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

MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF *BOTRYTUS CINEREA* CAUSING GRAY MOLD DISEASE OF LENTIL CROP FROM PAKISTAN

[a]Nawab Zada*, [a]Gulshan Irshad, [a]Farah Naz, [b]Asim Gulzar, [a]Muhammad Shahid [a]Muhammad F. Aslam, [a]Salman Ghuffar

[a]*Department of Plant Pathology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.*
[b]*Department of Entomology, PMAS-Arid Agriculture University Rawalpindi, Pakistan.*

A B S T R A C T

The lentil (*Lens culinaris*) is an edible pulse crop and belongs to family *Leguminosae*. Fungal diseases of lentil are the most important biological constraint to productivity. Out of which Botrytis Gray mold causes significant losses. The present investigation is carried out to characterize Botrytis gray mold on lentil crop. During the survey 11 isolates of *Botrytis Cinerea* causing grey mold disease was collected different lentil growing areas of Pothwar regions. The isolates were identified on the basis of cultural and microscopic features. After 6 days during cultural studies maximum growth was observed on Czepk and Malt extract medium (85 mm) and minimum growth was on PDA (74.1 mm). The colony color was varies from whitish to black grayish with fluffy texture. Microscopically *B. cinerea* produces gray mycelium with branched conidiophores that that have rounded apical cells bearing cluster of colorless or gray one celled, ovoid conidia. The conidiophores and cluster of conidia resembles as a grape-like cluster. The pathogenecity test confirmed all the isolates of *B. Cinerea* were able to cause the gray mold disease. The overall objective of this study is to morphologically characterize the *Botrytis cinera* that provides a due step for management of Botrytis grey mould disease of lentil crops.

Keywords: Lentil, *Botrytus cinerea*, Isolation, Morphological and cultural characterization

INTRODUCTION

The lentil (*Lens culinaris* L.) is an edible pulse crop and belongs to legume family *Leguminosae* (Stoilova *et al.,* 2013). The lentil was domesticated in Southern Turkey from where was exported to Europe and finally reached to Asia (Ali, 2010). It is cultivated worldwide about 4.2 million ha and producing 4.6 million tones with 110 kg/da average yield. The major lentil growing countries in the world are Canada, Turkey, Iran, China and Syria, whereas in South Asia are India, Nepal, Bangladesh and Pakistan (Ahlawat, 2012). Lentil, both in terms of quality and quantity is the second largest crop of legumes, grown in Pakistan after chickpea (Haq *et al*., 2011). Lentils growing areas in Pakistan in the Punjab province are mainly Gujranwala Chakwal, Gujrat, Jhang, Layyah, Mandi Bahauddin, Mianwali, Naroval, Rajanpur, Rawalpindi and Sialkot which contribute about 80% of cultivation of Lentils in Punjab, while in Bajaur Agency and Swat in Khyber Pukhtunkhawa The total area under which lentil crop in Pakistan is grown is 49 thousands hectare with production of 29.3 thousand tons having an average yield of 625 kg/ha (Rahman *et al*., 2013).

Lentil is a rich source of proteins, essential vitamins, minerals and important soluble and non-soluble dietary fibers (Haq *et al*., 2011). Botrytis grey mold caused by the pathogens viz. *Botrytis cinerea*, *B. fabae* are the two major diseases affecting the production of lentil in southern Australia as well as in Pakistan. Gray mould of lentil is favored by prolonged wet, cool conditions, often before flowering. However, it is most damaging if rain during pod results in pod infection and subsequent downgrading of lentil seed quality due to seed staining. Seasonal conditions and poor crop management play a significant role in disease outbreaks (Hawthorne *et al.*, 2012). Lentil is the dire need of the day and its production and yield is decreasing day by day due to destructive fungal diseases. The aim of this study is

* Corresponding Author:
Email: nawab_zada18@yahoo.com

conducting survey of gray mold disease from lentil growing areas located in pothwar region and also their morphological characterization by using different parameters and their pathogenicity for conformation.

MATERIALS AND METHODS

Two surveys of major lentil growing areas of Pothwar's region i.e. District Chakwal, Gujrat and Islamabad (NARC) were carried out at different growth stages to collect diseased samples on basis of symptomology (Appearance as grayish colored soft, necrotic leisons on leaves, stems, and flowers) and brought to Mycology Lab for further processing. Czapek Dox Agar (CDA) was prepared and sterilized and autoclaved at 121°C for 15 min. Infected stems, leaves and pods were cut into small pieces and surface sterilized using 1% Clorox for two minutes. Afterward the cut pieces were rinsed consecutively three times with sterilized distill water and dried on sterilized filter paper and placed on the Petri plates. Colonies were purified by using single spore method on different nutrient media to compare optimum growth on Potato Dextrose Agar (PDA), Czapek Dox Agar (CDA) and Malt Extract Agar (MEA) respectively. After 6 days the diameter of each colony was measured and identified on the basis of cultural and morphological characteristics accordingly on suitable media such as Czepk Dox Agar medium (CDA) by using taxonomic key (Ellis, 1971). In cultural characterization, colony color, colony texture, mycelial growth, colony margins and colony diameter were observed while in morphological characterization spore color; spore shape, spore size, mycelial color and mycelial septations were observed by preparing slides in Lacto-phenol blue under 10, 40 and 100X lens under microscope respectively. After obtaining the pure pathogens, pathogenicity test was conducted for the confirmation of vilulent pathogens. For this purpose Pathogenicity test was performed on healthy plant and detached lentil leaves grown in pots. Total 20 plants were inoculated and the remaining plants were left as control. The lentil shoots were inoculated with *Botrytis cinerea* with the conidial suspension 1×10^6 conidia per mL. Shoots sprayed with sterile distilled water were used as the control. The inoculated plants were kept for the symptoms developments. Symptoms developed on inoculated shoots were similar to those observed on the diseased plant in the field. The gray mold pathogen was re-isolated from the infected tissue and showed the typical colony of *B. cinerea* while the control shoots remained healthy. The highly virulent isolated pathogens were preserved for future use by using different silica gel.

RESULTS

During survey 11 isolates of pathogen were found causing gray mold disease on lentil. Most of the isolates were found to have similar cultural and morphological characteristics. They were identified as *Botrytis Cinerae.* All these isolates were preserved. Different types of fungal colonies were isolated and sub-cultured on different media for purification purpose. There were 11 isolates obtained after purification as shown in Table 1. These isolates were confirmed through morphological and cultural characteristics as shown in Table 2. Most of the isolates showed similarity in morphological and cultural characteristics. The isolated pathogens were preserved in silica gel. On the sixth day, all the isolates sporulated on all the three media but on Czepk-agar and malt extract medium maximum (85 mm) percent of sporulation was recorded as compared to PDA minimum growth was recorded (74.1 mm) as shown in Table 3. The growth pattern, appearance and colony color on all the three media were different from each other. The growth on the PDA was embedded mycelium, less sporulation with whitish color. On the Malt Extract Agar the color was noted grayish with fluffy mycelium and excessive sporulation. On the other hand on Czepk media the colony color was observed white or hyaline with light white margin, fluffy mycelium and aerial hyphae. The appearance and the color of the colonies showed differences on the three media developed different growth patterns as shown in Figure 1. The colonies were fluffy, radial and warty with different colors ranging from white, dirty white, grayish white or hyaline at first, becoming light gray, and dark gray. *B. cinerea* produces gray mycelium with branched conidiophores that that have rounded apical cells bearing cluster of colorless or gray one celled, ovoid conidia. The conidiophores and cluster of conidia resembles a grape-like cluster. The conidia observed were ellipsoidal or sometimes globose, smooth, often with a slightly protuberant hilum and unicellular as shown in figure 2. After isolation and purification pathogenicity test was performed to confirm that 11 isolated pathogens were highly pathogenic as shown in figure 3. The similar work was done by (Harrison, 1988 and Gossen *et al.,* 2007) for the conformation of isolated pathogens either these were virulent or not is a real cause or not.

DISCUSSION

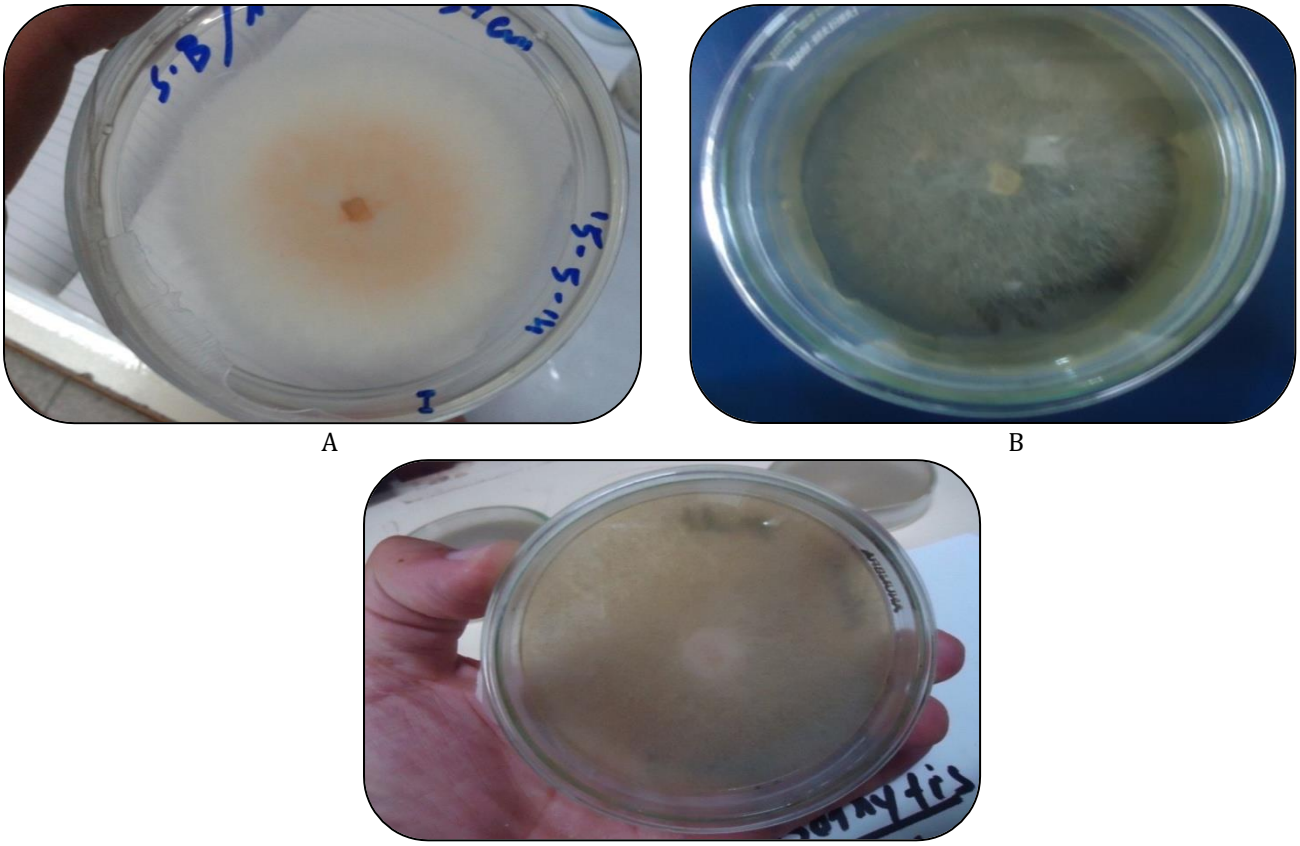
All isolated fungai were identified by indicating of their Cultural as well as microscopic characters and further classified using Bergey's fungal key (Ivanova and Bernadovicova, 2010). In the present study, all the isolates grew within a wide range of temperature of 5-30 C°. Maximum mycelial growth was observed at 20 C° and failed to grow at 35 C°. Result of this study is very similar to the findings of (Ahmed *et al.,* 2007). *B. cinerea* grew abundantly on Malt extract and Czepk medium between pH 4.0 and 6.5 with a maximum growth at pH 4.5. Similar results were found by (Hosen *et al*., 2010b). Conidiophores of *B. cinerea* were arisen from the hyphal mass and twisted type microfilament, which branches alternately. At the terminal end of these branches two types of conidia viz. macro and micro conidia arose in cluster form. Macro conidia of all the isolates were hyaline or pale brown in colour, single celled, oval, globose or short cylindrical and single or multinucleated. The abovementioned characteristics of *B. cinerea* were in conformity with the findings of other researchers (Acero, 2006). After Pathogenicity test result showed similarity with that of (Pande *et al.,* 2010) who artificially inoculated lentil by B. cinerea conidia and observed the conidia to be germinated within 6-8h on leaf surface. After 48h of incubation, B. cinerea found to penetrate through stomata of lentil leaf.

Table 1. List of Isolates Collected From Different Lentil Growing Areas of Pothwhar Regions.

S. No	Locations	No of Isolates	Names of Isolates
1	Lamra	4	GhLBr1 GhLBr2 GhLBr3 GhLBr4
2	Somri	3	GhSBr1 GhSBr2 GhSBr3
3	Dulat Nagar	4	GhDBr1 GhLDr2 GhLDr3 GhLDr4
4	Sukkho	-	
5	NARC	-	
Total	5	11	

Table 2. Cultural Characteristics of *Botrytis cinera*

S. No.	Isolates	Colony Color	Colony Texture	Average Colony Diameter in 6 days (mm)	Hypha Growth	Reverse
1	GhLBr1	Off-White	Slightly fluffy	82	Raised	Cream
2	GhLBr2	Whitish	Fluffy	74	Raised	Cream with light margin
3	GhLBr3	Slightly white	Fluffy	76	Slightly raised	Off- white
4	GhLBr1	Greyish white	Fluffy	80	Raised	Light pink
5	GhSBr2	Greenish black	Slightly fluffy	75	Slightly raised	Off-white
6	GhSBr3	Greyish White	Slightly fluffy	78	Aerial	Orange
7	GhSBr4	Greyish white	Fluffy	74	Slightly raised	Slightly dark
8	GhDBr1	Greyish white	Fluffy	78	Embedded	Cream
9	GhDBr2	Greenish black	Slightly fluffy	85	Slightly raised	Dark with hyaline margin
10	GhDBr3	Greyish black	Slightly fluffy	76	Aerial	Cream
11	GhDBr4	Greenish black	Fluffy	80	Raised	Light dark



A B C

Figure 1. *Botrytis cinera* growth on different cultural Media. (A) Potato Dextrose Agar, (B) Malt Extract Agar, (C) Czepk Media.

Table 3. Mean diameter (mm) of the colony of *Botrytis cinerea* on different Cultural media.

S. No	Isolates Name	Day 2	Day 4	Day 6
		Czepk		
01	P1	23.5	78.5	85.00
02	P2	16.5	64.5	85.00
03	P3	22.5	72.0	85.00
04	P4	18.6	63.9	85.00
05	P5	21.0	85.0	85.00
		PDA		
06	P1	14.5	53.3	77.6
07	P2	12.8	55.0	70.6
08	P3	14.8	56.1	74.1
09	P4	13.6	58.3	70.9
10	P5	22.8	66.0	78.9
		MEA		
11	P1	24.8	64.8	85.0
12	P2	29.4	70.0	85.0
13	P3	25.6	68.8	85.0
14	P4	18.4	66.0	78.0
15	P5	22.4	64.4	82.8

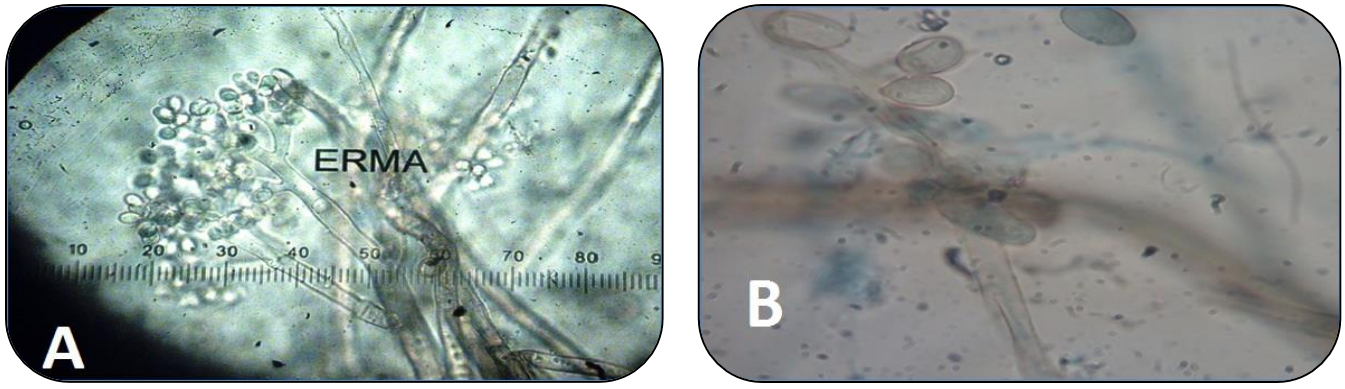


Figure 2. *Botrytis cinerea* conidia under Compound Microscope under 40X and 100 X (A & B).

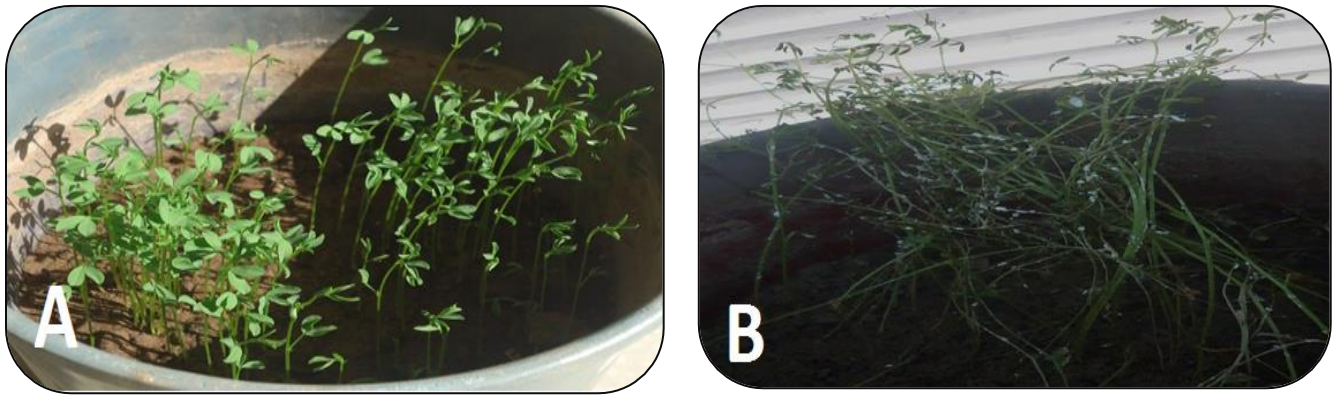


Figure 3. Pathogenicity test, (A) Growth of lentil in Pods (B) Pathogenicity symptoms on Plants.

CONCLUSION

A comparative study was performed to check which media is best suited for growth and as a result Czepk and malt extract was found to be best for carrying out fungal purification and their cultural and morphological study. Pathogenicity test was carried out using healthy lentil's detached leaves as well as whole plant .The result revealed all 11 isolates were found pathogenic.

ACKNOWLEDGEMENT

We are very thankful to the department of plant pathology PMAS Arid agriculture University Rawalpindi for conducting the experiment and also highly appreciated and thankful to the department for providing us the funds to conduct this research work.

REFERENCES

Acero F.J.F. 2006. Application of proteomics to the characterization of mechanisms pathogenicity in *Botrytis cinerea*: Use and evaluation of new fungicides. Ph.D thesis, The University of Cadiz, Spain.

Ahmed A.U., S. Pande., A.K. Basandrai, G.K. Kishore and J.N. Rao. 2007. Variation in isolates of *Botrytis cinerea* causing Botrytis gray mold in chickpea. Bangladesh J. Agril. Res, 32(1):135-143.

Ahlawat, I.P.S. 2012. Agronomy Rabi crops, Lentil. Division of Agronomy Indian Agricultural Research Institute, New Delhi – 110 012 Agronomy.

Ali, J. A.F., M. A. Arain and N.A. Shaikh. 2010. Genetic manipulation of lentil through induced mutations. Pak. J. Bot. 42 (5): 3449-3455.

Brouwer, J. B., B. Sharma, B. A. Malik, and G. D. Hill. 2000. Region 6: Asia-Pacific: Meeting the challenge. In: Linking research and marketing opportunities for pulses in the 21st century. Proceedings of the third international food legumes research conference. Current Plant science and biotechnology in agriculture.(Ed R. Knight) Kluwer Academic Publishers. 34:115-129.

Cokkizgin, A. and M. J. Y. Shtaya. 2013.Lentil: Origin, Cultivation Techniques, Utilization and Advances in Transformation. Agri. SCI. 1 (1): 55-62.

Diolez, A., F. Marches, D. Fortini, and Y. Brygoo. 1995. Botrytis, a long-terminal-repeat retroelement in

the phytopathogenic fungus Botrytis cinerea. Appl. Environ. Microbiol. 61: 103-108.

Ellis, M. B. 1971. Dematiacious hyphomycetes. CABI, Kew, England.608 pp.

Gossen, B.D., Lan. Z., Harrison. L.M., Holley. J and Smith. S.R. 1997. Survey of blossom blight of alfalfa on the Canadian Prairies. *Can. Plant Dis. Surv.*77: 88-89.

Haq, M. Z. U., S. Ahmad, M. A. Shad, S. Iqbal, M. Qayyum, A. Ahmad, D. L. Luthria and R. Amarowicz. 2011. Compositional studies of lentil (*Lens culinaris* Medik) cultivars commonly grown in Pakistan. Pak. J. Bot., 43 (3): 1563-1567.

Hawthorne, W., M. Materne, J. Davidson, K. Lindbeck, L. McMurray and J. Brand.2012. Lentil Disease Management Strategy. Southern pulse bulletin of Australia.50-55.

Hosen, M.I., A.U. Ahmed and M.R. Islam. 2010b. Physiological variability and in vitro antifungal activity against Botrytis cinerea causing botrytis gray mold of chickpea (*Cicer arietinum* L.).Spain J Agric Res., 8(3):750-756. http://dx.doi.org/10.5424/sjar/2010083-1274.

Ivanova, H., S. Bernadovicova. 2010. Species diversity of microscopic fungi on Austrian pines growing in urban greenery of Nitra town. *Folia Oecologica*, *37*(2):168.

Morsy, K. M. M. 2005.Induced Resistance against Damping-off, Root Rot and Wilt Diseases of Lentil. Plant Pathol. 33: 53-63

Muehlbauer, F.J. 1991. Use of introduced germplasm in cool season food legume cultivar development.

Pande, S., M. Sharma, G. Kishore, L.S. Krishna and U.M. Naga . 2010. Characterization of *Botrytis cinerea* isolates from chickpea: DNA polymorphisms, cultural, morphological and virulence characteristics. African Journal of Biotechnology, 9(46):7961-7967.

Pande, S., G. Galloway, P. M. Gaur, K. H. M. Siddique, H. S. Tripathi, P. Taylor, M. W. J. MacLeod, A. K. Basandrai, A. Bakr, S. Joshi, G. K. Kishore, D. A. Isenegger, R. J. Narayana and Smith, D., A.H.S. Onions. 1983. A comparision of some preservation techniques for fungi. *Trans. Br. Mycol. Soc*. 81 (3): 535-540.

Stoilova., T. Petrova, N. Penov And A. Krastanov. 2013. Antioxidant activity of lentil semolina extrudates using cathode voltammetry. BJAS. 19 (1): 15-21.

Williamson, B., Tudzynsk, B., Tudzynski, P., and van Kan, J. A. L. 2007. Botrytis cinerea: the cause of grey mould disease. Molecular Plant Pathology.8: 561 - 580.

www.planthealthaustralia.com.au/biosecurity/grains