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RESEARCH ARTICLE

Compatibility of various fluorescent pseudomonads isolates with different plant extracts

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SUMMARY

In integrated disease management, use of botanical extract or products is one of the effective components to reduce the management cost and eco-friendly. Compatibility of different plant extracts was tested against fluorescent pseudomonads isolates *in vitro* by poison food technique. The leaves of neem while bulb of garlic and onion were used for extracts. Fifteen fluorescent pseudomonads isolates were obtained on King's B medium from the rhizosphere and rhizoplane of plant roots. No inhibiting effect was observed in onion bulb extract, but garlic bulb and neem leaves extracts inhibited the growth of few isolates. Isolate FP-V did not grow under 10 and 15 per cent neem leaves extract. Whereas, FP-X was found to be sensitive *i.e.*, no growth at all the three concentrations (5, 10 and 15 %) of garlic bulb extract, FP-VIII isolate growth was only inhibited at 15 per cent garlic bulb extract.

Key Words : Isolation**,** Fluorescent pseudomonads isolates, Compatibility, Plant extracts

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This has lead to the search for alternate strategies for the management of plant pathogens. The microorganism isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species. Such plant associated microorganisms may make better bio-control agents because they are already closely associated with and adapted to the plant or plant part as well as the particular environmental conditions in which they must function. The screening of such locally adapted strains has yielded improved bio-control in some cases (Cook, 1993).

Biological control of the pathogen is one of the pivotal components of integrated disease management for sustainable agriculture, as it is long lasting and eco-friendly (Mukhopadhyay, 1987). In integrated disease management, use of botanical extract or products is one of the effective components to reduce the management cost and eco-friendly. Murali *et al.* (1999) reported that isolates of *P. fluorescens* sustain the growth and multiply appreciably in the presence of phytochemical Pesticide from the qualitative result of the effect of neem granules on the three fluorescent pseudomonas isolates by paper disc method in King's B broth culture. Rajappan *et al.* (2000) noticed no adverse effect of neem oil on *P. fluorescens*. Singh *et al.* (2000) found the beneficial effect of seed bacterization of *P. fluorescens* with aerial spray of neemazal a product of neem (*Azadirachta indica*) on control of powdery mildew of pea. Saravanan *et al.* (2003) reported the compatible reaction of strain of *P. fluorescens* with extracts of neem cake.

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MATERIAL AND METHODS

Collection of soil and plant samples :

Fifty soil and roots samples were collected from field crop of different locations of Patan and Banaskantha districts where the castor is commonly grown. Healthy plants of 60-75 days growth were carefully uprooted along with adhering soil and were carried to the laboratory in polythene bags. The soil particles loosely adhering to the roots were gently teased out and used for isolation of rhizosphere bacteria. Soil particles adhering tightly to the roots were allowed to go with the roots for isolation of rhizoplane bacteria.

Table A: Plant extracts tested for compatibility with fluorescent pseudomonads isolates *in vitro*

Sr. No.	Plant	Botanical name	Family	Plant part
1.	Neem	*Azadirachta indica* L.	Meliaceae	Leaf
2.	Garlic	*Allium sativum* L.	Lilliaceae	Bulb
3.	Onion	*Allium cepa* L.	Lilliaceae	Bulb

Isolation of fluorescent pseudomonads isolates :

Excess of soil adhering with roots was removed by gentle shaking. From each sample 10 g of closely associated rhizosphere was added to 250 ml flask containing 90 ml sterilized distilled water. For isolation of rhizoplane bacteria, roots were cut into approximately 2-3 cm long pieces and 10 g of root bits were then transferred to 90 ml sterilized distilled water. The flasks were placed on a rotary shaker for 1 hr to allow root associated bacteria to diffuse. Three replications were kept for each location and serial dilution of rhizosphere and rhizoplane samples were made up to 10^6. An aliquot of 0.1 ml from 10^6 dilution of each sample was spread plated over solidified King's medium B (Protease peptone No. 3 20.00 g, Dipotassium hydrogen phosphate 1.50 g, Magnesium sulphate $7H_2O$ 1.50 g, Agar 20.00 g, Glycerol 15.00 ml and Distilled water 1 lit.), selective medium on which preferentially fluorescent pseudomonads recovered under aseptic conditions. The plates were incubated at $30^o \pm 1^oC$ for 24-48 hrs. Colonies of different morphology were examined for their fluorescence under ultraviolet light (240-340 nm). The colony showing fluorescence was picked-up and was further purified by streaking on same medium plates. The purified cultures were finally transferred onto solid King's B medium and preserved at low temperature (4^oC) in refrigerator in the Department of Plant Pathology, C. P. College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, for further activities.

Identification :

All the rhizobacterial isolates were identified with the help of morphological, cultural and biochemical characteristics as per the "Bergey's Manual of Determinative Bacteriology." The cultures were tested for characters *viz.*, colony and cell morphology, gram reaction and urease activity.

Compatibility of fluorescent pseudomonads isolates with plant extracts :

Compatibility of different plant extracts (Table A) were tested against fluorescent pseudomonads isolates *in vitro* by poison food technique. The leaves of neem while bulb of garlic and onion were used for extracts.

Different parts of plant including leaves and bulb were first washed with sterilized distilled water. Weighed plant material was crushed in electrically operated mixer and grinder using 1 : 1 w/v amount of distilled water for 100 g of leaves and bulbs separately. The material was homogenized for five minutes and filtered through double layer sterilized muslin cloth. Then the filtrate was centrifuged at 5000 rpm for 15 minutes. The clear supernant was collected and was considered as cent per cent concentration (Standard solution).

For evaluation of compatibility of fluorescent pseudomonads isolates with plant extract, desired concentration (5.00, 10.00 and 15.00 %) were obtained by adding appropriate amount of standard solution of plant extract to 100 ml King's B medium (KMB) in conical flasks. Then about 20 ml extract mixed King's B medium was poured in sterilized Petri plates. A loopful of 24 hours old culture of fluorescent pseudomonads isolates inoculated centrally after solidification of these KMB plates. Three replications were kept for each treatment and plates were incubated at $30^o \pm 1^oC$ for 48 hours and finally growth of bacterial isolates recorded.

RESULTS AND DISCUSSION

The results of the present study as well as relevant discussions have been presented under following sub heads:

Isolation of fluorescent pseudomonads isolates from rhizosphere and rhizoplane :

Fifteen fluorescent bacterial isolates were isolated on selective medium *viz.*, King's B medium from the rhizosphere and rhizoplane of castor by dilution plating method (106 cfu ml^{-1}) after incubation period of 24-48 hours at $30^o \pm 1^oC$ and examined the fluorescence under ultraviolet light (200-340 nm). These isolates were designated as FP-I, FP-II, FP-III, FP-IV, FP-V, FP-VI, FP-VII, FP-VIII, FP-IX, FP-X, FP-XI, FP-XII, FP-XIII, FP-XIV and FP-XV. Out of 20 samples collected from ten villages of Patan district, nine fluorescent pseudomonads isolates (FP-I to FP-IX) were obtained, whereas six isolates (FP-X to FP-XV) were gained from 30 samples from seven villages of Banaskantha district. These results are in accordance with the methodology adopted by Vidhyasekaran and Muthamilan (1995), Gupta *et al.* (2000), Yeole and Dube (2001), Gholve and Kurundkar (2004), Samanta and Dutta (2004) and Sen *et al.* (2006).

Compatibility of fluorescent pseudomonads isolates with plant extracts :

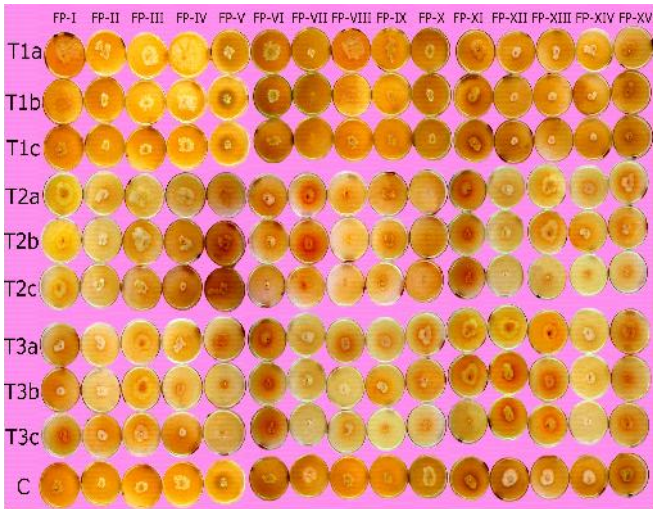
In integrated disease management, use of botanical extract or products is one of the effective components to reduce the management cost and eco-friendly. Therefore, obtained fluorescent pseudomonads isolates were evaluated for their compatibility with extracts of neem leaves, garlic and

Table 1: Compatibility of fluorescent pseudomonads isolates with various plant extracts

Sr. No.	Character	Isolates														
		FP-I	FP-II	FP-II	FP-IV	FP-V	FP-VI	FP-VII	FP-VIII	FP-IX	FP-X	FP-XI	FP-XII	FP-XIII	FP-XIV	FP-XV
1.	**Neem leaves**															
	5 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10 %	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
	15 %	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
2.	**Garlic bulb**															
	5 %	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	10 %	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
	15 %	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+
3.	**Onion bulb**															
	5 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	10 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15 %	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4.	Control	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Growth ; – = No growth

onion bulb at three concentration *viz.*, 5, 10 and 15 per cent. The results presented in Table 1 (Plate 1) exhibited that no inhibiting effect was observed in onion extract, but garlic and neem leaves extracts inhibited the growth of few isolates. Isolate FP-V did not grow at 10 and 15 per cent neem leaves extract.Whereas, FP-X was found to be sensitive *i.e.*, no growth was observed at all the three concentrations (5, 10 and 15 %) of garlic bulb extract, FP-VIII isolate growth was only inhibited at 15 per cent garlic bulb extract. The result are in accordance with results of Murali *et al.* (1999), Rajappan *et al.* (2000), Singh *et al.* (2000) and Sarvanan *et al.* (2003).



T_1 : Neem
T_2 : Garlic
T_3 : Onion,
C : Control

a : Neem
b : Garlic
c : Onion,

Plate 1: Compatibility of fluorescent pseudomonads isolates with various plant extracts

Conclusion :

Fluorescent pseudomonads isolates were assayed for their compatibility with plant extracts, as the use of plant extract is one of the effective components in integrated disease management to reduce the management cost and also eco-friendly. No inhibiting effect was observed in onion bulb extract, but garlic bulb and neem leaves extracts inhibited the growth of few isolates. Isolate FP-V did not grow at 10 and 15 per cent neem leaves extract. Whereas, FP-X was found to be sensitive *i.e.*, no growth was observed at all the three concentrations 5, 10 and 15 per cent of garlic bulb extract, FP-VIII isolate growth was only inhibited at 15 per cent garlic bulb extract.

REFERENCES

Bergey, D.H. and Breed, Robert S. (1957). "*Bergey's manual of determinative bacteriology*".Publisher: Baltimore, Williams and Wilkins Co. USA.

Cook, R.J. (1993). Making greater use of introduced microorganisms for biological control of plant pathogens. *Annual Rev. Phytopathol.*, **31**: 53-80.

Gholve, V.M. and Kurundkar, B.P. (2004). Efficacy of combined inoculation of *Pseudomonas fluorescens* and *Trichoderma viride* in water culture. *J. Mycol. & Plant Pathol.*, **34** (2): 292-293.

Gupta, A., Gopal, M. and Tilak, K.V.B.R.(2000). Rhizobacteria from field grown mungbean: isolation and identification. *Leg. Res.*, **23** (4): 241-244.

Mukhopadhyay, A.N. (1987). Biological control of soil borne plant pathogen by *Trichoderma* spp. *Indian J. Mycol. & Plant Pathol.*, **17** : 1-10.

Murali, G., Gupta, A. and Magu, S.P. (1999). *In vitro* testing of plant growth promoting rhizobacteria against a chemical and phytochemical pesticide. *Leg. Res.*, **22** (3) : 185-188.

Rajappan, K., Mariappan, V. and Kareem, A.A. (2000). Effect of solvent free EC formulation of neem oil on beneficial microorganisms and fungal pathogen of rice. *Ann. Plant Proec. Sci.*, **8** (1) : 98-100.

Samanta, S.K. and Dutta, S. (2004). Potential of native plant growth promoting rhizobacteria in the management of sclerotinia stem rot of mustard. *J. Mycol. & Plant Pathol.*, **34** (3): 761-768.

Saravanan, T., Muthusamy, M. and Marimutha, T. (2003). Development of integrated approach to manage the fusarial wilt of banana. *Crop Protec.*, **22** (9): 1117-1123.

Sen, S., Bose, T., Basu, K., Acharya, R., Samajpati, N. and Acharya, K. (2006). *In vitro* antagonistic effect of fluorescent Pseudomonas BRL-1 against *Sclerotium rolfsii*. *Indian Phytopathol.*, **59** (2): 227-230.

Singh, U.P., Prithiviraj, B., Singh, K.P. and Sarma, B.K. (2000). Control of powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*) by combined application of plant growth-promoting rhizobacteria and Neemazal TM. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz.*, **107** (1) : 59-66.

Vidhyasekaran, P. and Muthamilan, M. (1995). Development of formulation of *Pseudomonas fluorescens* for control of chickpea wilt. *Plant Disease*, **79** (8): 782-786.

Yeole, R.D. and Dube, H.C. (2001). Rhizobacterial fluorescent *Pseudomonas* isolates from four crop plants and their rhizosphere competence. *J. Mycol. & Plant Pathol.*, **31** (3): 273-276.

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