Plant Signaling & Behavior 6:7, 919-923; July 2011; ©2011 Landes Bioscience

Induction of systemic resistance in rice by leaf extracts of *Zizyphus jujuba* and *Ipomoea carnea* against *Rhizoctonia solani*

Sateesh Kagale,[1,†,*] Thambiayya Marimuthu,[1] Jayashree Kagale,[3] Balsamy Thayumanavan[2] and Ramasamy Samiyappan[1]

[1]Centre for Plant Protection Studies; [2]Centre for Plant Molecular Biology; Tamil Nadu Agricultural University; Coimbatore, Tamil Nadu India; [3]Department of Biotechnology; Brindavan College; Bangalore University; Bangalore, Karnataka India

[†]Current Address: Saskatoon Research Centre; Agriculture and Agri-Food Canada; Saskatoon, SK Canada

Key words: sheath blight, *Zizyphus jujuba*, *Ipomoea carnea*, *Rhizoctonia solani*, induced systemic resistance, antimicrobial compounds

Plants accumulate a great diversity of natural products, many of which confer protective effects against phytopathogenic attack. Earlier we had demonstrated that the leaf extracts of *Zizyphus jujuba* and *Ipomoea carnea* inhibit the in vitro mycelial growth of *Rhizoctonia solani,* and effectively reduce the incidence of sheath blight disease in rice.[7] Here we demonstrate that foliar application of the aqueous leaf extracts of *Z. jujuba* and *I. carnea* followed by challenge inoculation with *R. solani* induces systemic resistance in rice as evident from significantly increased accumulation of pathogenesis-related proteins such as chitinase, β-1,3-glucanase and peroxidase, as well as defense-related compounds such as phenylalanine ammonia-lyase and phenolic substances. Thin layer chromatographic separation of secondary metabolites revealed presence of alkaloid and terpenoid compounds in the leaf extracts of *Z. jujuba* that exhibited toxicity against *R. solani* under in vitro condition. Thus, the enhanced sheath blight resistance in rice seedlings treated with leaf extracts of *Z. jujuba* or *I. carnea* can be attributed to the direct inhibitory effects of these leaf extracts as well as their ability to elicit systemic resistance against *R. solani*.

Sheath blight disease of rice, caused by *Rhizoctonia solani,* has become a major production constraint in intensive rice cropping systems where semi-dwarf, nitrogen-responsive and high-yielding rice cultivars are grown. The disease causes an annual yield loss of upto 50%.[1] *R. solani* is both soil- and water-borne, and can infect more than 27 families of both monocot and dicot species.[2] Natural host genetic resistance to *R. solani* has not been recorded in cultivars or wild relatives of rice.[3] Several broad spectrum fungicides have been recommended for control of sheath blight, however, chemical method of disease management is neither practical due to high cost of fungicides nor sustainable as it can affect the balance of ecosystem by destroying beneficial microbial population. In addition, the environmental pollution problems associated with indiscriminate use of synthetic pesticides have prompted investigations on exploiting bio-pesticides of plant and microbial origin.

Plants accumulate an enormous variety of over 100,000 secondary metabolites,[4] which can act as pre-existing chemical inhibitors to invading pathogens and/or help strengthen defense response of host plant. The pre-formed infectional barriers in plants are generally referred to as "phytoanticipins;" whereas, the antimicrobial compounds that are synthesized de novo in response to pathogen attack are referred to as "phytoalexins."[5] Because of years of selective breeding leading to removal of natural products, the endogenous levels of phytoanticipins in commonly cultivated crop species are generally low and often not sufficient to fight pathogen attack, effectively.[4] Various weed species and wild relatives of crop plants that are not subjected to selective breeding are believed to contain higher levels of antimicrobial compounds, consistent with their ability to fight invading pathogens more effectively than cultivated crop species. Identification of such weed/plant species that are enriched with antimicrobial principles, isolation of bio-active compounds from them, and application in the form of concentrated formulations to crop plants can augment their disease resistance capability by directly inhibiting the growth of pathogen and inducing defense responses. Indeed, the antimicrobial properties of tissue extracts of several weed/plant species have been reported by a number of research groups world-wide, especially in Asia and Latin America.[6-13]

Earlier, we had evaluated the antimicrobial activity of leaf extracts of 16 different plant species belonging to 16 different families and demonstrated that leaf extracts of most of these plant species exhibit growth-inhibitory activities against *R. solani* and *Xanathomoas oryzae* pv. *oryzae (Xoo)*.[7] Among these, the

*Correspondence to: Sateesh Kagale; Email: kagale.sateesh@gmail.com
Submitted: 01/27/11; Accepted: 02/27/11
DOI: 10.4161/psb.6.7.15304

leaf extracts of *Datura metel* were found to be the most effective in inhibiting the mycelial growth and sclerotia formation of *R. solani,* and the growth of *Xoo*, as well as in reducing the incidence of sheath blight and bacterial blight diseases caused by these pathogens, respectively, under greenhouse condition.[7] We further demonstrated that rice seedlings treated with leaf extracts of *D. metel* accumulated significantly higher levels of pathogenesis-related (PR) proteins and other defense related compounds following challenge inoculation with *R. solani* or *Xoo*.[7] Our attempts to identify biologically active compounds from *D. metel* revealed the presence of a withanolide compound "daturilin" that exhibited remarkable antibacterial activity against *Xoo*.[7]

Apart from *D. metel*, two other plants species, *Zizyphus jujuba* and *Ipomoea carnea,* were found to possess remarkable antifungal activity against *R. solani*.[7] *Z. jujuba* is a thorny rhamnaceous plant that is widely distributed in Europe and South-eastern Asia. *I. carnea* of convolvulaceae family, commonly known as morning glory, is a toxic weed found in abundance in India, Brazil, the United States and other countries.[14] Both of these plant species have allelopathic effect and are commonly used in folklore medicine for curing multiple diseases.[15-18] The aqueous and methanol leaf extracts of *Z. jujuba* and *I. carnea* have been found to be highly effective in reducing in vitro mycelial growth, and therefore, sclerotia production of *R. solani*.[7] In the greenhouse experiments, rice seedlings sprayed with leaf extracts of *Z. jujuba* and *I. carnea* exhibited 44 and 34% reduction in severity of sheath blight disease over the control, respectively.[7] While these findings are encouraging, the mechanisms by which the leaf extracts of *Z. jujuba* and *I. carnea* modulate defense responses in rice have not yet been explored.

Plants are endowed with defense genes which remain quiescent or are expressed at basal levels in healthy plants. Activation of defense genes results in induction of systemic resistance in host plant; this defense response, designated as induced systemic resistance (ISR), plays an important role in development of disease resistance.[19] The onset of ISR in plants correlates with accumulation of phytoalexins and increased activity of PR proteins such as chitinases, β-1,3-glucanases and peroxidases;[20-23] consequently, PR proteins are generally used as ISR markers.[19] The classical inducers of ISR include both biotic and abiotic factors, including disease causing microorganisms themselves,[24,25] plant growth promoting rhizobacteria,[22,26] chemicals[27,28] and natural plant products.[7,10,12,13,29,30] Plant products have been considered as one of the major groups of compounds that induce ISR. To date, extracts of at least a few plant species have been reported to contain allelopathic substances which can act as elicitors and induce systemic resistance in host plants resulting in reduction or inhibition of disease development.[7,10,12,13]

In the present study, with the objective of understanding the mechanisms of disease suppression by leaf extracts of *Z. jujuba* and *I. carnea,* we investigated their ability to induce ISR in rice by analyzing the activities of ISR markers including PR-proteins and other defense enzymes involved in phenylpropanoid metabolism. The changes in activities of chitinase, β-1,3-glucanase, peroxidase, phenylalanine ammonia-lyase (PAL) and phenolic compounds induced in rice seedlings that were elicited with leaf extracts (at 1:10 dilution; w/v) of *Z. jujuba* or *I. carnea* and infected with *R. solani* were analyzed, and compared to changes in non-elicited and uninfected seedlings. Rice seedlings that were both elicited with leaf extracts of *Z. jujuba* or *I. carnea* and infected with *R. solani* accumulated significantly higher levels (2–5-fold) of ISR markers as compared to non-elicited and/or uninfected seedlings (**Fig. 1**). About two-fold increase in activities of ISR markers was also observed in seedlings that were either infected but not elicited or elicited but not infected; however, this increase was significantly lower than the changes in seedlings that were both elicited and infected (**Fig. 1**). Although the activity of all ISR markers began to increase around or after 24 h post-infection, at least two distinct induction patterns were observed. For instance, the activities of chitinase and phenolic substances gradually increased to reach maximum levels at 164 h post-infection (**Fig. 1A and E**); whereas, the activities of β-1,3-glucanase, peroxidase and PAL reached maximum levels at 72 to 96 h post-infection and decreased thereafter (**Fig. 1B–D**). The leaf extracts of *Z. jujuba* were found slightly more effective in inducing ISR markers than the leaf extracts of *I. carnea*. There was no significant change in the activity of ISR markers in control seedlings sprayed with sterile distilled water (**Fig. 1**). Collectively, these results suggested that the leaf extracts of *Z. jujuba* and *I. carnea* have the ability to induce systemic resistance in rice seedlings infected with *R. solani*. The fungitoxicity of the leaf extracts of *Z. jujuba* and *I. carnea* [7] combined with their ability to elicit ISR is possibly responsible for low sheath blight disease incidence observed in rice seedlings treated with these leaf extracts.[7]

The in vitro antimicrobial and in vivo disease inhibitory effects of natural plant products are generally attributed to the allelopathic substances present in them. However, very few attempts have been made to purify and characterize active principles from bio-active natural plant products. We have previously identified a withanolide compound from leaf extracts of *D. metel* which exhibited antibacterial activity against *Xoo*.[7] Both *Z. jujuba* and *I. carnea* are rich source of secondary metabolites including alkaloids, terpenoids, flavonoids and phenolic compounds.[31-35] To determine the composition of bio-active ingredients within the leaf extracts of *Z. jujuba* and *I. carnea*, we performed thin layer chromatographic separation of alkaloid, terpenoid and phenolic compounds. The partially purified compounds, as reported in **Table 1**, were tested for antifungal activity against *R. solani.* Interestingly, two different possibly terpenoid (Rf value 0.358 and 0.446) and an alkaloid (Rf value 0.784) compounds from *Z. jujuba* exhibited antifungal activity against *R. solani* (**Table 1**); suggesting that at least a part of the bio-active ingredients in the leaf extracts is composed of terpenoid and alkaloid compounds. Further characterization of these bio-active terpenoid and alkaloid compounds from *Z. jujuba* is essential to determine their chemical composition.

In conclusion, our results together with several other reports in the literature have established that natural plant products possess antimicrobial substances that can inhibit the growth of the pathogens and augment disease resistance capability of plants by eliciting ISR in host plants. In the immediate future,

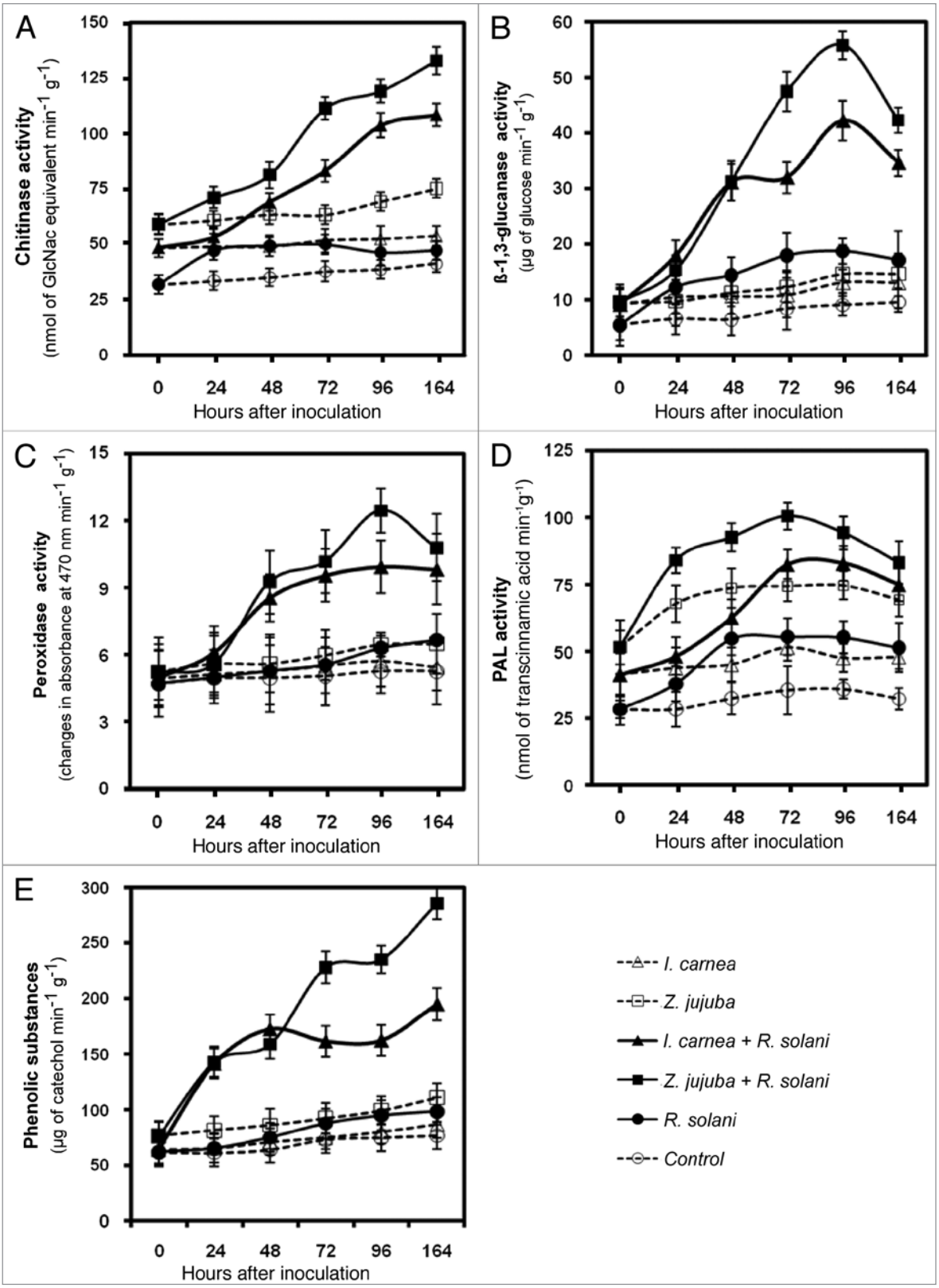

Figure 1. Activity of ISR markers and defense-related compounds in rice seedlings elicited with the leaf extracts of *Zizyphus jujuba* or *Ipomoea carnea* and challenge inoculated with *Rhizoctonia solani*. Total activity of chitinase (A), β-1,3-glucanase (B), peroxidase (C) phenylalanine ammonia-lyase (PAL; D) and phenolic substances (E) was analyzed in rice seedlings. The inoculation of rice seedlings with *R. solani* was performed 45 days after planting. Spraying of leaf extracts (1:10 dilution; w/v) of *Z. jujuba* or *I. carnea* was performed two days prior to inoculation. Tissue samples (sheath) from elicited and/or infected seedlings were collected for analysis at various time intervals.

Table 1. Thin layer chromatographic separation of secondary metabolites from leaf extracts of *Zizyphus jujuba* and *Ipomoea carnea*

Leaf extract	R_f value				Anti-fungal activity against *R. solani**
	Visible	Iodine vapors	UV-light	Spray reagent	
Phenolic substances[1]					
Z. jujuba	0.696	0.696	-	0.696	-
I. carnea	-	0.807	-	0.807	-
Terpenoid compounds[2]					
Z. jujuba	-	-	-	0.189	-
	0.358	0.358	0.358	0.358	5.1 mm
	-	-	-	0.446	3.7 mm
I. carnea	-	0.590	0.590	0.590	-
Alkaloid compounds[3]					
Z. jujuba	-	0.784	-	0.784	5.1 mm
I. carnea	-	0.806	-	0.806	-

*Inhibition zone diameter (mm) as mean of triplicate tests. [1]Solvent-acetic acid:chloroform (1:9); Spray reagent-Diazotised sulphanilic acid. [2]Solvent-methanol:chloroform (2:9); Spray reagent-10% vanillin-sulphuric acid. [3]Solvent-methanol:chloroform (1:1); Spray reagent-Drag endorffs reagent.

identification and characterization of additional novel bio-active compounds from natural plant products is essential for developing commercial formulations of potential use in controlling pathogenic diseases in crop plants.

Rice cultivar, IR-50 (susceptible to sheath blight) and virulent isolate of *R. solani* (RS7 Anastamosis group AG1),[36] were used in all experiments. The leaf tissues of *Z. jujuba* and *I. carnea* were collected from local areas around Coimbatore, India and aqueous extracts were prepared, as described previously in reference 7. Forty-five-day-old rice seedlings were sprayed with either aqueous leaf extracts (1:10 dilution) or sterile distilled water, two-days prior to inoculation with sclerotia of *R. solani*.[37] Sheath tissues from infected seedlings were collected at various time intervals, including 0, 24, 48, 72, 96 and 164 h after pathogen inoculation. The changes in the chitinase and peroxidase activities were determined by colorimetric assays, as described previously by Boller and Mauch,[38] and Hammerschmidt et al.[39] respectively. β-1,3-glucanase activity was assayed by the laminarin-dinitrosalicylic acid method.[40] PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm as described by Dickerson et al.[41] The amount of trans-cinnamic acid synthesized was calculated using its extinction coefficient of 9,630 M^{-1}. Estimation of phenolic substances was carried out as described previously in reference 7.

TLC was carried out on 20 x 20 cm glass plate coated with 0.5 mm thickness silica gel. Twenty microliters of *Z. jujuba* and *I. carnea* leaf extracts (1 g/ml) were spotted on each plate. The mixture of solvents comprising acetic acid:chloroform (1:9), methanol:chloroform (2:9) or methanol:chloroform (1:1) were used to develop the chromatograms for detection of phenolic, terpenoid or alkaloid compounds, respectively. The developed chromatograms were observed under visible, UV light and after exposing to iodine vapours. Additionally, the chemical class specific visualization spray reagents were used for detection of phenolic substances (Diazotized sulphanilic acid), terpenoids (10% vanillin-sulphuric acid) and alkaloids (Dragendorffs reagent). Preparative TLC was carried out using 2 mm thickness silica gel. The Rf value of each spot detected on the chromatogram was recorded. The silica gel corresponding to each spot was scraped off and the chemical compound was eluted using sterile water. The eluted compound was tested for its antimicrobial activity using the inhibition zone technique.[42]

Acknowledgments

We thank Dr. R. Nandakumar for helpful discussions on induced systemic resistance in plants and analysis of the activity of ISR markers. S.K. was recipient of Indian Council of Agricultural Research-Junior Research Fellowship (ICAR-JRF) for postgraduate study.

References

1. Savary S, Willocquet L, Elazegui FA, Teng PS, Pham Van D, Zhu D, et al. Rice pest constraints in tropical Asia: characterization of injury profiles in relation to production situations. Plant Dis 2000; 84:341.
2. Roy AK. Sheath blight of rice in India. Indian Phytopath 1993; 46:197-205.
3. Bonman JM, Khush GS, Nelson RJ. Breeding rice for resistance to pests. Annu Rev Phytopathol 1992; 30:507-28.
4. Dixon RA. Natural products and plant disease resistance. Nature 2001; 411:843-7.
5. VanEtten HD, Mansfield JW, Bailey JA, Farmer EE. Two Classes of Plant Antibiotics: Phytoalexins versus "Phytoanticipins." Plant Cell 1994; 6:1191-2.
6. Harish S, Saravanakumar D, Radjacommare R, Ebenezar EG, Seetharaman K. Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. BioControl 2008; 53:555-67.
7. Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R. Antimicrobial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. Physiol Mol Plant Pathol 2004; 65:91-100.
8. Yarmolinsky L, Zaccai M, Ben-Shabat S, Mills D, Huleihel M. Antiviral activity of ethanol extracts of *Ficus binjamina* and *Lilium candidum* in vitro. N Biotechnol 2009; 26:307-13.
9. Daayf F, Schmitt A, Belanger RR. Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of *Reynoutria sachalinensis*. Plant Physiol 1997; 113:719-27.
10. Cavalcanti FR, Resende MLV, Carvalho CPS, Silveira JAG, Oliveira JTA. Induced defence responses and protective effects on tomato against *Xanthomonas vesicatoria* by an aqueous extract from *Solanum lycocarpum* infected with *Crinipellis perniciosa*. Biol Control 2006; 39:408-17.
11. Chaijuckam P, Davis RM. Efficacy of natural plant products on the control of aggregate sheath spot of rice. Plant Dis 2010; 94:986-92.

12. Godard S, Slacanin I, Viret O, Gindro K. Induction of defence mechanisms in grapevine leaves by emodin- and anthraquinone-rich plant extracts and their conferred resistance to downy mildew. Plant Physiol Biochem 2009; 47:827-37.
13. Latha P, Anand T, Ragupathi N, Prakasam V, Samiyappan R. 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 2009; 50:85-93.
14. Austin DF, Huaman Z. A synopsis of Ipomoea (Convolvulaceae) in the Americas. Taxon 1996; 45:3-38.
15. Erenmemisoglu A, Kelestimur F, Koker AH, Ustun H, Tekol Y, Ustdal M. Hypoglycaemic effect of *Zizyphus jujuba* leaves. J Pharm Pharmacol 1995; 47:72-4.
16. Souleles C, Shammas G. Flavonoids from the leaves of *Zizyphus jujuba*. Fitoterapia 1988; 59:154.
17. Al-Reza SM, Bajpai VK, Kang S. Antioxidant and antilisterial effect of seed essential oil and organic extracts from *Zizyphus jujuba*. Food Chem Toxicol 2009; 47:2374-80.
18. Jain R, Jian N, Jain S. Evaluation of anti-inflammatory activity of ipomoea fistulosa. Asian J Pharmaceut Clin Res 2009; 2:64-7.
19. Heil M, Bostock RM. Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann Bot 2002; 89:503-12.
20. Dalisay RF, Kuc JA. Persistence of reduced penetration by *Colletotrichum lagenarium* into cucumber leaves with induced systemic resistance and its relation to enhanced peroxidase and chitinase activities. Physiol Mol Plant Pathol 1995; 47:329-38.
21. Xue L, Charest PM, Jabaji-Hare SH. Systemic induction of peroxidases, 1,3-beta-glucanases, chitnases and resistance in bean plants by binucleate Rhizoctonia species. Phytopathol 1998; 88:359-65.
22. Schneider S, Ullrich WR. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. Physiol Mol Plant Pathol 1994; 45:291-304.
23. Peer Rv, Niemann GJ, Schippers B. Induced resistance and phytoalexin accumulation in biological control of *Fusarium wilt* of carnation by Pseudomonas sp. strain WCS417r. Phytopathol 1991; 81:728-34.
24. Kombrink E, Schroder M, Hahlbrock K. Several "pathogenesis-related" proteins in potato are 1,3-beta-glucanases and chitinases. Proc Natl Acad Sci USA 1988; 85:782-6.
25. Chittoor JM, Leach JE, White FF. Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv. *oryzae*. Mol Plant-Microbe Interact 1997; 10:861-71.
26. Liu L, Kloepper JW, Tuzun S. Induction of systemic resistance in cucumber by plant growth-promoting rhizobacteria: duration of protection and effect of host resistance on protection and root colonization. Phytopathol 1995; 85:1064-8.
27. Boller T, Gehri A, Mauch F, Vogeli U. Chitinase in bean leaves: induction by ethylene, purification, properties and possible function. Planta 1983; 157:22-31.
28. Uknes S, Mauch-Mani B, Moyer M, Potter S, Williams S, Dincher S, et al. Acquired resistance in Arabidopsis. Plant Cell 1992; 4:645-56.
29. Sneh N, Balasubrahmanyam A, Sadhna P, Kapoor HC, Lodha ML. A systemic resistance inducing antiviral protein with N-glycosidase activity from *Bougainvillea xbuttiana* leaves. Indian J Exp Biol 2001; 39:600-3.
30. Kumudini BS, Shetty HS. Association of lignification and callose deposition with host cultivar resistance and induced systemic resistance in pearl millet to *Sclerospora graminicola*. Aust Plant Pathol 2002; 31:157-64.
31. Haraguchi M, Gorniak SL, Ikeda K, Minami Y, Kato A, Watson AA, et al. Alkaloidal Components in the Poisonous Plant, *Ipomoea carnea* (Convolvulaceae). J Agric Food Chem 2003; 51:4995-5000.
32. Tripathi M, Pandey MB, Jha RN, Pandey VB, Tripathi PN, Singh JP. Cyclopeptide alkaloids from *Zizyphus jujuba*. Fitoterapia 2001; 72:507-10.
33. Lee SM, Min BS, Lee CG, Kim KS, Kho YH. Cytotoxic triterpenoids from the fruits of *Zizyphus jujuba*. Planta Med 2003; 69:1051-4.
34. Patel AK, Singh VK, Moir AJ, Jagannadham MV. Biochemical and spectroscopic characterization of morning glory peroxidase from an invasive and hallucinogenic plant weed *Ipomoea carnea*. J Agric Food Chem 2008; 56:9236-45.
35. Nazif NM. Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity. Food Chem 2002; 76:77-81.
36. Sriram S, Raguchander T, Vidhyasekaran P, Muthukrishnan S, Samiyappan R. Genetic relatedness with special reference to virulence among the isolates of *Rhizoctonia solani* causing sheath blight in rice. Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 1997; 104:260-71.
37. Nandakumar R, Babu S, Viswanathan R, Raguchander T, Samiyappan R. Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol Biochem 2001; 33:603-12.
38. Boller T, Mauch F. Colorimetric Assay for Chitinase. Meth Enzymol 1988; 161:430-5.
39. Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol 1982; 20:73-82.
40. Pan SQ, Ye XS, Kuc J. A technique for detection of chitinase, beta-1,3-glucanase and protein patterns after a single separation using polyacrylamide gel electrophoresis or isoelectrofocusing. Phytopathol 1991; 81:970-4.
41. Dickerson DP, Pascholati SF, Hagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia-lyase and hydroxycinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol plant pathol 1984; 25:111-23.
42. Nene YL, Thapliyal PN. In: Fungicides in plant disease control. New Delhi: Oxford and IBH Publishing Co. 1979:415-6.