Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 25, No. (1) : 2023 : 91-96

ISSN-0972-3005

DOI No.: http://doi.org/10.53550/AJMBES.2023.v25i01.013

CULTURAL AND PHYSIOLOGICAL CHARACTERISTICS OF *ALTERNARIA SPP.* CAUSING LEAF SPOT ON MUSTARD (*ALTERNARIA BRASSICAE*)

MOIN KABIR[1], AKHILESH CHANDRAPATI[2] AND MEENAKSHI RANA[3]

Department of Plant pathology, Lovely Professional University, Phagwara 144 411, Punjab, India

(Received 3 August, 2022; Accepted 29 September, 2022)

Key words: *Morphology, Growth, pH, Temperature, Different media.*

Abstract– Mustard (*Brassica juncea*) is a major rabi oil seed crop out of total nine oil seeds crops in India. It belongs to family Brassicaceae and was introduced into China. Mustard is affected by various diseases i.e., *Alternaria* leaf spot, Downey mildew, Powdery mildew and White rust etc. *Alternaria* leaf spot is caused by various *Alternaria* species like brassicae, brassicicola, rafani, Alternata etc. in this experiment for testing the good growth of *Alternaria* spp. 7 different media are taken, i.e. rose bengal, czepak dox agar, potato dextrose agar, *Richards* synthetic media, corn meal, malt extract agar, oat meal agar. Among all the media malt extract agar was the best media for the growth of *Alternaria* spp. Also Richards media was quite good for the growth after malt extract. To check the factors that effect on growth of *Alternaria* spp. two different factors, i.e. pH and temperature have shown a significant change in growth of *Alternaria* spp. pH range taken from 4 to 9 and temperature from 15 to 35 °C. Best growth was observed with pH 5 and best growth observed at a temperature range between 23 to 25 °C. Weather conditions have a significant impact on the development of *Alternaria* spots on mustard. Warm weather conditions were found to be the primary that triggers for increased disease damage in some cruciferous species in this study.

INTRODUCTION

Rapeseed-mustard is a group of crops contributes to 32% of the total oilseed production in India, and it is the second largest indigenous oilseed crop. Out of 75.55 m tones of estimated rapeseed-mustard produced over 30.51 m ha in the world, India produces 7.36 m tones from 6.18 m ha with 1190 kg/ha productivity (As per Government of India Statistics, 2009). Despite considerable increase in the productivity and production under Technology Mission, huge amount of money is spent on the import of edible oil

Mustard is produced abundantly in India's northeastern and central states since it requires colder temperatures and lower humidity. The crop flourishes on light to heavy-loam soil and grows well in areas with rainfall ranging from 25 to 40 cm. Rajasthan is India's main and greatest producer of mustard, accounting for 43 percent of the country's total growth. As a rabi crop, it is also grown in Punjab, Haryana, Madhya Pradesh, Uttar Pradesh, Bihar, Assam, Jammu and Kashmir, Himachal Pradesh, and other states. It is also grown in Karnataka, Tamil Nadu, and Andhra Pradesh in south India. During 2008-2009, the Punjab region's cultivated area and production of this crop were 27000 hectares and 33000 tons, respectively

A wide gap exists between the potential yield and the yield realized at the farmer's field, which is largely because of the number of biotic and abiotic stresses to which the rapeseed-mustard crop is exposed. Among the biotic stresses, Alternaria blight disease caused by *Alternaria brassicae* (Berk.) Sacc. has been reported from all the continents of the world and is one among the important diseases of Indian mustard causing up to 47% yield losses with no proven source of transferable resistance in any of the hosts. Average yield losses range between 32-57 percent due to Alternaria blight that have been reported from Nepal. Mustard is also used as vegetable as (*Sarso da saag*) in Punjab and Haryana. The oil is consumed as edible food oil and the meal cake left over after the extraction of oil forms an

([1]M.Sc. Student, [2]M.Sc. Student, [3]Assistant Professor)

important cattle feed or is utilized as fertilizer for various grain crops. The oil content of the mustard seed ranges between 30 to 48% Mustard oil is an integral part of the human diet. The main chemical component of Mustard oil is allyl isocyanide, about 92% of oil. The mustard is having high nutrient value i.e carbohydrates 4.51g, dietary fiber 2g, protein 2.56g, sugar 2.56g, sugar 1.41g and fat 0.47g per 100 grams of seeds. The seeds are usually about 1 to 2 millimeters (0.039 to 0.079) in diameter and may be colored from yellowish white to black. Seeds contain numerous chemical constituents including phytoalexins (sinalexin, sinalbin A and B) sterols and steryl esters (primarily sitosterol and campesterol) and flavonoids (apigenin and chalone)

Alternaria affects most cruciferous crops, including broccoli and cauliflower (Brassica oleracea L. var. botrytis L.), field mustard and turnip (*B. rapa* L. (synonym: *B. campestris* L.), leaf or Chinese mustard (B. juncea), Chinese or celery cabbage (*B. pekinensis*), cabbage (*B. oleracea* var. capitata), rape (*B. campestris*), and radish (*Raphanus sativus*). *A. brassicae* and *A. brassicicola* are cosmopolitan in their distribution. *A. raphani* and *A. alternata* are widespread in the Northern hemisphere (Jasalavich *et al.,* 1995).

Major storage protein is cruciferin or 12S protein. Many oil-producing plants include a significant amount of protein, which has a lot of potential for human consumption. Canola oil is included (rapeseed). The high proportion of water-soluble proteins, or albumins, in these Brassica proteins accounts for 45–50% of the total protein (Appleqvist, 1972).

The disease is most noticeable 45 days after planting and most severe 75 days after sowing (Meena *et al.,*). The pathogenesis primarily affects the plant's aerial components. The first signs of the disease are black coloured points on the bottom leaves, which thereafter grow into circular to concentric spots of varying sizes. Following that, little dark patches with concentric rings can be seen on the plant's middle and upper leaves. The pathogen spreads by seeds and has an impact on plant parts in the soil. Severe infection causes leaves to fall out, and the plant produces smaller pods and seeds.

Family *Pleosporaceae, phylum Ascomycota, subphylum, Pezizomycotina, class Dothideomycetes*, subclass *Pleosporomycetidae*, and order Pleosporales are all home to *Alternaria brassicae*. Mycelium is short, septate, and branching, with multinucleate hyphal cells. Conidia are huge, ellipsoid to ovoid in shape, dark in colour, numerous cells, and beak shaped. Leaf spot or black leaf spot disease is another name for Alternaria blight of mustard. This disease primarily affects crops during the rainy season and in areas with high rainfall.

Due to the pathogen's seed-borne origin, dark leaf spot (*Alternaria brassicae*) is one of the most important diseases in crucifers, causing significant yield and quality loss in production. *Alternaria brassicae*, the causative agent of Alternaria blight of cauliflower (Vegetable) rapeseed-mustard (Oil seed), was investigated for variation in appearance and cultural traits across 32 representative Indian regional isolates. In vitro, all of the isolates had a lot of variation in terms of conidial length, width, and number of septa. The Uttar Pradesh isolate (CaAbU4) has the smallest conidia and the fewest septa. In diverse nutrient media, there was significant variance in mycelial development and sporulation among these isolates. On the same nutritional medium, none of the isolates grew or sporulated abundantly. However, all the cultures thrived on Potato Dextrose Agar, Cauliflower (Host) Agar medium, and Carrot Potato Agar.

MATERIALS AND METHOD

Isolation

Diseased mustard leaves were obtained from our Lovely Professional University field, and small, infected tissue was cut along with some healthy tissue using a sterile surgical blade. These items were sterilized in 1% sodium hypochloride (Naocl) for 10 seconds before being washed three times with distilled water in a sterilized laminar air flow chamber. These small pieces were then air dried on sterilized blotting paper, single pieces were maintained on PDA-containing petri plates, and finally were kept in a B.O.D incubator for seven days at Temperature 25 +2 degrees C and inspected at periodic intervals to identify fungus growth. Pure culture of the fungus was obtained by hyphal tip isolation method.

Effect of Different Media on Growth of *Alternaria* spp.

To conduct this experiment seven different media have been selected and grown on every single media to check the growth and morphological characteristics of *Alternaria brassicae*. Data have shown that Malt extract agar (78.5 mm) was the best

growing medium for *A. brassicae* as compared to PDA and Richards medium. The cultural characters of *A. brassicae* were conducted on the following seven different media viz. oat meal agar, potato dextrose agar, malt extract agar, corn meal agar, Czepak dox agar, Rose bengol, Richards synthetic media. Observation were taken on the radial growth of the pathogen (mm) when the fungi attained full growth at any one of media tested.

Effect of pH on Growth of Pathogen

PDA medium with a variety of pH ranges, including 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0, were developed in order to explore the impact of pH on the proliferation of pathogens. To change the pH of this PDA media to the correct level, acid (HCl) or alkali (NaOH) were added. Four replications were maintained for each pH and PDA media of the corresponding pH was separately poured into the sanitized petriplates. Then, using a sterile cork borer, a 5 mm disc of fungus was removed from a seven-day-old culture pathogen and implanted into the petriplate's centre.

Effect of Temperature on Growth on Pathogen

The virulent pathogen's growth was examined at five different temperatures: 15, 20, 25, 30, and 35 degrees Celsius. PDA media was produced and sterilized for this. Then, sanitized petriplates were filled with melted and cooled PDA medium. Five millimetre fungal discs of the pathogen were injected into the centre of the petriplate under aseptic circumstances using a seven-day-old culture. For each treatment, three replications were kept. When the pathogen reached complete development in the petriplate at any one treatment, these plates were then incubated at various temperatures and observations on radial growth were recorded.

RESULTS AND DISCUSSION

Physiological Studies

Effect of Temperature on Growth of Pathogen

To find the ideal temperature for pathogen growth, the pathogen was cultivated on the PDA medium at several temperatures, including 15, 20, 25, 30 and 35 °C. The outcomes revealed a substantial difference between the treatments.

Maximum pathogen development occurred at 25 °C (51mm), then at 20 °C. (31mm). At 35 °C, the growth was completely suppressed. The pathogen's optimal growth temperature was found to be 25 °C,

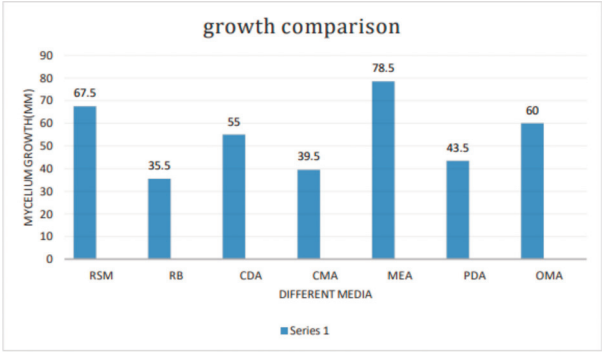

Fig. 1. Effect of different media on growth of *A.brassicae*

Table 1. Growth of *Alternaria brassicae* on different media.

Name of media	Diameter of colony (14th day)	Colony color & Margin
Richards synthetic media	67.5 mm	Regular (raddish)
Rose bengal	35.5 mm	Irregular (black)
Czepak dox agar	55 mm	Irregular (white)
Corn meal agar	39.5 mm	Irregular (white)
Malt extract	78.5 mm	Regular (black)
Potato Dextrose agar	43.5 mm	Regular (white)
Oat meal agar	60 mm	Irregular (white)

Table 2. Growth of *Alternaria brassicae* on different pH.

Different ph	Diameter of colony(mm)	Colony color & margin	Surface
pH 4	58.5	Brown to greenish (irregular)	cushion
pH 5	90	Olive brown (regular)	cushion
pH 6	35	Brownish (irregular)	cushion
pH 7	43.5	Brown to greenish (regular)	cushion
pH 8	40	Dark brown to pale brown (regular)	cushion
pH 9	67.5	Blakish (regular)	cushion

and growth was hindered by additional increases or decreases in temperature (Table 3)

A temperature range between 21 and 23°C with an optimum round 23 °C has been reported to be favourable for *A. brassicae* by various scientists. In the present study, growth of *A. brassicae* occurred between a temperature range of 5 to 30 °C Both growth and sporulation were excellent at temperatures ranging between 23 and 25 °C. However, 23 ºC was the best amongst all. These findings are in line with those of earlier works on *A.brassicae*. (Taber *et al*., 1968; Neergaard, 1945; Limasset, 1955; Changsri and Weber, I963; Czyzewska, 1969)

Effect of pH on Growth of Pathogen

The pathogen's ability to proliferate was examined in vitro under three acidic (pHs of 4, 5, 6, 8,) and one neutral (pH of 7) one basic conditions. The in vitro evaluation's findings revealed that the pathogen's radial development was at its greatest between pH - 5 (90 cm) and 4 (58.5mm). Then came pH 7 (43.5 mm), pH 8 (40mm), and pH 6 (35mm), all of which were equal to one another. 35 mm was the pathogen's smallest growth measurement at pH 6 (Table 2, Fig. 2).

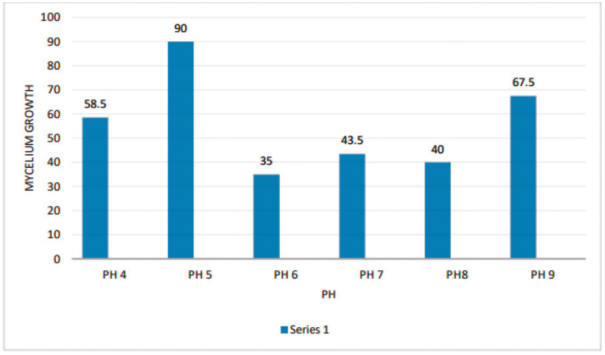

Fig. 2. Effect of different pH on growth of *A.brassicae*.

According to Somasekhara *et al*. (2016) The largest growth and sporulation of *Alternaria spp* was observed at pH 6, whereas the least growth was observed at pH 4 and 10 (no growth). The pH range of 5.5 to 7.5 is ideal for the growth of *Alternaria spp*, which causes cabbage leaf spot.

Out of seven media best result on growth in Malt extract agar, sporulation of the pathogen was excellent not only in media which supported excellent growth but also in media in which moderate growth occurred. on some media like, Czepak growth was poor but sporulation was good, but on the other hand corn meal agar growth and sporulation were poor, previous study have shown good growth on malt extract agar which justify my experiment about the experiment conducted on different media. Satisfactory growth on malt extract

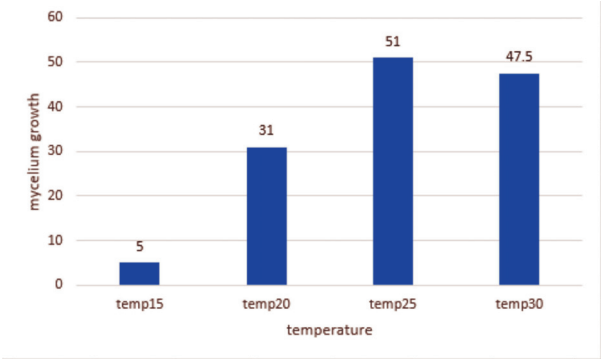

Fig. 3. Effect of different temperature on growth of *A.brassicae*.

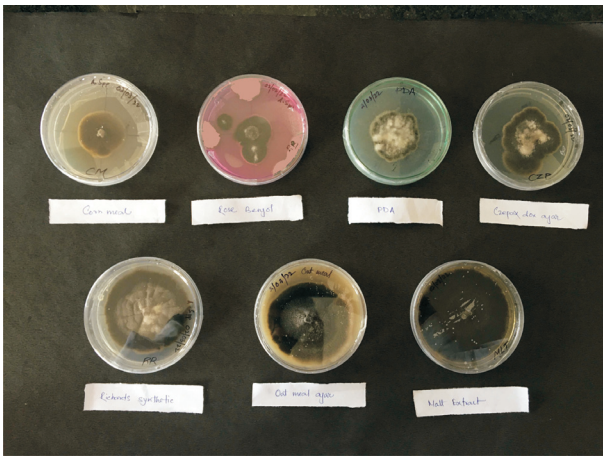

Plate 1. Radial growth of *A. brassicae* at different media.

Table 3. Growth of *Alternaria brassicae* in different temperature

Different temperature	Diameter of colony(mm)	Colony colour and margin
15 ºC	5	White mycelium only
20 ºC	31	Dark greenish (irregular, white)
25 ºC	51	Olive green (regular, white)
30 ºC	47.5	Dark green to olive green (regular, white)
35 ºC	NIL	No growth

medium as recorded are similar with the findings of Neergard (1945) and Taber *et al.* (1968)

Growth of *Alternaria brassicae* have been tested on 15, 20, 25, 30, 35 temperature and the best result have seen on temperature 25 °C. Variation of growth of *A. brassicae* is difficult between the temperature range from 22 to 28 °C. in my experiment optimum growth was 25 °C. A temperature range between 21 and 23 °C with an optimum round 23 °C has been reported to be favourable for *A. brassicae* by various scietists. In the present study, growth of *A. brassicae* occurred between a temperature range of 5 to 30 °C. Both growth and sporulation were excellent at temperatures ranging between 23 and 25°C. However, 23 ºC was the best amongst all. These findings are in line with those of earlier works on *A. brassicae*. (Taber *et al*., 1968; Neergaard, 1945; Limasset, 1955; Changsri and Weber, I963; Czyzewska, 1969)

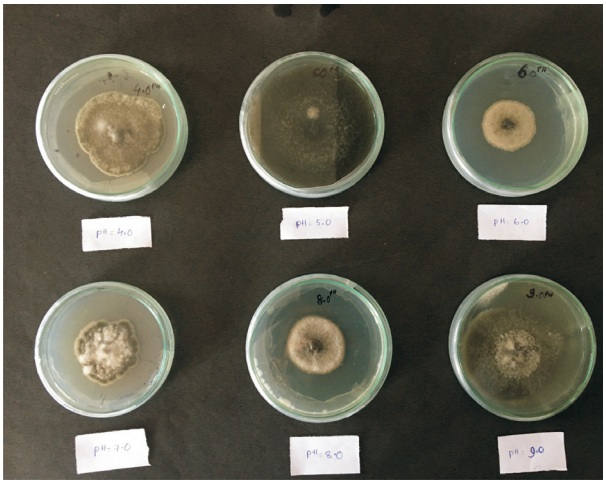

Plate 2. Radial growth of *A. brassicae* at different pH.

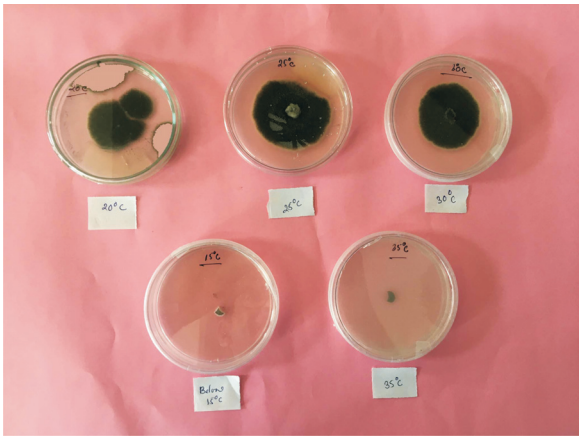

Plate 3. Radial growth of *A. brassicae* at different temperature.

Although the pathogen grew well at all pH levels tested (4-9), sporulation occurred only between pH values of 3.5 and 8.5. The extremely low and extremely high pH levels tested resulted in very poor growth and spore formation. Lower pH levels resulted in poorer growth and sporulation, which gradually improved to reach a peak at 5.0 pH after which growth declined. (Table 2). Normally when *A. brassicae* grows initial stage would slow, normally used media PDA contain 7.6 pH when it changes to 5.0 it grows tremendously faster than any other pH. Whereas full petri plates it takes 15 to 20 days to grow on pH 5.0 it filled petri plate within eight days. These findings are in close agreement with those obtained earlier on the same pathogen by Gupta *et al.* (1969); Welch *et al*. (I969).

REFERENCES

Bhat, H.A., Ahmad, K., Ahanger, R.A., Qazi, N.A., Dar, N.A. and Ganie, S.A. 2013. Status and symptomatology of Alternaria leaf blight (Alternaria alternata) of Gerbera (Gerbera jamisonii) in Kashmir valley. *African Journal of Agricultural Research.* 8(9): 819-823.

Bhowmik, T.P. 2003. Oilseed Brassicas: Constraints And Their Management. CBS publishers & Distributors.

Chahal, A. S. and Kang, M.S. 1979. Different levels of alternaria blight in relation to grain yield of brown sarson [mustard, India]. Note. *Indian Journal of Mycology and Plant Pathology.*

Chowdhury, S. 1937. Germination of fungal spores in relation to atmospheric humidity. *Indian Journal of Agricultural Science.* 7(4): 653-657.

Chowdhury, S. 1944. An Alternaria disease of safflower. Kadian, A. K. and Saharan, G.S. 1984. Studies on spore germination and infection of Alternaria brassicae of rapeseed and mustard. *J. Oilseed Res.* 1:183-188.

Ghosh, R., Barman, S., Khatun, J. and Mandal, N.C. 2016. Biological control of *Alternaria alternata* causing leaf spot disease of Aloe vera using two strains of rhizobacteria. *Biological Control.* 97: 102-108.

Gireesh Chand, U.N., Kumar, M. and Verma, S. 2007. Symptomatology, Etiology and Ecofriendly Management of *Alternaria. Ecofriendly Management of Plant Diseases.* 461.

Goyal, P., Chahar, M., Mathur, A. P., Kumar, A. and Chattopadhyay, C. 2011. Morphological and cultural variation in different oilseed Brassica isolates of *Alternaria brassicae* from different geographical regions of India. *Indian Journal of Agricultural Sciences.* 81(11) : 1052.

Humpherson Jones, F. M. and Phelps, K. 1989. Climatic factors influencing spore production in Alternaria brassicae and Alternaria brassicicola. *Annals of Applied Biology*. 114(3): 449-458.

Jasalavich, C. A., Morales, V. M., Pelcher, L. E. and Séguin-Swartz, G. 1995. Comparison of nuclear ribosomal DNA sequences from Alternaria species pathogenic to crucifers. *Mycological Research*. 99(5): 604-614.

Kadian, A. K. and Saharan, G.S. 1984. Studies on spore germination and infection of Alternaria brassicae of rapeseed and mustard. *J. Oilseed Res.* 1: 183-188.

Koley, S. and Mahapatra, S.S. 2015. Evaluation of culture media for growth characteristics of *Alternaria solani*, causing early blight of tomato. *J. Plant Path. Microbiol.* S: 1.

Kumar, A., Bhadana, N.K., Kumar, G. and Kumar, V. 2019. Chemical and Biological Management of *Alternaria* Leaf Spot of Aloe Vera. *Think India Journal.* 22(16): 1871-1893.

Kumar, V., Haldar, S., Pandey, K.K., Singh, R.P., Singh, A.K. and Singh, P.C. 2008. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of Alternaria solani in India. *World Journal of Microbiology and Biotechnology.* 24(7): 1003-1009.

Meena, P. D., Chattopadhyay, C. and Meena, R. L. 2011. Eco-friendly management of Alternaria blight in *Brassica juncea.* Indian Phytopathology.

Meena, P. D., Meena, R. L., Chattopadhyay, C. and Kumar, A. 2004. Identification of critical stage for disease development and biocontrol of Alternaria blight of Indian mustard (*Brassica juncea*). *Journal of Phytopathology.* 152(4): 204-209.

Meena, P. D., Rani, A., Meena, R., Sharma, P., Gupta, R. and Chowdappa, P. 2012. Aggressiveness, diversity and distribution of Alternaria brassicae isolates infecting oilseed Brassica in India. *African Journal of Microbiology Research.* 6(24): 5249-5258.

Narain, U., Kant, S. and Chand, Gireech, 2016. Characterization of species and parasitism in the genus Alternaria. Crop Diseases and Their Management: Integrated Approaches; Chand, G., Kumar, S., Eds, 385-391.

Patni, C. S., Kolte, S.J. and Awasthi, R.P. 2012. Efficacy of botanicals against Alternaria blight (*Alternaria brassicae*) of mustard. *Indian Phytopathology.*

Pramila, P. G., Tasleem, M., Taj, G., Mal, R. and Kumar, A. 2014. Morphological, cultural, pathogenic and molecular variability amongst Indian mustard isolates of Alternaria brassicae in Uttarakhand. *African Journal of Biotechnology.* 13(3).

Rahimloo, T. and Ghosta, Y. 2015. The occurrence of Alternaria species on cabbage in Iran. *Zemdirbyste Agriculture.* 102(3

Sarkar, S. K. and Sengupta, S. 1978. Lundqvist Three Body Interaction and Lattice Dynamics of Ionic Crystals. *Physica Status Solidi* (b). 87(2): 517-525.

Singh Saharan, G., Mehta, N. and Meena, P.D. 2016. Pathogen. In: Alternaria Diseases of Crucifers: Biology, Ecology and Disease Management (pp. 53-86). Springer, Singapore

Singh, Dalel, Prasad, Rajendra, Yadav, S. P. and Kumar, S.O.N.I.T. 2014. Characterization of Alternaria brassicae causing Alternaria blight of rapeseed mustard (*Brassica juncea* L.) and its management. *Supplement on Plant Pathology.* 9(3): 1207

Soledade, M., Pedras, C. and Smith, K. C. 1997. Sinalexin, a phytoalexin from white mustard elicited by destruxin B and Alternaria brassicae. *Phytochemistry*. ***46***(5): 833-837.

Tosi, L. and Zazzerini, A. 1985. Alternaria brassicae (Berk.) Sacc. on rape (*Brassica napus* L. subsp. oleifera DC.) and turnip rape (*Brassica campestris* L. var. oleifera DC.). *Informatore Fitopatologico*. 35(11): 43-44.

TU, D.V., Somasekhara, Y.M. and Shivaprakash, M.K. 2016. Cultural and physiological studies of *Alternaria brassicicola* (Schw.) Wiltshire of cabbage (*Brassica oleracea* var. capitata L.). *Mysore J. Agric. Sci.* 50(3): 529-534.

Waghe, K.P., Wagh, S.S., Kuldhar, D.P. and Pawar, D.V. 2015. Evaluation of different fungicides, bioagents and botanicals against Alternaria blight caused by Alternaria helianthi (Hansf) of sunflower. *African Journal of Agricultural Research.* 10(5): 351-358.