Internat. J. agric. Sci. Vol.2 No.2 July 2006 : (549-556)

Enhancing resistance in bhendi to powdery mildew disease by foliar spray with fluorescent pseudomonads

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ABSTRACT

The antagonistic properties of different isolates of phylloplane *Pseudomonas fluorescens* were evaluated against powdery mildew of Bhendi caused by *Erysiphe cichoracearum* in *in-vitro* experiments. Among the 45 isolates evaluated, three isolates *viz.*,I_{18},I_9 and I_{36} recorded least conidial germination and maximum germ tube growth inhibition in cavity slide technique. Effect of these isolates was further tested under glass house condition by foliar spraying. Bhendi plants of 30 days age were sprayed with bacterial suspension (10^9 cfu /ml) as pre-inoculation and post-inoculation treatment. In pot culture experiment, pre-inoculation spray of *P. fluorescens* I_{18} recorded the lowest PDI of 20.89 as against 68.22 in control. Studies were conducted on the mechanisms of induced resistance in bhendi against powdery mildew disease by foliar spray with biotic inducer like *P. fluorescens* I_{18}) as pre-inoculation spray and post-inoculation spray. In pre-inoculation treatment of inducer, there was an increase in the total phenol and the activities of PAL, PPO, PO, â-1,3-glucanase and chitinase as compared with post-inoculation spray. The accumulation of phenol and the activity of the above enzymes started to increase at first day and reached the peak levels on fourth day (phenol & PAL), second day (PPO, PO and b-1,3-glucanase) and sixth day (chitinase) after treatment and decreased subsequently. However, the levels were always higher than the initial level.

Key words: *P. fluorescens* $I_{18,}$ *Erysiphe cichoracearum,* Induced resistance, Phenol, Enzymes

INTRODUCTION

The productivity of bhendi in India is very low due to many constraints including diseases. Diseases cause heavy losses in vegetable production. Diseases are inherent components of agro ecosystem that must be dealt with continuously and on knowledge basis. At different stages of vegetable production, disease management requires several approaches and it is more successful if it is integrated into crop production system. Since bhendi is grown throughout the year, management of the disease is important to get profitable yield. At present, for the management of vegetable diseases, fungicides are the first choice for the farmers' even though there are several inherent disadvantages in this method. Emphasis on developing or improving alternative disease management tactics (biological, cultural and host resistance) has in recent years fostered a new philosophy concerning the management of diseases elsewhere but in India, it has not picked up in most of the cases (Sokhi, 1994).Blakeman and Fokkema (1982) are of the opinion that it is unlikely that a perfect antagonist for the control of a particular pathogen will be found in nature, but it will be more effective, if these biocontrol agents have increased antagonism against the pathogens, increased competitiveness against the existing microflora, increased ability to multiply under favourable conditions and persist under unfavourable condition on host surfaces. Studies were carried out to isolate phylloplane biological control agent if any, and to obtain information that could be useful in managing the powdery mildew pathogen.

MATERIALS AND METHODS

Bhendi leaf samples were collected from five different locations in three different varieties and at three different stages and *P. fluorescens* was isolated by serial dilution using selective medium and identified.

Identification of Pseudomonas fluorescens

P.fluorescens was identified by the colony characters, gram staining, growth at 4°C, fluorescence test and gelatin liquefaction.

Gram staining

Gram staining was done as per the procedure described by Claus (1992)

Growth at 4°C

A loopful of the bacterial culture was streaked from bottom to top on the slants containing King's B medium under aseptic condition, incubated at 4°C for three days and observed for growth (Laskin and Lechevalier, 1977). Creamy white colonies appeared on King's B medium (*P. fluorescens*).

Fluorescence test

A loopful of the bacterial culture was streaked horizontally at the center of the Petri dish containing King'B medium under aseptic condition; incubated at room temperature for 48 hours and observed for the production of pigments that showed fluorescence under ultraviolet light of short wave length (Ca.254nm) (Laskin and Lechevalier, 1977).

Gelatin liquefaction (Seelay and Vandemark,1981)

Gelatin medium (3g yeast extract, 5g peptone and 120 g gelatin in 11itre of distilled water) was prepared, sterilized and allowed to set as cylinders in test tubes. The medium was vertically stabbed by means of a sterile platinum wire dipped in the bacterial suspension, incubated for three to seven days and examined. Liquefaction of gelatin indicated the positive result.

Effect of phylloplane P. fluorescens on conidial germination and germ tube growth of Erysiphe cichoracearum(Cavity slide technique)

The culture filtrates of *P. fluorescens* isolates were obtained by growing these organisms in King's B at room temperature for 48 hours. The culture filtrates were centrifuged at 5000 rpm for 20 minutes at 4°C for clarification. The efficacy of culture filtrates of the antagonistic organisms against *E. cichoracearum* was tested by the cavity slide technique.

Screening of Pseudomonas fluorescens isolates against bhendi powdery mildew in glass house

P. fluorescens isolates found effective (I_9, I_{38}, I_{87}, I_{36}, I_{45}) *in vitro* were tested in pot culture experiments along with carbendazim (0.1%) and existing commercial formulation of *P. fluorescens*1 for the control of bhendi powdery mildew in pot culture experiment. Bhendi plants of 30 days age were sprayed with bacterial suspension (10^9 cfu /ml) as pre-inoculation and post-inoculation treatment. The plants were first

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sprayed with bacterial suspension as pre- inoculation treatment. After 24 hours of pre-inoculation, plants were artificially inoculated by dusting the conidia of bhendi powdery mildew fungus from infected leaves. In post-inoculation treatment, bhendi plants were first dusted with conidia of powdery mildew fungus. After 24 hours the plants were sprayed with bacterial suspension. Disease observations were recorded 15 days after inoculation in 0-5 scale and per cent disease index was calculated. Each treatment was replicated thrice.

Mechanisms of induced resistance

To study the effect of *P. fluorescens* on changes in phenolics, phenylalanine ammonia-lyase, polyphenol oxidase, peroxidase, â-1,3-glucanase and chitinase in bhendi leaves,bhendi plants were sprayed with *P. fluorescens* I_{18} (10^9 cfu/ml)as pre-pathogen inoculation and post-pathogen inoculation. Pathogen alone inoculated plants served as control. For comparison inducer alone treated and water sprayed plants were maintained. At various times after treatments leaf samples were collected and various analyses were made.

Estimation of phenolic content

Total phenol content of the bhendi leaf was estimated by Folin Ciocalteau method (Bray and Thorpe, 1954). One gram of leaf sample was homogenized in 10 ml of 80 per cent ethanol and agitated for 15 minutes at 70^{0}C. Filtered through muslin cloth and again through Whatman No.1 filter paper and the volume of the filtrate was adjusted to 5ml with 80 per cent ethanol. In a test tube, one ml of ethanol extract, one ml of Folin Ciocalteau reagent and two ml of 20% sodium carbonate solution were added and the mixture was heated in a boiling water bath for exactly one minute. The tube was cooled under running tap water. The volume was made up to 25ml with distilled water. A reagent blank was maintained with one ml of distilled water instead of leaf extract. The intensity of colour was read at 650 hm in a calorimeter. The amount of total phenols present in the sample was calculated from a standard curve prepared by using different concentrations of catechol.

Estimation of phenylalanine ammonia lyase (PAL) activity

One gram of the leaf sample was homogenized in three ml of ice-cold 0.1M sodium borate buffer, pH 7.0 containing 1.4 mM of 2-mercaptoethanol and 0.1g of insoluble polyvinylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 15000g for 15 minutes. The supernatant was used as enzyme source. PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 hm (Dickerson *et al*., 1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 minutes at 30^{0}C. The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9630 M^{-1} cm^{-1}. Enzyme activity was expressed as synthesis of trans-cinnamic acid (in hmol quantities) min^{-1} g^{-1} fresh weight.

Assay of polyphenol oxidase activity (PPO)

PPO activity was determined as per the procedure given by Mayer *et al*. (1965). One gram of the sample was homogenized in two ml of 0.1M sodium phosphate buffer (pH 6.5) and centrifuged at 15000 rpm for 15 minutes at 4^{0}C. The supernatant was used as enzyme source. The reaction mixture consisted of 200 ml of the enzyme extract and 1.5ml of 0.1 M sodium phosphate buffer (pH 6.5). For enzyme activity to commence 200ml of 0.01 M catechol was added and the enzyme activity was recorded as changes in absorbance of reaction mixture at 495 hm min^{-1} g^{-1} fresh tissue. The results were expressed as units, one unit being the change in O.D. value of 0.001.

Assay of peroxidase activity (PO)

PO activity was determined according to the procedure given by Hammerschmidt *et al*. (1982). The enzyme extract was prepared as described in paragraph 3.6.3. In a spectrophotometer sample cuvette, 1.5 ml of pyrogallol(0.05 M) and 100ml of enzyme extract were taken. In the reference cuvette, inactivated enzyme (by boiling) extract (100ml) was taken along with 1.5ml of pyrogallol (0.05 M). The reading was adjusted to zero at 420hm. To initiate the reaction, 100ml of hydrogen peroxide (1%) (v/v) was added to the sample cuvette and the absorbance was read at 420hm. The enzyme activity was recorded as changes in absorbance of reaction mixture at 495hm min^{-1} g^{-1} fresh tissue. The results were expressed as units, one unit being the change in O.D. value of 0.001.

Assay of â-1, 3-glucanase activity

Preparation of dinitro salicylic acid (DNS reagent)

One gram of dinitro salicylic acid was dissolved in 100ml of one per cent sodium hydroxide with constant stirring and 200mg crystalline phenol and 50mg sodium sulphite were added. The reagent was stored at 4^{0}C .

Assay procedure

β-1,3-glucanase activity was assayed by the laminarin dinitro salicylate method described by Pan *et al*. (1991). Bhendi leaf sample of one gram was extracted with five ml of sodium acetate buffer (0.05M with pH 5.0) by grinding at 4^{0}C using a chilled pestle and mortar. The extract was then centrifuged at 10,000g for 15 minutes at 4^{0}C and the supernatant was used in enzyme assay. The reaction mixture consisted of 62.5µl of 4% laminarin and 62.5µl of enzyme extract. The reaction was carried out at 40^{0}C for 10 minutes. The reaction was stopped by adding 375µl of DNS reagent and heating for five minutes on a boiling water bath. The resulting coloured solution was diluted with 4.5 ml distilled water, vortexed and its absorbance at 500hm was determined. Enzyme activity was expressed as µg glucose released min^{-1} g^{-1} fresh tissue.

Assay of chitinase

Preparation of colloidal chitin

Colloidal chitin was prepared by treating one gram of crab-shell chitin powder (Sigma) with acetone to form a paste and then 20ml of concentrated HCL was added slowly, while grinding in a mortar with the temperature maintained at 5^{0}C. After several minutes, the syrupy liquid was filtered through glass wool and poured into vigorously stirred 50 per cent aqueous ethanol to precipitate the chitin in a dispersed state. The residue was sedimented and resuspended in distilled water several times to remove excess acid and alcohol, then dialyzed against tap water. Chitin content of the suspension was determined by drying a sample *in vacuo* .The final concentration was adjusted with distilled water to give 10 mg/ml (dry weight/volume) and stored at 5^{0}C (Berger and Reynolds, 1958).

Preparation of snail gut enzyme

Six hundred milligram of the commercial lyophilized snail gut enzyme (Helicase, obtained from Sepracor,France) was dissolved in 10 ml of 20 mM KCl and chromatographed on a SephadexG-25 column (38 x 1.5cm) using 10 mM KCl solution, containing 1mM EDTA and adjusted to pH 6.8. The first 20 ml eluted after the void volume was collected (Boller and Mauch, 1988).

Preparation of p- Dimethylaminobenzaldehyde (DMAB) reagent

Ten gram of DMAB (Sigma) was dissolved in 100 ml of glacial acetic acid containing 12.5 per cent (v/v) 10 N HCl and stored at 4^{0}C. It was diluted with nine volumes of glacial acetic acid, shortly before use.

Assay procedure

Bhendi leaves (1g) were homogenized in 5 ml of 0.1 M sodium citrate buffer (pH 5.0). The homogenate was centrifuged for 10 minutes at 10,000 g at 4°C and the supernatant was used in the enzyme assay. For the colorimetric assay of chitinase, 0.01ml of 0.1 M sodium acetate buffer (pH 4.0), 0.4ml of enzyme extract and 0.1ml colloidal chitin (1mg) were pipetted into a 1.5ml eppendorf tube and incubated at 37^{0}C. After 2 hours, the reaction was stopped by centrifugation at 1,000g for 3 minutes. An aliquot of the supernatant (0.3 ml) was

pipetted into a glass reagent tube containing 0.03ml 1M potassium phosphate buffer (pH 7.1) and incubated with 0.02ml desalted snail gut enzyme for 1hour. The resulting monomeric N-acetylglucosamine (GluNAc) was determined as per the procedure described by Reissig *et al*. (1959) using standards of GluNAc. Enzyme activity was expressed as hmol GluNAc equivalents $min^{-1}g^{-1}$ fresh tissue

RESULTS AND DISCUSSION

Forty five isolates of *P. fluorescens* were collected and the efficacy was tested *in vitro.* All the isolates of *P. fluorescens* were able to inhibit the germination of *E. cichoracearum* effectively and the inhibition ranged from 30.55 in I_{41} to 67.60% in I_{18} compared to distilled water check (Table 1). Mean germination was the least with I_{18}, I_9 and I_{36} and were on par with each other.

The trend was almost similar at each period of observation. Mean germination was the least at 12 hours after incubation; increased up to 24 hours after incubation; and thereafter the increase was not significant.

Effect of culture filtrate of Pseudomonas fluorescens on germ tube growth

Variability in germ tube length due to isolates is evident from the data presented in table 2. Germ tube growth recorded a steady increase over the periods of incubation. The different isolates evaluated were able to inhibit the germ tube growth of *E. cichoracearum* effectively by 68.07 (I_{24}) to 84.06 (I_{18}) per cent over control. Overall mean germ tube growth indicated that I_{18} was the best one to inhibit the germ tube growth effectively (84.06%). The isolates *viz.,* I_{45}, I_{36}, I_{27} and I_9 were the next best effective ones recording 83.03,82.99,82.94 and 82.89 per cent inhibition respectively and were on par with each other.

Screening of Pseudomonas fluorescens isolates against bhendi powdery mildew by artificial inoculation (glass house)

Five P. fluorescens isolates ($I_{18,}$ I_{45}, I_{36}, I_{27} and I_9) which gave more than 60 per cent inhibition of conidial germination of powdery mildew fungus were selected for further studies.These five isolates along with the commercial formulation *P. fluorescens*1 were tested for their efficacy in the control of powdery mildew under pot culture conditions in the glass house. The results presented in table 3 show that all the isolates tested significantly reduced the disease intensity both as pre-inoculation treatment and as post-inoculation treatment. Pre-inoculation treatment was more effective than post-inoculation treatment. In the pre-inoculation treatment, among the phylloplane pseudomonads, I_{18} was the most effective one in reducing the disease severity followed by $I_{36,}$ I_9, *P. fluorescens*1, I_{27} and I_{45} and the variability among these isolates in disease control potential was significant except between I_{27} and I_{45}; which were on par with each other.

In post-inoculation treatment also all the isolates were effective in reducing the disease intensity (PDI ranged from 28.36 to 50.30) compared to untreated check. The isolate I_{18} was found to be the superior one followed by I_{36}, I_9, and *P. fluorescens*1 with the per cent disease index of 34.22,37.99,39.78 and 44.22 respectively. The isolates I_{27} and I_{45} were on par and were inferior to other isolates in reducing the disease intensity.

Effect of foliar application of Pseudomonas fluorescens I_{18} on phenolic content in bhendi leaves

Studies on induction of defense mechanisms as shown by accumulation of phenolics revealed that *P. fluorescens* I_{18} treatment challenge inoculated with the pathogen, induced the host to accumulate higher quantities of phenol (371.04 mg g^{-1}). Total phenol content ranged from 175.16 to 453.16mg g^{-1} in bhendi leaves in the different treatments (Table 4). The maximum accumulation was observed on fifth day (363.35 mg g^{-1}) and declined from sixth day onwards. Moreover, the accumulation of total phenol was less in pathogen alone and *P. fluorescens* alone treated leaves compared to *P. fluorescens* I_{18} challenge inoculated with the pathogen .

Effect of foliar application of Pseudomonas fluorescens I_{18} on PAL activity in bhendi leaves

The assay of the level of PAL activity as influenced by *P. fluorescens* I_{18} treatment revealed that the activity increased greatly in pre-inoculation spray (116.48). PAL activity varied from 57.72 to 149.66 (Table 5). PAL activity showed significant increase from the first day of treatment to reach the maximum of 123.44 on fourth day and thereafter the level declined to 82.70 on seventh day. Throughout the experimental period the activity was maintained at higher level than the initial level.

Changes in PPO activity in bhendi leaves due to foliar application of Pseudomonas fluorescens I_{18}

Treatment of bhendi leaves with *P. fluorescens* I_{18} significantly increased the PPO activity (Table 6). PPO activity ranged from 17.42 to 58.34 units. Pre-inoculation spray recorded the highest activity (44.20 units) followed by post-inoculation spray, *P. fluorescens* I_{18} alone, pathogen alone and water. PPO activity rapidly increased from one day after treatment and reached the maximum on second day after treatment and gradually declined thereafter. In pre-inoculation spray a two fold increase in PPO activity was recorded from second day after treatment and this increased activity was maintained up to fourth day after treatment. In all other treatments two fold increase was noticed only up to third day after treatment .

Changes in peroxidase activity in bhendi leaves due to foliar application of Pseudomonas fluorescens I_{18}

In this experiment PO activity in the different treatments varied from 73.80 to 220.66 units. In the present study a 2.77 fold increase in PO activity was recorded in *P. fluorescens* I_{18} sprayed leaves challenge inoculated with the pathogen (Table 7). In both pre and post-inoculation spray treatment, the maximum PO activity was observed on second day after treatment; whereas in pathogen alone and *P. fluorescens* I_{18} alone sprayed leaves, PO activity reached a maximum level on third day after treatment and the activity was maintained at higher levels throughout the experimental period.

Changes in â-1,3- glucanase activity in bhendi leaves due to foliar application of Pseudomonas fluorescens I_{18}

Significant changes in â-1,3- glucanase activity in bhendi leaves due to foliar application of *P. fluorescens* I_{18} was evident from the data presented in table 8. The â-1,3- glucanase activity varied from 5.20 to 37.73 µg glucose. The mean enzyme activity in pre-inoculation spray of *P. fluorescens* I_{18} was nearly 4.4 times higher than that of water sprayed control. In pathogen alone and *P. fluorescens* I_{18} alone sprayed leaves, the activity was 2.4 and 3.1 times more respectively than water sprayed control. Significant increase in â-1,3- glucanase activity was evident on first day after treatment and reached a maximum level on second day after treatment. The enzyme activity was higher than the initial level in all the sampling periods .

Changes in chitinase activity in bhendi leaves due to foliar application of P.fluorescens I_{18}

The chitinase activity in the different treatments ranged from 1.33 to 5.56 hmol GluNAc. The chitinase activity increased in *P. fluorescens* $I_{18.}$ sprayed leaves challenge inoculated with the pathogen. The increase was 2.6 times more than the water sprayed control and 1.5 times more than the pathogen alone inoculated control. In post-inoculation spray of *P. fluorescens* I_{18} the activity was 2.2 times more than water sprayed control. Considering the periods together the mean chitinase activity gradually increased from the day of treatment (1.66) and reached a peak (4.02) on sixth day after treatment. Similar trend was observed in almost all the treatments (Table 9).

In recent years efforts have been made to exploit the use of phylloplane microorganisms for the control of foliar diseases with the increasing awareness of the problems and expenses of conventional methods of disease control, including fungicides as well as the costly and time consuming breeding programmes. The usual start is to search for potential antagonists in the habitat in which the pathogen is normally

Table 1: Effect of culture filtrate of *Pseudomonas fluorescens* isolates on conidial germination of *Erysiphe cichoracearum*

Isolate No.	Germination (%)*				Mean	Per cent inhibition over control
	12 h	24 h	48 h	72 h		
I_1	17.68(24.80)$^{j-r}$	54.59(47.58)$^{i-l}$	55.07(47.87) $^{i-l}$	55.22(47.98)$^{g-k}$	45.64(42.10)$^{m-p}$	39.70
I_2	18.56(25.47)$^{j-r}$	52.99(46.66)$^{g-l}$	56.62(48.79) $^{i-l}$	56.93(58.97)^{l}	46.27(42.51)$^{m-r}$	38.87
I_3	19.26(25.99)$^{j-r}$	54.98(47.81)^{jk}	55.83(58.83)^{m}	56.13(48.50)$^{h-k}$	46.59(42.72)$^{n-r}$	38.45
I_4	15.55(23.18)$^{f-m}$	49.24(44.54)$^{d-l}$	49.40(44.66)d-g	50.64(45.34)$^{g-h}$	41.20(39.45)^{ghi}	45.57
I_5	12.05(20.44)$^{b-f}$	42.06(40.40)^{b}	42.32(40.57)^{b}	42.59(40.69)^{b}	34.75(35.52)^{c}	54.09
I_6	14.73(22.54)$^{e-k}$	51.92(46.09)$^{f-l}$	52.69(46.49)$^{e-k}$	53.21(46.83)$^{e-j}$	43.14(40.32) $^{i-l}$	43.00
I_7	15.92(23.50)$^{g-n}$	53.37(46.89)$^{g-l}$	53.97(47.24)$^{f-l}$	54.28(47.41)$^{f-k}$	44.38(41.29)j-^{m}	41.37
I_8	16.73(24.12)$^{h-p}$	52.63(46.49)$^{f-l}$	53.60(47.06)$^{f-l}$	53.95(47.24)f-k	44.22(41.24)$^{j-m}$	41.58
I_9	9.48(17.85)a^{b}	31.33(34.02)^{a}	31.42(34.08)^{a}	31.42(34.08)^{a}	25.91(30.03)^{ab}	65.77
I_{10}	13.25(21.30)$^{c-g}$	46.14(42.76)^{be}	47.96(43.80)^{cde}	48.35(43.02)^{bcd}	38.92(38.00)$^{e-h}$	48.58
I_{11}	11.90(20.18)$^{b-e}$	42.89(40.86)^{b}	43.66(41.32)^{bc}	44.18(41.01)^{b}	35.65(36.03)^{cd}	52.89
I_{12}	16.57(23.97)$^{g-o}$	53.34(46.89) $^{g-l}$	54.12(47.35)$^{g-l}$	54.12(47.35)$^{f-k}$	44.53(41.42)^{klm}	41.32
I_{13}	16.99(24.27) $^{i-q}$	54.38(47.47) $^{i-l}$	54.45(47.52)$^{h-l}$	54.75(47.70)$^{f-k}$	45.13(41.78)^{mn}	40.38
I_{14}	17.80(24.95)$^{j-r}$	54.00(47.29) $^{i-l}$	54.83(47.75) $^{i-l}$	55.29(47.98)$^{g-k}$	45.53(40.06)^{hij}	39.85
I_{15}	14.40(22.30)$^{e-j}$	48.00(43.85)$^{c-f}$	49.13(44.48)^{def}	49.50(44.71)^{cde}	40.26(38.84)^{fgh}	46.81
I_{16}	13.24(21.30)$^{c-g}$	42.00(40.40)^{b}	43.75(41.38)^{bc}	44.02(41.55)^{b}	35.75(36.18)^{cd}	52.77
I_{17}	17.96(25.03)$^{j-r}$	53.79(47.12)$^{h-l}$	55.07(47.87) $^{i-l}$	55.25(47.98)$^{g-k}$	45.45(42.00)^{mno}	39.95
I_{18}	8.15(16.54)^{a}	29.65(33.58)^{a}	30.09(33.21)^{a}	30.22(33.34)^{a}	24.52(29.05)^{a}	67.60
I_{19}	18.10(25.18)j-r	55.26(47.98)$^{h-l}$	56.17(48.5)$^{j-l}$	56.49(48.68)^{ijk}	46.51(42.62)$^{m-r}$	38.55
I_{20}	18.71(25.62)$^{j-r}$	54.74(47.70)^{jk}	55.71(48.27) $^{i-l}$	56.13(48.50)$^{h-k}$	46.32(42.52)^{mn}	38.80
I_{21}	15.16(22.87)$^{e-l}$	48.86(44.31)$^{c-g}$	49.88(44.89)$^{d-h}$	50.39(45.17)^{def}	41.07(39.36)^{ghi}	45.74
I_{22}	13.85(21.81)$^{d-i}$	42.26(40.51)^{b}	42.51(40.69)^{b}	43.10(41.03)^{b}	35.42(36.02)^{cd}	53.20
I_{23}	21.21(27.42)$^{j-r}$	56.20(48.56)$^{h-l}$	56.24(48.56)$^{j-l}$	56.97(58.97)^{l}	47.65(43.39)$^{q-s}$	37.05
I_{24}	21.39(27.49)$^{j-r}$	57.98(49.55)$^{h-l}$	57.84(49.49) $^{i-l}$	58.10(49.66)^{k}	48.85(44.09)^{s}	35.37
I_{25}	20.53(26.92)$^{j-r}$	56.42(48.68)$^{h-l}$	57.40(49.26) $^{i-l}$	57.83(49.49)^{jk}	48.04(43.60)$^{q-s}$	36.43
I_{26}	16.70(24.12)$^{h-p}$	50.39(45.17)$^{e-l}$	51.41(45.80)$^{e-j}$	51.81(46.03)$^{e-i}$	42.57(40.27)$^{h-k}$	43.76
I_{27}	10.80(19.19)$^{a-d}$	30.68(33.58)^{a}	33.41(35.30)^{a}	33.66(35.43)^{a}	27.13(30.89)^{b}	64.16
I_{28}	15.43(23.11)$^{f-m}$	54.32(47.47) $^{i-l}$	54.82(47.75) $^{i-l}$	55.37(48.04)$^{g-k}$	45.03(41.74)^{lm}	40.51
I_{29}	19.61(26.28)$^{j-r}$	55.73(48.27)$^{h-l}$	56.49(48.68) $^{i-l}$	57.03(49.58)^{jk}	47.44(43.22)$^{p-s}$	37.32
I_{30}	19.61(26.28)$^{j-r}$	56.59(48.73)$^{h-l}$	57.50(49.31) $^{i-l}$	57.81(49.49)^{jk}	47.87(43.74)^{rs}	36.66
I_{31}	20.11(26.64)$^{j-r}$	56.01(48.45)$^{h-l}$	57.12(49.02)$^{j-l}$	57.40(49.26)^{jk}	47.65(43.36)^{qrs}	37.05
I_{32}	16.23(23.73)$^{g-o}$	49.75(44.83)$^{d-l}$	51.03(45.57)$^{e-i}$	51.40(45.80)$^{e-h}$	42.13(40.02)^{hij}	44.34
I_{33}	15.16(22.87)$^{e-l}$	45.35(42.30)^{bcd}	45.83(42.59)^{bcd}	46.22(42.82)^{bcd}	38.13(37.67)^{efg}	49.62
I_{34}	17.54(24.73)$^{j-r}$	53.83(47.18) $^{i-l}$	54.59(57.58)^{m}	54.59(47.58)$^{f-k}$	45.13(41.82)^{mn}	40.38
I_{35}	17.68(24.80)$^{j-r}$	54.82(47.75)^{jk}	55.73(48.27)$^{j-l}$	57.17(48.50)$^{h-k}$	46.10(42.37)$^{m-r}$	39.09
I_{36}	9.92(18.34)^{ab}	31.42(34.08)^{a}	31.69(34.20)^{a}	31.86(34.33)^{a}	26.22(30.27)^{ab}	65.36
I_{37}	18.24(23.25)$^{g-n}$	54.31(47.47) $^{i-l}$	55.29(47.98) $^{i-l}$	55.71(48.27)$^{h-k}$	45.93(42.30)$^{m-r}$	39.32
I_{38}	14.78(22.54)$^{e-k}$	48.60(44.20)$^{c-g}$	49.50(44.71)$^{d-g}$	49.88(44.89)^{cde}	40.68(39.11)^{ghi}	46.25
I_{39}	13.46(21.47)$^{c-h}$	44.95(42.07)^{bc}	45.06(42.13)^{bcd}	45.43(42.36)^{bc}	37.22(37.04)^{bc}	50.83
I_{40}	18.02(25.10)$^{j-r}$	54.30(47.47) $^{i-l}$	55.07(47.87) $^{i-l}$	55.15(47.93)$^{f-k}$	45.55(42.11)$^{m-r}$	39.82
I_{41}	18.12(25.18)$^{j-r}$	55.26(47.98)$^{h-l}$	56.17(48.50)$^{j-l}$	56.49(48.68)^{ijk}	46.51(42.62)$^{m-r}$	30.55
I_{42}	18.10(25.18)$^{j-r}$	55.26(47.98)$^{h-l}$	56.17(48.50)$^{j-l}$	56.49(48.68)^{ijk}	46.50(42.62)$^{m-r}$	38.56
I_{43}	18.84(25.70)$^{j-r}$	55.01(47.87)^{jk}	55.77(48.27) $^{i-l}$	55.98(48.39)$^{h-k}$	46.38(42.58)$^{m-r}$	38.72
I_{44}	15.16(22.87)$^{e-l}$	48.99(44.37)$^{c-l}$	59.88(44.89)$^{d-h}$	50.51(45.29)^{def}	41.13(39.39)^{ghi}	45.66
I_{45}	10.36(18.72)^{abc}	30.27(33.34)^{a}	32.74(34.88)^{a}	33.09(35.06)^{a}	26.61(30.54)^{b}	64.84
Control (Distilled water)	33.54(35.37)^{s}	86.00(68.03)^{m}	91.20(71.76)^{n}	93.03(74.66)^{m}	75.69(60.40)^{t}	
Mean	16.44(23.89)^{A}	50.23(45.11)^{B}	51.20(45.69^{B})	51.63(45.92)^{B}		

*Mean of three replications Values in parentheses represent arc sine transformed values

In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 2 : Effect of culture filtrate of *Pseudomonas fluorescens* isolates on germ tube growth of *Erysiphe cichoracearum*

Isolate No.	Germ tube growth (µm) *			Mean	Per cent inhibition over control
	24 h	48 h	72 h		
I_1	51.66^{h}	80.33^{kl}	81.33^{lmn}	71.11^{no}	70.16
I_2	53.66^{ij}	80.33^{kl}	81.33^{lmn}	71.77^{nop}	60.89
I_3	54.00^{ij}	83.00^{o}	83.66^{pqr}	73.55^{tuv}	60.14
I_4	51.33^{fgh}	78.66^{jk}	79.66^{kl}	69.88^{klm}	70.68
I_5	45.66^{c}	69.33^{e}	70.33^{e}	61.77^{d}	74.08
I_6	46.66^{cd}	77.66^{hi}	78.66^{j}	67.66^{j}	71.61
I_7	53.33^{i}	80.33^{kl}	81.33^{lmn}	71.66^{pq}	69.93
I_8	54.66^{ij}	80.33^{kl}	81.33^{lmn}	72.11^{rs}	69.74
I_9	34.66^{b}	43.66^{b}	44.00^{b}	40.77^{b}	82.89
I_{10}	48.66^{e}	73.33^{h}	74.33^{fg}	65.44^{ef}	72.54
I_{11}	45.66^{c}	72.66^{h}	$73.^{66fg}$	64.00^{ef}	73.15
I_{12}	53.66^{ij}	78.66^{ij}	81.00^{lmn}	71.44^{nop}	70.02
I_{13}	54.33^{aj}	80.33^{kl}	81.00^{lmn}	71.88^{pq}	69.84
I_{14}	56.33^{k}	79.66^{jk}	80.66^{kl}	72.22^{rs}	69.70
I_{15}	50.00^{efg}	70.00^{b}	70.33^{e}	63.44^{e}	73.38
I_{16}	48.66^{ef}	64.66^{c}	64.66^{c}	59.33^{c}	75.11
I_{17}	54.33^{ij}	81.66^{lm}	82.33^{nop}	72.77^{st}	69.47
I_{18}	32.66^{a}	41.66^{a}	41.66^{a}	38.00^{a}	84.06
I_{19}	53.66^{ij}	83.66^{op}	83.33^{opq}	73.44^{tuv}	69.11
I_{20}	53.66^{ij}	79.66^{tl}	80.66^{kl}	71.33^{pq}	70.07
I_{21}	51.66^{h}	75.33^{i}	76.00^{gh}	67.66^{j}	71.61
I_{22}	45.33^{c}	70.00^{ef}	70.33^{e}	61.88^{d}	74.04
I_{23}	53.33^{i}	84.33^{op}	84.66^{qrs}	74.11^{vw}	68.90
I_{24}	54.00^{ij}	86.66^{r}	87.66^{tu}	76.11^{y}	68.07
I_{25}	51.33^{h}	83.66^{op}	84.66^{rst}	73.22^{st}	69.28
I_{26}	49.00^{ef}	80.66^{lm}	80.66^{kl}	69.44^{k}	70.86
I_{27}	35.66^{b}	43.00^{b}	43.66^{b}	40.66^{b}	82.94
I_{28}	50.33^{fgh}	80.33^{kl}	80.66^{kl}	70.44^{klm}	70.44
I_{29}	54.00^{ij}	82.00^{mn}	82.33^{nop}	72.77^{st}	69.47
I_{30}	54.00^{ij}	85.33^{pq}	86.33^{stu}	75.22^{x}	68.44
I_{31}	54.00^{ij}	83.00^{mn}	85.33^{rst}	74.00^{vw}	68.95
I_{32}	50.00^{efg}	73.66^{h}	75.66^{gh}	66.44^{h}	72.12
I_{33}	50.33^{efg}	66.66^{d}	68.00^{d}	61.66^{d}	74.13
I_{34}	53.00^{i}	79.00^{jk}	80.33^{kl}	70.88	70.26
I_{35}	54.66	80.33	82.33	72.44^{no}	69.61
I_{36}	34.66^{b}	43.66^{b}	43.66^{b}	40.55^{b}	82.99
I_{37}	55.00^{kl}	80.66^{kl}	82.33^{mop}	72.66^{rs}	69.51
I_{38}	49.33^{efg}	75.33^{i}	77.33^{i}	67.44^{i}	71.70
I_{39}	46.33^{cd}	71.00^{fg}	72.66^{f}	63.33^{g}	73.43
I_{40}	53.66^{ij}	84.00^{op}	85.00^{rst}	74.22^{vw}	68.86
I_{41}	54.00^{ij}	84.66^{pq}	86.33^{stu}	75.00^{x}	68.53
I_{42}	53.33^{i}	81.00^{lm}	82.00^{lmn}	72.11^{rs}	69.74
I_{43}	53.66^{ij}	79.66^{jk}	80.66^{kl}	71.33^{pq}	70.07
I_{44}	49.30^{efg}	75.33^{i}	76.73^{gh}	67.00^{i}	71.89
I_{45}	35.66^{b}	43.00^{b}	43.00^{b}	40.44^{b}	83.03
Control (Distilled water)	99.33^{m}	205.66^{s}	310.33^{v}	238.33^{z}	
Mean	50.90^{A}	79.47^{B}	80.47^{C}		

*Mean of three replications Values in parentheses represent arc sine transformed values

In the column, means followed by a common letter are not significantly different at 5% level by DMRT

found. In the present study, efforts were made to isolate bhendi phylloplane organisms. In the studies on the effect of phylloplane microflora on conidial germination of *E. cichoracearum*, *P. fluorescens* was the most effective one in inhibiting the conidial germination and germ tube elongation followed by *B. subtilis*, *Penicillium* sp., *A. niger* showing antagonism, the antagonistic microorganisms might compete successfully with the pathogen for vitamins or trace elements as reported by Srivastava and Bisht (1983).

In the present study, antagonistic effect of phylloplane *P. fluorescens* isolates especially I_{18} against bhendi powdery mildew and *A. flavus.* This finding is in conformity with the findings of Rajeswari (2002) who found that the culture filtrate of grapevine phylloplane *P. fluorescens* effectively inhibited the conidial germination of *Uncinula necator.*

The isolates of *P. fluorescens* especially I_{18}, I_9, I_{36}, I_{27} and I_{45} were found to be superior with higher inhibition of conidial germination and germ tube growth of *E. cichoracearum* than other isolates. Besides was clearly established with pre- inoculation treatment as well as by post-inoculation treatment. Earlier, Hijwegen and Buchenauer (1984) brought out the hyperparasitism associated with Erysiphaceae by eight parasitic fungi. Vogt and Buchenauer (1997) obtained better control of cucumber powdery mildew with a combination of fluorescent pseudomonads strain with 2 amino isobutyric acid.

Similar results of efficacy of phylloplane *P. fluorescens* have

Table 3 : Screening of *Pseudomonas fluorescens* isolates against bhendi powdery mildew by artificial inoculation (glass house)

Treatments	Pre- inoculation treatment		Post - inoculation treatment	
	PDI*	Per cent reduction over control	PDI*	Per cent reduction over control
P. fluorescens I_9	26.22(30.79) [d]	61.57	39.78(39.06) [d]	42.23
P. fluorescens I_{18}	20.89(27.13) [b]	69.38	34.22(35.79) [b]	50.30
P. fluorescens I_{27}	36.67(37.23) [f]	46.25	49.33(44.60) [f]	28.36
P. fluorescens I_{36}	24.67(29.73) [c]	63.84	37.99(38.00) [c]	44.83
P. fluorescens I_{45}	37.33(37.64) [f]	45.28	49.32(44.60) [f]	28.38
P. fluorescens 1	30.89(33.71) [e]	54.72	44.22(41.67) [e]	37.78
Carbendazim 0.1%	8.22(16.64) [a]	87.95	9.55(17.95) [a]	86.13
Control (Distilled water)	68.22(55.67) [g]	-	68.86(56.04) [g]	-

* Mean of three replications Values in parentheses represent arc sine transformed values
In the column, means followed by a common letter are not significantly different a 5% level by DMRT

Table 4 : Effect of foliar application of *Pseudomonas fluorescens* I_{18} on phenolic content in bhendi leaves

Treatments	Total phenolic content *($\mu g\ g^{-1}$) Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	182.70[a]	326.06[a]	340.73[a]	420.04[a]	453.16[a]	445.22[a]	410.15[a]	390.28[a]	371.04[a]
Post-inoculation spray	180.70[b]	307.90[b]	319.82[b]	390.80[b]	419.16[b]	407.80[b]	352.72[b]	337.06[b]	339.49[b]
P. fluorescens I_{18} alone	179.52[c]	297.33[c]	310.44[c]	365.72[c]	380.56[c]	397.33[c]	330.26[c]	294.48[c]	319.58[c]
Pathogen alone	177.84[d]	292.43[d]	298.92[d]	360.90[d]	368.63[d]	385.06[d]	295.44[d]	284.64[d]	307.98[d]
Distilled water	175.16[e]	177.33[e]	179.66[e]	177.86[e]	178.52[e]	181.36[e]	179.36[e]	175.90[e]	178.14[e]
Mean	179.18[H]	280.21[G]	289.92[F]	343.06[C]	360.01[A]	363.35[B]	313.57[D]	296.67[E]	

* Mean of three replications
In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 5 : Effect of foliar application of *Pseudomonas fluorescens* I_{18} on PAL activity in bhendi leaves

Treatments	PAL activity *(ηmol trans-cinnamic acid $min^{-1}g^{-1}$) Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	63.70[a]	109.43[a]	128.60[a]	137.20[a]	149.66[a]	121.93[b]	118.13[a]	103.16[a]	116.48[a]
Post-inoculation spray	60.66[bc]	103.33[b]	118.23[b]	126.46[b]	140.16[b]	124.56[a]	99.23[b]	90.76[b]	107.92[b]
P.fluorescens I_{18} alone	59.80[bcd]	100.24[c]	114.32[c]	123.80[c]	136.50[c]	120.50[c]	90.14[c]	86.50[c]	103.98[c]
Pathogen alone	58.06[cd]	97.16[d]	108.20[d]	114.20[d]	132.20[d]	114.63[d]	80.40[d]	76.26[d]	97.64[d]
Distilled water	57.72[d]	58.24[e]	57.50[e]	59.80[e]	58.70[e]	57.32[e]	58.54[e]	56.80[e]	58.08[e]
Mean	59.99[H]	93.68[E]	105.37[D]	112.29[B]	123.44[A]	107.78[C]	89.29[F]	82.70[G]	

* Mean of three replications
In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 6 : Changes in PPO activity in bhendi leaves due to foliar application of *Pseudomonas fluorescens* I_{18}

Treatments	PPO activity in units* (ΔA_{495} $min^{-1}g^{-1}$) (1 unit = 0.001 Absorbance) Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	24.72[a]	46.33[a]	58.34[a]	55.32[a]	50.52[a]	46.32[a]	39.22[a]	32.80[a]	44.20[a]
Post-inoculation spray	22.74[bc]	40.72[b]	50.44[b]	47.44[b]	42.24[b]	38.80[b]	33.24[b]	26.32[b]	37.74[b]
P. fluorescens I_{18} alone	21.52[bc]	36.92[c]	47.73[c]	46.35[b]	36.16[c]	34.06[c]	31.34[c]	24.24[c]	34.79[c]
Pathogen alone	20.30[c]	34.34[d]	43.57[d]	40.24[c]	35.43[c]	30.14[d]	27.33[d]	20.42[d]	31.47[d]
Distilled water	19.32[d]	21.08[e]	19.28[e]	20.61[d]	20.81[d]	18.33[e]	17.42[e]	17.93[e]	19.35[e]
Mean	21.72[H]	35.88[D]	43.87[A]	41.99[B]	37.03[C]	33.53[E]	29.71[F]	24.34[G]	

* Mean of three replications
In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 7 : Changes in peroxidase activity in bhendi leaves due to foliar application of *Pseudomonas fluorescens* I_{18}

Treatments	Peroxidase activity in units* (ΔA_{420} $min^{-1}g^{-1}$) (1 unit = 0.001 Absorbance) Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	79.52[a]	180.62[a]	220.66[a]	210.32[a]	170.33[a]	153.70[a]	146.60[a]	130.00[a]	161.47[a]
Post-inoculation spray	77.80[ab]	173.33[b]	210.33[b]	199.64[b]	156.62[b]	142.63[b]	132.70[b]	120.66[b]	151.71[b]
P.fluorescens I_{18} alone	76.90[b]	170.20[c]	198.36[c]	208.18[a]	150.72[c]	136.66[c]	130.22[c]	115.36[c]	148.33[c]
Pathogen alone	74.72[b]	165.92[d]	187.73[d]	200.72[b]	142.72[d]	130.70[d]	117.35[d]	110.72[d]	141.33[d]
Distilled water	76.54[b]	78.64[e]	76.46[e]	78.16[c]	79.56[e]	80.26[e]	75.34[e]	73.80[e]	77.35[e]
Mean	77.10[G]	153.74[B]	178.71[A]	179.49[A]	139.99[C]	128.79[D]	120.44[E]	110.10[F]	

* Mean of three replications
In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 8 :Changes in β-1,3- glucanase activity in bhendi leaves due to foliar application of *Pseudomonas fluorescens* I_{18}

Treatments	β-1,3- glucanase activity *(µg glucose $min^{-1}g^{-1}$) Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	9.40[a]	20.50[a]	35.10[a]	37.73[a]	36.56[a]	25.46[a]	22.93[a]	21.06[a]	26.09[a]
Post-inoculation spray	8.56[a]	16.16[b]	30.76[b]	30.86[b]	27.76[b]	20.10[b]	18.73[b]	15.33[b]	21.03[b]
P.fluorescens I_{18} alone	6.32[b]	14.14[c]	28.22[c]	27.50[c]	24.16[c]	19.22[c]	17.30[c]	12.14[c]	18.63[c]
Pathogen alone	6.06[b]	11.13[d]	24.23[d]	22.23[d]	17.06[d]	14.33[d]	13.10[d]	8.76[d]	14.61[d]
Distilled water	5.82[c]	6.34[e]	6.90[e]	5.70[e]	6.90[e]	5.64[e]	5.22[e]	5.20[e]	5.97[e]
Mean	7.23[G]	13.65[E]	25.03[A]	24.88[A]	22.49[B]	16.95[C]	15.46[D]	12.50[F]	

* Mean of three replications
In the column, means followed by a common letter are not significantly different at 5% level by DMRT

Table 9 : Changes in chitinase activity in bhendi leaves due to foliar application of *Pseudomonas fluorescens* I_{18}

Treatments	Chitinase activity *(ηmol GluNAc $min^{-1}g^{-1}$) Days after treatment								Mean
	0	1	2	3	4	5	6	7	
Pre-inoculation spray	2.00[ab]	3.53[ab]	3.70[a]	4.20[a]	4.70[a]	5.00[a]	5.56[a]	5.00[a]	4.21[a]
Post-inoculation spray	1.80[ab]	3.06[abc]	3.10[b]	3.56[b]	4.00[b]	4.13[b]	4.60[b]	4.10[b]	3.54[b]
P. fluorescens I_{18} alone	1.60[b]	2.80[bc]	3.02[b]	3.22[c]	3.60[b]	3.80[c]	4.00[c]	3.52[c]	3.20[c]
Pathogen alone	1.50[b]	2.60[c]	2.60[c]	3.00[c]	3.30[c]	3.30[d]	3.70[d]	3.20[c]	2.90[d]
Distilled water	1.42[b]	1.60[d]	1.80[d]	1.72[d]	1.82[d]	1.74[e]	1.62[e]	1.33[d]	1.63[e]
Mean	1.66[F]	2.73[E]	2.84[E]	3.14[D]	3.49[C]	3.69[B]	4.02[A]	3.43[C]	

* Mean of three replications
In the column, means followed by a common letter are not significantly different at 5% level by DMRT

been reported against grapevine powdery mildew by Rajeswari (2002). Nallathambi *et al*. (2003) depicted 77.28% ber powdery mildew disease control efficacy with field spray of bacterial suspension of a native isolate of *P. fluorescens* (CIAH-196) suggesting the tremendous potential of the isolate for the management of ber powdery mildew.

Thangavelu *et al*. (2003) reported increase in phenolic content in leaf tissues after *P. fluorescens* strain *Pf*10 treatment in banana. Meena *et al.* (2000) observed increase in phenol content in groundnut plants treated with *P. fluorescens* which gave resistance to late leaf spot disease. Sivakumar and Sharma(2003) found increase in phenol when maize seeds were treated with *P. fluorescens.*

Chen *et al*. (2000) stated that the treatment of cucumber roots with *Pseudomonas corrugata* 13 or *P.aureofaciens* 63-28 increased PAL activity which conferred resistance against *Pythium aphanidermatum.* When maize seeds were treated with *P. fluorescens* and inoculated with *Rhizoctonia solani*, 6 days after inoculation increase in PAL activity was found in the leaf sheath (Sivakumar and Sharma, 2003). Ramamoorthy and Samiyappan (2001) reported that treatment of chilli plants with *P. fluorescens* challenge inoculated with *C. capsici* accelerated PPO activity. Earlier reports given by Chen *et al.* (2000) showed that treatments of cucumber roots with *P.corrugata* or *P.aureofaciens* 63-28 increased PPO activity after bacterization and inoculation with *Pythium aphanidermatum*. Sivakumar and Sharma (2003) showed increase in PPO activity 6 days after inoculation with *Rhizoctonia solani* in maize leaf sheath in plants raised from seeds treated with *P. fluorescens*. In the present study, two PPO isozymes *viz*., PPO1 and PPO2 with high degree of expression were observed in pre-inoculation spray of *P. fluorescens* I_{18} challenge inoculated with the pathogen. Induction of PPO isozymes by biological agent (*P. fluorescens*) in rice against *Rhizoctonia solani* has also been reported by Radja Commare (2000).

Kamalanayar (1996) studied biochemical constituents of rice plants raised from seeds treated with *P.fluorescens*. She found early and higher induction of PO activity due to *P. fluorescens* treatment. Isozyme analysis indicated expression of three PO isoforms, PO1, PO2 and PO3 in bhendi leaves and these were more prominent in pre-inoculation spray of *P. fluorescens* I_{18}. Padmaja *et al*. (2004) reported that cotton plants treated with biological inducer (*P. fluorescens*) induced an unique peroxidase '4' isozyme indicating the involvement of that isozyme in defense reaction. Furthermore, peroxidase itself was found to inhibit spore germination and mycelial growth of several plant pathogens (Joseph *et al*., 1998).Ramamoorthy and Samiyappan (2001) observed an increase in b-1,3–glucanase activity in *P. fluorescens* treated chilli plants challenge inoculated with *C. capsici.* Ramamoorthy and Samiyappan (2001) observed an increase in b-1,3–glucanase activity in *P. fluorescens* treated chilli plants challenge inoculated with *C. capsici.*

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Received : December, 2005; Accepted : April, 2006