Evaluation of Plant Extracts Against Rice Blast Disease Caused by *Pyricularia grisea*

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Abstract

This study was carried out to determine the effect of plant extracts such as Siam weed, Eucalyptus, Swallow-wort, Neem, Lemongrass, Basil, Oleander and Golden trumpet on rice blast disease fungus *Pyricularia grisea in vitro* and *in vivo*. Percentages of disease control for rice blast with different plant extract spraying times (2 days before inoculation, 2 days after inoculation and after symptom appearance, *i.e.* 5 to 7 days after inoculation) were compared to find out the most effective spraying time. The experiments were conducted at the Department of Plant Pathology during May 2015 to August 2016. Antifungal activities of different plant extracts were evaluated on mycelial growth and spore germination using poison food technique and five effective plant extracts were selected for the next experiment. The results indicated all the tested plant extracts had antifungal effect with inhibition percent (10% - 39%) on mycelial growth and spore germination (67% - 88%). Among the extracts, Siam weed showed the maximum inhibition percent on both mycelial growth and spore germination followed by Eucalyptus, Swallow-wort and Neem extracts. In the greenhouse test, the test variety Shwe Thwe Yin was used to evaluate the effect of five selected plant extracts at different spraying times on rice blast disease by inoculating the plants at 21 days after sowing. The results indicated that Siam weed extract gave the higher disease control percentage (36%) compared with other treatments such as Swallow-wort (32%) and Eucalyptus (31%) at 11 days after inoculation. Spraying of plant extracts at 2 days before inoculation showed the highest disease control in comparison to other two spraying times.

Key words: Plant extracts, rice blast, *Pyricularia grisea*

Introduction

Rice blast disease is caused by the fungus, *Pyricularia grisea* and its outbreak has been a serious threat to rice production worldwide (Koutroubas *et al.* 2009). Rice blast disease is more severe in irrigated rice grown in temperate regions or at high elevations in the tropics, and in rainfed upland rice (Bonman 1992). In Myanmar, occurrence of rice blast disease has been recorded on monsoon rice of Ayeyarwady. The disease has also been found sporadically in the fields of Yezin Agricultural University farm and surrounding areas during the monsoon rice growing season of 2002 (Toe *et al*. 2003) and a severe leaf blast epidemic occurred in 2002 - 2003 at Yezin especially at dry (Mar-Jun) and cool (Nov-Feb) seasons (Naing 2004).

Several management practices such as cultural control, host resistance, chemical control, biological control, etc. have been used for the management of rice blast disease. Although there were many practices reported for rice blast management, most of the farmers in developing countries mainly rely on synthetic chemicals because of their quick response. However, prolong use of chemical fungicides may cause high risk to human health, environmental hazard and development of fungicide resistance to pathogen. Many researchers have reported that the fungal toxic property of plant-based extracts to manage plant diseases as an alternative way instead of using chemical fungicides.

The extracts of many plants possess active constituents which have either direct antimicrobial activity or induce host defense response thereby re-

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sulting in reduction of disease development (Schneider and Ullrich 1994). Furthermore, the plant tissues contain secondary components - medicinally active compounds (viz. flavonoids, tannins, saponins, phenols, etc.) that are toxic to pathogens (Gurjar *et al.* 2012). Plant extracts were also reported to provide sustainable disease management solutions especially in organic farming where synthetic fungicides are non-tolerable. Therefore, this study was carried out to determine the effect of different plant extracts against *P. grisea,* causal organism of rice blast, *in vitro* and to evaluate the effective spraying time of selected plant extracts against the rice blast disease *in vivo*.

Materials and Methods

The experiments were conducted at the Department of Plant Pathology, Yezin Agricultural University (YAU) from May 2015 to August 2016.

Isolation and identification of the pathogen

Rice blast infected samples including fresh lesions were cut into small pieces and surface sterilized in 95% ethyl alcohol for 30 seconds and 2% NaOCl for two minutes. Next, these pieces were washed with sterilized water and placed on moist filter paper in petridishes at room temperature to induce sporulation. After one day incubation, conidia from the surface of the lesion were spread onto water agar with a sterilized loop and incubated overnight. Germinated conidia were isolated and transferred onto potato dextrose agar (PDA) according to Xia *et al.* 1993. Colony characters and morphological characteristics of conidia were identified under the microscope. The pathogens were subcultured to obtain a pure culture and stored in refrigerator for further experiments.

Preparation of extracts

Fresh leaves of tested plants, namely Siam weed (*Chromolaena odorata*), Eucalyptus (*Eucalyptus globolus*), Swallow-wort (*Calotropis procera*), Neem (*Azadirachta indica*), Lemongrass (*Cymbopogon citratus*), Basil (*Ocimum gratissimum*), Oleander (*Nerium oleander*) and Golden trumpet (*Allamanda cathartica*) were collected from YAU campus. Plant extracts were prepared according to the method of Barreto *et al.* (2002) with slight modifications. Test plant materials were air dried, separately powdered with a blender and 100% concentration of plant extracts was obtained by soaking 100 grams of each plant part in 100 ml of methylated spirit (95% ethyl alchol + 5% methyl alchol). The mixtures were kept at room temperature for 48 hr in sterilized conical flasks covered with aluminum foil to prevent evaporation. After that, the extracts were poured into the flasks through sterilized muslin cloth and filtered again through sterilized filter paper (<10µ). The plant extracts were stored in the refrigerator at 4°C for further studies.

Inoculum preparation

Production of *P. grisea* spores were carried out by growing the fungus on PDA plates and incubated at room temperature for 8 - 10 days. Before pouring the PDA into plates, streptomycin (40 µg l^{-1}) was added to avoid bacterial contamination. After incubation period, mycelium were scratched with sterilized tooth brush and kept under 12 hr light and 12 hr dark at 26°C for 5 - 6 days to induce sporulation. Spores were harvested by rubbing with sterilized paint brush to the surface of the fungal colony. Afterwards, spore suspension was filtered through a sterilized muslin cloth and the concentration was determined by using haemacytometer (Suryadi *et al.* 2013).

Study - 1: Determination on inhibition effect of different plant extracts on mycelial growth and spore germination of *Pyricularia grisea in vitro*

Effect of different plant extracts on mycelial growth of *P. grisea in vitro*

Inhibition effect of eight different plant extracts on mycelial growth of *P. grisea* was determined by using poison food technique. Stock solutions of different plant extracts (400 µl of each) were spread to form a thin film on the solidified PDA plates by using sterilized L-shaped glass rod. The PDA plates were previously marked with two perpendicular lines at the bottom to indicate the center of the plates. Potato dextrose agar medium without plant extract was served as control. A mycelial disc of 7 mm diameter from 10 day old culture of *P. grisea* was aseptically

transferred to the center of the PDA-extract medium plate and incubated at room temperature (Amadioha

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of thes core}}{\text{No. of observation} \times \text{highest number in rating}} \times 100$$

$$\text{Percent disease control} = \frac{\text{Score of control - Score of treatment}}{\text{Score of control}} \times 100$$

2000). There were 10 treatments *i.e.* 8 different plant extracts, ethanol and control. Completely randomized design (CRD) with 4 replications was used for this experiment.

Data collection and analysis

Leaf blast infection types were recorded from five leaves per replication at 8 days after inoculation (DAI) and 11 DAI. Leaf blast severities were recorded and disease scores were rated by using Standard Evaluation System 0-9 scale developed by International Rice Research Institute (IRRI 1996) (Table 3.2). The percent disease index (PDI) and percent disease control were determined by using the following formulae (McKinney 1923 and Pascual *et al.* 2000).

The data were statistically analyzed by using statistic version 8.0 and means were compared with least significant different (LSD) at 5% level.

Results and Discussion

The results of *P. grisea* growth on PDA amended with plant extracts showed that all tested plant extracts had a positive effect on inhibiting mycelial growth (Figure 1 and 2). Among the tested plant extracts, Siam weed extract gave the highest mycelial growth inhibition (38.61%) at 15 DAI, while the lowest mycelial growth inhibition effect (10.32%) was found in both extracts of Basil and Oleander. Manjappa (2015) reported that growth of *P. grisea* was exhibited to the maximum extent (85.6%) by methanol extract of Siam weed. It was also observed that Eucalyptus extract showed (25.07%) mycelial growth inhibition followed by extract of Swallow-wort (24.9%) but not significantly different from each other. Zhou *et al.* (2016) proved that Eucalyptus oil has broad-spectrum inhibitory effects on *M. grisea* and its treatment may cause remarkable morphological and structural changes of hypha. Khanzada *et al.* (2012) stated that the aqueous extract of Swallow-wort failed to inhibit the mycelial growth of *Magnaporthe oryzae*. However, in this study, ethanol extract of Swallow-wort inhibited 24.9% on the mycelial growth of fungus. Parekh *et al.* (2006) reported that water is used as universal solvent to extract plant products with antimicrobial activity but plant extracts in organic solvents have been found to give more consistent antimicrobial activity compared to water extract. Neem extract showed the higher mycelial inhibition (17.1%) than Golden trumpet extracts (14.92%), Lemon grass

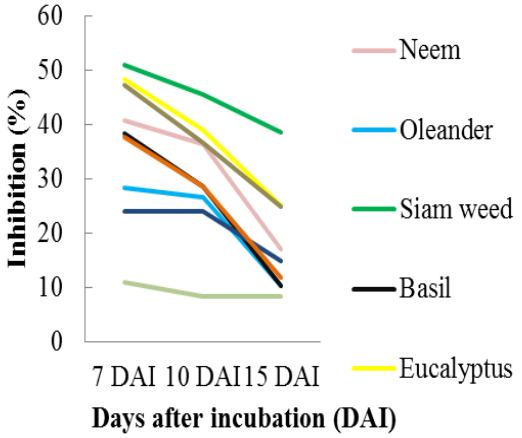

Figure 1. Inhibition effects of eight plant extracts on mycelial growth of *P. grisea* at 7-15 days

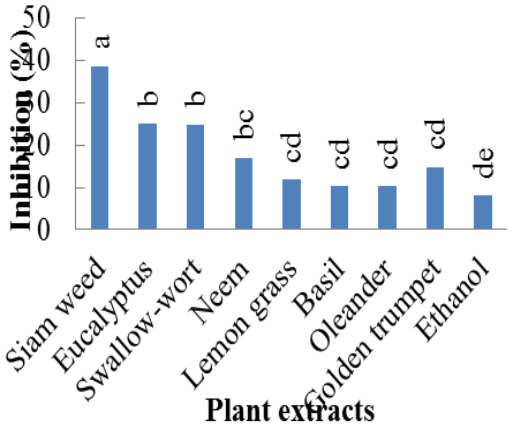

Figure 2. Inhibition effects of eight plant extracts on mycelial growth of *P. grisea* at 15 days after incubation

Table 1 Effect of eight plant extracts on spore germination of *P. grisea* after 12 hrs incubation

Treatments	Germination percent (%)*	Inhibition percent (%)*
Siam weed	0.00 d**	88.00 a
Eucalyptus	2.79 cd	79.33 b
Swallow-wort	12.66 b	67.67 d
Neem	10.22 bc	70.77 cd
Lemon grass	8.80 bc	71.67 cd
Basil	11.76 b	68.33 d
Oleander	5.44 bcd	76.33 bc
Golden trumpet	12.97 b	67.33 d
Control	89.63 a	0.00 e
$LSD_{0.05}$	**7.89**	**6.42**
Pr>F	<0.0001	<0.0001
CV (%)	**18.54**	**5.71**

*** Mean of three replications**
**** Means followed by the same letter in the same column are not significantly different at 5% level**

(11.91%), Basil and Oleander (10.32%) but they were not significantly different from each other (Figure 2). Several researchers have ascertained that alcohol extract of Neem, n-hexane extracts of Siam weed, Lemongrass and Basil showed the significant inhibition on mycelial growth of *P. oryzae* (Adeosun and Onasanya 2015).

It was observed that all the tested plant extracts had significant inhibition effect on the spore germination of *P. grisea* compared with control (Table 1). Among the tested plant extracts, Siam weed extract gave the maximum inhibition effect (88%) followed by Eucalyptus (79.33%), Oleander (76.33%) and Lemongrass (71.67%) on spore germination but there were no significant differences from each other. However, Golden trumpet extracts showed the lowest inhibition effect (67.33%) on spore germination of *P. grisea*. Neem extracts showed the higher inhibition effect (70%) than Basil (68.33%), and Swallow-wort (67.67%) extracts but they were not significantly different from one another. These findings are in line with Shunying *et al*. (2005), who explained that the phytochemical constituents of each extract had antifungal activity associated with the presence of phenolic compounds - monoterpenes and sesquiterpene hydrocarbons. These phenolic compounds were responsible for denaturing enzymes that could restrict the amino acids involved in spore germination (Nychas 1995). Moreover, variations in the activity of phytochemicals can also be effected by the climatic and edaphic variations in the geographic locations of growth of the plant (Pallant 2010).

Among the five plant extracts tested, the least disease index at 8 DAI was 31.36% in Siam weed extract sprayed plants followed by Eucalyptus (39.75%), Neem (43.7%) and Swallow-wort (44.2%). Maximum disease index (61%) at 8 DAI was recorded in control. The results showed that after application of plant extracts against the disease after 11 DAI, the plant extracts - Siam weed, Swallow-wort and Eucalyptus showed the best results with disease index of 52.6%, 57.26% and 59.01%, respectively. The disease index recorded in control was 81.2% at 11 DAI (Table 2). These results are in close agreement to those reported by Manjappa

Table 2 Disease score and index of rice blast disease affected by selected five plant extracts with three different spraying times

Plant extracts	Disease score*		Disease index (%)*	
	8 DAI	11DAI	8DAI	11DAI[x]
Control (T_1)	5.62 a**	7.31 a	60.99 a	81.21 a
Siam weed (T_2)	2.99 d	4.73 e	31.36 d	52.60 e
Eucalyptus (T_3)	3.58 cd	5.31 cd	39.75 c	59.01 cd
Swallow-wort (T_4)	3.98 bc	5.16 de	44.2 bc	57.26 de
Neem (T_5)	3.93 bc	5.76 bc	43.70 bc	63.95 bc
Lemongrass (T_6)	4.51 b	6.16 b	50.12 b	68.35 b
$LSD_{0.05}$	**0.75**	**0.49**	**7.49**	**5.47**
Spraying times				
2 days before inoculation (S_1)	3.67 b	5.50 b	40.74 b	61.06 b
2 days after inoculation (S_2)	4.13 ab	5.78 ab	45.19 ab	64.20 ab
After symptoms appear (S_3)	4.49 a	5.93 a	49.14 a	66.00 a
$LSD_{0.05}$	**0.53**	**0.34**	**5.30**	**3.87**
Pr > F				
Treatments (A)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Spraying times (B)	0.0094	0.0507	0.0108	0.0472
A × B	0.7201	0.0297	0.3848	0.0292
CV (%)	**18.87**	**8.99**	**17.37**	**8.97**

*** Mean of three replications**
**** Means followed by the same letter in the same column are not significantly different at 5% level**
DAI[x] = Days After Inoculation

(2015), who observed that Siam weed extracts showed lower disease index of leaf blast under field condition. Significant differences in disease index between each of all the plant extracts and control were found at 8 DAI and at 11 DAI. However, no significant differences were recorded among the plant extracts. Moreover, the application of five plant extracts significantly reduced the disease index in all spraying times compared to control (Table 2). Highly significant differences in disease index were found among plant extracts and times of spraying at 8 DAI. At 11 DAI, highly significant differences in disease index were recorded among plant extracts, among times of spraying and in the interaction between plant extracts and times of spraying (Table 2). This result suggested that the three plant extracts responded differently to the times of spraying. The efficacy of phytochemicals contained in plant tissues will be influenced by the age of the plant or plant parts. Extraction methods involve separation of medicinally active fractions of plant tissue from inactive components by using selective solvents and extraction technology. Quality

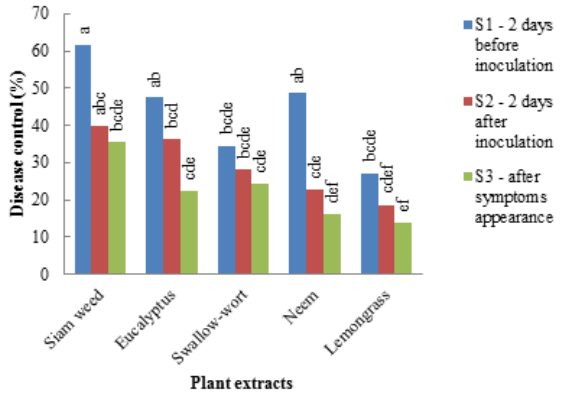

Figure 3. Effect of five plant extracts and spraying times on rice blast disease at 8 days after inoculation

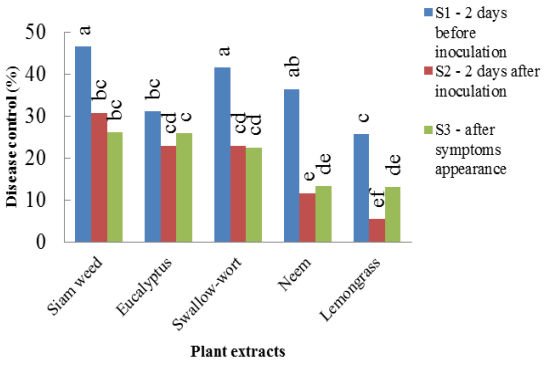

Figure 4. Effect of five plant extracts and spraying times on rice blast disease at 11 days af-

of plant extract depends on plant material, extraction method and choice of solvent, which will also depend on target compounds (Green, 2004).

The effect of different plant extracts on rice blast disease was shown in the figure 3 and 4. Siam weed extract reduced the disease ranging from 35.6% to 61.78% at 8 DAI. These extract reduced the disease in a range of 26.1% to 46.61% compared to the control at 11 DAI. Application of plant extracts at 2 days before inoculation gave the significant disease control in the range of 27.1% to 61.78% as compared with 2 days after inoculation (18.7% - 39.94%) and after symptoms appearance (13.94% - 35.6%) (Figure 3). In the mean disease control (%) of all the spraying times in each plant extracts, the highest one (45.77%) was found in Siam weed and the lowest one, (19.94%) was in Lemongrass at 8 DAI. At 8 DAI, in the mean disease control (%) of all the plant extracts in each spraying time, the highest one, (43.96%) was found in 2 days before inoculation followed by (29.2%) in 2 days after inoculation and (22.51%) in after symptoms appearance (Figure 3). However, in the mean disease control of all the plant extracts in each spraying time, the highest one, (36.26%) was found in 2 days before inoculation at 11 DAI. In other words, among the different spraying times, application of plant extracts at 2 days before inoculations

gave good results against the disease (Figure 4). This finding was consonant with that of Kamalakannan *et al.* (2001), who reported that spraying of plants extract such as Neem (*Azadirachta indica*), Common jujube (*Zizypus jujube*) and Mesquite (*Prosophis juliflora*) at pre inoculation was comparatively most effective than post inoculation in reducing disease index of *P. grisea*. Amadioha (2000) stated that delay in treatment of *P. grisea* inoculated rice with Neem extracts until after disease symptom appearance decreased the efficiency of extracts. The finding of the present investigation is an important step towards crop protection strategies because the use of plant extracts are environmentally non-pollutive, indigenously available, easily accessible, largely non phytotoxic, easily biodegradable and non-toxic to human.

References

Adeosun, B. O. and O. R. Onasanya. 2015. Efficacy of n-hexane plant extracts in the control of rice blast diseas. *Applied Tropical Agriculture*, 20 (1), pp. 37 - 41.

Amadioha, A. C. 2000. Controlling rice blast *in vitro* and *in vivo* with extracts of *Azadirachta indica. Crop Protection*, 19 (5), pp. 287 - 290.

Barreto, M., A. T. Critchley and C. J. Straker. 2002. Extracts from seaweeds can promote fungal growth. *Journal of basic microbiology*, 42 (5), pp. 302 - 310.

Bonman, J. M. 1992. Blast In: Compendium of Rice Diseases (Eds. RK Webster, PS Gunnel), *The American Phytopathological Society*, St. Paul Minnesota, USA, pp. 14 - 16.

Green, R. J. 2004. Antioxidant activity of peanut plant tissues. Masters Thesis. North Carolina State University. USA.

Gurjar, M. S., S. Ali, M. Akhtar and K. S. Singh. 2012. Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, 3 (3), pp. 425 - 433.

Kamalakannan, A., V. Shanmugam, M. Surendran and R. Srinivasan. 2001. Antifungal properties of plant extracts against *Pyricularia grisea,* the rice blast pathogen. *Indian Phytopathology*, 54 (4), pp. 490-492.

Khanzada, M. and G. S. Shah. 2012. *In-vitro* evaluation of fungicides, plant extracts and bio-controlagents against rice blast pathogen *Magnaporthe oryzae* Couch. *Pakistan Journal of Botany* 44 (5), pp. 1775 - 1778.

Kiraly, Z. 1974. Methods in plant pathology: with special reference to breeding for disease resistance. Elsevier Scientific Publishing Company, New York, 212p.

Koutroubas, S. D., D. Katsantonis, D. A. Ntanos and E. Lupotto. 2009. Blast disease influence on agronomic and quality traits of rice varieties under Mediterranean conditions. *Turkish Journal of Agriculture and Forestry*, 33 (5), pp. 487 - 494.

Manjappa, K. 2013. Evaluation of Antifungal Properties of Eupatorium (*Chromolaena odorata* L.) Plant Exstract Against *Pyricularia oryzae* Causing Blast Disease in Rice Crop. *Asean Journal of Pharmaceutical Science and Technology*, 5 (1), pp. 79 - 81.

McKinney, H. H. 1923. A new system of grading plant diseases. *J. Agric. Res*, 26 (2), pp. 195 - 218.

Medwid, R. D. and D. W. Grant. 1984. Germination of *Rhizopus oligosporus* sporangiospores. *Applied and environmental microbiology*, 48 (6), pp. 1067 - 1071.

Naing, T. A. A. 2004. Rice Production in Myanmar and effects of intensification on crop health. Cuvillier. Doctoral Dissertation. University of Kassel. pp. 81.

Nychas, G. J. E. 1995. Natural antimicrobials from plants. In New methods of food preservation, *Springer US,* pp. 58 - 89.

Pallant A. C. 2010. Bioactivity of alkaloidal fraction of *Tabernaemontana elegans* Stapf. Thesis (*Magister Scientiae*) submitted to the Department of Pharmacology, Faculty of Health Sciences, University of Pretoria.

Pandey, D. K., N. N. Tripathi, R. D. Tripathi and S. N. Dixit. 1982. Fungitoxic and phytotoxic properties of the essential oil of *Hyptis suaveolens. Journal of Plant Diseases and Protection*. 89, pp. 344 - 349.

Panse, V. G. and P. V. Sukhatme. 1954. Statistical methods for agricultural workers. *Statistical methods for agricultural workers. New Delhi, India,* 347p.

Parekh, J., D. Jadeja and S. Chanda. 2006. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology*, 29 (4), pp. 203 - 210.

Pascual, C. B., T. Toda, A. D. Raymondo and M. Hyakumachi. 2000. Characterization by conventional techniques and PCR of *Rhizoctonia solani* isolates causing banded leaf sheath blight in maize. *Plant Pathology*, 49 (1), pp. 108 - 118.

Schneider, S. and W. R. Ullrich. 1994. Differential induction of resistance and enhanced enzyme activities in cucumber and tobacco caused by treatment with various abiotic and biotic inducers. *Physiological and Molecular Plant Pathology*, 45 (4), pp. 291 - 304.

Shunying, Z., Y. Yang, Y. Huaidong, Y. Yue and Z. Guolin. 2005. Chemical composition and antimicrobial activity of the essential oils of *Chrysanthemum indicum*. *Journal of Ethnopharmacology*, 96 (1), pp. 151 - 158.

Suryadi, Y., D. N. Susilowati, E. Riana and N. R. Mubarik. 2013. Management of rice blast disease (*Pyricularia oryzae*) using formulated bacterial consortium. *Emirates Journal of Food and Agriculture*, 25 (5), pp. 349 - 357.

Toe, S., T. Zin, E. G. Hlaing, T. S. Kyaw, Y. H. Aung, S. N. Win, Z. W. Win, Z. and M. Kyaing. 2003. Reaction of some rice varieties to rice leaf blast caused by *Pyricularia griesea*. Report for Special Research Project, Dept. of Plant Pathology, YAU.

Xia, J. Q., J. G. Correli, F. N. Lee, M. A. Marchetti and D. D. Rhoads. 1993. DNA fingerprinting to examine microgeographic variation in the *Magnaporthe grisea* (*Pyricularia grisea*) population in two rice fields in Arkansas. *interactions*, *28*, 29p.

Zhou, L. J., F. R. Li, L. J. Huang, Z. R. Yang, S. Yuan and L. H. Bai. 2016. Antifungal Activity of *Eucalyptus* Oil against Rice Blast Fungi and the Possible Mechanism of Gene Expression Pattern. *Molecules*, 21 (5), 621p.