Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

MANAGEMENT OPTIONS FOR MINIMIZING ASCOCHYTA BLIGHT (*ASCOCHYTA RABIEI*) RISK THROUGH NOVEL RESISTANCE SOURCES AND FUNGICIDES IN CHICKPEA (*CICER ARIETINUM* L.)

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A B S T R A C T

Chickpea (*Cicer arietinum* L.) is the second largest pulse crop cultivated worldwide. Ascochyta blight caused by *Ascochyta rabiei* is the major constraints to chickpea production across the continent including Pakistan. The pathogen *Ascochyta rabiei* is highly variable. Chickpea cultivars contain limited resistance to pathogen due to their potential for sexual recombination. Investigations were conducted for the identification resistant sources against *A.rabiei.* Sixty-six chickpea accessions and one susceptible variety were tested against *A. rabiei*. However, four accessions TG-1427, Star Channa, PARB913/CH03 and PARB913/CH02 showed resistant reaction, eight accessions moderately resistant reaction, eleven accessions developed moderately susceptible reaction whereas twenty two accessions recorded susceptible reaction, moreover remaining twenty one accessions exhibited highly susceptible reaction with maximum ratings ≥ 9. Ten fungicides were tested against *A. rabiei* at three concentrations (3g, 5g and 7g/liter of water) on the susceptible cultivars (AUG-424). Application of Pyraclostrobin and Azoxystrobin proved most effective and expressed minimum disease incidence 8.37 and 10.97% respectively on comparison to control 77.31%. Results of the present investigation will help the farming community and researchers for timely management of *A. blight*. Resistant accessions that were identified in this study will be useful for developing blight resistant cultivars.

Keywords: Ascochyta blight, Azoxystrobin, Pyraclostrobin, resistance source and sexual recombination.

INTRODUCTION

Chickpea (*Cicer aritinum* L.) is the edible pulse crop particularly in African and Asian countries (Kanouni *et al.,* 2011; Gan *et al.,* 2006). It is the self-pollinated, diploid and annual legume crop which ranks third after bean (*Phaseolus vulgaris* L.) and field pea (*Pisum sativum* L.) worldwide with the production of 8.8 million tons and cultivated on an area of 9.6 million ha with the average yield potential of 920 kg ha^{-1} food (Varshney *et al.,* 2013; FAO, 2018). Chickpea is commonly grown on small to large area for the purpose of food and cash crop. Seeds, pods and immature shoots are used as the vegetable by humans due to its valued nutritive seed with maximum protein contents ranged 25.3-28.0% which is better than other legumes quality, likewise green gram, black gram and pigeon pea (Singh *et al*., 1993).

Numerous chickpea genotypes are cultivated all over the world and Kabuli and Desi are most popular among all genotypes. It is cultivated in different agro-ecological conditions worldwide due to their medicinal use for bronchitis, cholera, flatulence, sunstroke, aphrodisiac, catarrh, diarrhea and warts. West Asia is native for chickpea cultivation and currently grown in fifty-five

Submitted: August 10, 2022
Revised: November 12, 2022
Accepted for Publication: November 30 19, 2022
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countries. Chickpea is the nutritious food for bodybuilding as it makes the body muscles more strong; moreover, it can be utilized for livestock as fodder crop (wood and Grusak, 2007).

It is mainly considered for being attacked by numerous pathogen such as fungal (67), bacterial (3), viral (2), nematodes spp. (80) and few mycoplasma-like organism in the world. Typical characteristic symptoms of these attacks are fusarium wilt, leaf blight, collar rot, root rot, powdery and downy mildew. Numerous fungal species including Aspergillus spp. *A.alternata* and *A.porri* have been reported in chickpea fields (Prajapati *et al*., 2017). Among all diseases leaf blight of chickpea is the most destructive disease and it causes potential threat for the successful cultivation of chickpea crop by causing 20% agricultural spoilage (Mehta and Pandey, 2016). Numerous attacks of leaf blight disease have been reported worldwide and it caused t5-47% loss of potential yield in Pakistan (Shrestha *et al*., 2005). Disease development starts from the leaves and lesions appears on stem and pods with > 1.25 mm diameter. *A.blight* lesions are commonly surrounded by the chlorotic tissues (Peever *et al*., 2012). Disease progression and blight infection occurs from 25-50 °C and it requires 6-h leaves wetness with the 16-20°C optimum temperature. Increase in relative humidity leads to the maximum disease severity (Davidson and Kimber, 2007). Cloudiness and wet weather favors disease development and transmission. Fungal pathogen survives on infected seeds and contaminated debris of chickpea (Chang *et al*., 2007).

Different management strategies such as botanical, biological, chemical and essential oils have been studied to control chickpea leaf blight disease. Presence of partial resistance against favorable weather conditions and maximum inoculum pressure is mainly responsible for devasting disease. Thus, it is compulsory to combine the application of foliar fungicides and other management practices to overcome *A.rabiei*. In the view of chickpea leaf blight disease importance, this study was conducted with the objective of determining resistant source against *A. alternata* and its management through the application of fungicides.

MATERIALS AND METHOD

Experimental area: Present study was designed in the field area of Arid Zone Research Institute, Bhakkar, Punjab, Pakistan (31.6344° N, 71.1202° E). The climate of study area is arid where average temperature remains 24.6 °C whereas, the annual rainfall is 213 mm. November was the driest month with 2 mm rainfall.

Experimental design: Sixty-six chickpea accessions were tested against Ascochyta blight. One-meter-long rows were used to grow the entries. After two test entries, a susceptible variety, Aug-424, was employed as a check and the procedure repeated. Spraying plain water and covering with a transparent plastic sheet were used to adjust the temperature and humidity. The highest blight disease developed between 14 and 18°C and at a humidity level of more than 80%. The genotype AUG-424 served as repeated checks among all genotypes.

Data collection: Experimental data of the number of wilted plants in each row for each genotype were collected on weekly basis and disease incidence was determined by using international standard scale 1-9 (ICARDA, 2003).

Management of *Ascochyta rabiei* through fungicides: Ten fungicides at the concentrations of 1.5g, 2.5g and 3.5g/liter of water were evaluated against *A. rabiei* under vivo conditions. Experiment was laid out in randomized complete block design (RCBD) by adopting standard row to row and plant to plant spacing. Three sprays at the interval of fifteen days were used and the data of the disease reduction was obtained after seven and fourteen days of each spray. First spray application was done after the appearance of characteristics symptoms. IHT-401 Hand sprayer was used for the application of fungicides on genotypes. Application of fungicides was started after the appearance of initial disease symptoms. Disease data were recorded by following visual observation and rating scale as described by Iqbal *et al.* (2005).

STATISTICAL ANALYSIS

Data were subjected to The Fisher's Least Significant Difference (LSD) test was used to compare the results of an analysis of variance (ANOVA) with fungicide treatments. SAS statistical software was used to conduct each and every statistical test. (SAS institute, 1990).

RESULTS AND DISCUSSION

Screening against *Ascochyta rabiei*: The most crucial component of an integrated disease management plan is identifying the source of disease resistance. *A. rabiei* resistance in chickpea germplasm is extremely low worldwide. (Reddy and Singh, 1984). Sixty six chickpea accessions were tested against the isolate of *A. rabiei*. Accessions showed a variety of responses from resistant to highly susceptible reaction. Firstly, typical disease symptom with some scattered lesions was recorded on susceptible cultivar Aug 242 which further developed extensive lesions leading to drying of the branches, severe defoliation and ultimately death of the complete plant.

Minimum disease severity index was noted on TG 1427 and the maximum on Aug-242. Disease rating scale showed that none of the tested genotype showed highly resistant or immune response against *A. rabiei*. This may be due to the presence of maximum disease inoculum pressure (Akhtar *et al.,* 2009). Moreover, four accessions showed resistant response, eight were moderately resistant, eleven were the moderately susceptible, and twenty two accessions recorded susceptible reaction while as most of the tested accession 21 exhibited highly susceptible reaction. *A.blight* disease incidence was initiated during the month of February whereas the Maximum disease was recorded during the month of March and April (*Basandri et al.,* 2007) (Figure 2).

In this perspective chickpea accessions with resistant or moderately resistant reactions against *A. rabiei* are good and may be tested for agronomic characteristics or used in breeding programs to develop commercial cultivars. Results of present study are supported by the findings of Atta *et al.* (2006) and Shah *et al.* (2005).

Table 1. Rating scale

Ratings	Reaction	Description
1	Immune	No symptoms
2	Highly Resistant	spot or depression on small tissue
3	Resistant	Elongated spot
4	Moderately Resistant	Coalescent spot
5	Tolerant	Girdling of stem
6	Moderately susceptible	Breaking of stem
7	Susceptible	Downward lesion growth from stem breaking point
8	Highly Susceptible	Complete plant is nearly to die
9	Highly susceptible	Complete plant died

Table 2. Fungicides used in the experiment against *Ascochyta rabiei*

Sr.	Commercial name	Molecule	Chemical formula	Manufacturer’s
1	Cabrio Top	Pyraclostrobin 5% + Metiram 55%	$C_{19}H_{18}ClN_3O_4$	FMC Pvt. Pakistan
2	Amistor Top	Azoxystrobin+ Difenoconazole	$C_{22}H_{17}N_3O_5$+ $C_{19}H_{17}Cl_2N_3O_3$	Sygenta Pakistan
3	Shincar	Carbendazim	$C_9H_9N_3O_2$	FMC Pvt. Pakistan
4	Antracal	Propineb 700 g/kg	$C_5H_8N_2S_4Zn$	Bayer Crop Sciences, Karachi, Pakistan
5	Success 40	Chlorothalonil+ Metalaxyl	$C_8Cl_4N_2$ + $C_{15}H_{21}NO_4$	Arysta life sciences, Pakistan
6	Nativo	Tebuconazole 50%+ Trifloxystrobin 25% w/w	$C_{16}H_{22}ClN_3O$ + $C_{20}H_{19}F_3N_2O_4$	Bayer Crop Science, Karachi, Pakistan
7	Alliete	Aluminum tris (O-ethyl phosphonate)	$C_6H_{18}AlO_9P_3$	Bayer Crop Science, Karachi, Pakistan
8	Curzate M	Mancozeb+ Cymoxanil	$C_7H_{10}N_4O_3$	Arysta life sciences, Pakistan
9	Dithane M	Mancozeb	$C_8H_{12}MnN_4S_8Zn$	Dow agro sciences
10	Thiulux	Copper Oxychloride	Cu2(OH)3Cl	Sygenta Pvt. Pakistan

Screening of Fungicides against *Ascochyta rabiei:* Despite recent genetic advancements leading to the creation of resistant cultivars, *A. rabiei* still poses a significant global production barrier for chickpeas. Even with the cultivation of disease-resistant cultivars, loss of the yield potential is a linear function of disease incidence (Fig.1). Fungicides must be used promptly in order to reduce disease risks and increasing yield potential (Macleod and Galloway, 2002).

Ten fungicides were evaluated against *A. rabiei* under field conditions. Among all fungicides Cabrio Top expressed significant results with minimum disease inhibition (8.37) followed by Amister Top (10.97), Shincar (14.61), Antracal (16.20), Success (20.08), Nativo (23.14), Alliete (26.78), Curzate M (33.33), Dithane M (39.44) and Thiulux (41.81) on comparison to control (77.31%).

During the impact of interaction among fungicides and their concentration on the development of *Ascochyta rabiei* Cabrio Top expressed minimum disease incidence (10.42), (8.41), (6.28) at all concentrations (Conc.1, Conc.2, Conc.3) followed by Amister Top (12.61, 10.77, 9.54), Shincar (16.53, 14.57, 12.72), Antracal (18.52, 15.41, 14.66), Success 40 (24.77, 19.54, 16.35), Nativo (25.60, 23.43, 20.38), Alliete (29.65, 26.33, 24.35), Curzate M (36.39, 34.28, 29.43), Dithane M (43.33, 39.69, 35.31) and Thiulux (45.38, 41.52, 38.54) on comparison to control 77.27 respectively. Earlier studies revealed that in Saskatchewan, Canada, Ascochyta blight disease incidence was reduced to 8% by two applications of Chlorothalonil (Chongo *et al.,* 2003a).

Results of the contemporary are supported by earlier studies (Table.6). In contemporary studies, Cabrio Top expressed minimum disease incidence as it contains Pyraclostrobin which inhibits multi sites of different enzymes; moreover, it has curative and protectant characteristics and is highly systemic, resulting in long-term effectiveness. It preserves normal leaf area, prevents mycelial development, respiration, and spore germination, and maximizes average production potential (Younas *et al.,* 2021). The majority of the countries that produce chickpeas use chlorothalonil extensively. Mancozeb has also been utilised in Australia and regions that produce chickpeas. (MacLeod and Galloway, 2002), Canada (Chongo *et al.,* 2003a,b), and Israel (Shtienberg *et al.,* 2000) against Ascochyta blight. Tebuconazole, carbendazim, and difenoconazole, three fungicides that have also been studied against A. rabiei, are now being used sparingly in the subcontinent. (Gaur and Singh, 1996b), Australia (Kimber and Ramsey, 2001)

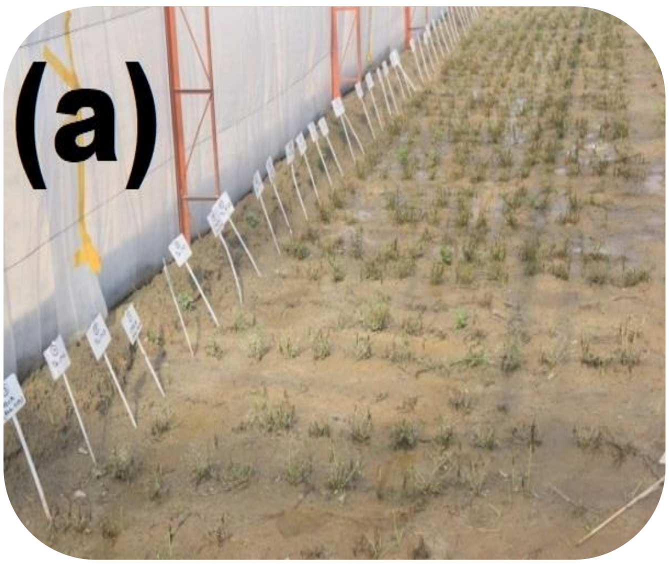

Figure 1. Picture of the Blight Trial under controlled conditions (a) and blight symptoms (b)

Table 3. Screening of chickpea accessions against Ascochyta blight

	Disease Response	Accessions	Total
1	Highly resultant (HR)	0	0
2	Resistant (R)	TG-1427, Star Channa, PARB913/CH03 and PARB913/CH02	4
3	Moderately resistant (MR)	CH-29/11, Bittal-2016, PARB913/CH01, PARB913/CH04, Thal-2020, PARB913/CHK01, PARB913/CHK02 and NIAB CH-2016	8
4	Moderately susceptible (MS)	PARB913/CH06, PARB913/CH08, PARB913/CH10, BRC-448, TG-1620, TG 1903, TG 1911, PARB913/CH12, PARB913/CH14, PARB913/CH16, and PARB913/CHK15	11
5	Susceptible (S)	TG 1501, TG 1507, TG1510, TG 1613, TG 1616, TG 1617, TG 1618, TG 1620, TG-1711, TG- 1801, TG-1812, TG 1817, TG- 1820, TG-1825, TG 1829, PARB913/CHK12 and PARB913/CHK13 TG 1713, TG- 1802, TG-1815, TG-1818, TG 1826 ,	22
6	Highly susceptible (HS)	TG-1621, TG-1622, TG-1623, TG-1626, TG-1703, TG-1707, TG-1710, TG- 1714, TG-1716, TG-1718, TG-1806, TG-1813, TG-1708, TG-1814 TG-1702, TG-1704, TG-1712, TG-1715, TG-1717, TG-1805, TG-808, and Aug-424 (check)	21

Table 4. Assessment of fungicides against *Ascochyta rabiei*

Treatments	PDI (%)	SD
Cabrio Top	8.37±0.60k	1.80
Chlostrobin	10.97±0.46j	1.40
Shincar	14.61±0.55i	1.65
Antracal	16.20±0.59 h	1.77
Success 40	20.08±1.16g	3.50
Nativo	23.14±0.75f	2.27
Alliete	26.78±0.77e	2.33
Curzate M	33.33±1.02d	3.08
Dithane M	39.44±1.16c	3.48
Thiulux	41.81±0.99b	2.98
Control	77.31±0.45a	1.36
LSD	0.78	

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P<0.05).

Table 5. Impact of Interaction between fungicides and their concentration on disease expression

Treatments	Reduction in Disease severity (%)		
	Concentration 1	Concentration 2	Concentration 3
Cabrio Top	10.42q	8.41r	6.28s
Chlorostrobin	12.61p	10.77q	9.54qr
Shincar	16.53n	14.57o	12.72p
Antracal	18.52m	15.41no	14.660
Success 40 wsp	24.77jk	19.54lm	16.35n
Nativo	25.60ij	23.43k	20.38l
Alliete	29.65h	26.33i	24.35jk
Curzate M	36.39f	34.28g	29.43h
Dithane M	43.33c	39.69e	35.31fg
Thiolux	45.38b	41.52d	38.54e
Control	77.21a	77.27a	77.47a
LSD	1.61		

*Mean values in a column sharing similar letters do not differ significantly as determined by the LSD test (P<0.05).

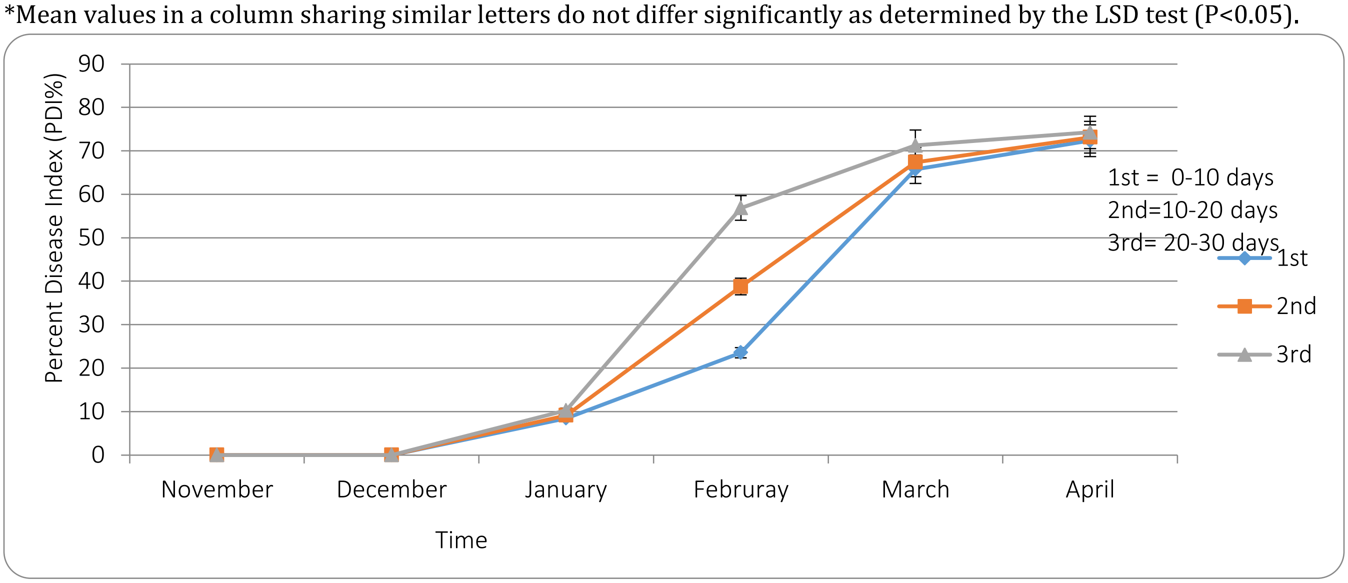

Figure 2. Impact of Interaction between the time and Disease development

Table 6. Results of the Fungicides tested against Ascochyta blight in different chickpea growing areas of the world in foliar application

Sr.	Fungicide	Potency	Test location	Reference
1	Pyraclostrobin	Excellent	UK	Chongo *et al.* (2003a,b)
2	Azoxystrobin	Excellent	UK	Chongo *et al.* (2003a,b)
3	Carbendazim	Very good	Iran	Sharafeh and Banihashemi, (1992)
		Good	India	Singh *et al*. (1992)
		Poor	India	Gaur and Singh, (1985)
		Very good	Egypt	Abdel Kader *et al.* (1989)
		Poor	Aus	Kimber and Ramsey, (2001)
4	Chlorothalonil	Excellent	Pak	Bashir and Ilyas, (1986)
		Very good	India	Gaur and Singh, (1985)
		Excellent	Australia	MacLeod and Galloway, (2002) Kimber and Ramsey, (2001),
		Very good	UK	Chongo *et al.* (2003 a,b)
		Very good	ICARDA	Reddy and Singh, (1990 a,b)
5	Tebuconazole	Very good	Israel	Shtienberg *et al.* (2000)
6	Mancozeb	Poor	Pak	Bashir and Ilyas. (1986)
		Good	Iran	Sharafeh and Banihashemi, (1992)
		Fair	Aus	MacLeod and Galloway, (2002)
		Poor	UK	Chongo *et al.* (2003 b)
		Fair	Egypt	Abdel Kader *et al*. (1989)

ACKNOWLEDGEMENT

The authors are highly grateful to Punjab Agricultural Research Borad (PARB), Lahore for financial support under PARB Project No. 913 “Enhancement of mungbean and gram production in Thal through development of improved genotypes and technologies to reduce pulse import bill”. The authors are also thankful to Arid Zone Research Institute Bhakkar, Punjab, Pakistan for carrying out research activities in its experimental area.

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Khalid Hussain	:	Planning of research experiment and provided resources
Niaz Hussain	:	Conducted research experiment
Abdul Ghaffar,	:	Data collection and research paper writing
Muhammad Younas,	:	Data collection and reviewed literature
Mohammad Irshad	:	Data interpretation
Muneer Abbas	:	Analyzed results
Farah Shabir	:	Proof read
Mohammad Nadeem	:	Selection of appropriate chemicals