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Lactuca sativa mediated chitinase activity and resistance in pearl millet against *Sclerospora graminicola*

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Pearl millet seeds were treated with latex extracts (L1- 50 µl, L2- 100 µl and L3- 200 µl) of *Asclepias curassavica, Calotropis gigantea*, *Croton* sp., *Eucalyptus alba*, *Morus alba*, *Nerium odoratum, Lactuca sativa* and *Tridax procumbens* were applied on pearl millet seeds for 3 and 6 h to evaluate their role in growth promotion and downy mildew resistance. Latex extracts of all tested plants improved vegetative parameters when compared with the control. *L. sativa* (L2) at 3 h showed 38 and 18% increase in seed germination and vigor, respectively, over the control. Plant height, shoot fresh and dry weight, number of tillers and leaf surface area also increased upon latex treatment. Green house studies indicated that *L. sativa* required four days after challenge inoculation to build up maximum resistance and offered 54% protection against downy mildew. Resistance induction was evident with increased activities of phenylalanine ammonia lyase (PAL) (four fold), peroxidase (POX) (three fold) and chitinase (three fold) in *L. sativa* (L2) treated seedlings challenged with the pathogen as compared to control seedlings.

Key words: Downy mildew, induced resistance, *Lactuca sativa,* pearl millet, plant extracts, *Sclerospora graminicola.*

INTRODUCTION

The knowledge on biochemical basis of resistance has become a major area of investigation to understand the possible defense mechanisms during pathogen infection. The disease resistance in plants is acquired by conventional fungicides and during recent years, eco-friendly approaches using biotic agents have been carried out in an attempt to build up durable resistance in host plants. Among the new biological approaches, induction of plant's own defense system is considered as one of the most promising alternative strategy for crop protection (Heil and Bostock, 2002; Walters et al., 2005; Anderson et al., 2006; Walters and Fountaine, 2009). Numerous studies have shown the appearance of defense responses against several important plant diseases (Abo-Elyousr and El-Hendawy, 2008).

Various studies have focused on developing eco-

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friendly, long lasting and effective biocontrol methods such as use of plant products (Latha et al., 2009; Modafar et al., 2012) and biocontrol agents for the management of plant diseases (Alizadeh et al., 2012; Chowdappa et al., 2013). Microbial biocontrol agents (Naher et al., 2012; Senthilraja et al., 2013) and plant extracts (Amadioha, 2003; Bowers and Locke, 2004; Nisha et al., 2012; Pal et al., 2013; Sangeetha et al., 2013) have been found efficient against a wide range of pathogens. Numerous plant extracts have been explored as natural resistance inducers like *Azadirachta indica* against *Alternaria* leaf spot (Guleria and Kumar, 2006), *Datura metel* against *Rhizoctonia solani*, *Xanthomonas oryzae* and *Alternaria solani* (Kagale et al., 2004; Latha et al., 2009).

Latex is a stable dispersion of naturally occurring polymer of micro particles in an aqueous medium. It is also rich in enzymes like proteases, glucosidases, chitinases and lipases. It has been proved to be a source of natural fungicides (Barkai-Golan, 2001) which is regarded as safe and effective against various diseases of banana, papaya and other fruits. Latex extracted from several plants has shown a strong antifungal activity against *Botrytis cinerea*, *Fusarium* sp. and *Trichoderma* sp. (Barkai-Golan, 2001) and evidences concerned with likely participation of laticifer proteins (LP) in the plant defense mechanisms (Agrawal and Konno, 2009) are available. Though widespread studies have been done on isolation and characterization of different proteins in latex, restricted information exists on the use of latex as elicitor in inducing resistance against phytopathogens.

Based on the above reports we hypothesized that latex extract derived from latex plants might contain certain metabolites which can effectively manage pearl millet downy mildew disease in an eco-friendly manner. Pearl millet (*Pennisetum glaucum* [L.] R. Br) is one of the important crop (D'Andrea et al., 2001) cultivated in about 25 million ha (Naylor et al., 2004) in semi-arid tropical zone of the world. India alone accounts for 11.2 million ha with a total annual production of 7 million tonnes (Khairwal et al., 2007). The crop is a staple cereal of 90 million people and also used as fodder (Singh and Shetty, 1990). Downy mildew is caused by *Sclerospora graminicola* resulting in high economic losses which can be attributed to disease development at early stages, poor tillering and ear-head malformation (Singh and Shetty, 1990). The disease was known in most of the pearl millet-growing regions, but remained sporadic until the high-yielding hybrids with susceptible parental lines were introduced (Singh, 1995). A number of new resistant varieties and hybrids developed have turned into highly susceptible owing to breakup of host resistance and continuous change in pathogen's nature (Chaudhry et al., 2001). This has drawn the attention of pearl millet growers (Ball, 1983) in search of new line of resistance factors against this devastating disease.

The growing demand on isolation of bioactive molecules from different parts of the plant, paved way for the use of latex and its utility in inducing disease resistance in plants. However, limited information on the role of the latex in induction of resistance in plants is available. An attempt has been made in the present study to evaluate some latex-producing plants for their ability to induce systemic resistance in pearl millet against downy mildew disease. Further study is required to determine the nature of the factors controlling the induced resistance.

MATERIALS AND METHODS

Collection of seed samples

Seeds of pearl millet cultivar cv. 7042S highly susceptible to downy mildew pathogen were obtained from All India Co-ordinated Pearl Millet Improvement Project (AICPMIP), Agricultural Research Station, Mandore, Rajasthan, India and were used throughout the study.

Pathogen and inoculum preparation

The downy mildew pathogen *S. graminicola* maintained on its susceptible host 7042S under greenhouse conditions was used as a source of inoculum throughout the study. The leaves of pearl millet infected with downy mildew disease were collected in the evening hours. The collected leaf samples were washed under running tap water to remove the remnants of sporulation and dust particles, they were blot dried, cut into 1-2 cm pieces and placed on Petri dishes lined with moist blotters and incubated over night. Next day, fresh sporangia were harvested into sterile distilled water (SDW) and the concentration of sporangia was adjusted to 4×10^4 zoospores/ml in SDW using haemocytometer which served as inoculum for all the experiments (Safeeulla, 1976).

Plant material and latex collection

Latex yielding plants viz., *Asclepias curassavica*, *Calotropis gigantea* (Asclepiadaceae), *Croton* sp. (Euphorbiaceae), *Eucalyptus alba* (Myrtaceae), *Morus alba* (Moraceae), *Nerium odoratum* (Apocynaceae), *Lactuca sativa* and *Tridax procumbens* (Asteraceae) were collected in and around Mysore District. The plant materials were washed under running tap water, blot dried and small downward v-shaped incisions were made at the base of the stem to collect the latex extracts (droplets). 50, 100 and 200 µl latex extracts (L1, L2 and L3) of each plant were immediately mixed with 10 ml of methanol containing 1% phosphoric acid (Sessa et al., 2000). The latex extracts (L1, L2 and L3) of each plant were evaporated to dryness and suspended in 10 ml sterile distilled water (SDW) which served as inducer for seed treatment.

Seed treatment

Pearl millet seeds of 7042S cultivar were surface sterilized with 0.02% mercuric chloride for 5 min and thoroughly washed for 2-3 times in SDW. After surface sterilization, the seeds were soaked in inducers (L1, L2 and L3) prepared as mentioned above and then incubated at 28 ± 2°C in an incubator rotary shaker at 100 rpm (rotation per minute) for 3 and 6 h, respectively. After incubation,

the seeds were air-dried aseptically. Seeds soaked in SDW served as control.

Effect of latex extracts on seed germination and seedling vigor

Germination test was carried out by paper towel method (ISTA, 2003). The latex extracts treated (L1, L2 and L3) seeds were placed on moist germination sheets equidistantly and another presoaked paper towel was placed on the first one in order to hold the seeds in position, rolled and wrapped with polythene to prevent drying and incubated for seven days at 25 ± 2°C. After seven days of incubation, seed germination and seedling vigor were analyzed (Abdul Baki and Anderson, 1973). The experiment consisted of four replicates of 100 seeds in all the treatments and repeated thrice.

$$\text{Percent germination} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds planted}} \times 100$$

Vigor Index = (Mean root length + Mean shoot length) × Germination (%)

Effect of latex extracts on growth parameters of pearl millet under greenhouse conditions

Based on percent germination results, the L2 treatments of all the latex extracts for 3 h time duration was continued for further studies. Evaluation of growth promotion under greenhouse conditions was carried out in pearl millet cv. 7042S seeds treated with L2 treatments for 3 h time duration. After treatment, the seeds were blot-dried and sown in earthen pots (9 x 12 cm) filled with sand, soil and manure in the ratio of 1:2:1. The experiment consisted of four replicates per treatment with 10 pots in each replicate (10 seeds/ pot) and repeated thrice. The pots were maintained at 25 ± 2°C with 95% RH (relative humidity) and watered regularly. Seeds treated with SDW served as control. 30 days after sowing, seedling height, fresh and dry weight of shoot, leaf surface area and number of basal tiller per plant were measured and recorded accordingly.

Effect of latex extracts on pearl millet-downy mildew disease under greenhouse conditions

The pearl millet seeds primed with latex extract and distilled water treatments for 3 h time duration were sown in earthen pots filled with autoclaved soil (1:2:1 ratio of sand, soil and manure). Leaf-whorls of two-day old seedlings were inoculated with a suspension of 4 x 10^4 zoospores/ml of *S. graminicola* by whorl inoculation method (Singh and Gopinath, 1985). Each treatment consisted of four replicates of 10 pots per replication with 10 seedlings per pot of 14 inch diameter. The pots were arranged in a randomized complete block design (RBD), maintained under greenhouse conditions (90 to 95% RH, 20 to 27°C). Plants were observed daily and were rated diseased when they showed any one of the typical symptoms of downy mildew, that is, sporulation, chlorosis, stunted growth or malformation of the earheads. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of downy mildew disease. The experiment was repeated thrice with four replicates of 100 plants each. Downy mildew disease protection was calculated using the formula:

$$\text{Downy mildew disease protection} = \frac{C-T}{C} \times 100$$

Where, C, is percent downy mildew disease incidence in control; T-

percent downy mildew disease incidence in treated plants.

Spatio-temporal time gap studies

Spatio-temporal time gap studies were carried out in order to understand the nature of disease protection offered by seed treatment and maintaining spatio-temporal separation between inducer treatments and the pathogen inoculation (Amruthesh et al., 2005). The susceptible pearl millet seeds (7042S) treated with latex extract of *L. sativa* (L2) along with control seeds were sown in autoclaved potting medium as mentioned above and arranged in RBD. Two-day-old seedlings were challenge inoculated with zoospore suspension of *S. graminicola* (4 x 10^4 zoospore/ml) by whorl inoculation method with a time gap of 1, 2, 3, 4, 5 and 6 days of emergence in different set of plants. Plants were maintained under greenhouse conditions as mentioned above and were observed for typical symptoms of downy mildew, that is, sporulation, chlorosis, stunted growth or malformation of the earheads at every 15-day time intervals and were rated for disease when they showed any one of the typical downy mildew symptoms. At the end of 60 days, disease incidence was recorded as the percentage of plants showing symptoms of DM disease. The experiment was repeated thrice with four replicates of 100 plants each.

Biochemical studies

Sampling of seedlings

Effect of seed treatment on the activity of defense enzymes like phenylalanine ammonia-lyase, peroxidase and chitinase activity during host-pathogen interaction in pearl millet was carried out. Susceptible pearl millet seeds treated with L2 (*L. sativa*) treatments for 3 h time duration were placed on petri plates lined with moistened blotter discs. Two day old seedlings were harvested at different time intervals (0, 2, 4, 6, 8, 12, 18, 24, 48 and 72 h) after challenge inoculation with *S. graminicola* (4 x 10^4 zoospres/ml) and were stored at -30°C until used for further studies. SDW treated seedlings served as control.

Estimation of phenylalanine ammonia lyase (PAL) activity

Two-day-old pearl millet seedlings (1 g) of all the samples were homogenized in 1 ml of ice cold 25 mM Tris buffer, pH 8.8, containing 32 mM of 2-mercaptoethanol in a prechilled pestle and mortar. The extract was centrifuged at 10,000 rpm for 25 min at 4°C and the supernatant was used as enzyme source. Reaction mixture containing 0.5 ml of enzyme extract was incubated with 1 ml of 25 mM Tris-HCl buffer, pH 8.8 and 1.5 ml of 10 mM L-phenylalanine in the same buffer for 2 h at 40°C. The activity was stopped using 5 N HCl (Geetha et al., 2005). PAL activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Enzyme activity was expressed as µmol of trans-cinnamic acid min/mg protein/h.

Estimation of peroxidase (POX) activity

Two-day-old pearl millet seedlings (1 g) of all the samples were macerated with 0.2 M sodium phosphate buffer (pH 6.5) in a prechilled pestle and mortar. The homogenate was centrifuged at 10,000 rpm for 15 min at 4°C to get the supernatant. Peroxidase (POX) activity was determined following the method of

Table 1. Effect of seed treatment of latex extract on pearl millet for 3 h on seed germination and seedling vigor.

Plant extract	Plants extracts at different modes					
	Percent germination			Seedling vigor		
	L 1	L 2	L 3	L 1	L 2	L 3
Asclepias curassavica	81±2.41^{bc}*	88±1.82^{ab}	80±2.27^{c}	1188±20.14^{c}	1254±30.38^{de}	1170±29.07^{c}
Calotropis gigantea	88±1.08^{ab}	93±0.91^{a}	88±1.58^{ab}	1352±20.35^{ab}	1418±26.28^{ab}	1304±25.41^{ab}
Croton sp.	87±2.41^{abc}	93±1.29^{a}	87±0.91^{ab}	1320±37.02^{ab}	1394±23.84^{abc}	1296±35.69^{ab}
Eucalyptus alba	83±1.73^{abc}	88±1.35^{ab}	82±0.9^{1bc}	1186±42.47^{c}	1272±12.51^{de}	1154±23.54^{c}
Lactuca sativa	92±1.87^{a}	95±1.35^{a}	90±2.12^{a}	1416±15.11^{a}	1482±29.87^{a}	1384±30.35^{a}
Morus alba	81±2.73^{bc}	85±2.34^{b}	80±0.91^{c}	1190±27.95^{c}	1208±39.86^{e}	1174±27.67^{c}
Nerium odoratum	84±2.41^{abc}	90±1.82^{ab}	83±0.57^{bc}	1245±35.80^{bc}	1298±25.22c^{de}	1240±26.54^{bc}
Tridax procumbens	86±1.29^{abc}	91±1.58^{ab}	84±0.91^{abc}	1282±24.50^{bc}	1340±31.48^{bcd}	1228±23.64^{bc}
Control	78±1.29^{c}	78±1.29^{c}	78±1.29^{c}	916 ± 20.12^{d}	916 ± 20.12^{f}	916 ± 20.12^{d}

*Mean from four repeated experiments with four replicates of 100 plants per treatment in each experiment. *Values are means ± SD of four replicates.

Hammerschmidth et al. (1982). The reaction mixture of 3 ml consisted of 0.25% (v/v) guaiacol in 10 mM potassium phosphate buffer (pH 6.9) containing 10 mM hydrogen peroxide. 5 µl of crude extract was added to initiate the reaction, which was followed calorimetrically at 470 nm (Hitachi 2000). PO activity was expressed as the increase in absorbance at 470 nm/mg protein/min.

Estimation of chitinase

One gram of seedlings of each sample were macerated using 0.05 M sodium acetate buffer, pH 5.2 (1 ml/g fresh weight) and acid washed glass beads at 4°C in prechilled mortar and pestle. The homogenate were centrifuged at 10,000 rpm for 30 min at 4°C (Himac Centrifuge, HITACHI) and the supernatant was used as crude extract. Chitinase was assayed following the method of Isaac and Gokhale (1982) with N-acetyl glucosamine (Sigma) as standard. Colloidal chitin in 0.05 M sodium acetate buffer (pH 5.2), purified from chitin (Sigma-Aldrich, USA) was used as a substrate as suggested by Skujins et al. (1965). Monomers of N-acetyl glucosamine released after incubation were measured spectrophotometrically at 585 nm using dimethyl amino benzaldehyde reagent (Reissig et al., 1955). The enzyme activity was expressed in terms of nmol/min/mg protein.

Protein estimation

Protein content in the crude extracts was estimated by dye binding method (Bradford, 1976) using bovine serum albumin (Sigma) as a standard.

Statistical analysis

Data from four replicates were analyzed for each experiment and subjected to arcsine transformation and analysis of variance (ANOVA) using SPSS Inc. 16.0. Significant effects of treatments were determined by magnitude of F values ($P \leq 0.05$). Treatment means were separated by Tukey's HSD test.

RESULTS

Effect of inducers on seed germination and seedling vigor

The latex extract(s) as inducer treatment to the pearl millet susceptible seeds significantly enhanced the seed germination and vigor in general. However, the percent germination and vigor differed greatly with treatments and time duration of treatments.

The seeds treated for 3 h with L2 treatment showed higher seed germination and vigor followed by other treatments viz., L1 and L3 when compared with all the treatments at 6 h (Tables 1 and 2). Among the L2 treatment for 3 h, *L. sativa* showed highest seed germination (95%) and vigor (1482) followed by *C. gigantea* with 93% germination and 1418 vigor. The germination and vigor of pearl millet seeds in response to latex extracts of other plants were higher when compared with the control which showed 78% germination and 916 vigor.

Effect of latex extracts on growth parameters of pearl millet under greenhouse conditions

The latex extract (L2) treatment on seeds for 3 h were further tested for their efficiency on growth promotion by evaluating different growth parameters like plant height, shoot fresh weight, shoot dry weight, leaf surface area and number of basal tillers per plant.

The data on vegetative parameters revealed a significant increase upon inducer treatment. Among the different plants tested, L2 of *L. sativa* treated seeds recorded highest readings in all the growth parameters with 39.2 cm height, 14.5 g shoot fresh weight, 4.5 g shoot dry weight, 39.6 cm^2 leaf surface area and 4.5 number of basal tillers per plant followed by *C. gigantea, Croton* sp., *T. procumbens, N. odoratum, E. alba, A. curassavica* and *M. alba.*

The distilled water treated seeds showed 26.4 cm height, 8.2 g shoot fresh weight, 2.8 g shoot dry weight, 30.6 cms^2 leaf surface area and 3.2 number of basal tillers per plant (Table 3).

Table 2. Effect of seed treatment of latex extract on pearl millet for 6 h on seed germination and seedling vigor.

Plant Extracts	Plants extracts at different modes					
	Percent germination			Seedling vigor		
	L 1	L 2	L 3	L 1	L 2	L 3
Asclepias curassavica	79±0.91^{bc}*	79±1.82^{d}	74±1.58^{bc}	980±10.97^{b}	1024 ± 30.08^{cde}	844±26.04^{c}
Calotropis gigantea	85±1.08^{a}	87±1.08^{ab}	82±1.29^{a}	1178±36.22^{a}	1214±41.90^{ab}	1124±21.89^{ab}
Croton sp.	82±1.47^{ab}	85±0.91^{abc}	80±1.82^{ab}	1148±34.14^{a}	1190±22.66^{ab}	1106±36.34^{ab}
Eucalyptus alba	80±0.91^{abc}	81±0.91^{cd}	76±0.91^{abc}	986±16.45^{b}	1078±61.22^{bcd}	872±13.19^{c}
Lactuca sativa	85±0.91^{a}	89±0.91^{a}	82±0.91^{a}	1205±12.29^{a}	1253±26.47^{a}	1185±30.70^{a}
Morus alba	75±0.91^{c}	78±1.29^{d}	72±1.29^{c}	886±10.43^{b}	976±28.87^{de}	820±11.83^{c}
Nerium odoratum	81±1.29^{ab}	82±1.29^{bcd}	79±2.04^{abc}	1116±32.17^{a}	1154±35.97^{abc}	1023±29.14^{b}
Tridax procumbens	82±1.58^{ab}	83±1.29^{bcd}	79±2.41^{abc}	1148±20.89^{a}	1162±43.17^{abc}	1040±25.88^{b}
Control	78±1.29^{bc}	78±1.29^{d}	78±1.29^{abc}	916±20.12^{b}	916±20.12^{e}	916±20.12^{c}

*Mean from four repeated experiments with four replicates of 100 plants per treatment in each experiment. *Values are means ± SD of four replicates.

Table 3. Effect of latex extract treatment on plant height, shoot fresh weight, shoot dry weight, leaf area and number of basal tillers under green house conditions after 30 DAS.

Plant extracts	Height (cm)*	Shoot fresh weight/plant (g)*	Shoot dry weight/ plant (g)*	Leaf surface area (cm^2)*	No. of basal tillers/plant*
Asclepias curassavica	27.9±0.26^{g}	9.6±0.09^{g}	3.2±0.09^{g}	32.8±0.34^{e}	3.4±0.09^{d}
Calotropis gigantea	35.6±0.14^{b}	13.6±0.09^{b}	4.2±0.09^{b}	38.5±0.23^{b}	4.1±0.12^{ab}
Croton sp.	32.4±0.12^{c}	12.6±0.09^{c}	3.9±0.09^{c}	37.3±0.09^{c}	4.1±0.07^{ab}
Eucalyptus alba	29.1±0.13^{f}	10.8±0.05^{f}	3.3±0.09^{f}	34.8±0.34^{d}	3.4±0.09^{d}
Lactuca sativa	39.2±0.20^{a}	14.5±0.09^{a}	4.5±0.09^{a}	39.6±0.20^{a}	4.5±0.15^{a}
Morus alba	26.5±0.12^{h}	8.9±0.09^{h}	3.1±0.09^{h}	34.8±0.21^{d}	3.2±0.09^{d}
Nerium odoratum	30.5±0.09^{e}	11.6±0.09^{e}	3.5±0.05^{e}	36.5±0.18^{c}	3.6±0.12^{cd}
Tridax procumbens	31.7±0.13^{d}	12.2±0.12^{d}	3.7±0.10^{d}	37.4±0.23^{c}	3.9±0.09^{bc}
Control	26.4±0.09^{h}	8.2±0.12^{i}	2.9±0.09^{i}	30.8±0.43^{f}	3.2±0.13^{d}

*Mean of three repeated experiments with four replicates of 100 plants per treatment in each experiment. Means within columns sharing the same letters are not significantly different according to Tukey's HSD test at P ≤ 0.05.

Effect of latex extracts on pearl millet downy mildew disease under greenhouse conditions

The potential of latex extracts in managing the pearl millet downy mildew disease was assessed under green house conditions by treating the pearl millet susceptible seeds with L2 treatments for 3 h. Significant ($P \leq 0.05$) disease protection was observed in seedlings raised from latex extract treatments (Table 4). Among the latex extracts, *L. sativa* offered maximum (54%) disease protection against downy mildew disease followed by 43 and 42% by *C. gigantea* and *Croton* sp. respectively. The least disease protection of 8% was found with *M. alba* (Table 4). The control plants showed 100% disease incidence.

Spatio-temporal time-gap studies

Spatio-temporal time gap studies were carried out in order to study the systemic nature of resistance offered by susceptible pearl millet seeds upon treatment with latex extract of *L. sativa* followed by pathogen inoculation at different time intervals. Since L2 treatment of *L. sativa* showed higher disease protection under greenhouse conditions, it was further used for time gap studies. The spatio-temporal time gap studies showed varied degrees of downy mildew disease protection depending upon the time interval between the inducer treatment and challenge inoculation. The downy mildew disease protection on the first day was 28%, which subsequently increased to a maximum of 56% on the fourth day after inoculation

Table 4. Effect of latex extract treatment (L2) on percent downy mildew disease protection.

Plant extracts	Percent downy mildew disease protection
Asclepias curassavica	12.9[g]
Calotropis gigantea	43.2[b]
Croton sp.	41.5[c]
Eucalyptus alba	16.6[f]
Lactuca sativa	54.6[a]
Morus alba	8.5[h]
Nerium odoratum	32.7[e]
Tridax procumbens	39.7[d]

Mean of four repeated experiments with four replicates of 100 plants per treatment in each experiment. Means within columns sharing the same letters are not significantly different according to Tukey's HSD test at $P \leq 0.05$.

and it sustained upto 6-day-time gap period (Figure 1). The results showed that, disease resistance was noticed as early as 24 h and maximum disease resistance developed when a time gap of 4 days was given between latex treatment and pathogen inoculation and was maintained throughout the cropping period.

Enzyme assay

PAL

Two-day-old seedlings raised from latex extract (L2- *L. sativa*) treated susceptible seeds when subjected to estimation of PAL activity at regular time intervals after post inoculation with the pathogen showed a sequential increase in activity at time points tested when compared with that of control. The maximum activity of 378.29 was

achieved as early as 4 hpi in latex extract treated seedlings which decreased thereafter maintaining higher activity than the control at all time points tested. The uninoculated latex extract-treated seedlings also showed same pattern of enzyme activity as that of latex extract treated inoculated seedlings with maximum activity of 253.46 at 4 hpi when compared with susceptible uninoculated (92.94) and inoculated control (79.21). The enzyme activity of uninoculated control varied at all time points which upon inoculation with the pathogen showed decreased activity (Figure 2).

POX

A significant difference in POX activity was observed between the treated and control seedlings and also between the different time intervals. POX enzyme activities increased in susceptible seedlings after latex extract (L2- *L. sativa*) treatment when compared with control plants. Latex extract treatment induced the POX activity in both inoculated and uninoculated susceptible seedlings as early as 2 hpi. The rate of increase was more pronounced in the treated seedlings after inoculation with the pathogen which reached maximum at 8 hpi with 61.06 units when compared with treated uninoculated (43.98). The induced activity of POX however either decreased thereafter or more or less remained constant at all other time points tested. Susceptible control showed lower activity when compared with latex extract treated which further decreased or remained constant on challenge inoculation (Figure 3).

Chitinase

The chitinase activity in all the samples tested showed sequential increase up to 24 h time interval. The chitinase activity estimated in latex extract (L2- *L. sativa*) treated pearl millet seedlings at different time intervals prior to inoculation revealed high activity at 24 hpi (5.21) and low at 0 hpi (2.33). An increase in chitinase activity over control was recorded in susceptible treated seedlings immediately after pathogen exposure. The percentage of increase in enzyme activity was maximum in induced resistant inoculated at 24 hpi (6.32). The activity in the untreated susceptible seedlings after inoculation showed decreased activity when compared with uninoculated control (Figure 4). Though all the samples showed high activity at 24 hpi, the activity declined thereafter.

DISCUSSION

In the present study, latex extracts of seven different plants were tested for their ability to induce resistance against pearl millet-downy mildew disease. The effort revealed the efficacy of latex extracts at low concentration (L2- 100 µl) in escalation of plant growth and inhibition of downy mildew severity in pearl millet when compared with L1, L3 and control. However, the seeds treated with latex extract of *L. sativa* (L2) for 3 h showed maximum seed germination and seedling vigor when compared with 6 h treatment and all other treatments. Our results are in agreement with that of Shivakumar et al. (2009) and Chandhrashekara et al. (2010) who have showed that seed germination and seedling vigor accelerated in pearl millet seeds treated with plant extracts of *Datura* and *Viscum*. Experiments on vegetative growth parameters like plant height, shoot fresh and dry weight, leaf area and tillering capacity by latex extracts showed better results as compared to the untreated control (Table 3). Comparable raise in vegetative parameters in various crops and also in pearl millet using plant extracts (Shivakumar et al., 2009; Chandhrashekara et al., 2010) and various other indu-

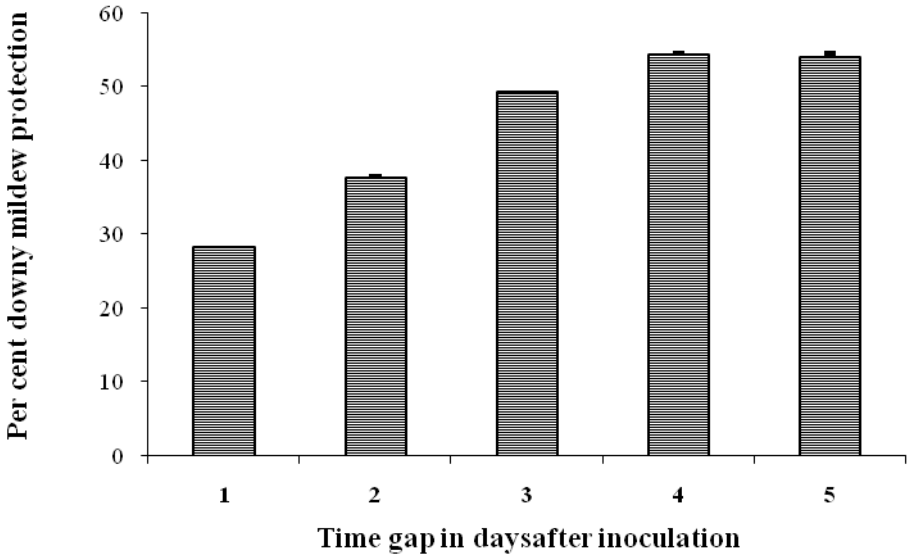

Figure 1. Nature of resistance by *L. sativa* (L2) treatment with spatio-temporal separation of the inducer and pathogen inoculation. The bars represent standard error.

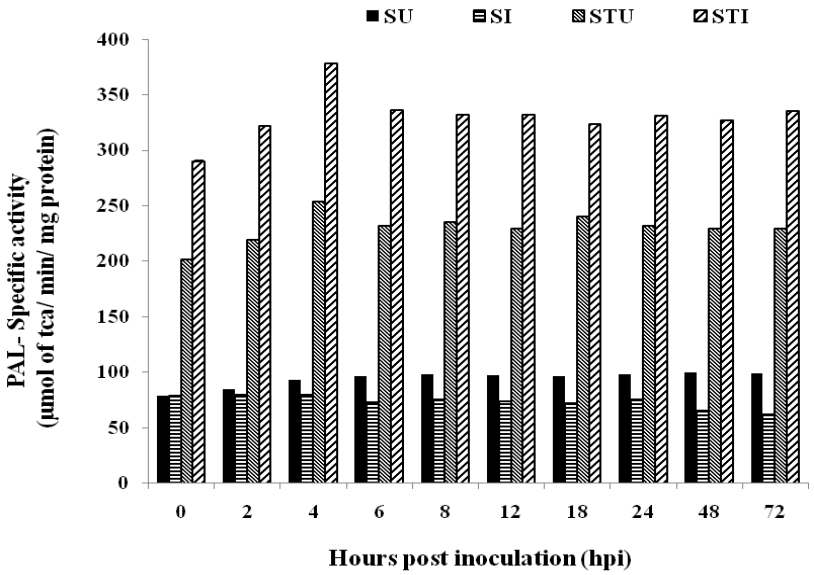

Figure 2. Temporal pattern of accumulation of the phenylalanine ammonia lyase (PAL) enzyme in pearl millet seedlings upon seed treatment with latex extract (L2- *L. sativa*). Bars represent standard errors. SU- susceptible uninoculated; SI- susceptible inoculated; STU- susceptible treated uninoculated; STI- susceptible treated inoculated.

cers has been reported (Amruthesh et al., 2005; Pushpalatha et al., 2007; Sudisha et al., 2011).

Green house studies on temporal and spatial separation of the pathogen and inducer action showed that at least four days were vital to put up maximum resistance against *S. graminicola.* Leaf extracts of spinach and rhubarb induced systemic resistance in cucumber to anthracnose disease caused by *Colletotrichum lagenarium* (Doubrava et al., 1988). Parallel studies on pearl millet under green house conditions have been proven to create resistance towards downy mildew pathogen after treatment with unsaturated fatty acids (Amruthesh et al., 2005), plant extracts (Chandhrashekara et al., 2010; Shiavkumar et al., 2009), raw cow milk and

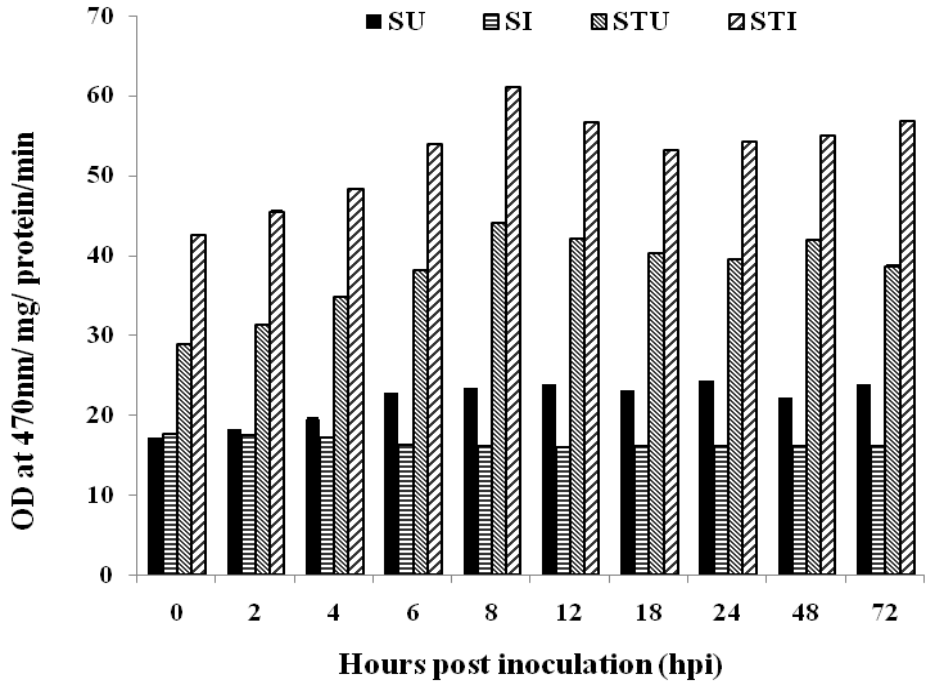

Figure 3. Temporal pattern of accumulation of the peroxidases (POX) enzyme in pearl millet seedlings upon seed treatment with latex extract (L2- *L. sativa*). Bars represent standard errors. SU- susceptible uninoculated; SI- susceptible inoculated; STU- susceptible treated uninoculated; STI- susceptible treated inoculated.

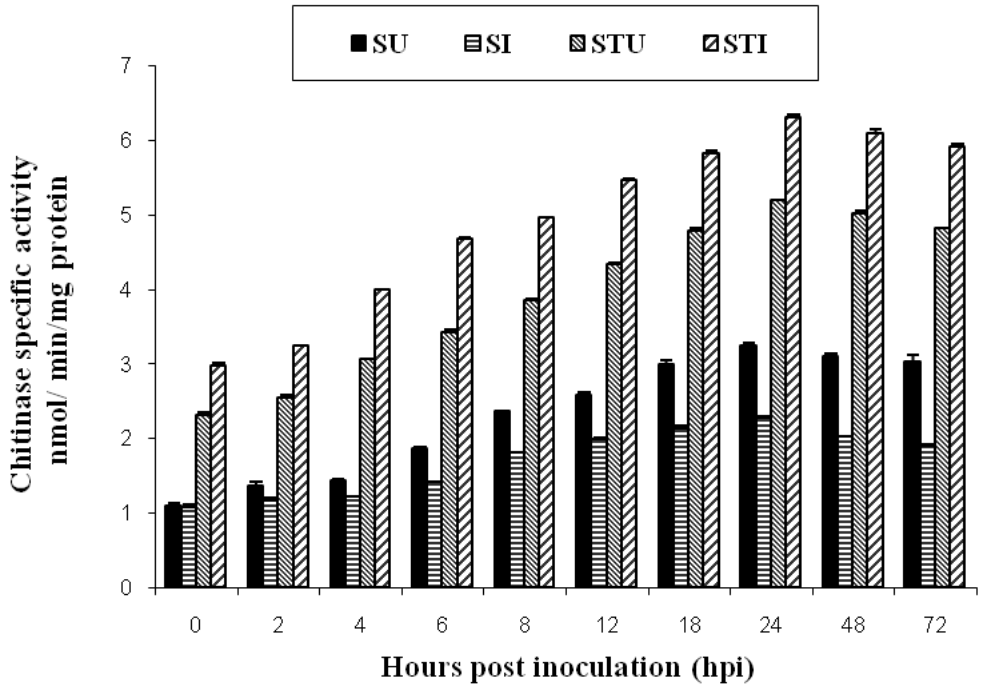

Figure 4. Temporal pattern of accumulation of the chitinase enzyme in pearl millet seedlings upon seed treatment with latex extract (L2- *L. sativa*). Bars represent standard errors. SU- susceptible uninoculated; SI- susceptible inoculated; STU- susceptible treated uninoculated; STI- susceptible treated inoculated.

amino acids (Sudisha et al., 2011). Our results are also consistent with those obtained by other investigators where natural plant products have suppressed plant pathogens leading to resistance towards diseases and consequently, increasing growth parameters and seed yield (Nakatani, 1994; Mohamed et al., 2006; Mohamed and El-Hadidy, 2008). Baraka et al. (2006) reported that extracts of rosemary leaves and *Citrullus colocynthis* fruits, leucarna seeds and alfalfa roots reduced significantly lupine root infection by *F. oxysporum*, *Fusarium solani*, *Rhizoctonia solani* and *Macrophomina phaseolina*. Active principles present in plant products may either act on the pathogen directly (Baraka et al., 2006) or induce resistance in host plants resulting in reduction of disease development (Narwal et al., 2000; Paul and Sharma, 2002; Kagale et al., 2004).

The changes in the activity of defense related enzymes like chitinase, PAL and POX in reaction to pathogen invasion have been illustrated in various plant-pathogen systems (van Loon and van Strien, 1999). The time course analyses of chitinase, PAL and POX activities were made in two-day-old seedlings of non-inoculated and inoculated pearl millet seedlings after inclusion with latex extract of *L. sativa* (L2) for 3 h at different time intervals starting from 0 to 72 hpi.

PAL, a key enzyme of the phenylpropanoid biosynthesis pathway is involved in the biosynthesis of plant defense-related secondary metabolites including salicylic acid, phytoalexins and lignin-based polymers (La Camera et al., 2004), leading to the production of physical and chemical barriers against pathogen infections. In the present study, PAL activity was induced in *L. sativa* (L2) treated seedlings as early as 2 hpi and receded after reaching a peak at 4 hpi. The enzyme activity remained higher at all time points tested when compared with the control and uninoculated seedlings. Similar outcome of increased PAL activity has been recorded against downy mildew pathogen in pearl millet treatment with oligosaccharide of *Trichoderma* spp. (Nandini et al., 2013) and other pathogens in rice on treatment with aqueous extract of *Datura metel* leaves (Kagale et al., 2004), ethanol extracts of *Cymbopogan citrus* and *Ocimum sanctum* (Pal et al., 2013), carrot with sea weed (Jayaraj et al., 2008) and tomato with zimmu leaves (Latha et al., 2009) and ulvans (oligosaccharide) of green algae (*Ulvan lactuca*) (Modafar et al., 2012). Corresponding initial raise and subsequent decline in PAL activity are presented in other incompatible host-pathogen interactions (Borner and Grisebach, 1982; Ralton et al., 1988). An incompatibility thus generated subsequent to latex extract inclusion in susceptible pearl millet seeds suggests that in latex extract induced defense of pearl millet, PAL might be involved in triggering phenylpropanoid pathway resulting in discharge of toxic phytoalexins at the site of *S. graminicola* penetration (Geetha et al., 2005).

Numerous studies have revealed the involvement of greater POX activity with induced resistance in plants against fungal, bacterial and viral pathogens (Hammerschmidt et al., 1982; Reuveni et al., 1990; Hassan and Buchenauer, 2007). POX are recognized to contribute plant defense mechanisms, by catalyzing oxidative polymerization of simple phenols to lignin, synthesis of antimicrobial oxidized phenols, oxidative cross-linking of plant cell walls and also generation of active oxygen species (Lamb and Dixon, 1997; Mehdy, 1994). In the present investigation, POX activity increased in latex extract treated seeds which further increased upon pathogen infection as compared to the control. The figures specify that the level of POX activity remained high at 8 hpi after which the enzyme activity gradually dropped in both infected and uninfected samples. Increase in POX activity is reported in chilli treated with cerebrosides of *F. oxysporum* (Naveen et al., 2013), *Brassica carinata* treated with BABA (β-aminobutyric acid) (Cahavan et al., 2013) against *Colletotrichum capsici* and *Alternaria brassicae*. Similary, there are several other reports on increased POX activity in response to pathogen infection and elicitor treatment as studied by Jayaraj et al. (2008) (*Ascophyllum nodosum* extract: carrot against *A. radicina* and *Botrytis cinerea*), Latha et al. (2009) (Zimmu extract: tomato against *A. solani*), Cawood et al. (2010) (*Agapanthus africans* extract, wheat against *Puccinia triticina*), Nisha et al. (2012) (*Vitex nigundo* extract: rice against *X. oryzae*), Pal et al. (2013) (*C. citrus* and *O. sanctum* extract: rice against *R. solani*) and Senthilraja et al. (2013) (*Pseudomonas fluorescens* bioformulation: groundnut against *sclerotium rolfii*). According to our results, POX can be suggested as typical marker of ISR-mediated defense reaction in pearl millet plants.

Chitinases are present constitutively at a low level in some plants, which is induced by wounding, infection with pathogens (Majeau et al., 1990; Robey et al., 1990) or by abiotic elicitors (Boller et al., 1983) and during plant developmental processes like embryogenesis or fruit ripening. Expression of higher levels of hydrolases like chitinases has been proved to provide enhanced resistance to fungal pathogens (Kasprzewaka, 2003). Chitinase induction after pathogen attack provides protection directly by degrading fungal cell wall components and indirectly by releasing some elicitors from the decaying fungal cell wall that excite other plant defense mechanisms like phytoalexin accumulation in the host (Edreva, 2004). An increased level of chitinase activity was evident on seed treatment with latex extract of *L. sativa* as compared to the control. The activity was induced immediately after challenge inoculation and reached highest at 24 hpi and decreased thereafter. Our results are comparable with those obtained in many other crops on treatment with *Ocimum* extracts (Colpas et al., 2009), zimmu extracts (Latha et al., 2009; Sangeetha et

al., 2013) and *Agapanthus africans* extracts (Cawood et al., 2010), plant growth promoting fungi- *Trichoderma harzianum* (Naher et al., 2012) and plant growth promoting bacteria like *P. fluorescens* (Senthiraja et al., 2013). Bioactive compounds of plants act as elicitors and induce resistance in host plants following reduced disease development (Vidhyashekaran et al., 1992). Plants that are characterized by reduced chitinase activity are significantly more vulnerable to attack by unspecific fungi under natural conditions (Heil et al., 1999) and plants apparently tend to reduce the activity of these enzymes when they are not required due to the presence of other defense mechanisms (Heil et al., 1999, 2000). We therefore assume that activities of these three enzyme classes give an ecologically relevant measure of a plant's overall resistance to pathogens.

Additional investigations are requisite to instigate the bioactive constituents of *L. sativa* involved in bringing about resistance in pearl millet against downy mildew. It is recommended that latex extract induced activity of PAL and PO enzymes in pearl millet, which might have resulted in increased biosynthesis and metabolism of phenols which might have protected pearl millet. In conclusion, the application of latex extracts *L. sativa* can possibly lead to certain defense responses in pearl millet, through elicitation of defense responses induced by its components and its concentration. Further studies are essential to determine the components and concentration for application/seed treatment. The potential of latex extract as elicitors of resistance in plants could be a surplus for other chemical control of diseases in future that cannot be discarded.

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REFERENCES

Abdul B, Anderson JD (1973). Vigor analysis in soybean seed by multiple criteria. Crop Sci. 13:630-633.

Abo-Elyousr KA, El-Hendawy HH (2008). Integration of *Pseudomonas fluorescens* and acibenzolar-S-methyl to control bacterial spot disease of tomato. Crop Prot. 27:1118-1124.

Agrawal AA, Konno K (2009). Latex: A model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. Ann. Rev. Ecol. Evol. Syst. 40:311-31.

Alizadeh H, Behboudi K, Ahmadzadeh M, Javan-Nikkhah M, Zamioudis C, Pieterse CMJ Bakker PAHM (2013). Induced systemic resistance in cucumber and *Arabidopsis thaliana* by the combination of *Trichoderma harzianum* Tr6 and *Pseudomonas* sp. Ps14. Biol. Cont. 65:14-23.

Amadioha AC (2003). Evaluation of some plant extracts against *Collectotrichum lindemuthianum* in cowpea. Act. Phytopathol. Entomol. Hung. 38(3-4):259-265.

Amruthesh KN, Geetha NP, Lyngs Jorgensen HJ, de Neergaard E, Shetty HS (2005). Unsaturated fatty acids from zoospores of *Sclerospora graminicola* induce resistance in pearl millet. Eur. J. Pl. Pathol. 111:125-137.

Anderson CR, Brzezinski MA, Washburn L, Kudela R (2006). Circulation and environmental conditions during a toxigenic *Pseudo-nitzschia australis* bloom in the Santa Barbara Channel, California. Mar. Ecol. Prog. 327:119-133.

Ball SL (1983). Pathogenic variability of downy mildew (*Sclerospora graminicola*) on pearl millet - Host cultivar reactions to infection by different pathogen isolates. Ann. Appl. Biol. 102:257-264.

Baraka MA, Omar SA, El-Barougy, Ebtehage, Zian AH (2006). Controlling seedling damping-off, root rot and wilt diseases of lupine (*Lupinus albus* L.). Agric. Res. J. Suez Canal Univ. 6:57-68.

Barkai-Golan R (2001). Post harvest diseases of fruits and vegetables. Development and control. Elsevier, Netherlands, pp.418.

Boller T, Mauch AGF, Vogeli U (1983). Chitinase in bean leaves: induction by ethylene, purification, properties, and possible function. Planta 57:22-31.

Borner H, Grisebach H (1982). Enzyme induction in soybean infected by *Phytophthora megasperma f.sp. glycinea*. Arch. Biochem. Biophys. 217:65-71.

Bowers JH, Locke JC (2004). Effect of formulated plant extracts and oils on population density of *Phytophthora nicotianae* in soil and control of *Phytophthora* blight in the greenhouse. Pl. Dis. 88:11-16.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72:248-254.

Cahavan V, Bhargava S, Kamble A (2013). Temporal modulation of oxidant and antioxidative responses in *Brassica carinata* during b-aminobutyric acid-induced resistance against *Alternaria brassicae*. Physiol. Mol. Pl. Pathol. 83:35-39.

Cawood ME, Pretorius JC, van der Westhuizen AJ, Pretorius ZA (2010). Disease development and PR-protein activity in wheat (*Triticum aestivum*) seedlings treated with plant extracts prior to leaf rust (*Puccinia triticina*) infection. Crop Prot. 29:1311-1319.

Chandhrashekar, Niranjan RS, Manjunath G, Deepak S, Shetty, HS (2010). Seed treatment with aqueous extract of *Viscum album* induces resistance to pearl millet downy mildew pathogen. J. Pl. Int. 5(4):283-291.

Chaudhry Z, Feroz I, Ahmed W, Rashid H, Mizra B, Qureshi A (2001). Varietal response of *Lycopersicon esculentum* to callogenesis and regeneration. J. Biol. Sci. 1:1138-1140.

Chowdappa P, Mohan Kumar SP, Jyothi Lakshmi, M, Upreti KK (2013). Growth stimulation and induction of systemic resistance in tomato against early and late blight by *Bacillus subtilis* OTPB1 or *Trichoderma harzianum* OTPB3. Biol. Cont. 65(1):109-117.

Colpas FT, Schwan-Estrada KRF, Stangarlin JR, Ferrarese MDL, Scapim CA, Bonaldo SM (2009). Induction of plant defense responses by *Ocimum gratissimum* L. (Lamiaceae) leaf extracts. Summa Phytopathol. 35(3):191-195.

D'Andrea M, Daniels J, Arredondo P, Ivey MB, Ivey AE, Locke DC (2001). Fostering organizational changes to realize the revolutionary potential of the multicultural movement: An updated case study. In: Ponterotto JG, Casas JM, Suzuki LA, Alexander CM (eds) Handbook of multicultural counseling, Thousand Oaks, CA, Sage, pp.222-254.

Doubrava NS, Dean RA, Kuc J (1988). Induction of systemic resistance to Anthracnose caused by *Colletotrichum lagenarium* in cucumber by oxalate and extracts from spinach and rhubarb leaves. Physiol. Mol. Pl. Pathol. 33:69-79.

Edreva A (2004). A novel strategy for plant protection: Induced resistance. J. Cell Mol. Biol. 3:61-69.

Geetha NP, Amruthesh KN, Sharathchandra RG, Shetty HS (2005). Resistance to downy mildew in pearl millet is associated with increased phenylalanine ammonia lyase activity. Funct. Pl. Biol. 32(3):267-275.

Guleria S, Kumar A (2006). *Azadirachta indica* leaf extract induces resistance in sesame against *Alternaria* leaf spot disease. J. Cell Mol.

Biol. 5:81-86.
Hammerschmidth R, Nickles EM, Kuc J (1982). Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colleototrichum lagenarium*. Physiol. Pl. Pathol. 20:73-82.
Hassan MAE, Buchenauer H (2007). Induction of resistance to fire blight in apple by acibenzolar-S-methyl and DL-3-aminobutyric acid. J. Pl. Dis. Protect. 114:151-158.
Heil M, Bostock RM (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Ann. Bot. 89:503-512.
Heil M, Fiala B, Boller T, Linsenmair KE (1999). Reduced chitinase activities in ant plants of the genus *Macaranga*. Naturwissenschaften 86:146-149.
Heil M, Hilpert A, Kaiser W, Linsenmair E (2000). Reduced growth and seed set following chemical induction of pathogen defence: Does systemic acquired resistance (SAR) incur allocation costs? J. Ecol. 88:645-654.
Isaac S, Gokhale AV (1982). Autolysis: A tool for protoplast production *Aspergillus nidulans*. Trans. Br. Mycol. Soc. 78:389-394.
ISTA (2003). International Rules for Seed Testing. International Seed Testing Association (Chapter V).
Jayaraj J, Wan A, Rahman M, Punja ZK (2008). Sea weed extract reduces foliar diseases on carrot. Crop Prot. 27:1360-1366.
Kagale S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2004). 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. Pl. Pathol. 65:91-100.
Kasprzewaka A (2003). Plant chitinases - Regulation and function. Cell Mol. Biol. Lett. 8(3):809-824.
Khairwal IS, Rai KN, Yadav OP, Bhatnagar SK (2007). Pearl millet cultivars. Proceedings of All India Coordinated Pearl Millet Improvement Project annual meeting, Indian Council of Agricultural Research, Mandor, Jodhpur, pp.342-344.
La Camera S, Gouzerh G, Dondt S, Hoffmann L, Fritig B, Legrand M, Heitz T (2004). Metabolic reprogramming in plant innate immunity the contributions of phenylpropanoid and oxylipin pathways. Immunol. Rev. 198:267-284.
Lamb C, Dixon RA (1997). The oxidative burst in plant disease resistance. Ann. Rev. Pl. Physiol. Pl. Mol. Biol. 48:251-275.
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. Cont. 50:85-93.
Majeau N, Trudel J, Asselin A (1990). Diversity of cucumber chitinase isoforms and characterization of one seed basic chitinase with lysozyme activity. Pl. Sci. 68:9-16.
Mehdy MC (1994). Active oxygen species in plant defense against pathogens. Pl. Physiol. 105:467-472.
Modafar CE, Elgadda M, Boutachfaiti RE, Abouraicha E, Zehhar N, Petit E, Alaoui-Talibi ZE, Courtois B, Courtois J (2012). Induction of natural defence accompanied by salicylic acid-dependant systemic acquired resistance in tomato seedlings in response to bioelicitors isolated from green algae. Scientia Horticulturae 138:55-63.
Mohamed IAI, Bauiomy MAM, Ibrahim ASA (2006). Efficacy of different natural products as safe management of guar damping-off disease in Egypt. Egypt J. Phytopathol. 34:1-15.
Mohamed NH, El-Hadidy AM (2008). Studies of biologically active constituents of *Verbascum eremobium* Murb. and its inducing resistance against some diseases of cucumber. Egypt. J. Phytopathol. 36:133-150.
Naher L, Tan SG, Yusuf UK, Ho CL, Siddiquee S (2012). Activities of chitinase enzymes in the oil palm (*Elaeis guineensis* Jacq.) in interactions with pathogenic and non-pathogenic fungi. Pl. Omics J. 5(4):333-336.
Nakatani N (1994). Antioxidative and antimicrobial constituents of herb and species. In:Charalambous G (eds) Species Herbs and Edible Fungi. Elsevier, Amsterdam, pp. 251-271.
Nandini B, Hariprasad P, Niranjana SR, Shetty HS, Geetha NP (2013). Elicitation of resistance in pearl millet by oligosaccharides of *Trichoderma* spp. against downy mildew disease. J. Pl. Inter. 8(1):45-55.
Narwal S, Balasubrahmanyam A, Sadhna P, Kapoor H, Lodha ML (2000). A systemic resistance inducing antiviral protein with N-glycosidase activity from *Bougainvillea xbuttiana* leaves. Indian J. Exp. Biol. 39:600-603.
Naveen J, Hariprasad P, Chandra Nayaka S, Niranjana SR (2013). Cerebroside mediated elicitation of defense response in chilli (*Capsicum annuum* L.) against *Colletotrichum capsici* infection. J. Pl. Inter. 8(1):65-73.
Naylor RL, Falcona WP, Goodman RM, Jahn MM, Sengooba T, Tefera H, Nelson RJ (2004). Biotechnology in the developing world:a case for increased investments in orphan crops. Food Policy 29:15-44.
Nisha S, Revathi K, Chandrasekaran R, Kirubakaran SA, Sathish-Narayanan S, Stout MJ, Senthil-Nathan S (2012). Effect of plant compounds on induced activities of defense-related enzymes and pathogenesis related protein in bacterial blight disease susceptible rice plant. Physiol. Mol. Pl. Pathol. 80:1-9.
Pal TK, Bhattacharya S, Chakraborty K (2013). Induction of systemic resistance in rice by leaf extract of *Cymbopogan citrus* and *Ocimum sanctum* against sheath blight disease. Arch. Appl. Sci. Res. 3(1):392-400.
Paul PK, Sharma PD (2002) *Azadirachta indica* leaf extract induces resistance in barley against leaf stripe disease. Physiol. Mol. Pl. Pathol. 61:3-13.
Pushpalatha HG, Mythrashree SR, Radhakrishna Shetty, Geetha NP, Sharathchandra RG, Amruthesh KN, Shetty HS (2007). Ability of vitamins to induce downy mildew disease resistance and growth promotion in pearl millet. Crop Prot. 26:1674-1681.
Ralton JE, Howlett BJ, Clarke AE, Irwin JAG, Imrie B (1988). Introduction of cowpea with *Phytophthora vignae*:inheritance of resistance and production of phenylalanine ammonia- lyase as a resistance response. Physiol. Mol. Pl. Pathol. 32(1):89-103.
Reissig JL, Strominger JL, Leloir LF (1955). A modified calorimetric method for the estimation of N-acetylamino sugars. J. Biol. Chem. 217:959-966.
Reuveni O, Shlesinger DR, Lavi U (1990). *In vitro* clonal propagation of dioecious *Carica papaya*. Pl. Cell Tis. Org. Cult. 20:41-46.
Robey D, Broglie K, Cressman R, Biddle P, Chet I, Broglie R (1990). Activation of a bean chitinase promoter in transgenic tobacco plants by phytopathogenic fungi. Pl. Cell 2:99-1007.
Safeeulla KM (1976). Biology and control of the downy mildews of pearl millet, sorghum and finger millet. Wesley Press, Mysore, India.
Sangeetha G, Thangavelu R, Usha Rani S, Muthukumar A (2013). Antimicrobial activity of medicinal plants and induction of defense related compounds in banana fruits cv. Robusta against crown rot pathogens Biol. Cont. 64:16-25.
Senthilraja G, Anand T, Kennedy JS, Raguchander T, Samiyappan R (2013). Plant growth promoting rhizobacteria (PGPR) and entomopathogenic fungus bioformulation enhance the expression of defense enzymes and pathogenesis-related proteins in groundnut plants against leafminer insect and collar rot pathogen. Physiol. Mol. Pl. Pathol. 82:10-19.
Sessa RA, Bennett MH, Lewis MJ, Mansfield JW, Beale MH (2000). Metabolite profiling of sesquiterpene lactones from *Lactuca species*. J. Biol. Chem. 275(35):26877-26884.
Shivakumar PD, Geetha HM, Shetty HS (2009). Induction of systemic resistance in pearl millet (*Pennisetum glaucum*) against downy mildew (*Sclerospora graminicola*) by *Datura metel* extract. Crop Prot. 28:783-791.
Singh SD (1995). Downy mildew of pearl millet. Pl. Dis. 79:545-550.
Singh SD, Gopinath R (1985). A seedling inoculation technique for detecting downy mildew resistance in pearl millet. Pl. Dis. 72:425-428.
Singh SD, Shetty HS (1990). Efficacy of pearl millet (*Pennisetum glaucum*). Ind. J. Agri. Sci. 60:575-581.
Skujins JJ, Potgieter HJ, Alexander M (1965). Dissolution of fungal cell walls by a streptomycete chitinase and β-(1,3)-glucanase. Arch. Biochem. Biophys. 111:358-364.
Sudisha J, Kumar A, Amruthesh KN, Niranjana SR, Shetty HS (2011). Elicitation of resistance and defense related enzymes by raw cow

milk and amino acids in pearl millet against downy mildew disease caused by *Sclerospora graminicola*. Crop Prot. 30:794-801.
van Loon LC, van Strien EA (1999). The families of pathogenesis-related proteins, their activities and comparative analysis of PR-1 type proteins. Physiol. Mol. Pl. Pathol. 55:85-97.
Vidhyashekaran P, Borromeo ES, Mew TW (1992). *Helminthosporium oryzae* toxin suppresses phenol metabolism in rice plants and aids pathogen colonization. Physiol. Mol. Pl. Pathol. 41:307-315.
Walters D, Walsh D, Newton A, Lyon G (2005). Induced resistance for plant disease control:Maximizing the efficacy of resistance elicitors. Phytopathol. 95:1368-1373.
Walters DR, Fountaine JM (2009). Practical application of induced resistance to plant diseases:An appraisal of effectiveness under field conditions. J. Agri. Sci. 147:523-535.