were loaded onto the microtitre plate and this was incubated overnight at room temperature. The plate was then unloaded, washed three times with phosphate buffer saline+tween surfactant and blocking agent (200 μL) was then loaded and for 30 min. It was then unloaded and washed, after which cross-absorbed primary antiserum (100 μL) was loaded into the wells and the plate was incubated at 37°C for 1 h. The plate was then washed; secondary antibody (swine anti rabbit conjugated with alkaline phosphatase) was loaded into the wells and incubated at 37°C for 1 h. The washing procedure was repeated and *p*-nitrophenyl phosphate substrate was loaded. The plate was examined after 30 min using a microtitre plate reader (Dynatech MR5000).

RESULTS

Effect of seed treatment with Sanosil and *Pseudomonas fluorescens*: Seed treatment with Sanosil at 1.0 or 1.5% decreased the BCMV incidence to 14 and 25%, when compared to 91% in the untreated plants in screenhouse experiments and to 17 and 14% incidence compared to 52% in the untreated plants (Table 1). Extracts from such leaves produced a typical necrotic reaction on indicator plants in 14 days although those from apparently healthy leaves also showed mild necrotic reactions on indicator plants confirming the presence of BCMV. ELISA results showed higher absorbance in the infected sample extracts whereas the treated samples showed lower

Table 1: Effect of sanosil seed treatment on infection and concentration of BCMV in French bean

	Percent infection		ELISA reading* (A_{410} nm) Mean±SD	
(%) Concentration of sanosil	Screen house	Field	Screen house	Field
0	91	52	0.27±0.03	0.25±0.05
0.5	73	29	0.23±0.01	0.19±0.05
1.0	14	17	0.22±0.01	0.14±0.03
1.5	25	14	0.25±0.01	0.12±0.08
LSD at 5% level	20	9		

*Negative Control (NC)0.06 0.06, Positive Control (PC) 0.39 0.18, Threshold value (2x NC) 0.12 0.12

Table 2: Effect of *P. fluorescens* seed treatment on infection and concentration of BCMV

Treatments	Percent infection*	ELISA reading**(A_{410} nm) Mean±SD
Control	53	0.42±0.01
0.1% *P. fluorescens* treatment	10	0.33±0.03

*Significant at 5% level when tested by t-test, ** Negative Control (NC) = 0.06, Positive Control (PC) = 0.35, Threshold value (2xNC) = 0.12

Table 3: Effect of seedling treatment with sanosil on incidence and concentration of BCMV in French bean

		Percent infection		ELISA reading* (A_{410} nm) Mean±SD	
Treatments	Concentration (%)	Screenhouse	Field	Screenhouse	Field
Control		91	52	0.15±0.02	0.23±0.04
Sanosil treatment 24 h before	0.5	30	33	0.015±0.02	0.20±0.05
BCMV inoculation	1.0	00.6	26	0.14±0.03	0.19±0.02
	1.5	11	17	0.21±0.02	0.21±0.04
Sanosil treatment immediately	0.5	56	39	0.12±0.30	0.23±0.09
followed by BCMV inoculation	1.0	55	41	0.15±0.07	0.17±0.03
	1.5	00.6	53	0.13±0.01	0.19±0.04
Sanosil treatment after 24 h	0.5	13	45	0.13±0.05	0.09±0.01
BCMV inoculation	1.0	27	31	0.12±0.01	0.01±0.01
	1.5	11	20	0.13±0.05	0.08±0.05
CD 0.05		5*	Not significant		

*Negative Control (NC) 0.06 0.05, Positive Control (PC) 0.49 0.21, Threshold value (2x NC) 0.12 0.10

Table 4: Effect of seedling treatment with *P. fluorescens* on BCMV incidence and concentration of virus in screen house

Treatments	Percent incidence	ELISA reading* (A_{410} nm) Mean±SD
Control	53	0.42±0.01
P. fluorescens treatment immediately followed by BCMV inoculation	13	0.34±0.01
Before 24 h BCMV inoculation	19	0.33±0.03
After 24 h BCMV inoculation	8	0.30±0.04

*Negative Control (NC) = 0.06, Positive Control (PC) = 0.35, Threshold value (2x NC) = 0.13

absorbance values than control (Table 1). Treatment with *P. fluorescens* significantly reduced the BCMV incidence to 10% when compared to 53% in control (Table 2). In the indicator plant test, necrotic reactions were observed. Apparently healthy leaves produced mild necrotic reactions and were ELISA-positive (Table 2).

Seedling treatment with Sanosil and *P. fluorescens*: Seedling treatment with sanosil at 1 or 1.5%, 24 h before inoculation with the virus decreased the incidence of BCMV to 6 or 11%, respectively compared to the control (91%). With Sanosil treatments immediately following BCMV inoculation, those at 0.5 or 1.0% concentration were less effective than 1.5%, in reducing the BCMV incidence and when applied 24 h after virus inoculation Sanosil reduced the disease incidence to 11 or 27% depending on the concentration. In field conditions the treatment did not decrease disease incidence (Table 3).

Indicator plant test and ELISA: Indicator plant tests showed necrotic reactions five days after inoculation, confirming the presence of BCMV in treated plants. ELISA results were also positive (Table 3).

P. fluorescens also decreased the incidence of BCMV to 8-19% compared to control (53%) (Table 4). All the treatment schedules with this organism were effective in reducing the disease incidence. Indicator plant tests and ELISA proved the presence of virus in apparently healthy leaves (Table 4).

DISCUSSION

The seed treatment was found to be effective in reducing the bean mosaic virus incidence in the screenhouse as well as in field trials. The observation indicated the possibility of induction of some factors that persist and protect the seedlings from BCMV infection.

However, virus replication in the inoculated plant was not affected as evident from the indicator plant test and ELISA. So sanosil seed treatment may just mask the expression of symptoms caused by BCMV. Even seedling treatment with sanosil produced the same effect. Several chemicals are known to mask the symptoms of viral infections. Thus, the application of dodecylbenzene sulfonate to half leaves of tobaccoc Cv. xanthi 'nc' plants before *Tobacco mosaic tobamovirus* (TMV) inoculation was shown[20] to result in decrease in lesion number and size as well as virus content. The plant stand in treated blocks, however, was good and apparently the yield was not affected.

Treatment of seeds and seedlings with *P. fluorescens* was also effective in reducing the incidence of BCMV on French bean. Strains of plant growth rhizobacteria *P. fluorescens,* applied to seeds reduced the numbers of symptomatic plants when CMV was inoculated onto cotyledons[11]. While Kloeppen and Schroth[21] reported the efficiency of *P. fluorescens* as a biocontrol agent and as a plant growth-promoting microbe. They found that *P. fluorescens* was not effective in reducing the concentration of virus, but that the effect of the virus on the host could be minimized and higher yield could be obtained when compared to untreated plants. Induction of resistance by *P. fluorescens* strain CHA0 in tobacco against TNV[22].

The results are promising for the use of Sanosil or *P. fluorescens* in reducing the severity of BCMV infection in French bean and getting better yield, in the absence of any effective control strategies against virus diseases.

REFERENCES

1. Bos, L., 1971. *Bean common mosaic virus* (CMI/AAB) Description of Plant Virus, pp: 4.
2. Muniyappa, V., 1976. Studies on a mosaic disease of French bean (*Phaseolus vulgaris* Linn.). Madras Agric. J., 63: 69-70.
3. Capoor, S.P., D.G. Rao and D.M. Sawant, 1985. A note on a viral disease of French bean. Ind. Phytopathol., 38: 152-154.
4. Sastry, K.I.S., A. Satyanarayana, S.J. Singh and R. Rajendran, 1981. Screening of different bean cultivars against *Bean common mosaic virus.* Pulse Crop Newslett., pp: 130.
5. Bergstrom, G.C., M.C. Johnson and J. Kuc, 1982. Effect of local infection of cucumber by *Colletotrichum lagenarium*, *Pseudomonas lachrymans* and *Tobacco necrosis virus* on systemic resistance to *Cucumber mosaic virus.* Phytopathology, 72: 922-926.
6. Hsich, S.T., Y.L. Ksu and T.Y. Lai, 1967. Study on milk and its effective constituents as TMV inhibitors. Annu. Rep.Tobacco Res. Inst.Taiwan, pp: 171-176.
7. Manicham, K. and K. Rajappan, 1999. Field efficiency of plant extracts and chemicals against Greengram leaf curl disease. Ind. J. Virol., 15: 35-37.
8. Van Loon, L.C., P.A.H.M. Bakker and C.M.J. Pieterse, 1998. Systemic resistance induced by rhizosphere bacteria. Ann. Rev. Phytopathol., 36: 453-483.
9. Liu, L., J.W. Kloepper and S. Tuzun, 1995. Induction of systemic resistance in cucumber by plant growth promoting rhizobacteria: Duration of protection and effect of host resistance on protection and root colonization. Phytopathology, 85: 1064-1068.
10. Leeman, M., J.A. Van pelt, F.M. Denouden, M. Heinsbroek, P.A.H.M. Bakker and B. Schippers, 1995. Induction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *P. fluorescens.* Phytopathology, 85: 1021-1027.
11. Raupach, G.S., L. Liu, J.F. Murphy, S. Tuzun and J.W. Kloepper, 1996. Induced systemic resistance in Cucumber and Tomato against *Cucumber mosaic virus* using Plant Growth-Promoting Rhizobacteria (PGPR). Plant Dis., 80: 891-894.
12. Viswanathan, R. and R. Samiyappan, 1999. Induction of systemic resistance by Plant Growth Promoting Rhizobacteria against red rot disease in sugarcane. Sugar Technol., 1: 67-76.
13. Burdman, S., E. Turkevitvh and Y. Okan, 2000. Recent Advances in the use of Plant Growth Promoting Rhizobacteria (PGPR) in Agriculture, Subbarao, N.S. and Dommergues, (Eds.). Microbial Interaction in Agriculture and Forestry (Science Publishers Inc.) USA, 2: 229-249.
14. Ongena, M., F. Daayf, P. Jacques, P. Thonart, N. Benhamou, T.C. Paulitz and R.R. Belanger, 2000. Systemic induction of phytoalexins in cucumber in response to treatments with fluorescent *Pseudomonads.* Plant Pathol., 49: 523-530.
15. Ramamoorthy, V., R. Viswanathan, T. Raghuchander, V. Prakassam and R. Samiyappan, 2001. Induction of systemic resistance by Plant Growth Promoting Rhizobacteria in crops plants against pests and diseases. Crop Prot., 20: 1-11.
16. Niranjan Raj, S., N.P. Shetty and H.S. Shetty, 2003. Seed-bio-priming with *P. fluorescens* isolates, enhances growth of pearl millet plants and induce resistance against downy mildew. Intl. J. Pest Manage., pp: 1-9.
17. Vidhyashekaran, P. and M. Muthamilan, 1995. Development of *P. fluorescens* for control of chickpea wilt. Plant Dis., 79: 782-786.

18. King, E.O., M.K. Ward and D.E. Raney, 1954. Two simple media for the demonstration of phyocyanin and fluorescein. J. Lab. Clin. Med., 4: 301-307.
19. Hobbs, H.A., D.V.R. Reddy, R. Rajeshwari and A.S. Reddy, 1987. Use of direct antigen coating and protein A coating ELISA procedure for detection of three peanut viruses. Plant Dis., 71: 747-749.
20. Hellwald, K.H. and H. Buchenauer, 1989. Induction of resistance in tobacco leaves to *Tobacco mosaic virus* by dodecylbenzenesulfonate. J. Phytopathol., 125: 279-282.
21. Kloepper, J.W. and M.N. Schroth, 1978. Plant Growth Promoting Rhizobacteria in radish. In: Proc. 4th Intl. Conf. Plant Pathogenic Bact. Gibert-Clarey, France, pp: 879-882.
22. Maurhofer, M., C. Hase, P. Meuwly, J.P. Metaraux and G. Defago, 1994. Induction of systemic resistance of *Tobacco necrosis virus* by the root-colonizing *Pseudomonas fluorescens* CHAO: Influence of the *gacA* gene of pyoverdine production. Phytopathology, 84: 139-146.