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Original Research Article

ANALYSIS OF DIFFERENT ALLELOPATHIC PLANT EXTRACTS AND FUNGAL METABOLITES ON RICE TO CONTROL RICE GRAIN DISCOLORATION

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***Abstract*:** *Rice (Oryza sativa L.) is a grain. It is the seed of grass species. It is used as a cereal and a staple food in many countries. It is an annual plant, a monocot, and its chromosome number is 24. Rice crop faces many biotic and abiotic stresses that cause different diseases. Rice discoloration causes the main quality defect in rice, decreasing the demand for export rice. Grain discoloration of rice was established to be a severe disease in Pakistan and other rice-producing countries, causing huge damage to the yield and quality of the seed, declining the commercial significance of the crop. The disease has been detected as widespread with the introduction of high-yielding varieties and cultural management practice.*

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Keywords: *Aspergillus*; Blast; Disease; Monocot; Rice

Introduction

Rice is a very ancient cereal crop known to our forefathers. It deeply involves the life and cultures of people of different regions and continues till now. It has been cultivated for 10,000 years in the history of humanity, longer than any other cereal crop. There are almost 120,000 varieties of rice. When seen, the history Chinese know about its cultivation, then reported in Sri Lanka, and India. In China, extensive archeological evidence points to the middle Yangtze and upper Huai Rivers as the country's two earliest places of *O. sativa* cultivation. Rice and farming implements dating back at least 8,000 years have been found. Cultivation spread down these rivers over the following 2,000 years. It was then passed into Greece and areas of the Mediterranean. Then it is spread towards Europe and North America by travelers. It was brought into India by Alexander. From India it moved by the Muslims to Spain in 700s. Some resources said it was cultivated from 1500-1000 BC in Indian subcontinents. The grain sample found in Mohenjo-Daro in Pakistan is the oldest, dating back to about 2500 B.C. Rice is genetically very diverse, which is why it has different tastes, colors, taste, aromas, etc. One can imagine the diversity that International Rice Research Institute, Philippines has a repository of approximately 124,000 different lines to date. This is the largest worldwide collection (Irri.org). The diversity in rice germplasm started from a few single wild lines to more than one lac different genotypes, and this journey has enormous knowledge to share and spread. In more than a hundred countries, thousands of numerous varieties are sown. Every line has its trait, history and story (Bernhardt, 1999). Super Kernel Basmati rice is famous Pakistani rice for export due to its aroma, aromatic compounds, and good taste. Its grains naturally contain about 0.09 ppm of this aromatic chemical compound, which is about 12 times more than non-basmati rice varieties, giving basmati its distinctive spicy fragrance and flavor. In Pakistan, 95 percent of the basmati rice cultivation occurs in the Punjab province, where total production was 2.47 million tons in 2010(Rice export and Global Market). Basmati 370 (Pak Basmati), Super Basmati (Best Aroma), Basmati Pak (Kernal), 386 or 1121 basmati rice, Basmati 385, Basmati 515, Basmati 2000 and Basmati 198 (Ashfaq *et al*., 2017).

Material and Methods

Sample collection and preservation

Samples are collected from the rice fields at the maturity stages of the rice when the penicle emerges to the ripening stage. After collection, the samples dried in the shade using newspaper to absorb the moisture. Then these samples are preserved for later use in the lab work.

Media Preparation

MEA media plates were prepared to identify the pathogen. MEA artificial media plates were prepared for the fungal pathogen.

Inoculation

Took the preserved sample and surface sterilized it with 1% sodium hypo chloride solution. Took inoculum with sterilized forceps and inoculated the media plates to grow the causal agent artificially in the lab. MEA media plates inoculated to grow fungal pathogens. MEA media plates were kept in the growth chamber at 27ºC for three to seven days.

Identification and Purification

For identification, the fungal-grown Petri plates were sent to the first fungal culture bank in the Institute of the Agricultural Sciences University of the Punjab Lahore.

Extraction of Fungal Metabolites

Fungal metabolites were prepared to control rice discoloration. The fungus *Trichoderma harzianum* was noticed effective control agent against the disease. To prepare fungal metabolites, two liquid growth media, potato dextrose broth and malt extract broth, were used. The inoculum of *Trichoderma harzianum* was taken from the first fungal culture bank from the Institute of Agricultural Sciences at the University of the Punjab Lahore. MEA media plates were prepared and inoculated with Trichoderma harzianum and placed in the incubator at 25±2ºC for seven days. After seven days, a good growth of *Trichoderma harzianum* arrived on media plates. Took 1000 ml flask, prepared 400 ml malt extract broth, autoclave it, cut 5mm of Trichoderma harzianum growth from media plates, and placed it into the broth. Incubate it at 25±2ºC for fifteen days in an incubator shaker. After fifteen days of injection of *Trichoderma harzianum,* filter it with a muslin cloth to separate fungal growth and metabolites. The filtrate is then stored in the flask at 4ºC for later use.

Results and Discussion

Various pathogens might be involved in infecting rice grains and causing discoloration. Organic farming is the demand of the current world introducing biological control. For many years agricultural chemicals have been disturbing the environment and posing serious health hazards. Hence in this work, we tried to introduce some biological sources of disease control in one of the important field crops, rice. Different biological extracts and fungal metabolites were used to control rice dis-coloration (Table 1; Figure 1). We used *Trichoderma harzianum* and plant extracts of *Allium sativum, Azadirachta indica* and *Curcuma longa*, which have been long used for biocontrol (Abdel-Fattah *et al*., 2007). The studies showed that Trichoderma inhibits fungal diseases through Antibiosis, Parasitism, and competition. However, this does not exclude the possible involvement of other antagonism sources (Chethana *et al*., 2012). The present study observed that the presence of fungi in seed causes discoloration, which is supported by (Javaid and Anjum, 2006). Fungi-caused discoloration is known to invade the seed coat, endosperm, and embryo, resulting in germination failure. The role of *Curvularia in* inhibiting germination has been reported by Hage (2003). The adverse effect of *Curvularia* on the discoloration of cereals has been reported in recent years. Species of *Curvularia*, although reduced as a surface contaminant, were also responsible to produce aflatoxins and have also been known to deteriorate rice grain. Ghosh (1951) reported that *F. moniliforme* and *B. oryzae* reduced the germinability of seeds. Bicca et al. (1998) examined ten rice cultivars grown in non-saline tidal zones of the Patuakhali district were examined to identify seed-borne fungi and their 41 effects on germination. They found that seed germination is decreased with increased seed infection regardless of the rice cultivars tested. A high negative significant correlation was obtained between all isolated fungi and seed germination in the laboratory for all seed samples tested. The maximum biocontrol was observed with *Trichoderma harzianum*.

Table 1: *Trichoderma harzianum* inhibition zone of *Curvularia lunata*

Concentration %	Replicates	Total area of fungi covered (cm)	Inhibition zone (cm)
20	Control	8	0
	R1	7	1
	R2	6.5	1.5
	R3	7	1
40	Control	8	0
	R1	7	1.5
	R2	8	2
	R3	8	2.5
60	Control	7.5	0
	R1	6	3
	R2	7	2.5
	R3	6.5	3
80	Control	8	0
	R1	7	3.5
	R2	7.5	3
	R3	7	3.5

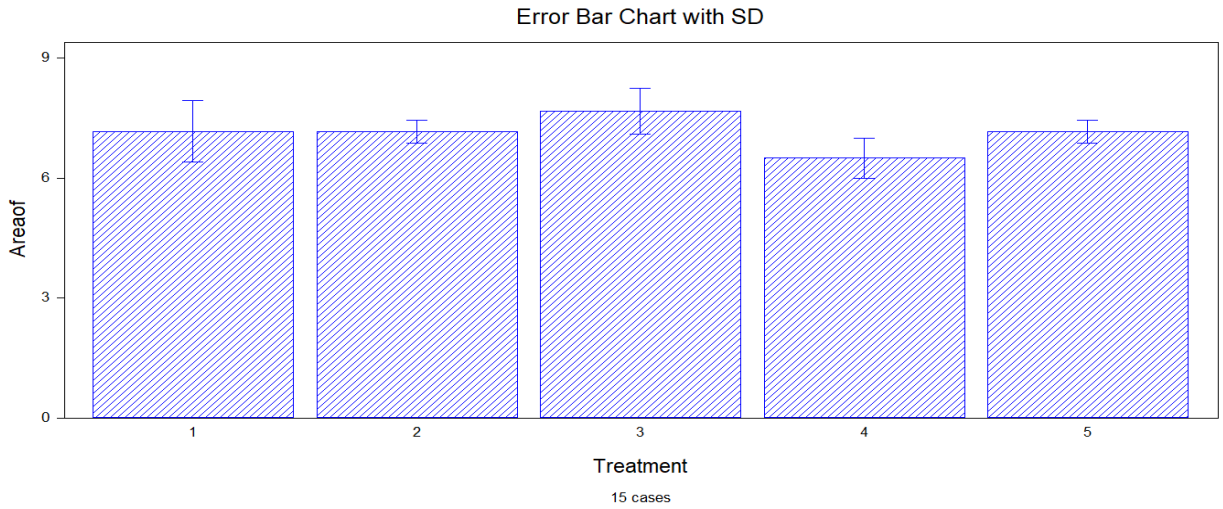

Fig 1: Diagram showing error bar against *Trichograma harzianum*

Conflict of interest

The authors declared absence of conflict of interest.

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