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

ASSESSMENT OF MUSTARD GERMPLASM FOR THE SOURCE OF RESISTANCE/ SUSCEPTIBILITY AGAINST ALTERNARIA LEAF SPOT DISEASE UNDER NATURAL FIELD CONDITIONS

[a]Muhammad R. Bashir*, [a]Ahsan M. U. Din, [b]Ahsan Raza, [a]Hafiz S. B. Mustafa, [a]Qamar A. T. Khan, [a]Muhammad Zubair, [a]Ahmad N. Gill, [a]Muhammad Anwar

[a] *Oilseeds Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*
[b] *Department of Plant Pathology, UAF, Sub-Campus, Burewala, Pakistan.*

A B S T R A C T

The current research trial was conducted at Oilseeds Research Institute (ORI), Faisalabad for resistance/ susceptibility source against a devastating Brassica disease *i.e.* Alternaria leaf spot. In this field research trial, thirteen (13) varieties/ advanced lines of brassica were assessed during 2019-20 and 2020-21 by adopting statistical design namely RCBD (Randomized Complete Block Design) at the experimental research field of (ORI), Faisalabad under the umbrella of Ayub Agricultural Research Institute Faisalabad. In 2019-20, one advance line *viz.* RBJ-15013 exhibited resistant response with 8.00% disease incidence with rating 1 while six lines *i.e.* RBJ-15017, RBJ-16007, RBJ-17003, BRJ-17075, BRJ-17078 and Super Raya expressed moderately resistant reaction under rating 2. Likewise, four varieties/advanced lines exhibited moderately susceptible (MS) response whereas one advanced line KJ-294 expressed susceptible response (rating 4) and KJ-159 showed highly susceptible (HS) response by 85.67% disease incidence with rating 5. During 2020-21 one advanced line exhibited highly susceptible response with (81.70%) disease incidence with rating 5 while one of the advance line *i.e.* KJ-159 (71.80%) showed susceptible response (rating 4). Similarly, eight advanced lines *viz.* RBJ-15017, RBJ-17003, BRJ-17075, Super Raya, RBJ-17005, KJ-294, 18CBJ005 and KJ-284 exhibited moderately susceptible (MS) response (rating 3). Likewise, two variety/ advance lines RBJ-15013 and BRJ-17078 exhibited moderately resistant response whereas one of the advanced line *i.e.* RBJ-16007 (8.47%) showed resistant response (rating 1). It had been concluded that resistant varieties/ advanced lines are the most appropriate and economical disease management approach against Alternaria Leaf Spot Disease of brassica.

Keywords: Source of Resistance/ susceptibility, *Brassica juncia*, Alternaria leaf spot, natural field conditions.

INTRODUCTION

Brassica is the most pivotal genus of the Brassicaceae family that consists of 37 different species (Cartea *et al.,* 2011; Kristal and Lampe, 2002). Brassica is a rich in vitamin C and E, that have the potential to prevent degenerative diseases (Higdon *et al.,* 2007; Lampe and Peterson, 2002). It has high nutritional value as it possesses 2.86 g of proteins, 27 kcal energy, 4.67 g carbohydrates, 0.42 g total fiber, 1.64 mg iron, 1.32 g sugar, 20 mg sodium, and essential vitamins including vitamin C 70 mg, thiamine 0.08 mg and riboflavin 0.11 mg etc. (USDA, 2008). According to FAO total area of brassica in the world under cultivation is 35357 hectares with the production of 76802 tons, and yield 2.17 tons/per hectare whereas in Pakistan total cultivated area is 227.8 hectares, production 209.5 tons with an annual yield of 0.92 tons per hectares (FAOSTAT, 2020). The mustard is attacked by a miscellaneous group of pathogens comprising fungi, bacteria and viruses however *Alternaria brassicae* is causal agent of Alternaria blight is much devastating in mustard/brassica growing regions of biosphere (Ali *et al.,* 2016). *A. brassicae* retards the sprouting and diminish the value of oil obtained from brassica (Meena

Submitted: March 28, 2022
Revised: May 17, 2022
Accepted for Publication: June 05, 2022
* Corresponding Author:
Email: mrizwan1526@gmail.com

et al., 2016). The severity of the disease assault reduces the length of the pod, the number of seeds per pod, the risk of seed infection, the weight per thousand seeds, and the amount of oil in the pods. Based on the area, the infection reduces mustard yields by 25% to 75%, depending on the species (Singh *et al.,* 2015).

Numerous Alternaria species, *Alternaria brassicae* (Berk) Sacc. is the highly important and crop deteriorating causal agent of oilseed crops (Saharan *et al*., 2016) that causes huge losses in India (Singh and Kalia, 2021; Singh *et al.,* 2021) while in Pakistan it has caused about 30-50% losses (Hassan *et al.,* 2022).

The initial symptoms of *A. brassicae* are the development of brown to black necrotic lesions on young leaves along with greyish spots on stem. Severely infected plant parts, under epidemic conditions become shriveled, dried, premature opening and finally dropping of leaves (Kumar *et al.,* 2014). The cool and foggy climate regions are favorable for the growth, infection of *A. brassicae* and disease development as compared to other warm regions (Sivasubramanian *et al.,* 2019). Pathogen causes infection on different plant organs and symptoms appear on petioles, siliques, florescence, true leaves, stems, seeds and cotyledons (embryonic leaves) etc. (Saharan *et al.,* 2016; Al-lami *et al.,* 2019).

The characteristic symptoms including dark brown lesions, concentric rings, yellow halo on stem and leaves can be observed on plants infected with *Alternaria brassicicola*. The process of photosynthetic reaction diminishes in optimum conditions that lead to necrotic lesions and death of plants (Hansen, 1997; Nowakowska *et al.,* 2019). First symptom seems as minute yellow spots on older leaves and twigs (verma and verma, 2010).

Leaf spot disease caused by *Alternaria brassicae* is known as most vicious disease in mustard plants (Ghose *et al.,* 2008). Different non-traditional (biological agents, plant activators, ionic content, plant extract) and traditional (fertilizers and fungicides application, seed treatments, tillage operations and soil drenching) methods are used to manage disease infection (Bhatt *et al.,* 2009). The uncountable plentiful use of chemicals is not only destroying the planting materials nevertheless it is also reducing the oil quality. Thus, it is dire need to find out a source of resistance/ susceptibility from available genotypes/germplasm of mustards at ORI (Oilseeds Research Institute), Faisalabad against *A. brassicae* in natural field (Bisht *et al.,* 2015). Furthermore, source of rapeseed mustered resistant genotypes is an ample and climate-friendly tactic to enhance yield under divers climatic conditions (Singh *et al.,* 2015). For this purpose, the current research was planned to find out a source of resistance from available mustard genepool against *A. brassicae* in natural field conditions.

Aim of present research: This trial was conducted for source of resistance/ susceptibility among available mustard gene pool through *in-vivo* normal situations against *A. brassicae*.

MATERIALS AND METHODS

The current research on Brassica against Alternaria Leaf Spot was conducted during 2019-20 and 2020-21 at Oilseeds Research Institute, Faisalabad. Thirteen (13) advanced lines of *Brassica napus* germplasm namely RBJ-15017, RBJ-16007, RBJ-17003, BRJ-17075, RBJ-15013, BRJ-17078, Super Raya, RBJ-17005, 18CBJ001, KJ-294, 18CBJ005, KJ-284, KJ-159 etc. were collected from Oilseeds Research Institute, Faisalabad. Two to Three seeds were grown with dibbler by adjusting R×R and P×P distance *i.e.* 3.5 cm and 42 cm respectively whereas R×R length was adjusted 12ft or 365cm accordingly. In the present research/ experiment Statistical RCBD (Randomized Complete Block Design) used by adopting three repeats. Small seedling was removed manually by pulling after 15 day of germination to maintain the plants healthy for growth and development. The experiment was monitored on a regular basis for both cultural and other operations, such as the removal of weeds, the application of fertilizer, the application of irrigation or watering at the appropriate time, and the collection of data. To ensure that the research goals were being met, the mustard plants were examined on a regular basis for disease.

DATA RECORDING AND STATISTICAL ANALYSIS

The DI (Disease Incidence) percent data was recorded with ten (10) days of interval with the help of disease rating scale (Table 1) after appearance of disease symptoms and it will be continued till harvesting of experiment/research trial with the help of following formula and rating scale of Hussain and Thakur, (1963) was used for quantitative disease measurement (Table 1). The STAT 8.1 statistical analysis software (SAS Institute, 1990) was used however, the means were separated by using the Fisher's Least Significant Difference (LSD) test by considering P = 0.05% probability level (Steel *et al.,* 1997).

$$\text{Disease Incidence (D. I. \%)} = \frac{\text{No. of Infected Plants}}{\text{Total No. of Observed Plants}} \times 100$$

Table 1. *Alternaria brassicae* disease rating scale for recording percent disease incidence

Scale for Disease Rating	Percent area infection on leaves	Resistance/ susceptibility Response	Remarks
0	Nil	Immune	I
1	1-10	Resistant	R
2	11-25	Moderately Resistant	MR
3	26-50	Moderately Susceptible	MS
4	51-75	Susceptible	S
5	76-100	Highly Susceptible	HS

(Hussain and Thakur, 1963)

RESULTS

During 2019-20, one advanced line viz. RBJ-15013 exhibited resistant response with 8.00% D.I. (Disease Incidence) with rating 1 while six lines i.e. RBJ-15017 (20.67%), RBJ-16007 (18.00%), RBJ-17003 (19.33%), BRJ-17075 (15.67%), BRJ-17078 (16.67%) and Super Raya (19.67%) exhibited moderately resistant reaction (rating 2). Likewise, four mustard varieties/ advance lines *viz.* RBJ-17005, 18CBJ001, 18CBJ005 and KJ-284 showed moderately susceptible response with (38.33%), (46.67%), (29.00%) and 34.67 percent disease incidence (rating 3). One advanced line KJ-294 expressed a susceptible response (rating 4) whereas KJ-159 expressed highly susceptible disease response i.e. 85.67 percent under rating 5 as it is used as spreader (Table 2). During 2020-21 one variety/ advanced line namely 18CBJ001 exhibited highly susceptible response with (81.70%) disease incidence with rating 5 while one line namely KJ-159 (71.80%) showed susceptible response (rating 4). Similarly, eight advanced lines viz. RBJ-15017, RBJ-17003, BRJ-17075, Super Raya, RBJ-17005, KJ-294, 18CBJ005 and KJ-284 exhibited moderately susceptible response with 26.60%, 31.63%, 27.83%, 36.60%, 31.33%, 41.30%, 27.40% and 38.23% DI correspondingly (rating 3). Similarly, 2 varieties/ lines i.e. RBJ-15013 and BRJ-17078 exhibited moderately resistant response with (15.67%) and (11.87%) DI under rating 2 while one advanced line i.e. RBJ-16007 (8.47%) showed resistant response (rating 1). (Table No. 3).

DISCUSSION

In mustard cultivating areas of the world Rapeseed is attacked by diverse group of pathogens including bacteria, fungi and viruses but mustard leaf spot by *Alternaria brassicae* is very hazardous (Ali *et al.,* 2016). Some of the most deadly strains of virulent pathogens, including *A. brassicae, A. brassicicola* and *A. japonica,* have been implicated in the decline in oil quality and yield due to the disease's attack by these pathogens (Verma and Saharan, 1994). *Alternaria brassicae* produced enormous losses from 45-55% in rapeseed under favorable climatic/ disease development stages (Meena *et al.,* 2016). The casual pathogen diminishes the photosynthesis rate, demolish immature pods, boosts the flowering bunches as well as production of shriveled seeds are the characteristic symptomatology of disease (Bashir *et al.,* 2019). The characteristic symptomatology of *Alternaria brassicae* comes under lower and older leaves, afterward appears on stem and pods. Furthermore, tan color small spots become visible that subsequently enhances in size to create lesion. The profuse lesions on leaves and pods instantly decrease the photosynthetic rate (Singh *et al.,* 2015). Similarly, dark lesions could be seen on stem in the mustard seedling stage although brown and black spots on hypocotyls also decreased regular plant growth (Higdon *et al.,* 2007). Favorable temperature is 30˚C for mycelial growth on host plant whereas *A. brassicae* can survive at 25-35˚C. Likewise, RH also plays an imperative character in the growth of mycelium as well as sporulation (Meena *et al.,* 2016).

Conventional natural selection methods take a long time to breed resistant rapeseed plants, but evaluation of germplasm for the source of resistance/susceptibility under natural field conditions through convention breeding is reliable, climate resilient, present for a long time, and applicable to different pathotypes (Harloff *et al.,* 2010). Consequently, in present research thirteen advanced lines rapeseed mustard germplasm namely RBJ-15017, RBJ-16007, RBJ-17003, BRJ-17075, BRJ-17078, Super Raya, RBJ-17005, 18CBJ001, 18CBJ005, KJ-284, KJ-294 and KJ-159 were used against leaf spot of brassica. It was observed that six advanced lines/ varieties *i.e.* RBJ-15017 (20.67%), RBJ-16007 (18.00%), RBJ-17003 (19.33%), BRJ-17075 (15.67%), BRJ-17078 (16.67%) and Super Raya (19.67%) expressed a moderately resistant response (rating 2) but

only one advanced line *viz.* RBJ-15013 exhibited resistant response with 8.00% disease incidence while during subsequent year eight advanced lines exhibited moderately susceptible response but two variety/ advanced line namely RBJ-15013 and BRJ-17078 exhibited moderately resistant response.

Table 2. Brassica advanced lines/varieties response to Alaternaria leaf spot disease under natural conditions 2019-20

Sr. No.	Varieties/ Advanced lines	Disease Incidence	Rating	Response
1	RBJ-15017	20.667 ef	2	MR
2	RBJ-16007	18.000 f	2	MR
3	RBJ-17003	19.333 f	2	MR
4	BRJ-17075	15.667 fg	2	MR
5	RBJ-15013	8.000 g	1	R
6	BRJ-17078	16.667 fg	2	MR
7	Super Raya	19.667 ef	2	MR
8	RBJ-17005	38.333 cd	3	MS
9	18CBJ001	46.667 c	3	MS
10	KJ-294	65.333 b	4	S
11	18CBJ005	29.000 de	3	MS
12	KJ-284	34.667 d	3	MS
13	KJ-159	85.667 a	5	HS

Table 3. Brassica advanced lines/varieties response to Alaternaria leaf spot disease under natural conditions 2020-21

Sr. No.	Varieties/ Advanced lines	Disease Incidence	Rating	Response
1	RBJ-15017	26.600 e	3	MS
2	RBJ-16007	8.467 f	1	R
3	RBJ-17003	31.633 de	3	MS
4	BRJ-17075	27.833 e	3	MS
5	RBJ-15013	15.667 f	2	MR
6	BRJ-17078	11.867 f	2	MR
7	Super Raya	36.600 cd	3	MS
8	RBJ-17005	31.333 de	3	MS
9	18CBJ001	81.700 a	5	HS
10	18CBJ005	41.300 c	3	MS
11	18CBJ005	27.400 e	3	MS
12	KJ-284	38.233 cd	3	MS
13	KJ-159	71.800 b	4	S

The results of contemporary research trial are almost similar with the findings of Subhani *et al.,* (2018) that was conducted on screening of fifteen (15) cultivars of Brassica *i.e.* Punjab canola, Toria selection-A, Bulbul 98, Faisal canola, Rainbow, Oscar, BSA, DGL, CON III, CON II, Legend, Excel, Dunkeld, Cyclone, Shirale and reported that Punjab canola exhibited moderately susceptible (MS) response under field conditions. Similarly, Hussain *et al.* (2018) also used 10 varieties/ germplasm *viz.* 8CBN002, 8CBN001, 10CBN005, 11CBN003, 11CBN001, 11CBN0011, 11CBN009, Chakwal Sarsoon and 12CBN003 under natural conditions by adopting split plot statistical design and found 10CBN005 advance line exhibited tolerance whereas 8CBN-002 showed susceptible response in comparison with the accessions of brassica mustard plants. Likewise, Bisht *et al.* (2015) evaluated 240 rapeseed mustard varieties in natural environment against brassica and observed that 07 varieties/ advance lines *i.e.* IC255498, IC326253, IC296685, IC339589, IC335847, IC417020 and IC339597 expressed resistant reaction against leaf spot disease of mustard.

CONCLUSION

It has been determined that the germplasm of rapeseed mustard at ORI-Faisalabad exhibited a moderately resistant response in screening trial against Alternaria leaf spot disease during the current research trial. It is possible that this germplasm could be used in future

breeding programs to develop resistant genotypes, which could then be commercialized in farmer's fields, provided that these genotypes exhibited other desirable qualities.

REFERENCE

Verma, P.R. and G.S. Saharan. 1994. Saskatoon Research Station Technical Bulletin. Monograph on Alternaria diseases of crucifers,.162:

Steel R.G.D., J.H. Torrie, D.A. Dicky, 1997 - Principles and procedures of statistics. A biometrical approaches. 3rd Ed. McGraw Hill Book Co. Inc. Singapore pp: 204- 227.

Ali, M., W. Muhammad and I. Ali. 2016. Yield of oilseed Brassica (*napus* and *juncea*) advanced lines as influenced by boron application. Soil Environment, 35(1): 30-34.

Bashir, M. R., A. Mahmood, M. Atiq, M. Yaseen, N. A. Rajput, M. Aftab, A. M. U. Din, M. Mohsan and Q. A. T. Khan. 2019. Assessment of genetic variability in mustard against Alternaria leaf spot disease through natural field conditions. Pakistan Journal of Phtyopathology, 31(2): 211-216.

Bhatt, R., R.P. Awasthi and A.K. Tewari. 2009. Management of downy mildew and white rust Diseases of mustard. Pantnagar Journal of Research, 7(1): 54-58

Bisht, K.S., M. Rana, K. Gairola, B.C. Sharma, A.K. Tewari and R.P. Awasthi. 2015. Screening of brassica germplasm for resistance to major diseases of rapeseed mustard. Supplement on Genetics and Plant Breeding. 10(4): 2111-2119

Cartea, M.E., M. Lena, M. Francisco, P. Velasco and J. Sadowski. 2011. Basic information on vegetable Brassica crops. Genetics, Genomics and Breeding of vegetables Brassicas, 1-33.

Dunder, M., A. Adhikari, B. Kontz, A. Varenhorst, T. Nleya, E. Byamukama, and F. Mathew. 2017. First report of Alternaria black spot caused by *Alternaria alternata* on *Brassica carinata* in South Dakota. Plant Disease, 101:1951.

FAOSTAT, Food and Agriculture Organization of United States of America. 2020. Production, Crops http://www.fao.org/faostat/en/#data/QC.

Ghose, K., S. Dey, H. Barton, G. J. Loake and D. Basu. 2008. differential profiling of selected defense-related Genes induced on challenge with *Alternaria brassicicola* in resistant white mustard and their comparative expression pattern in susceptible India Mustard. Molecular Plant Pathology, 9(6): 763-775.

Al-lami, H. F. D., M. P. You and M. J. Barbetti. 2019. Role of foliage component and host age on severity of Alternaria leaf spot (caused by *Alternaria japonica* and *A. brassicae*) in canola (*Brassica napus*) and mustard (*B. juncea*) and yield loss in canola. Crop and Pasture Science, 70: 969–980.

Hansen. 1997. Somatic hybrids between brassica oleracea l. And *sinapis alba* l. With resistance to *Alternaria brassicae* (berk.) Theoretical and Applied Genetics, 94: 1078–1085.

Harloff, H. J., S. Lemcke, J. Mittasch, A. Frolov, J. G. Wu, F. Dreyer, G. Leckband and C. Jung. 2010. A mutation screening platform for rapeseed (*Brassica napus* L.) and the detection of sinapine biosynthesis mutants. Theoretical and Applied Genetics, 124: 957–969.

Higdon, J.V., B. Delage, D.E. Williams and R.H. Dashwood. 2007. Cruciferous vegetables and Human Cancer risk: epidemiological evidence and mechanistic basis. Pharmacological Research, 55: 224-236.

Humpherson-Jones, F. M. and K. Phelps. 1989. Climatic factors influencing spore production in *Alternaria brassicae* and *Alternaria brassicicola*. Annals of Applied Biology**,** 114: 449-458

Hussain, A. and R. N. Thakur. 1963. Some sources of resistance to Alternaria blight of rapeseed and mustard. Indian Oilseed Journal, 7: 259-261.

Hussain, T., M. A. Tariq, R. Ansar, M. Tariq and A. Sher. 2018. Screening of breeding lines of *Brassica napus* L. tolerant to grain shattering. Asian Journal of Agriculture and Biology, 259-264.

Kolte, S. J. 1985. Diseases of Annual Edible Oilseed Crops. Vol II: Rapeseed-Mustard and Sesame Diseases, CRC Press, Boca Raton, USA, 135.

Kristal, A.R. and J.W. Lampe. 2002. Brassica vegetables and prostate cancer risk: a review of the epidemiological evidence. Nature and Cancer, 42: 1-9.

Lampe, J. W. and S. Peterson. 2002. Brassica, biotransformation and cancer risk: genetic polymorphisms alter the preventive effects of cruciferous vegetables. The Journal of Nutrition, 132: 2991-2994.

Meena, P.D., R. P. Awasthi, C. Chattopadhyay, S. J. Kolte and A. Kumar. 2016. Alternaria blight: a chronic disease in rapeseed mustard. Journal of Oilseed

Brassica. 1: 1-11.

Nowakowska M., M. Wrzesińska, P. Kaminski, W. Szczechura, M. Lichocka and M. Tartanus. 2019. *Alternaria brassicicola–brassicaceae* pathosystem: insights into the infection process and resistance mechanisms under optimized artificial bio-assay. European journal of Plant Pathology, 153: 131–151.

Dalel, S., R. Prasad and B. Bairwa. 2014 Screening of Brassica Germplasm and Bredding material against *Alternaria brassicae* causing Alternaria blight under Artificial condition, Journal of Pure and Applied Microbiology, 8(6): 91-102.

Saharan, G.S., N. Mehta, P.D. Meena and P. Dayal. 2016. 'Alternaria diseases of crucifers: biology, ecology and disease management. Springer, 17-51.

Singh, D., R. Prasad and B. Bairwa. 2015. Screening of brassica germplasm and breeding material against *Altenaria brassicae* causing Alternaria blight under artificial condition. Journal of Pure and Applied Microbiology, 8(6): 1-5.

Sivasubramanian R., P. Deepak and K. Jagreet. 2019. Near-Complete Genome Assembly of *Alternaria brassicae*—A Necrotrophic Pathogen of Brassica Crops. Molecular Plant Microbe Interactions, 32: 928-930.

USDA.2008. States Department of Agriculture (USDA), Agricultural Research Service. National Nutrient Database for standard Reference, Release 21.

Subhani, A., M. Asif, M. Atiq, M. Imran, A. Hameed, A. Qadus, S. Ali, A. Sultan, M. Akmal, M. H. Haq, U. Farooq and N.A. Rajput. 2018. Progressive impact of Alternaria blight on antioxidants enzyme of mustard leaves after infection of *Alternaria brassicae* to induce resistance. International journal of Bioscience, 13(4): 417-426.

Verma N. and S. Verma. 2010. Alternaria diseases of vegetable crops and new approaches for its control. Asian Journal of Biological Science, 1: 681–692.

Woudenberg, J. H., J. Z. Groenewald, M. Binder and P. W. Crous. 2013. Alternaria redefined. Studies in Mycology, 75: 171-212.

Singh N. and P. Kalia. 2021. Screening of germplasm for Alternaria leaf spot resistance in yellow sarson. International Journal of Plant Protection, 1(2): 116-117.

Singh, K.P., P. Kumari and P.K. Rai, 2021. Current Status of the Disease-Resistant Gene(s)/QTLs, and Strategies for Improvement in *Brassica juncea.* Frontiers in Plant Science,12: 61740

Hassan, R. A., S. Ali, M. S. Zaheer, H. H. Ali, J. Iqbal, A. Habib, M. A. Nadeem and M. Z. Mumtaz. 2022. *In-vitro* and *in-vivo* evaluation of different fungicides against leaf blight causing fungus *Alternaria cucumerina* in bitter gourd. Journal of the Saudi Society of Agricultural Sciences, 21: 208-215.

Kumar, D., N. Maurya, Y.K. Bharati, A. Kumar, K. Kumar, K. Srivastava, G. Chand, C. Kushwaha, S.K. Singh and R.K. Mishra. 2014. Alternaria blight of oilseed Brassicas: A comprehensive review. African Journal of Microbiology and Research,

Contribution of Authors:

Muhammad R. Bashir	:	Planned the research and wrote the manuscript
Ahsan M. U. Din	:	Supervised and supported in conducting the research
Ahsan Raza	:	Recorded the experimental data
Hafiz S. B. Mustafa	:	Wrote the Abstract.
Qamar A. T. Khan	:	Helped in data collection
Muhammad Zubair	:	Reviewed Material and Methods Sections
Ahmad N. Gill	:	Helped in performing the research
Muhammad Anwar	:	Helped in data statistical analysis