EUROPEAN ACADEMIC RESEARCH
Vol. III, Issue 7/ October 2015

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

Efficacy of different Botanical extracts on the linear colony growth of the *Helminthosporium oryzae*

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Abstract:

Brown spot of rice caused by H. Breda, attacks the plant at any growth stage and it appears on the foliage as scattered brown spot that coalesces and result in withering and yellowing of the leaves. This fungal disease lowers the seed germination, causing rotting of seeds and roots that ultimately reduce yield by 16% to 43%. So, this disease test different, botanicals extracts under in vitro condition against H. oryzae. The method followed was based on survey and sampling of diseased seed samples from different districts Larkana, isolation and purification of fungi from these samples were conducted and performed pathogenicity test of the most predominant isolated fungus (H. oryzae)

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five different botanical extracts such as Neem (Azadirachta indica), Akk (Calotropi sprocera), Garlic (Allium sativum), Datura (Datura stramonium) and ginger (Zingiber officinale) across three concentrations (5, 10 and 15 ml) against the same fungal pathogen were studied in CRD with three replications using food poisoning technique. Likewise, rhizobacteria was isolated from soil of rice plants and its 25 isolates were studied for their antagonistic effect on colony growth of H. oryzae using PDA as a growth medium. The results showed that five different botanical extracts such as Neem (Azadirachta indica), Akk (Calotropi sprocera), Garlic (Allium sativum), Datura (Datura stramonium) and ginger (Zingiber officinale) across three concentrations (5, 10 and 15 ml) against the same fungal pathogen were studied in CRD with three replications using food poisoning technique Likewise. The in-vitro efficacy of five botanicals against H oryzae at different doses revealed that the ginger (00.00 mm) and garlic were found highly effective at their highest doses (2.75 mm) in reducing the linear colony growth of the fungus followed by Dhatura (4.62 mm) neem (20.00 mm) and Akk (13.37 mm) respectively as compared to control (38.12mm). These studies will be helpful for researchers and farming community in future for better management of this disease.

Key words: Brown spot of rice, Pathogenicity, Botanical extracts

INTRODUCTION

Paddy or Rice crop (*Oryzae sativa* L.) belonging to Poace family ("true grass") the domesticated species of genus oryzae. Its native place ranges from tropical and subtropical southern Asia, while the African rice *Oryzae glaberrima* is native to West Africa. Being third largest cash crop after wheat and cotton and also major export item of Pakistan. High quality rice, with typical aroma and taste, to meet both export and domestic demand is grown in the country (Habib *et al*., 2012). Reported that rice as traditional medicinal plant in India. Rice can also be used as ointment to treat skin disorders. In paste form or

molded into balls and applied to boils, sores, swellings and skin blemishes. Steamy gummy rice is often taken to treat tolerance upsets, heart-burn and indigestion. Extracts from brown rice have been used to care for breast and abdomen cancer and warts. Rice in paste form is also used to treat indigestion, nausea and diarrhea in India (Umadevi *et al*., 2012).

INSECT PESTS AND DISEASES OF RICE CROP

Arain, (2013) reported that rice cultivation is commonly encountered by different biotic factors including insect pests: yellow stem borer, pink stem borer, leaf hopper, leaf folder, grass hopper, stem borer, aphid and jassid and diseases like brown spot disease rice blast, and bacterial leaf blight etc, which adversely affect its yield per unit area.

BROWN SPOT OF RICE

Importance:

One of the most commonly occurring and dangerous diseases; Brown spot causes both quantity and quality losses in rice crop that may range from 5-45% loss in the crop yeild. Seed blight is also seen and may leads to 10-57% seedling transience. The major epidemic disease caused by *Helminthosporium oryzae* was firstly observed during 1918-19 in India, at the Krishna-Godavari delta and while next epidemic was observed during the year of 1942 in India and Bangladesh Singh, (2013

Pathogen:

Shabana *et al*., (2008) reported that *Helminthosporium oryzae* Breda as the causal pathogen which belongs to family *Dematiaceae*, order *Moniliales* and sun-division *Deuteromycotina***.** This fungus is both inter- and intra-cellular within mesophyll tissue of the leaves.

Morphology:

Ginnis *et al*., (1986) reported that microscopic morphological symptoms of brown spot disease as brown spots or pigmented, fungi-like on irregular shape colonies are extremely fast penetatraing, effuse, and grey to black in color at ends, smooth to finely roughened and germination only from the ends (*Bipolar*). Malkhan *et al.,* (2013) studied the effectiveness of different botanicals extracts on dropping seed borne disease and rising germination of collected rice seed sample and reported Neem and chirata (1:1) extracts increased seed germination up to 65 to 67%. Even as, Jitendiya *et al.,* (2013) reported that acoruscalamus extract at 20% concentration alone showed 79.99 % degradation of linear colony growth. Aqueous extract of acoruscalamus showed highest percentage in reducing the disease by 45.29 % as compared with control. Although, Malkan *et el.,* (2012) reported that the overzealous and indiscriminate use of most of the artificial fungicides have created different types of environmental and toxicological problems. Similarly. Manimegalai and Ambikapathy, (2012) reported the popularity of botanical extracts is once more growing and some plant products are being used worldwide as green pesticides used as antimicrobials against storage pests because of their comparatively safe status and wide recognition by the consumers.

MATERIALS AND METHODS

Survey and sampling

Diseased samples consisting of seeds of IR-6, IR-8, IR-9, Super Basmati, Shahkar, DR-82, DR-83, Dokri Basmati, Basmati 270, Basmati 385, Super Karnal, Danglo hybrid, IRRI-09, IRRI-06, Rachana hybrid and Maharani Hybrid rice varieties, were collected from district Larkana, surrounding areas. These rice varieties are commonly cultivated in district of Sindh province.

These samples were placed in paper begs which were properly labeled and brought to the laboratory for isolation of disease causing fungi.

Isolation and purification of fungi

Isolation of different fungi were carried out from infected grains and panicles. Discolored grains were collected from the experimental field of RRI, Dokri and NIA, Tandojam. The diseased grains were separated from panicles and kept in petri plates. Seeds were surface sterilized with 5% Sodium Hypochlorite solution for two minutes and placed on fresh Potato Dextrose Agar (PDA) medium. Fifteen sterilized seeds were kept in three PDA plates (10 seeds/plate) and these plates were incubated at 28^0C for 5-7 days. Fungus was purified by taking small bit of fungus and transfering them in next PDA plate. Identification of the fungus was done by studying colony characteristics, spore shape and size following the method described in the Technical Bulletin of the Seed Borne Diseases and Seed Health Testing of Rice (Agarwal, (1989) and Ellis, 1980). The pure cultures of the isolated fungus were preserved at 5^0C on PDA slants for further studies

$$\text{Colonization (\%)} = \frac{\text{Number of seeds colonized by pathogen}}{\text{Total number of seed studied}} \text{x } 100$$

Pathogenicity test of the most predominant isolated fungus (*Helminthosporium oryzae*)

Pathogenicity test of the most frequent fungus i.e. *H. oryzae* was conducted in the green house on apparently 4 healthy varieties and 1 highly susceptible variety basmati 370. The nursery was sowed in sterilized earthen pots and then inoculated with fresh culture of the fungus. Inoculations were done as, 10 ml spore suspension of the fungus was spray below the growing tips of seedlings stage.

Effect of different botanical extracts on the linear colony growth of Helminthosporium oryzae

Five different botanical extracts (Plant extracts) were tested *in-vitro*; such as Neem (*Azadirachta indica*), Akk (*Calotropi sprocera*), Garlic (*Allium sativum*), Datura (*Datura stramonium*) and ginger (*Zingiber officinale*) (Table. 2). The basal medium was amended with three different doses of each extract such as (5, 10, & 15ml). For the preparation of aqueous extract, 100 gm fresh leaves of each plant were macerated in 100 ml of sterilized water with the help of pestle and mortar. The macerated plant extract was first passed through four layered sterilized muslin cloth and then filtered through Whatman's filter paper. The extract obtained was considered standard and was stored in freezer for further studies in laboratory. The sterilized PDA (potato dextrose agar) medium was poured into the sterilized petri plates of 9 cm diameter. These petri plates were allowed to solidify and different doses of plant extracts were poured into the PDA media with the help of sterilized pipette. Than inoculation of actively growing culture of *H. oryzae* (7 days old) were kept in each Petri plate with the help of sterilized inoculation needle. All these petri plates were then transferred to incubator at 28±1°C and data of mycelial growth of test fungus and inhibition zone by test plant extracts were recorded after 48 hours till 7 days of inoculation. The experiment was conducted in completely randomized block design (CRD), with three replications in each treatment. Control was similarly carried out with only difference that plant extracts replaced by PDA media. This experiment was conducted as described by Ahmed *et al.,* (2013).

Table 02. The botanical extracts & their doses used

S. No	Name of Botanicals extreact	No	Dose in ml/100 ml medium
01	**Neem (*Azadirachta indica*)**	I II III	5 ml in 100 ml medium 10 ml in 100 ml medium 15 ml in 100 ml medium
02	**Garlic (*Allium sativum*)**	I II III	5 ml in 100 ml medium 10 ml in 100 ml medium 15 ml in 100 ml medium
03	**Akk (*Calotropi sprocera*)**	I II III	5 ml in 100 ml medium 10 ml in 100 ml medium 15 ml in 100 ml medium
04	**Ginger (*Zingiber officinale*)**	I II III	5 ml in 100 ml medium 10 ml in 100 ml medium 15 ml in 100 ml medium
05	**Datura (*Datura stramonium)***	I II III	5 ml in 100 ml medium 10 ml in 100 ml medium 15 ml in 100 ml medium

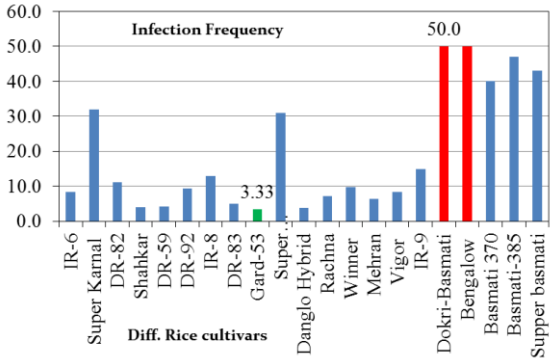
RESULTS AND DISCUSSION

Survey and sampling

Diseased samples consisting of seeds of IR-6, IR-8, IR-9, Super Basmati, Shahkar, DR-82, DR-83, Dokri-Basmati, Basmati-370, Basmati-385, Super Karnal, Danglo hybrid, Rachana hybrid and Maharani Hybrid rice cultivars, were collected from (RRI) Dokri and some areas of District Larkana. That is commonly cultivated cultivars. These samples were collected in paper begs which were properly labeled and brought to the laboratory for isolation of pathogen.

Response of different rice varieties against seed borne fungi

Association of seed borne microflora with 21 rice seed varieties collected from rice growing areas of Sindh showed that maximum infection i.e 50% was found on variety Bangalo and minimum % was observed on Shahkar (**Graph** . 01).



Graph. 01. Response of different rice varieties against seed borne fungi

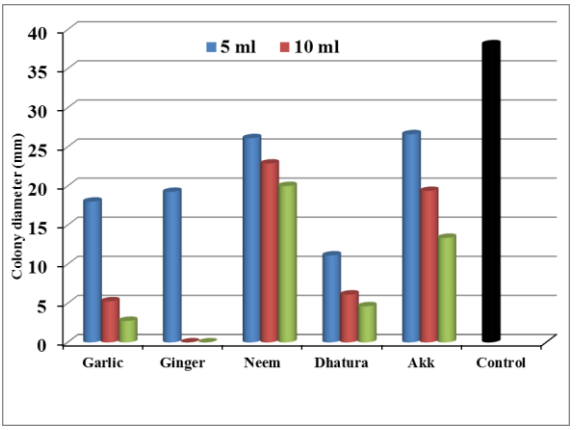
Pathogenicity test of *Helminthosporium oryzae*

The results shows that the pathogenicity test of the most predominant fungus (*H. oryzae)* was conducted in the green house apparently 2 highly susceptible available rice varieties (Basmati-370 and Bangalo) for the confirmation of etiology of the disease. These varieties then inoculated with fresh culture of the fungus. Inoculated into the nursery plants confirmation the *H. oryzae* was successfully re-isolated from the brown spot of rice diseased inoculated plants and was proved that the *H. oryzae* is the actual cause of the disease.

In-vitro evaluation of botanicals extracts against *Helminthosporium oryzae*:

Five different plant extracts were tested *in-vitro* conditions for their efficacy against *Helminthosporium oryzae* at different doses: i.e 5%, 10% and 15%. The botanical Neem (*Azadirachta indica*), Akk (*Calotropi sprocera*), Garlic (*Allium sativum*), Datura (*Datura stramonium*) and Ginger (*Zingiber officinale*). All botanical extracts significantly reduced the linear colony growth of *Helminthosporium oryzae* ($p<0.000$). The result shows that the most effective doses were 10% and 15%. While the best effect of Ginger extracts was (0mm) which reduced linear colony

growth such as Garlic was (2.75mm) reduced linear colony growth. At the same time medium effective was found in Datura (4.62) which reduced the linear colony growth and Neem and Akk were lowest effective found linear colony growth of fungus at highest doses the Neem was reduced linear colony growth (20mm). While Akk was (13.5mm) reduced linear colony growth. (Graph. 2) Respectively all the botanical extracts at their respective dose significantly retarded the growth of fungus as compared to control (38.5 mm).



Graph 2: Effect of selected botanical extracts on the linear colony growth of H. Oryzae

Six fungal infective agents were isolated from the 13 different varieties of rice i.e Dokri-Basmati, winner Hybird, Super Karnel, IR-06, IR-08, DR-59,DR-82,DR-83,DR-92, Karnel Basmati, Denglo Hybird and Guard- 53 hybird were severely infected with *Helminthosporium oryzae* followed by *Tillatia spp* and a lower rate of fungal infection from *Alternaria, spp* in the rice seeds collected from the variety Dokri-Basmati and Bengalow whereas a lower severity of fungal infection was recorded in Guard-53 in the present study area. At the same time as, other fungal agents commonly seen during the present investigation were *Helminthosporium oryzae* (52.91 *Curvularia*

spp:(16.66%)*, Alternaria, spp:(11.25%), Fusarium spp:(2.16%), Penisillium spp:*(1%) *and Tillatia spp:(4.91%).* This might be due to association of different fungal flora such as *Bipolaris oryzae, Curvularia spp, Alternaria spp; Penisillium spp; and Fusarium spp*. or it may be due to varietal genetic characterization to germinate. %) which was close agreement with the findings of (Naeem *et al.,* 2001 and Hafiz *et al.,* 2013).

The results of our studies showed that maximum disease severity of brown spot of rice was recorded in Dokri-Basmati, Bungalow, and Basmati-385 Supper basmati, and Basmati-270 and medium infection recorded Super Karnal Super karnal/Kainat, IR-9, IR-and DR-82 and less then infection recorded of rice variety of Winner, DR-92, Vigor, IR-6, Rachna, Mehran, DR-83, DR-59, Shahkar, Danglo Hybrid and Gard-53 According to studies of Jha *et al.* (2004) disease severity in direct sown plants than in transplanted crop. Monitoring of permanent sites maintained for five years indicated that zero tillage practice adopted in wheat crop had no significant effect on brown spot in subsequent rice crop.

Basmati-270 and Bangalow varieties inoculated with *Helminthosporium oryzae* developed typical distinctive symptoms of the fungal population. The leaves of plants inoculated with *Helminthosporium oryzae* showed brownish to reddish margin within 25 days inoculation. Our results are in close agreement . Farid *et al.* (2002) who found five important pathogenic fungi *Helminthosporium oryzae* in rice seed samples collected from three different locations. During present investigation, *Helminthosporium oryzae* appeared as the powerful destructive pathogen on the basmati-270 and Bangalow varieties, the disease appeared in 70% inoculated plant population after 21 days inoculation with 24% typical symptoms of the disease.The association of seed-borne fungi of rice also have been reported by a different researchers (Mew and Gonzales, 2002; Webster and Gunnell. 1992) reported that

5-54% of world rice crop was troubled by *Helminthosporium oryzae*. Singh *et al*., (1979) and Mia *et al*. (2001) who found 20-50% yield loss, respectively. At the same time as. Among the fungal disease, brown leaf spot of rice incited by *Bipolaris oryzae* is a main disease occurring in nearly all the Paddy crops growing areas of the world causing 7% yield loss across all lowland rice production situations in south and Southeast Asia (Savary *et al*., 2000).

As the chemical fungicides are much exclusive and have heath hazardous effects on human health and environment, so different alternative of chemical fungicides were tried to control the brown spot of rice. For this purpose, we have used five different botanicals extracts under *in-vitro* condition for their efficacy against *Helminthosporium oryzae* at different dose. Among all the tested botanical extracts, the ginger and garlic were found highly effective in reducing the linear growth of the fungus followed by Datura and Akk. At the same time, Neem was found less effective in reducing the linear growth of fungus. Ganguly, (1994) obtained that good inhibitory effect of *Azadirachta indica* against *Helmithosporium oryzae*. dela *et al*. (2006) studied brown spot disease of rice caused by *Bipolaris oryzae* to examined the effect of two essential oils (EOs) from *Lippia geminata* and *Cymbopogon jwarancusa* on in vitro growth and sporulation of these two pathogens. A significant reduction in both leaf spot and stalk rot phases of brown spot with increase in N levels from 0-180 kg/ha, irrespective of cultivars has been observed (Sunder *et al*., 2005). Application of sulphur-coated urea or urea super granules at 87-110 kg N/ha and neem cake + urea significantly reduced brown spot (Vidhyasekaran *et al*., 1983; Viswanathan and Kandiannan, 1990)

CONCLUSION

Six fungi *viz, Helminthosporium oryzae ,Curvularia, Alternaria, Fusarium, Penicillium, Tilletia sp* were associated with seeds of different (21) rice cultivars. *Helminthosporium oryzae* was appeared as the predominant fungus. Pathogenicity tests indicated that Basmati-270 and Bangalo were found highly susceptible against *Helminthosporium oryzae. Ginger and Garlic and Dhatura were found more effective in suppressing the linear colony growth of the fungus as compared to other botanicals tested.*

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