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Induction of systemic resistance in green gram against leaf blight caused by *Macrophomina phaseolina* (Tassi.) Goid

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Studies were conducted to develop the sustainable plant protection strategies against leaf blight of green gram incited by *Macrophomina phaseolina* by using plant extracts and fungicides under *in vitro* and *in vivo* conditions. *In vitro* studies indicated that plant leaf extracts of *Adenocalymma alliaceum* (77.20%) and *Allium* sp. (74.71%) demonstrated the highest inhibition of mycelia growth of *M. phaseolina* at 10% concentration. The results revealed the significant performance by foliar spray of carbendazim (0.1%), mancozeb (0.2%) and leaf extracts of *A. alliaceum* (10%), *Allium* sp. (10%) was more effective in reducing leaf blight disease in mungbean plants under greenhouse conditions. Further, induction of defense enzymes, such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), phenols and also chlorophyll content were studied. The enzyme accumulation was greater in treated plants as compared to untreated control. The present study revealed the induced systemic resistance (ISR) in enhancing the disease resistance in green gram plants against leaf blight disease by the application of plant extracts and fungicides.

Key words: *Macrophomina phaseolina,* induced systemic resistance (ISR), *Adenocalymma alliaceum, Allium* sp.

INTRODUCTION

Pulses are rich in protein content (18 to 32%), which plays an important role in human and animal nutrition. Among pulses, green gram [*Vigna radiata* (L.) Wilczek] is an important pulse crop grown extensively in Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh and Tamil Nadu. India is a major pulse growing country of the world with an area and production of 22.47 Mha and 13.11 million tons, respectively, thus accounting for about one-third and one-fourth of the total area and production under pulses, respectively (Department of Economics and Statistics, 2008-09). During the same production year (2008-09), mungbean was grown on an area of 158.7 thousand ha with the production and productivity of 46.2 thousand tons and 291 kg/ha, respectively in Tamil Nadu.

Vidhyasekaran and Arjunan (1978) reported a new blight disease in mungbean caused by *Macrophomina phaseolina* (Tassi.) Goid in Tamil Nadu. It is an important seed-borne fungal pathogen causing different types of symptoms viz., seedling blight, root rot, charcoal rot, wilt,

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stalk rot, stem blight, fruit rot, seedling decay and leaf blight in crop plants. A number of plant species have been reported to possess natural substances that are toxic to many fungi causing plant diseases. The presence of antifungal compounds in higher plants has long been recognized as an important factor imparting resistance to diseases (Mahadevan, 1982; Lyon et al., 1995).

Induced resistance may provide an alternative approach to plant protection especially for problems not satisfactorily controlled by various fungicides (Schoenbeck 1996). Induced systemic resistance (ISR) is defined by the systemic protection of plants by the enhancement of the plant's defensive capacity against a broad spectrum of pathogens that is acquired after appropriate inducing of infection by a pathogen. Induced systemic resistance (ISR) activates multiple defense mechanisms that include increased activity of pathogenesis related (PR) proteins like peroxidase (PO) (Maurhofer et al., 1994), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), phenols and also chlorophyll contents. Although, several effective pesti-cides have been recommended for use against this pathogen, they are not considered to be long-term solutions due to concerns of expense, exposure risks, fungicide residues, toxicity to non-target organisms and other health and environmental hazards. Therefore, recent efforts have been focused on developing eco-friendly safe, long lasting and effective against many plant pathogens for the management of plant diseases.

The present study was aimed at induction of various defense related enzymes by plant products and fungicides in response to infection by the pathogen.

MATERIALS AND METHODS

Plant materials and pathogens

The seeds of green gram (CO-3) were obtained from the Department of Pulses, Tamil Nadu Agricultural University (TNAU), Coimbatore, India. The pathogen was isolated from the green gram leaves showing typical leaf blight symptoms of *Macrophomina* by using potato dextrose agar (PDA) medium and it was identified as *M. phaseolina*.

Preparation of plant extracts

Plant extracts were prepared as described by Shekhawat and Prasada (1971). Fresh leaves of thirty different plant species were collected in and around Tamil Nadu Agricultural University campus and used for extraction. The leaves were first washed with distilled water and finally with sterile water, ground in a pestle and mortar by adding sterile water at the ratio of 1:1 (w/v). The macerate was squeezed using cotton wool to get the extract. The extracts were filtered through two layers of muslin cloth, through Whatman No.1 filter paper and finally through the bacteriological filter. This formed the standard plant extract solution (100%). This was further diluted with the sterile distilled water (v/v) to have the required 20% concentration.

Efficacy of plant extracts against *M. phaseolina* under *in vitro* conditions

The agar-well diffusion method was used to determine the antimicrobial activity of the extracts (Russell and Fur, 1977; Irobi et al., 1996). The potato dextrose agar (PDA) medium was poured into sterilized plates and allowed to cool. The wells were formed in the Petri plates on four sides using cork borer. Then, the 20% plant extract (100 µl) was delivered into wells (8mm diameter) of the already seeded PDA medium. The plates were inoculated with *M. phaseolina*. The diameter of the mycelial colony was measured 10 days after inoculation. The medium without plant extracts served as control and the mean percentage of inhibition was worked out.

Green house studies

Preparation of fungal spore suspension (leaf blight) and inoculation

The spore suspension was prepared by adding 20 ml of sterile distilled water to the Petri plates containing leaf blight culture and this was mixed well and filtered through muslin cloth. The spore suspension was used to spray onto the green gram plants. A pot culture study was conducted with greengram (variety CO-3) for management of *Macrophomina* blight with the effective plant extracts and fungicides. The plant extracts and fungicides which proved effective under laboratory condition in inhibiting the mycelial growth were selected for the scheduled spray.

Seeds of greengram variety CO-3 (10 seeds/plot) were sown in pots containing garden soil and sand in 3:1 ratio. The plants were inoculated with suspension of *Macrophomina phaseolina* on healthy leaves after 20 days of sowing.

The treatment spray was given after 7 days of pathogen inoculation. Three replications were maintained in each treatment; each replicate consisted of six pots. The experiments were conducted using randomized block design on a greenhouse bench. Leaves from sprayed and unsprayed plants were collected at 0, 1, 3, 5 and 7 days intervals.

Enzyme extraction

The leaf tissues collected from plants were immediately homogenized with liquid nitrogen. One gram of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1 M (pH 7.0) at 48°C. The homogenate was centrifuged for 20 min at 8000 rpm. Protein extract prepared from leaves were used for the estimation of PO, PPO, PAL and phenol.

Spectrophotometric assay

Peroxidase (PO)

PO activity was assayed spectrophotometrically (Hartee, 1955). The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract and 0.5 ml of 1% H_2O_2. The reaction mixture was incubated at room temperature (28 ± 1°C). The change in absorbance at 420 nm was recorded at 30 s interval for 3 min and the boiled enzyme preparation served as blank. (Hammerschmidt et al., 1982).

Polyphenoloxidase (PPO)

PPO activity was determined according to the procedure of Mayer

et al. (1965). The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5) and 20 ml of the enzyme extract. To start the reaction, 200 ml of 0.01 M catechol was added and the the absorbance was read at 495 nm.

Phenylalanine ammonia lyase (PAL)

The PAL assay was conducted as per the method described by Ross and Sederoff (1992). The assay mixture containing 100 ml of enzyme, 500 ml of 50 mM Tris HCl (pH 8.8) and 600 ml of 1mM L-phenylalanine was incubated for 60 min. The reaction was arrested by adding 2 N HCl. Later 1.5 ml of toluene was added, vortexed for 30 s, centrifuged (1000 rpm, 5 min) and toluene fraction containing trans-cinnamic acid was separated. The toluene phase was measured at 290 nm against the blank of toluene. A standard curve was drawn with graded amounts of cinnamic acid in toluene as described by Ross and Sederoff (1992).

Total phenols

The content of the total phenols present in the leaves was estimated following the procedure of Bray and Thorpe (1954). Fresh leaf samples of 0.5 g weight were blended with 10 ml of 80% ethanol and boiled at 50°C for 30 min. The extracts were filtered through cheese cloth and then through Whatman No. 41 filter paper and centrifuged at 8000 rpm for 10 min. The volume was made up to 10 ml with ethanol. An of one ml was taken in a series of boiling tubes and made up to 3 ml with distilled water. To this, one ml of Folin Ciocalteu reagent and two ml of 20% sodium carbonate were added. The tubes were heated for one min in a boiling water bath and cooled in running water. The solution was diluted to 10 ml with distilled water and the intensity of the blue colour was measured at 660 nm in a spectrophotometer against a blank (a blank was maintained with 3 ml of distilled water instead of the extract and the colour was developed as described above) for which three replications were maintained. Catechol was used for preparing the standard graph from which the amount of phenol in the given sample was calculated. All the enzyme activities and the content of total phenols were expressed as catechol/g of fresh tissue.

Chlorophyll

The samples were collected at 24 h and 72 h after last spraying under pot culture experiment. The leaves collected on respective time intervals were used for analysis of chlorophyll. Total chlorophyll contents were estimated by the procedure of Yoshida et al. (1971). About 500 mg of leaf samples were weighed and ground with 10 ml of acetone (80%) in a pestle and mortar. It was centrifuged at 8000 rpm for 10 min. The supernatant solution was collected, made upto 25 ml using acetone (80%) and read at 652 nm for total chlorophyll contents. The 80% acetone was used as blank. Total chlorophyll contents were expressed as mg g^{-1} of fresh weight.

Statistical analysis

The data were statistically analyzed (Gomez and Gomez, 1984) and the treatment means were compared by Duncan's Multiple Range Test (DMRT). The package used for analysis was IRRI-Stat version92-a developed by International Rice Research Institute Biometrics Units, The Philippines.

RESULTS

Efficacy of plant extracts against *M. phaseolina* under *in vitro* conditions

Cold water extracts of 30 plant species were screened against the leaf blight pathogen, *M. phaseolina*. Only two plant extracts, zimmu (*Allium cepa* L. and *Allium sativum* L.) and garlic creeper (*Adenocalymma alliaceum*) were found to be toxic to the fungus (Table 1) and were significantly superior (P = 0.05) to other plants extracts in reducing the colony growth of *M. phaseolina*. This clearly indicated that the botanicals (garlic creeper and zimmu) have antifungal property.

Evaluation of fungicides and plant extracts against *M. phaseolina* under greenhouse conditions

Fungicides, carbendazim (0.1%) and mancozeb (0.2%), leaf extracts of *A. alliaceum* (10%) and *Allium* sp. (10%) were tested for their efficacy against *M. phaseolina.* All the treatments significantly reduced the percent disease index (PDI) (by 37 to 94%) as compared to untreated controls, and also increased the yield (by 8 to 14 g/plant) (Table 2).

Activity of defense-related enzymes

The study of disease resistance in green gram plants treated with fungicides and plant extracts revealed the higher activity against leaf blight pathogen. The induction of PO, PPO, PAL and total phenol contents in the fungicide (carbendazim 0.1% and mancozeb 0.2%) and leaf extracts of *A. alliaceum* (10%) and *Allium* sp. (10%) treated plants gradually increased from the first day of inoculation, reached its maximum on the fifth day and declined on the seventh day after inoculation (Tables 3, 4, 5 and 6). In all treatments, foliar application of fungicides (carbendazim 0.1% and mancozeb 0.2%) and leaf extracts of *A. alliaceum* (10%) and *Allium* sp. (10%) treated plants recorded the maximum total chlorophyll at 45 and 55 DAS recording 0.72 and 0.93 mg/g, respectively as against the healthy control which recorded 0.52 mg per g fresh weight of leaf tissue (Table 7).

DISCUSSION

In the present study, the tested plant extracts and fungicides showed antifungal activity against the growth of *M. phaseolina* under *in vitro* and *in vivo* conditions. There are reports that the zimmu leaf extract has antimicrobial activity against some fungal (*Aspergillus flavus*, *Curvularia lunata*, *Alternaria solani*) and bacterial (*Xanthomonas oryzae* pv. oryzae, *Xanthomonas*

Table 1. Antimicrobial activity of plant extracts against *M. phaseolina.*

Plants used to prepare extract (leaf)*	Radial mycelial growth (mm)	Percent inhibition over control
Eucalyptus (*Eucalyptus globulus*)	87.74[c] (9.36)	2.48
Vettukaya poodu (*Tridax procumbens* L.)	87.55[c] (9.35)	2.48
Vasambu (*Acorus calamus* L.)	87.85[c] (9.37)	3.81
Pepper mint (*Mentha piperata* L.)	86.55[c](9.30)	3.81
Spear mint (*Mentha spicataa*)	87.42[c](9.34)	2.84
Kalmegh (*Andrographis paniculata* Wall. Ex Nees)	84.23[c] (9.17)	6.39
Nerium (*Nerium oleander* L.)	87.42[c] (9.34)	2.84
Karisilakanni (*Eclipta alba*)	87.52[c] (9.35)	2.73
Garlic creeper [*Adenocalymma alliceum* (Lam.) Miers]	20.51[a] (4.52)	77.20
Euphorbia (*Euphorbia cyathopora*)	86.55[c] (9.30)	3.81
Zimmu *(Allium spp.)*	22.75[a] (4.76)	74.71
Long pepper *(Piperum longum L.)*	73.51[b] (8.57)	18.30
Keelanelli *(Phyllanthus emblica)*	86.12[c] (9.28)	4.28
Melanelli *(Phyllanthus niruri L.)*	86.51[c] (9.30)	3.80
Neem *(Azadirachta indica A.Juss)*	77.14[b] (8.78)	14.26
Indian mulberry *(Morinda citrifolia L.)*	89.53[c] (9.46)	0.50
Polyalthia *(Polyalthia longifolia Sonn.)*	88.12[c] (9.38)	2.06
Lantana *(Lantana camera L.)*	87.56[c] (9.35)	2.68
Butterfly pea *(Clittoria ternatea L.)*	89.42[c] (9.45)	0.62
Thorn apple *(Datura stramonium)*	87.12[c] (9.33)	3.17
Lemon grass *(Cympopogon flexuosus Spreng.*)	87.42[c] (9.34)	2.84
Solanum *(Solanum torvum L.)*	85.52[c] (9.24)	4.95
Thudhuvalai *(Solanum trilobatum L.)*	86.12[c] (9.28)	4.28
Tulsi *(Ocimum sanctum)*	85.25[c] (9.23)	5.25
Periwinkle (*Catharanthus roseus (L) G. Don*)	86.12[c] (9.24)	4.28
Seemai karuvel *(Prosopis juliflora)*	77.12[b] (8.78)	14.29
Notchi *(Vitex negundo L.)*	85.12[a] (9.22)	5.40
Sweet basil (*Ocimum basilicum* L.)	89.93[a] (9.48)	0.00
Bougainvillea (*Bougainvillea spectabilis*)	87.75[c] (9.36)	2.47
Coleus (*Coleus aromaticus* (Lour.) Spreng.)	86.23[c] (9.28)	4.16
Control	89.98[c] (9.48)	-

Figures in parentheses are square root transformed values, means followed by a common letter are not significantly different at P = 0.05 by DMRT, *1 g of leaf in 1 ml of double distilled water.

campestris pv. *malvacearum* and *Xanthomonas axonopodis* pv. citri) pathogens (Satya et al., 2005; Sandosskumar et al., 2007; Latha et al., 2009). The suppression of fungal growth by filtered extracts of *Allium* spp. might have occurred due to the presence of antifungal substances viz., methyl and n-propyl compounds (Virtane and Matikkala, 1959).

Similarly, Gautam et al. (2003) screened 24 species of family asteraceae against *M. phaseolina*. Maximum inhibition of *M. phaseolina* was caused by *Eclipta alba* followed by *Launea aspleniifolia*. Though spraying of fungicide is hazardous to health and environment, disease epidemics needs fungicidal spray to prevent the fast spread. Hence, biopesticides can be recommended when the disease is in the initial stage and when the climate is conducive for further increase a fungicidal spray can be recommended. Two sprays of carbendazim were highly effective against Macrophomina blight of green gram (Tiwari and Kotasthane, 1984). Carbendazim (Rathore, 2006) and benomyl act against castor blight (Das, 1988), mancozeb against leaf blight of bottle gourd (Vaishnav and Patel, 1992).

The present study suggests that the foliar application of fungicides and plant extracts may help to overcome the

Table 2. Green house evaluation of fungicides and plant extracts against leaf blight (*M. phaseolina*).

Treatment	Percent disease index (PDI)	Reduction (%)	Yield/plant (g)
Mancozeb (0.2%)*	5.33[a] (13.34)	94.09[a]	14.00[b] (3.87)
Carbendazim (0.1%)*	10.66[b] (19.05)	88.19[b]	13.50[c] (3.80)
Adenocalymma alliaceum (10%)**	56.66[d] (48.82)	37.28[d]	8.00[e] (3.00)
Allium sp. (10%)**	48.00[c] (43.85)	46.86[c]	8.50[d] (3.08)
Control (healthy)	0.00 (1.28)	0.00	14.95[a] (3.99)
Control (inoculated)	90.33[f] (72.13)	0.00	5.00[g] (2.44)

Mean of three replicates; figures in parentheses are square root transformed values. Means followed by a common letter are not significantly different at the 5% level by DMRT. *Single spray; **two spray.

Table 3. Changes in peroxidase activity in *M. phaseolina* challenged greengram plants under pot culture .

Treatment	Peroxidase activity (changes in absorbance/min/g fresh weight at 420 nm)				
	Days after inoculation				
	0	1	3	5	7
Mancozeb (0.2 %)	6.16[a]	8.50[a]	10.38[a]	9.59[b]	7.60[a]
Carbendazim (0.1 %)	6.28[a]	7.45[b]	8.68[b]	7.73[d]	6.20[b]
Adenocalymma alliaceum (10%)	5.92[a]	7.78[b]	8.14[c]	7.25[d]	6.14[b]
Allium sp. (10%)	5.91[a]	7.41[b]	9.87[a]	10.54[a]	8.05[a]
Control (healthy)	3.90[d]	4.00[e]	4.10[e]	4.32[e]	4.29[c]
Control (inoculated)	4.49[c]	4.55[d]	4.59[d]	4.60[e]	4.60[c]

Mean of three replicates; Means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4. Changes in polyphenol oxidase activity in *M. phaseolina* challenged green gram plants under pot culture.

Treatment	Polyphenol oxidase activity (changes in absorbance/min/g fresh weight at 470 nm)				
	Days after inoculation				
	0	**1**	**3**	**5**	**7**
Mancozeb (0.2%)	1.01[c]	1.39[a]	2.07[a]	3.27[a]	2.52[a]
Carbendazim (0.1%)	1.02[c]	1.37[a]	1.96[b]	2.09[b]	1.84[b]
Adenocalymma alliaceum (10%)	0.94[d]	1.13[b]	1.54[c]	1.96[b]	1.44[c]
Allium sp. (10%)	0.87[e]	0.93[c]	1.11[ef]	1.35[c]	1.02[de]
Control (healthy)	1.12[b]	1.15[b]	1.17[e]	1.18[d]	1.13[d]
Control (inoculated)	1.31[a]	1.32[a]	1.36[d]	1.37[c]	1.39[c]

Mean of three replicates; means followed by a common letter are not significantly different at the 5% level by DMRT.

pathogen infection by increasing levels of defense related enzymes, phenolic and chlorophyll substances. Green gram plants treated with fungicides and plant extracts showed maximum increases in PO, PPO, PAL, phenolics and also chlorophyll contents. The association between higher levels of defense related enzymes and greater disease resistance has been reported by several workers. Higher levels of PO, PPO, PAL and phenolics have been reported to be effective against various fungal diseases (Sundaramoorthy et al., 2012; Latha et al., 2009).

Table 5. Changes in phenylalanine ammonia lyase activity in *M. phaseolina* challenged greengram plants under pot culture.

Treatment	Polyphenol oxidase activity (Changes in absorbance/min/g fresh weight at 470 nm)				
	Days after inoculation				
	0	1	3	5	7
Mancozeb (0.2%)	0.40^{ab}	0.41^{ab}	0.42^{b}	0.38^{ab}	0.35^{a}
Carbendazim (0.1%)	0.42^{a}	0.43^{a}	0.45^{a}	0.36^{a}	0.32^{b}
Adenocalymma alliaceum (10%)	0.38^{b}	0.37^{b}	0.36^{c}	0.32^{b}	0.31^{b}
Allium sp. (10%)	0.32^{c}	0.35^{b}	0.37^{c}	0.32^{b}	0.31^{b}
Control (healthy)	0.25^{e}	0.26^{c}	0.27^{d}	0.23^{c}	0.22^{c}
Control (inoculated)	0.18^{f}	0.19^{d}	0.20^{d}	0.17^{d}	0.15^{d}

Mean of three replicates; means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 6. Changes in phenol content in *M. phaseolina* challenged greengram plants under pot culture.

Treatment	Phenol content (mg/g fresh weight of leaf tissue)				
	Days after inoculation				
	0	1	3	5	7
Mancozeb (0.2%)	2.32^{b}	2.10^{c}	2.21^{c}	2.81^{a}	2.60^{a}
Carbendazim (0.1%)	2.46^{a}	2.52^{a}	2.72^{a}	2.82^{a}	2.63^{a}
Adenocalymma alliaceum (10%)	2.01^{c}	2.30^{b}	2.42^{b}	2.51^{b}	2.26^{b}
Allium sp. (10%)	1.61^{d}	1.71^{d}	1.81^{e}	1.95^{c}	1.74^{c}
Control (healthy)	1.72^{d}	1.82^{d}	1.92^{d}	1.73^{e}	0.64^{c}
Control (inoculated)	1.02^{e}	1.14^{e}	1.24^{f}	1.30^{f}	1.15^{d}

Mean of three replicates; means followed by a common letter are not significantly different at the 5% level by DMRT.

Table 7. Changes in total chlorophyll content in *M. phaseolina* challenged green gram plants under pot culture.

Treatment	Total chlorophyll content (mg / g)		
	45 DAS*	55 DAS	Increase over healthy control
Mancozeb (0.2%)	0.67^{b}	0.85^{b}	0.33^{a}
Carbendazim (0.1%)	0.72^{a}	0.93^{a}	0.41^{a}
Adenocalymma alliaceum (10%)	0.59^{c}	0.75^{c}	0.23^{b}
Allium sp. (10%)	0.50^{d}	0.62^{d}	0.10^{b}
Control (healthy)	0.47^{d}	0.52^{e}	0.00
Control (inoculated)	0.40^{e}	0.43^{f}	0.00

*Days after sowing; mean of three replicates; means followed by a common letter are not significantly different at the 5% level by DMRT.

Conclusion

The spraying of plant extracts and fungicides was found to be effective in controlling the leaf blight caused by *M. phaseolina*. Higher levels of PO, PPO, PAL and phenols by plant extracts and fungicides may contribute collectively to induced resistance in green gram against *M. phaseolina*. The present study clearly indicated that plant extracts and fungicides showed the maximum effects on reduction of *M. phaseolina* under *in vitro* and greenhouse

conditions.

REFERENCES

Bray W, Thorpe V (1954). Analysis of phenolic compounds of interest in metabolism. Methods Biochem. Anal. 1:27-52.

Das ND (1988). Effect of different sources of carbon, nitrogen and temperature on the growth and sclerotial production of *Macrophomina phaseolina* (Tassi.) Goid causing root rot or charcoal rot disease in castor. Indian J. Plant Pathol. 6:97-98.

Gautam K, Rao PB, Chauhan SVS (2003). Efficacy of some botanicals of the family compositae against *Rhizoctonia solani* (Kuhn.). J. Mycol. Pl. Pathol. 33(2):230-235.

Gomez KA, Gomez AA (1984). Statistical Procedure for Agricultural Research. John Wiley and Sons, New York, NY, USA.

Hartee EF (1955). Haematin compounds. In: Peach K, Tracy M, editors. Modern methods of plant analysis. New York: Springer-Verlag. pp. 97-245.

Hammerschmidt R, Nuckles EM, Kuc J (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20:73-82.

Irobi ON, Moo-Young M, Anderson WA (1996). Antimicrobial activity of Annatto (Bixa orellana) extract. Int. J. Pharmacogn. 34: 87-90.

Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R (2009). Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. Biol. Control. 50: 85-93.

Lyon GP, Beglinski T, Newton AC (1995). Novel disease control compounds: The potential to immunize plants against infection. Plant Pathol. 44: 407-427.

Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G (1994). Induction of systemic resistance of tobacco to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHAO: Influence of the gacA gene and of pyoverdine production. Phytopathology 84:139-146.

Mahadevan A (1982). Biochemical aspects of plant disease resistance part preferred

inhibitory substances prohibition. Today and Tomorrow Printers and Publishers, New Delhi.

Mayer AM, Harel E, Shaul RB (1965). Assay of catechol oxidase, a critical comparison of methods. Phytochemistry 5:783-789.

Rathore BS (2006). Management of diseases of greengram with fungicides. J. Mycol. Pl. Pathol. 36(2):138-141.

Ross WW, Sederoff RR (1992) Phenylalanine ammonia lyase from loblolly Pine:

Purification of the enzyme and isolation of complementary DNA clone. Plant Physiol. 98:380-386.

Russell AD and Fur JR (1977). Antibacterial activity of a new chloroxylenol preparation containing ethylene diamine tetra acetic acid. J. Appl. Bacteriol. 43: 253-260.

Sandosskumar R, Karthikeyan M, Mathiazhagan S, Mohankumar M, Chandrasekar G, Velazhahan R (2007). Inhibition of Aspergillus flavus growth and detoxification of aflatoxin B1 by the medicinal plant zimmu (*Allium sativum* L. and *Allium cepa* L.). World J. Microb. Biot. 23: 1007-1014.

Satya VK, Radhajeyalakshmi R, Kavitha K, Paranidharan V, Bhaskaran R, Velazhahan R (2005). In vitro antimicrobial activity of zimmu (*Allium sativum* L.A- *Allium cepa* L.) leaf extracts. Arch. Phytopathology Plant Protect. 38: 185-192.

Schoenbeck F (1996) Induced resistance: Mechanisms and evaluation. In: Lyr H, Russel P, Sisler HD, editors. Modern fungicides and antifungal compounds. Andover, UK: Intercept Ltd. pp. 447-450.

Shekhawat PS, Prasada R (1971). Antifungal properties of some plant extracts and inhibition of spore germination. Indian Phytopath. 24:800-802.

Sundaramoorthy S, Raguchander T, Ragupathi N, Samiyappan R (2012). Combinatorial effect of endophytic and plant growth promoting rhizobacteria against wilt disease of capsicum annum L. caused by *Fusarium solani*. Biol. Control. (60) 59-67.

Tiwari A, Kotasthane SR (1984). Chemical control of fungal foliar diseases of mungbean. Indian Phytopath. 37:383.

Vaishnav MU, Patel VA (1992). Fungicidal control of *Macrophomina phaseolina* (Tassi.) Goid causing leaf blight of bottle gourd *in vitro* and *in vivo* conditions. p. 89.

Vidhyasekaran P, Arjunan G (1978). Studies on leaf blight of urdbean caused by *Macrophomina phaseolina*. Indian Phytopath. 31: 361-362.

Virtanen AI, Matikkala EJ (1959). The isolation of S-methylcysteine-sulphonxide and s-n-propylcysteine sulphoxide from onion (*Allium cepa*) and the antibiotic activity of crushed onion. Acta. Chem. Scand. 13:1898.

Yoshida S, Farno DA, Rock JH, Gomez KA (1971). Laboratory manual for physiological studies in rice. IRRI News Letter, p. 70.