In vitro potential of some aqueous plant extracts for the management of brown spot of rice

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Abstract

The use of plant extracts is gaining importance in plant disease management as injudicious application of chemical fungicides has led to environmental concerns. There is dire need to explore potential alternatives to minimize the harmful effects of chemical pesticides. Therefore, the present work was designed to investigate the effect of different botanicals *viz.* ginger (*Zingiber officinale*), neem (*Azadirachta indica),* calotropis (*Calotropis gigantean*), garlic (*Allium sativum*), and datura (*Datura stramonium*) against *Helminthosporium oryzae*, the pathogen of brown spot of rice under lab conditions. Four concentrations (2, 5, 10, and 20 mL L^{-1}) of each extract were used in three replications along with an untreated control. A 5 mm piece of actively growing fungal culture was placed aseptically on the treated and untreated potato dextrose agar (PDA) medium. These Petri plates were incubated at 27 ºC for 15 days. The results revealed significant inhibition of fungal growth due to different extracts over control. Ginger extracts showed 94.11% reduction in mycelial growth followed by 82.13% inhibition produced by the neem extracts. Similarly, the extracts of calotropis, garlic, and datura were found effective over control with 78.86%, 76.25%, and 63.61% inhibition, respectively. These results suggest the antifungal potential of these extracts against *H. oryzae*. Their use can assist the ecofriendly management of brown spot disease of rice.

Keywords: Brown spot, Management, Plant extracts, Rice.

Introduction

Rice (*Oryzae sativa* L.) contributes significantly in the agro-based industry of Pakistan. It is one of the important cereal crops with a significant share in value-added agriculture and GDP of the country. During 2020-21, rice crop was planted on an area of 3,335 thousand hectares, which is 9.9% more than the last year acreage of this crop. Pakistan witnessed record rice production (8.419 million ton) this year (GOP, 2020-21). Rice is also one of the primary sources of foreign exchange among agricultural commodities of the country (Salim *et al*., 2003; Zahid *et al*., 2005). A large proportion of masses, particularly in rural Sindh, depend on rice for their livelihood. However, the erratic marketing policies deprive complete benefits to rice growers. As a result, the area under rice cultivation and its production is at stack. Rice crop is being affected by environmental and management factors throughout the world (Ngala, 2013). The important abiotic constraints hampering rice yield include water availability, soil salinity and fluctuations in temperature (Defoer *et al.*, 2002). Biotic factors like diseases and insect pests are also major challenges for rice growers (Bux *et al*., 2013; Javaid *et al.,* 2019; Soomro *et al*., 2020). Climate change adds more uncertainties (Dramé *et al*., 2013). Similarly, the emergence of new biotypes of diseases like brown spot, sheath blight, rice blast, and bacterial leaf blight has also threatened its production in rice tracts. Among these diseases, the importance of brown spot of rice is well recognized worldwide (Imran *et al*., 2020). Historically, it has resulted in the Great Bengal famine in 1942 (Barnwal *et al*., 2013). It is prevalent in the areas with inadequate irrigation supply and nutritional disproportion particularly nitrogen deficiency (Baranwal *et al*., 2013). Plant age, soil conditions, sowing method and time, temperature, light, and moisture also affect its development. The pathogen (*H. oryzae*) attacks the crop from seedling to milky stage. The affected nursery can often be recognized from a distance by scorched appearance due to the death of the seedlings. Its severe infection on grains is considered unfit for human consumption. The fungus is reported to survive in soil and infected plant parts including stubbles, straw, and grains for 2-3 years, which act as a source of primary inoculum. Some weed hosts have also been reported as inoculum reservoirs (Biswas *et al*., 2008). Cloudy weather favors disease development. Minimum temperature of 27–28.5 ºC, and relative humidity 90–99%, are the most conducive factors for the dispersal of primary and secondary inoculum of brown spot of rice Host plant resistance is the most efficient and economical method for plant management.

The use of plant-based products especially neem is well popular at commercial level (Gurjar, 2012; Dahar *et al.*, 2017). The presence of biologically active compounds in plant material can be exploited for plant disease management. These products can be applied against a number of diseases as foliar sprays, seed treatments and soil amendments (Javaid and Khan 2016; Ali *et al.*, 2020; Khan and Javaid, 2020a; Banaras *et al.*, 2021; Jabeen *et al.,* 2021). This will not only reduce use of chemical fungicides but will pave the way for ecofriendly control of plant pathogens. Although use of disease resistance is the most effective, economical and farmer friendly approach but chemical control through fungicides is the most preferred practice at farm level. However, their indiscriminate use has harmed environment and human life. It is need of the hour to minimize the use of chemical pesticides. To safeguard our natural sources, it is imperative to cope plant diseases with alternative and environment friendly measures (Shoaib *et al*., 2018; Khan and Javaid, 2020b: Khan *et al*., 2021). Keeping in view the importance of botanicals to control plant pathogen, present work was carried out to evaluate aqueous extracts of some plants against brown spot of rice in lab conditions.

Material and Method

Collection of disease samples

Infected rice samples were brought from the farmers' field. The samples were cleaned and sliced into small pieces with some healthy portion. The shards were surface sterilized with 5.0% sodium hypochlorite solution for two minutes, then rinsed with water and blotter dried. These infected fragments were placed in Petri plates containing PDA medium. Then the Petri plates were incubated at 27 ºC for 15 days. The preliminary isolation from the infected pieces was purified on fresh potato dextrose agar medium. The morphological features of the purified culture were observed under a microscope and identified with the help of illustrated genera of imperfect fungi fourth edition (Barnett and Hunter, 1998).

***In vitro* antifungal activity of the botanicals**

Present work was conducted to evaluate the effects aqueous extracts of various plants *viz.* neem, garlic, ginger), calotropis and datura for *in vitro* management of brown spot of rice. The experimental material including leaves of neem, calotropis and dhatura, while rhizome of ginger and garlic bulbs were brought to the lab. The material was cleaned and washed thoroughly in tap water and air-dried. Two hundred and fifty grams (250 g) of each plant material was weighed and crushed. Then 100 mL of sterile distilled water was added to the paste in 500 mL beaker and stirred vigorously. This mixture was kept overnight and then filtered. The efficacy of these extracts was evaluated with poison food technique. The extracts were applied in four treatments i.e. 2, 5, 10 and 20 mL L^{-1} of potato dextrose agar medium. All these doses were added to the sterilized molten PDA medium. Each treatment was replicated thrice by using completely randomized design. The Petri dishes containing PDA medium without any plant extracts served as control. A 5 mm piece of seven days old culture of *H. oryzae* was placed aseptically on the treated PDA medium. All these Petri plates were incubated for 15 days at 27 °C. The observations were made until the control plates were filled with mycelial mat of the test fungus. The fungal growth in treated Petri plates were compared with control and percent reduction was calculated following Sireesha and Venkateswarlu (2013).

$$\text{Inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Fungal growth in control}} \times 100$$

Statistical analysis

The observations were made on daily basis and means of the replicated data were calculated. As soon as, all the requirements of data analysis were fulfilled, ANOVA was performed followed by LSD test. The data collected on fungal growth and its inhibition over control was analyzed statistically using Statistix 8.1 version.

Results and Discussion

The effect of various concentrations of plant extracts on colony growth of *H. oryzae* are presented in Table 1. It is evident from these results that 2 mL L^{-1} concentration of all plant the extracts had the least inhibition as compared to the other tested doses of extracts over control ($P \leq 0.05$). However, 2 mL L^{-1} concentration of ginger extracts produced higher inhibition of growth as compared to the other extracts at same concentrations. Similarly, results revealed that ginger extract at its all doses was very effective against the pathogen and produced significantly higher inhibition as compared to the other extracts. Our findings are in accordance with those of Abdullai *et al.* (2020) who reported the efficacy of ginger against fungal and bacterial diseases of rice. The results of present work rates neem extract second in reducing the fungal growth on nutrient media. These results are in conformity with the studies of Devi and Chhetry (2013) and Kumar *et al*. (2015) that neem products are the best in reducing brown spot in laboratory as well as in field conditions. Similarly, Ahmed (2002) proved that 1:1 dilution of neem and garlic extracts reduced brown spot of rice incidence. It is also clear from present results that extracts of calotropis, garlic and dhatura were effective against the tested pathogen at their higher concentrations ($P<0.05$). Waheed *et al.* (2016) have attributed the antifungal potential of calotropis to presence of large number of biologically active compounds like saturated and unsaturated fatty acids and aromatic carboxylic acid. Antifungal activity of garlic extracts against some plant pathogenic fungal flora has been illustrated by various earlier workers (Appiah-Kubi and Aidoo, 2016; Kutawa *et al*., 2018; Ferdosi *et al.,* 2021). Bio-efficacy of datura extract containing some biologically active compounds against plant pathogenic fungus like *Fusarium* sp. and *Alternaria* sp. has been tested by Jalander and Gachande (2012).

The mean mycelial inhibition in fungal growth exhibited by all extracts as compared to control has been presented in Fig. 1. The result showed that all the tested extracts reduced the fungal growth as compared to control. However, it is clear that the Petri plates containing ginger extract produced least fungal growth depicting maximum reduction as compared to control which was followed by neem extracts. It was noticed that the fungal growth was very slow and retarded in all Petri plates containing ginger and neem extracts as compared to the vigorous growth of the fungus in the untreated control. The extracts of calotropis, garlic and dhatura were also found significantly effective as compared to control, however, their efficacy was comparatively lower than the ginger and neem extracts. The present findings of suppressing the brown spot pathogen with the use of plant extracts are in conformity with those of Iwuagwu *et al*. (2020).

The percent mycelial inhibition produced by the different concentrations are presented in Table 2. The results indicate that at 2 mL L^{-1} concentration of ginger extract showed 89.48% inhibition which was the highest among all tested botanicals at the same concentration. The other doses of ginger *i.e.* 5, 10 and 20 mL L^{-1} exhibited 91.55%, 96.65%, and 99.19% reduction in fungal growth respectively which was higher than the inhibition produced by the other extracts at same doses. Neem extract showed more percent inhibition as compared to calotropis, garlic and dhatura. Calotropis and garlic ranked as third and fourth in terms of the percent reduction in fungal growth on PDA. Among all the tested botanicals, dhatura extract showed more fungal growth which indicates that it was least effective against the tested pathogen. The results depict the efficacy of plant extracts against brown spot of rice and are in accordance with those of Choudhury *et al*. (2020). Overall percent reduction in the fungal growth produced by the tested doses of ginger, neem, calotropis, garlic and dhatura extracts ($P \leq 0.05$) has been summarized in Fig. 2. Among all the tested botanical, the ginger extracts showed 94.11% overall mean mycelial inhibition followed by neem 81.13% and minimum percent reduction of 63.61% was noticed in dhatura extracts. Plants possess a number of biologically active compounds with antimicrobial properties. The characterization of these compounds has shown their potential as an alternative to chemical fungicides (Han *et al*., 2018). The application of plant-based products for pest and disease management in crops at farm level will reduce the impact of hazardous chemicals in current climate change scenario. In this regard five aqueous extracts were evaluated to determine their potential against rice brown spot in lab conditions. The results revealed that all tested botanicals significantly reduced the progression of fungal growth on nutrient media. This suggests the presence of antifungal substances in these extracts. It is evident from our results that ginger extracts were found very effective against *H. oryzae*. Presence of several components particularly gingerol and shagelol in ginger rhizome are considered responsible for their antibacterial and antifungal properties (Chen *et al.,* 2008; Atai *et al*., 2009; Supreetha *et al*., 2011). Ginger essential oils are also important constituents of ginger with wide range of antimicrobial properties. (Abdullahi *et al*., 2020) have characterized eighty-two different biologically active molecules from ginger rhizome and thirteen of those compounds proved very effective against different fungal and bacterial plant pathogens of rice. Use of different parts of neem lants as an antimicrobial remedy is well documented. Alzohairy (2016) and Javaid *et al.* (2020) reported the presence of nimbin, nimbidin, nimbolide, and limonoids as biologically active molecules in neem which modify genetic pathways and other activities to control disease. Our results also depicted the efficacy of neem extracts in reducing the mycelial growth of *H. oryzae* as compared to control. The present results are also in conformity with those of Al-Hazmi (2013) who found higher concentrations of neem leaf extract very effective in controlling *Helminthosporium* sp. Similarly, Farooq *et al.* (2015) observed that seed treatment with neem and almanda leaf extract effectively reduced the brown spot incidence. Garlic, calotropis and dhatura constitute a number of biologically active molecules. Extracts of various parts of these plants have been used against a number of fungal and bacterial pathogens. The present study showed that higher doses (10 and 20 mL L^{-1}) of calotropis, garlic, and dhatura extracts proved very beneficial against brown spot pathogen in lab conditions. The studies conducted by Razu and Hossain (2015) showed low severity of brown spot in plots sprayed with garlic and clove extracts. The presence of Allicin (sulfoxide group) in garlic suppresses the fungal activities (Gurjar *et al*., 2012). The results presented in Table 2 indicated that the calotropis showed overall reduction of 78.86% while garlic and dhatura with 76.25% and 63.61% reduction in mycelial growth respectively were grouped and in fourth and fifth ratings in this study. Our findings suggest that extracts of ginger and neem are very effective against brown spot of rice. The management of rice diseases especially brown spot of rice using plant extracts has also been reported by various other researcher (Dutta *et al*., 2004; Madhusudan, 2004; Harlapur *et al*., 2007;

Sena *et al.*, 2013; Choudhury *et al.*, 2020). The application of plant extracts is an important crop disease management strategy based on natural and environmental tactics against various plant pathogens (Javaid *et al*., 2020). Being cost effective, their use is considered farmer friendly. Apart from this, their easy and rapid degradation does not harm environment as compared to the synthetic chemical pesticides, thus their use promotes non residual and ecofriendly disease management approach (Naz *et al*., 2018). The presence of phenolic compounds is also an important constituent of plant extracts. These botanicals produced the highest (94%) mean mycelial reduction over control. It is also very important to create awareness among farming community to adopt eco-friendly measure for diseases management. At the same time, the large-scale production of these antifungal products must be ensured. Pakistan must set regulation of testing for every type of corn before circulation into the market.

Table 1: Effect of various botanicals concentrations on growth of *Helminthosporium oryzae*.

Botanicals	Fungal growth (mm) at different concentration of plant extracts			
	2 mL L^{-1}	5 mL L^{-1}	10 mL L^{-1}	20 mL L^{-1}
Ginger	0.49 e	0.38 e	0.15 e	0.04 d
Neem	1.30 d	1.07 d	0.68 d	0.24 c
Calotropis	1.51c	1.17 d	0.84 d	0.35 c
Garlic	1.61c	1.43 c	1.10 c	0.23 c
Dhatura	2.39 b	1.86 b	1.50 b	0.95 b
Control	4.66 a	4.50 a	4.49 a	4.63 a
SE	0.0514	0.0876	0.1091	0.0875
LSD	0.1119	0.1909	0.2376	0.1907

Means with different letters in a column are significantly different at $P \leq 0.05$.

Table 2: Percent reduction in colony growth of *Helminthosporium oryzae* due to different concentrations of the botanicals.

Botanicals	**Reduction in fungal growth at various concentrations of plant extracts (%)**			
	2 mL L^{-1}	**5 mL L^{-1}**	**10 mL L^{-1}**	**20 mL L^{-1}**
Ginger	89	92	97	99
Neem	72	76	85	95
Calotropis	68	74	81	92
Garlic	65	68	76	95
Datura	49	59	67	79

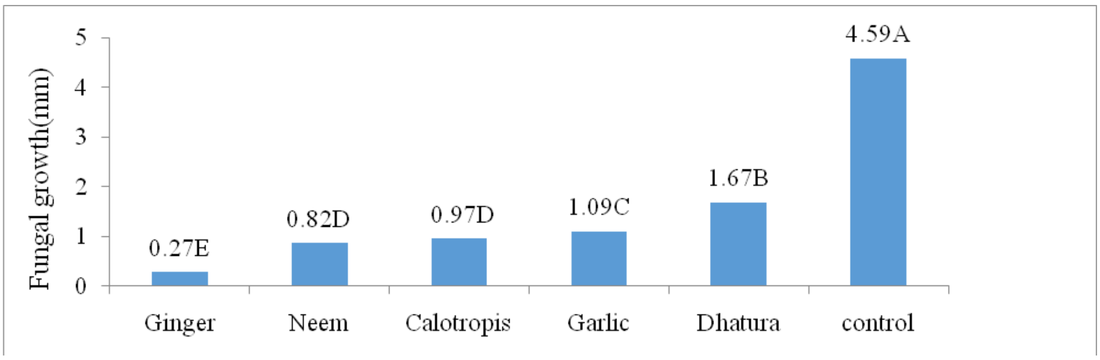

Fig. 1: Effect of plant extracts on mean colony growth of *Helminthosporium oryzae.*

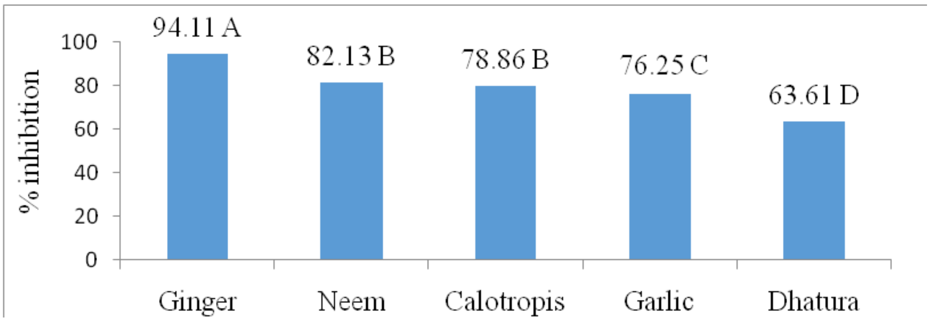

Fig. 2: Overall mean percentage inhibition in fungal growth on PDA by botanicals.

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