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SCREENING OF ADVANCED GERMPLASM AGAINST *ASCOCHYTA RABIEI* AND ITS MANAGEMENT

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A B S T R A C T

Ascochyta blight is caused by *Ascochyta rabiei*, one of the most prevalent chickpea diseases worldwide. Losses could reach as high as 100%, and this damage could significantly affect both seed quality and production. The most effective way for the management of this disease is genetic resistance. Seventeen chickpea varieties/lines were screened against a virulent isolate of *Ascochyta rabiei* in controlled environmental conditions. Advance Line-2, Bittle-2016, and Noor-2009 within the germplasm exhibit a moderately resistant reaction. TG-1415 and Advance Line-1 demonstrate a moderately susceptible response. On the other hand, Noor-2013, Star-Channa, and Rohi display a highly susceptible response. In the poisoned food technique among applied treatments at 50 ppm, Diphenoconazole proved be best, with a 46% reduction in the growth of the pathogen, followed by propiconazole and Azoxystrobine+Diphenoconazole with 45.5 % and 45%, respectively. In the mean while in glasshouse evaluation at 50ppm, Diphenoconazol proved best with a minimum disease severity of 24%, followed by propiconazole and Metalaxyl with a 25.9% and 27% reduction in disease severity, respectively.

Keywords: Chickpea; *Ascochyta rabiei;* Ascochyta blight; Screening; Germplasms

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INTRODUCTION

Chickpea, (*Cicer arietinum L.,)* belongs to the family *Fabaccea* and subfamily *Faboideae*. It is a self-pollinated field leguminous crop that produces two seeds in each legume. It is an annual diploid crop with 12 chromosomes and a genome of 740M (Nawaz et al., 2022). Chickpea is a significant protein, carbohydrate, and mineral source for animal and human consumption across chickpea-growing regions worldwide. It is called the "poor man's meat" because it provides protein to the world's growing populations (Saabale et al., 2020). It is the third-most significant pulse crop globally, after soybeans and peas, with an annual global production of roughly 14.78 million tons. Chickpea is primarily cultivated in developing countries, with a current cultivation area of approximately 14.25 million hectares (Otekunrin et al., 2021). India and Australia are major contributors, accounting for 60% and 14% of global production, respectively (FAO, 2010). Other significant producers include Pakistan, Turkey, Myanmar, Ethiopia, and Iran. Chickpeas cover 2,079 thousand hectares in Pakistan and yield 545 thousand tons annually (Economic Survey of Pakistan, 2020-21). The primary cultivation of chickpeas, up to 90%, occurs in rain-fed areas of Punjab Province. Main districts for chickpea cultivation include Mianwali, Khushab, Muzaffargarh, Rajanpur, Dera Ghazi Khan, Bhakkar, Chakwal, Faisalabad, Jhang, and Layyah (Ahmad, 2015). Although the environment in these districts is highly suitable for chickpea cultivation, best yields are reduced by fungal diseases such as chickpea wilt and chickpea blight (Ali et al., 2012). They are highly destructive diseases affecting chickpea crops in temperate regions of the Indian subcontinent and worldwide. (Mallikarjuna et al., 2017). Chickpea blight, caused by *Ascochyta rabiei*, is a significant foliar disease affecting

chickpea globally, leading to potential losses of grain yield and quality reaching 100%(Pande et al., 2011). The causal organism belonging to the Ascochyta genus is airborne, waterborne, and seed-borne. Young seedlings with seed-borne infections display brown lesions at the base of the stem, which progressively enlarge, encircling the stem and eventually leading to plant mortality (Nene, 1982). Optimal conditions for disease spread involve humid, cool, and cloudy climates (Pande et al., 2011). Notably, in Pakistan, outbreaks of this disease have caused significant reductions in output, prompting growers in the irrigated regions of Punjab to shift to alternative crops (Wohor et al., 2022). Genetic resistance is the favored strategy due to its efficacy and cost-effectiveness in tackling biotic stresses. When combined with a fungicide regimen, chickpea varieties exhibiting enhanced resistance offer a viable approach. Understanding the pathogen's variability in population is vital in predicting blight incidence in chickpea crops. The knowledge about resistance in available germplasm and effectiveness of current available fungicide could provide valuable baseline information to develop disease management strategies. Therefore, the objectives of the study were to identify resistant sources against Ascochyta blight within the available chickpea germplasms and Evaluation of the efficacy of existing fungicides against Ascochyta blight disease.

MATERIAL AND METHOD

Isolation and purification

Samples of chickpea plants infected with Ascochyta blight were collected from Bhakkar, Punjab, Pakistan. The samples were disinfected with 1% sodium hypochlorite solution for duration of 3-5 minutes and then washed twice with sterilized distilled water for 3-5 minutes. After this, samples were dried on filter paper and directly positioned on the surface of acidified CSMA (40 g chickpea flour, 20 g dextrose, 20 g agar per litre of water). The culture plates were then placed in the incubator, set at a temperature of 20-22°C for 14 days, with alternating cycles of 12 hours of darkness and fluorescent light. Once the pycnidia were observed, they were collected using a sterilized needle and transferred to 1.5ml tubes filled with distilled water. Following this, they were vortexed and spread onto a 2% water agar medium. After two days, a single germinating spore was selected from the water agar and transferred to CSMA. Each isolate was subsequently cultured and multiplied on CSMA plates for further use.

Pathogenicity test

A pathogenicity test was carried out on susceptible chickpea germplasm (Thall 2006) within controlled environmental conditions. Fifteen days old *A. rabiei* culture was used for inoculation. Inoculum was prepared and quantified by using hemocytometer (10^5spore/ml). After the plants were inoculated, they were covered with a clear polyethylene sheet for a period of 48 hours. The assessment of disease symptoms was performed fifteen days post-inoculation, employing a rating scale ranging from 1 to 9 (Aslam et al., 2021). Re-isolation of the pathogen was done from the diseased plants to confirm the identity of the pathogen and establish the Koch's postulates.

Screening of germplasm

The 17 chickpea genotypes were collected from AARI Faisalabad and AZRI Bhakkar. Seeds were disinfected and were kept in water for 24 hours for good seed germination. Every germplasm had three replications. Seeds were sown in pots (10- $300cm^3$) containing a mix (3:1) of sterile sandy soil and were kept in a glasshouse under natural light at 24°C ± 1°C until the moment of inoculation. Highly aggressive isolate of *A rabiei*, was used as inoculum. Inoculum was prepared and sprayed as described in previous section. Disease reaction was measured in individual plants 14 days after inoculation, according to (Nasir *et al.* 2000). The severity of the disease in each variety was measured over 14 days after infection and the average score was calculated.

In vitro evaluation of fungicide against *A. rabiei*

The efficacy of four fungicides, namely Difenoconazole, Propiconazole, Metalaxyl, and Azoxystrobin + Difenoconazole, was evaluated in inhibiting the colony growth of *A. rabiei* by using poisoned food technique on two different concentrations, i.e., 20 ppm and 50 ppm. The prepared concentrations were added in falcon tubes. CSMA medium was poured into falcon tubes by respective concentration, and 40 ml of the medium was added to each 50 ml falcon tube for two Petri plates. Each treatment was replicated four times by following a completely randomized design. Disks, measuring 7 mm in diameter, were taken from an actively growing *A. rabiei* culture using a sterile cork borer. These disks were placed at the centre of each plate. The plates were subsequently incubated in an environment maintained at 20 ± 2°C until the *A. rabiei* culture in the control plate had fully developed, following the procedure outlined by (Mahmood et al., 2015). To calculate the percentage inhibition of colony growth, the colony diameter of the treated (poisoned plates) and control plates was measured and compared using the formula provided.

Percentage Inhibition = $\frac{C-T}{C} \times 100$

C= Colony diameter of control

T= Colony diameter of treatment

The assessment of fungicides in a controlled laboratory setting followed a completely randomized design (CRD), with each treatment having three replications. The data acquired were then analyzed using ANOVA to ascertain the primary and interactive impacts of the treatments.

Evaluation of fungicides in glass house assay for *A. rabiei*

Fungicides that were effective during in vitro evaluation by poisoned food technique were further tested in a glasshouse for the control of chickpea blight disease by foliar application at different concentrations, i.e., 10 ppm, 20 ppm, and 50 ppm. For the glasshouse evaluation of treatments, a single variety (Thall-2006) that was susceptible to blight was planted in a completely randomized design (CRD), with three replicates. Within each block, there were three pots for each treatment. All the replicates were subjected to inoculation with *A. rabiei* (at a concentration of 5 $\times 10^5$ spores/ml) until the disease became visible. As soon as disease symptoms started to appear, the treatments were applied using a sprayer with the necessary formulations.

Data regarding disease severity index was recorded by the formula suggested by by (Hassan et al., 2012).

$$(\text{DSI \%}) = \frac{\text{Total of all ratings}}{\text{No.of Plants examined}} \text{ x } \frac{100}{\text{Max. Disease rating}}$$

To evaluate the interactive effects of different treatments, data were subjected to ANOVA and LSD statistical analysis tests (Shah et al., 2010).

RESULTS

Screening of germplasm

The analysis showed that all genotypes were affected by *Ascochyta rabiei*, and there were no single plants that showed resistant behavior against these isolates. The germplasm was ranked from moderately resistant to highly susceptible. The diseased plants showed similar symptoms to those previously reported by (Aslam, Shah et al. 2021). Screening results showed that Advance Line-2 showed the moderately resistance effect with a disease severity rating of 5. Advance Line-1 and TG-1415 were found to be moderately susceptible with a disease severity rating of 6. Germplasm of Noor-2013, TG-1427, Bhakkar-2011, TG-1626, Thall-2020, TGK-1508, Bittle-2016, BRE-446, BWP-21 BRE-525, and Noor-2009 were found to be susceptible with a disease severity rating of 7. While Star-Channa and Rohi showed a highly susceptible reaction against the pathogen with a disease severity rating of 8. No seedling without any lesions was found except control (Table 1).

Table 1. Level of resistance/ susceptibility of chickpea lines/germplasm against *A. rabiei* pathogen in glass house condition.

S. No	Germplasm	Disease Severity	Scale/Rating	Rank/Class
1	Noor-2013	67.58	7	HS
2	Advance Line-2	34	5	MR
3	Advance Line-1	41.45	6	MS
4	TG-1415	42.69	6	MS
5	TG-1427	52.76	7	S
6	Thall-2006	0	1	N
7	Bhakkar-2011	58.54	7	S
8	TG-1626	57.31	7	S
9	Thall-2020	60.63	7	S
10	TGK-1508	65.21	7	S
11	Star-Channa	76.3	8	HS
12	Bittle-2016	67.05	7	MR
13	BRE-446	65.74	7	S
14	Rohi	77.2	8	HS
15	BWP-21	66.87	7	S
16	BRE-525	64.89	7	S
17	Noor-2009	68.39	7	MR

In vitro* evaluation of fungicide against *A. rabiei

The *in- vitro* evaluation of fungicides at concentrations i.e.20 ppm, and 50ppm against *A. rabiei* reported a significant reduction in the colony growth of pathogen as compared to control. The Diphenoconazole at 50ppm concentration proved to be best with 46% reduction in growth of pathogen, followed by propiconazole and Azoxystrobine+Diphenoconazole with 45.5% and 45% respectively. Hence the fungicides Metalaxyl and Mancozeb proved to be less effective (Figure 1).

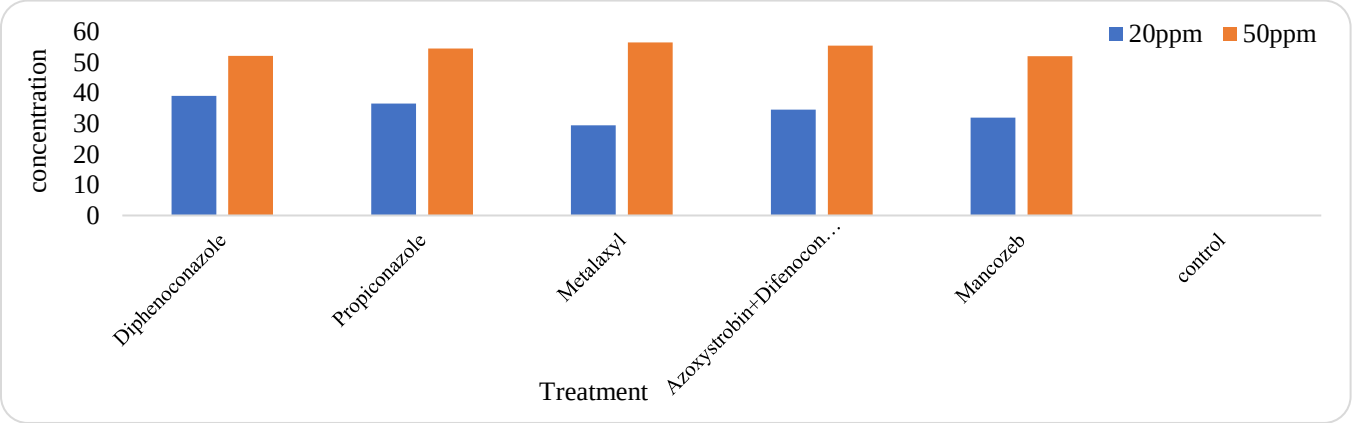

Figure1. Efficacy of different fungicides against chickpea blight in laboratory.

Evaluation of Fungicides in glass house assay for *A.rabiei*

The in-vivo evaluation of all applied treatments showed a significant reduction in disease severity. The fungicide Diphenoconazol with 50ppm concentrations proved to be best with minimum disease severity 24% followed by propiconazole and Metalaxyl with reduction in disease severity 25.9% and 27% respectively. While Diphenoconazole+Azoxystrobin at same concentration 28.5%, were least effective. The positive control had 53.76% disease severity, while the negative control had 0.00% disease severity (Figure 2).

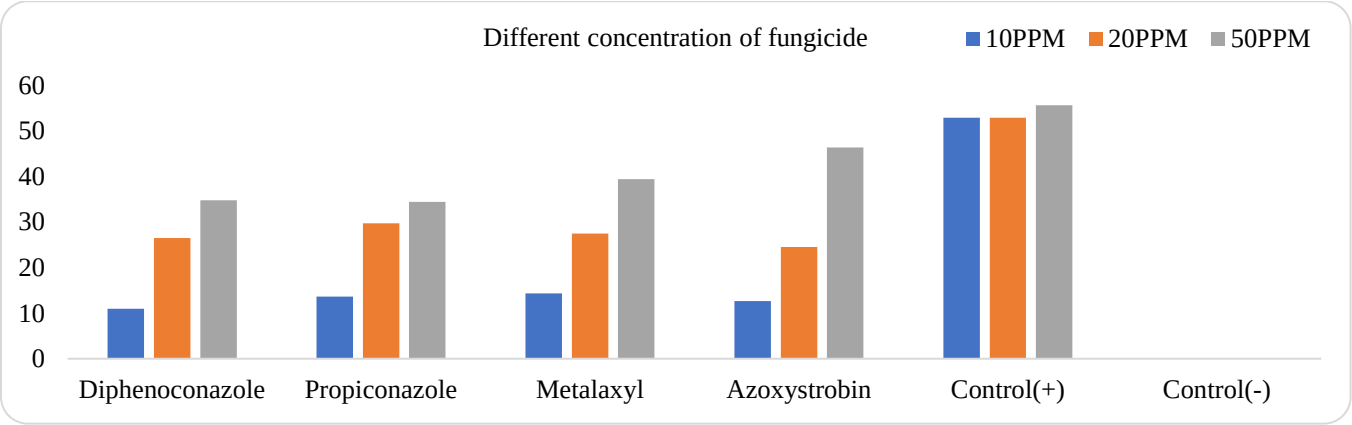

Figure.2. Efficacy of different fungicides against chickpea blight in glass house.

Discussion

Chickpea is economically important crop of Pakistan with production of average 565 thousand ton annually. The production of chickpeas faces many challenges. Most important Ascochyta blight, which is caused by *Ascochyta rabiei*, this disease cause 100% yield loses due to the increasing prevalence of favorable weather conditions.

Most previous research showed that resistant cultivars lose their resistance to *A. rabiei* when new physiological races form, and this leads to the establishment of new sources of resistance (Jabbar et al., 2014). Most genotypes were discovered to be extremely sensitive to susceptibility during screening. This indicates that most of the chickpea germplasm lacked resistance genes. In present work some of

the advance lines revealed resistance. This was confirmed by the previous findings on the subject of resistance in chickpea against blight by different renowned workers (Reddy and Singh, 1984).The germplasm screening also revealed varying reactions to different germplasm which could help to determine their resistance ranking. It was discovered that the germplasm TGK-1508, TG-1427, Bre-446, Noor-2013, BWP-21 and BRE-525 exhibited a susceptibility to isolate. On the other hand, Advance Line-2 displayed moderate resistance against isolate while Star-Channa and Rohi were also highly susceptible in their reaction's profiles. There were varying degrees of susceptibility and resistance among the selected genotypes for virulence analysis, which could account for these results. A similar study was conducted by (Iqbal et al 2010) involved screening one hundred and forty-five genotypes against both Ascochyta blight and fusarium wilt disease where most germplasm exhibited highly susceptible or susceptible responses. When (Bokhari et al., 2011) evaluated the level of resistance displayed by 10 gramme cultivars, they discovered that the majority of germplasm were susceptible under field conditions. Various techniques applicable for AB resistance screening in greenhouse and field settings had already been reported such as those by (Nene 1982; Pande et al 2010; Nasir et al.2000; Du et al 2012).

In this investigation, chemical agents were employed to ascertain a cost-effective and practical source for farmers confronted with the absence of resistant sources in the field against Ascochyta blight. The assessment of various treatments utilizing poisoned food techniques and greenhouse experimentation revealed the effectiveness of diverse fungicides in suppressing the growth of the pathogen. The findings indicated that Diphenoconazole exhibited a statistically significant difference compared to alternative fungicides. Specifically, Diphenoconazole emerged as the most efficacious fungicide, demonstrating a 46% reduction in pathogen growth in the poisoned food technique. Propiconazole exhibited a 45.5% reduction, and the combination of Azoxystrobin and Diphenoconazole proved effective with a 45% reduction in colony growth of the pathogen. Conversely, Metalaxyl and Mancozeb demonstrated comparatively lower efficacy, resulting in a 43% and 42% reduction in pathogen growth in the poisoned food technique.

Similarly, in the greenhouse evaluation, Diphenoconazole once again emerged as the most effective fungicide, yielding a minimum disease severity of 24%, followed by propiconazole and metalaxyl, which exhibited reductions in disease severity of 25.9% and 27%, respectively. Diphenoconazole combined with Azoxystrobin reduced disease severity by 28.5% but proved to be the least effective in the greenhouse setting. Our study conclusively demonstrates that the fungicides Diphenoconazole and propiconazole exhibit heightened efficacy in both greenhouse and in vitro conditions.Chemical control was proved to be very effect against blight disease previously as well. For example, another study identified chlorothalonil, zineb, captan, antracol, propiconazole, penconazole, and thiabendazole as effective in controlling the spread of Ascochyta blight (Ahmad et al., 2021). Under in vitro conditions, Aliette fungicide successfully controlled chickpea blight, showing significant inhibition, and aligning with the current research. The timing of chemical application was emphasized by Chongo et al. (2003a), highlighting the importance of reducing losses caused by A. rabiei. Notably, the application of chlorothalonil at two different stages led to an 8% incidence reduction compared to a 45% incidence in the control treatment (Chongo et al., 2003a). Gan et al. (2006) supported these findings, advocating for foliar application along with integrated management as an effective approach for disease management in chickpea. Demirci et al. (2003) found that chlorothalonil and azoxystrobin, while not performing well in vitro, demonstrated effectiveness under field conditions against A. rabiei. Shtienberg et al. (2000) emphasized the importance of protective fungicides such as zineb, Bordeaux mixture, and captan in disease reduction, though they were less effective on susceptible cultivars. Recent years have seen the identification of new fungicides effective against A. rabiei, including boscalid, pyraclostrobin, difenoconazole, azoxystrobin, tebuconazole, and mancozeb, aligning with the current research (Gan et al., 2006). MacLeod and Galloway (2002) highlighted the successful use of mancozeb in Australia, Canada, and Israel for controlling chickpea blight, which was corroborated by the positive performance of mancozeb in the present research. MacLeod et al. (2002) reported on the effectiveness of carbendazim (now banned), difenoconazole, and tebuconazole in various regions, further supporting the effectiveness of these fungicides against *A. rabiei*. Overall, the study provides insights into integrated management strategies for controlling Ascochyta blight on chickpea germplasm in Pakistan.

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