# Qualified studies on the pathogenic variability of *Alternaria brassicae*, the causal agent of blight disease of rapeseed-mustard


## Abstract

Alternaria blight of mustard caused by *Alternaria brassicae* is one of the major disease which causes an economic yield losses in Uttar Pradesh. Total ten (10) isolates of *Alternaria brassicae* were collected from different host from different states of India and characterized for pathogenic variations. The incubation period was maximum in *Brassica carinata* (8.82) followed by *B. napus* (8.70), *B. nigra* (7.86), *B. juncea* (6.00), while it was minimum in *B. campestris* var. yellow sarson (5.83) for all the isolates. The number of spots per leaf recorded maximum on *Brassica juncea* (17.66) followed by *B. napus* (12.66), *B. nigra* (9.66), *B. campestris* var. yellow *sarson* (9.00), while it was minimum in *B. carinata* (7.66) at 80 days after sowing (DAS). The size of spots per leaf recorded maximum on *Brassica juncea* (14.89 mm) followed by *B. campestris* var. yellow *sarson* (12.48 mm), *B. nigra* (7.89 mm), *B. napus* (6.96 mm), while minimum in *B. carinata* (5.12 mm) at 80 DAS. The number of spots on pods recorded maximum on *Brassica juncea* (14.66) followed by *B. campestris* var. yellow *sarson* (12.66), *B. nigra* (10.66), *B. napus* (7.66), and *B. carinata* (6.33) at 105 DAS. The results revealed that maximum PDI was noted on *Brassica juncea* (66.70) followed by *B. campestris* var. yellow *sarson* (62.99), *B. nigra* (60.25), *B. napus* (29.85), while minimum in *B. carinata* (24.42).



**Key words:** Rapeseed-mustard, *Alternaria brassicae,* Isolates, Pathogenic variability


## 1. INTRODUCTION

In India, rapeseed-mustard is an important group of edible oilseed crops that contributes around 26.1 per cent of the total oilseed production. Indian mustard (*B. juncea*) is one of the major oilseed crops cultivated in India. Rapeseed-mustard is cultivated on 5.96 million hectares with production of 8.32 million tonnes and productivity of 1397 kg/ha [1]. Rapeseed-mustard is the second most important oilseed crop after groundnut and accounts for nearly 30.7 per cent of the total oilseed production in the country. In spite of higher yield potential, diseases are major constraints, of which Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw) Wiltshire is one of the most severe and yield destabilizing factor reduction from 35 to 70 per cent [3, 4, 10, 14]. The disease also adversely affects quality by reducing seed size, impairing seed colour and oil content [2, 11]. In oilseed Brassicas, the symptoms of disease

caused by *A. brassicae* appear primarily in the seedling stage on cotyledons and hypocotyls in the form of small light brown lesions which at adult stage affect leaves, leaf petiole, stem, inflorescence, siliquae and seeds. Studies on pathogen variability have to be the foundation for development of pre-breeding populations as strategic defense mechanism. Severity of Alternaria blight on oilseed Brassica differs among regions and also Brassica crops within a region might be due to existence of variability among the isolates of *A. brassicae*. The existence of pathogen diversity among *A. brassicae* isolates has already been reported by earlier workers.

Variability studies are important to document the changes occurring in populations and individuals as variability in physiological traits indicate the existence of different pathotypes. *Alternaria* blight severity on oilseed Brassicas differ season to season, region to region and also individual crop to crop in India. This might be due to the existence of variability among geographically similar isolates of *A. brassicae*. Considering the importance of the crop, destructive nature of disease and unavailability of information, the study was undertaken to find out the variation in pathogenic variability among eight isolates of *A. brassicae* collected from different geographical location of India. The variability is a well known phenomenon in genus *Alternaria* and may be noticed as changes in spore shape and size, growth and sporulation, pathogenicity etc. diversity appears even in single spore isolates.

## 2. MATERIALS AND METHODS

### 2.1 Collection, isolation and purification

Ten isolates collected from different *Brassica* spp. during the crop season of 2018-19 from different growing locations of India (Assam, Bihar, Uttar Pradesh, Tripura, Odisha, Haryana, West Bengal, Uttarakhand, Tamil Nadu and Chattisgarh) were cultured on PDA and further purification was done using single spore isolation. The samples were kept in sterile polythene bags and rough dry paper envelops, especially meant for this purpose. Envelops were marked clearly mentioning the location, variety and date of collection. The collected samples were dried for 24 hours under shade in order to remove excess surface moisture in laboratory, and then the samples were repacked. These samples were kept in Biological Oxygen Demand (B.O.D.) incubator at 6-8$^0$C temperature for further study. The present sample was cultured on Potato Dextrose Agar (PDA) and further purification was done using single spore isolation. The culture was preserved in the refrigerator at 4°C for further studies (Table 1).

**Table 1: Diseased sample collected from different places of India**

| Place of collection | Host | Plant parts | Isolate No. |
|---|---|---|---|
| Sonitpur (Assam) 26.67$^0$N,92.85$^0$E | *Brassica juncea* | Leaf | $Abr_1$ |
| Samastipur (Bihar) 25.85$^0$N, 85.78$^0$E | *Brassica rapa* | Leaf | $Abr_2$ |
| Ayodhya (Uttar Pradesh) 26.70$^0$N, 82.13$^0$E | *Brassica rapa* | Leaf | $Abr_3$ |
| Lembucherra (Tripura) 23.90$^0$N, 91.31$^0$E | *Brassica juncea* | Leaf | $Abr_4$ |
| Bhubaneswar (Odisha) 20.29$^0$N, 85.82$^0$E | *Brassica juncea* | Leaf | $Abr_5$ |
| Hissar (Haryana) 29.14$^0$N, 75.72$^0$E | *Brassica juncea* | Leaf | $Abr_6$ |
| Mohanpur (West Bengal) 21.83$^0$N, 87.42$^0$E | *Brassica juncea* | Leaf | $Abr_7$ |
| Dehradun (Uttarakhand) 30.31$^0$N, 78.03$^0$E | *Brassica juncea* | Leaf | $Abr_8$ |
| Coimbatore (Tamil Nadu) 11.01$^0$N, 76.95$^0$E | *Brassica juncea* | Leaf | $Abr_9$ |
| Bilaspur (Chattisgarh) 22.07$^0$N, 82.14$^0$E | *Brassica juncea* | Leaf | $Abr_{10}$ |

**2.2 Pathogenic variability**

The experiment was conducted at Student Instructional Farm situated at main campus of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.) during season 2018-19 for pathogenic variability of ten isolates on different *Brassica* spp. (Table 2) to study incubation period, number of spots on leaves and pods, size of spots and disease severity on leaves on leaves. The seeds of five varieties of different rapeseed-mustard species were treated with Mancozeb @ 0.25% per kg.

**Table 2: *Brassica* spp. for pathogenic variability**

| Botanical name | Common name | Local name |
|---|---|---|
| *Brassica juncea* | Indian mustard | Rai, Laha |
| *Brassica campestris* | Turnip rape | Yellow *sarson* |
| *Brassica nigra* | Black mustard | Banarsi rai |
| *Brassica napus* | Gobhi *sarson* | Gobhi *sarson* |
| *Brassica carinata* | Karan rai | Ethiopian mustard |

**2.3 Selection of host differentials**

Five different *Brassica* spp. viz. *Brassica juncea, B. campestris, B. carinata, B. napus* and *B. nigra* by single drop inoculation method (10 µl/leaf) at 40 days after sowing (DAS). The average

number of spots/leaf from five leaves were counted at 80 days after sowing (DAS) and spot size (mm) were also recorded. Similarly, number of spots was also counted from five siliquae at 105 DAS. The experiment was conducted in three replications.

## 3. RESULTS AND DISCUSSION

Incubation period on *B. juncea* ranged from 3 to 4 days. The data presented in table 3 revealed that incubation period maximum in isolate $Abr_{10}$ (6.00), while minimum in $Abr_3$ (4.00). In *B. carinata* incubation period ranged from 7.76 to 8.82 days. The incubation period was maximum in isolate $Abr_{10}$ (8.82), while minimum in $Abr_3$ (7.76). Incubation periods on *B. napus* ranged from 7.72 to 8.70 days. It was maximum in isolate $Abr_{10}$ (8.70), while minimum in $Abr_3$ (7.72). In *B. nigra* incubation period ranged from 6.48 to 7.86 days. The incubation period was maximum in isolate $Abr_8$ (7.86), while minimum in $Abr_3$ (6.48). Incubation period in *B. campestris* ranged from 4.48 to 5.83 days. The incubation period was maximum in isolate $Abr_9$ (5.83), while minimum in $Abr_3$ (4.48). On an average the incubation period was maximum in, *B. carinata*, followed by *B. napus*, *B. nigra*, *B. juncea* and *B. campestris* var. yellow *sarson*. The results are in agreement with earlier workers [5, 6, 12, 13].

The number of spots on *B. juncea* ranged from 11.66 to 17.66. The data presented in table 4 revealed that number of spots was maximum in isolate $Abr_3$ (17.66) However it was minimum in isolates $Abr_2$ (11.66). Number of spots on *B. carinata* ranged from 3.66 to 7.66. The numbers of spots were maximum in isolate $Abr_4$ (7.66), while minimum in $Abr_{10}$ (3.66). In *B. napus* the average number of spots ranged from 7.66 to 12.66. The number of spots was maximum in isolate $Abr_3$ (12.66), while minimum in $Abr_{10}$ (7.66). In *B. nigra*, number of spots ranged from 5.00 to 9.66. Isolate $Abr_3$ showed maximum (9.66) number of spots, while minimum in $Abr_4$ (5.00). The average number of spots ranged from 5.33 to 9.00 in *B. campestris* var. yellow *sarson*. The maximum spots were noted in isolate $Abr_3$ (9.00), while minimum in $Abr_5$ (5.33). The results are in agreement with earlier workers [6, 9].

The size of spots on *B. juncea* ranged from 7.18 to 14.89 mm. The data presented in table 5 revealed that size of spots were maximum in isolate $Abr_3$ (14.89) whereas, isolate $Abr_{10}$ showed minimum (7.18) spot size on *B. juncea* at 80 days. Size of spots ranged from 3.12 to 5.42 mm in *B. carinata*. The size of spots was maximum in isolate $Abr_7$ (5.42), while minimum in $Abr_5$ (3.12). On *B. napus* the size of spots ranged from 5.12 to 6.96 mm. The size of spots was maximum in isolate $Abr_3$ (6.96), while minimum in $Abr_{10}$ (5.12). The size of spots ranged from

4.83 to 7.89 mm in *B. nigra*. The size of spots was maximum in isolate $Abr_3$ (7.89), while minimum in $Abr_{10}$ (4.83). In *B. campestris* var. yellow *sarson* the size of spots ranged from 5.12 to 12.48 mm. The size of spots was maximum in isolate $Abr_6$ (12.48), while minimum in $Abr_{10}$ (5.12). These findings were further supported by other workers [6]. The size of spots (mm) on leaves ranged from 3.05 to 9.26 mm in different genotypes [9].

The number of spot from five pods counted at 105 days after sowing on different *Brassica* spp. The data presented in table 6 revealed that number of spots on *B. juncea* ranged from 7.66 to 14.66. The number of spots were maximum in isolate $Abr_3$ (14.66) However, number of spots on pods was minimum in isolate $Abr_{10}$ (7.66) at 90 DAS. Number of spots on *B. carinata* ranged from 3.00 to 6.33. The number of spots was maximum in isolate $Abr_3$ (6.33), while minimum in $Abr_{10}$ (3.00). In *B. napus* the number of spots ranged from 4.33 to 7.66. The number of spots was maximum in isolate $Abr_7$ (7.66), while minimum in $Abr_{10}$ (4.33). The number of spots in *B. nigra*, ranged from 5.33 to 10.66. Isolate $Abr_3$ showed maximum (10.66) number of spots, while minimum in $Abr_{10}$ (5.33). The number of spots ranged from 6.66 to 12.66 in *B. campestris* var. yellow *sarson*. The maximum spots were noted in isolate $Abr_3$ (12.66) However, number of spots on pods was minimum in isolate $Abr_{10}$ (6.66). These measurements were found within the range of the measurements of the number and size of spots on *Brassica* spp. described by various earlier workers [6, 7, 8, 9].

The per cent disease intensity (PDI) ranged from 44.73 to 66.70 in *B. juncea*. The data presented in table 7 revealed that maximum PDI was found in isolate $Abr_3$ (66.70), whereas, it was lowest in isolate $Abr_2$ (44.73). In *B. carinata* the PDI ranged from 12.74 to 24.42. The PDI was maximum in isolate $Abr_3$ (24.42), while minimum in $Abr_1$ (12.74). Per cent disease intensity ranged from 16.77 to 29.85 in *B. napus*. The PDI were maximum in isolate $Abr_3$ (29.85), while minimum in $Abr_{10}$ (16.77). The average PDI ranged from 43.74 to 60.25 in *B. nigra*. Maximum PDI was noted in isolate $Abr_7$ (60.25), while minimum in $Abr_2$ (43.74). In *B. campestris* var. yellow *sarson* PDI ranged from 44.95 to 62.99. The PDI was maximum in isolate $Abr_3$ (62.99), while minimum in $Abr_2$ (44.95). The results revealed that maximum PDI was noted on *Brassica juncea* followed by *B. campestris* var yellow *sarson, B. nigra, B. napus* and *B. carinata*. Concurrent with present findings [6, 7, 8].

**4. CONCLUSION**

In present studies significant variation in pathogenic variability was observed in *A. brassiace* isolates irrespective to geographical locations and *Brassica* spp. The incubation period was maximum in *Brassica carinata* (8.82) followed by *B. napus* (8.70), *B. nigra* (7.86), *B. juncea* (6.00), while it was minimum in *B. campestris* var. yellow sarson (5.83) for all the isolates. The number of spots per leaf recorded maximum on *Brassica juncea* (17.66) followed by *B. napus* (12.66), *B. nigra* (9.66), *B. campestris* var. yellow *sarson* (9.00), while it was minimum in *B. carinata* (7.66) at 80 days after sowing (DAS). The size of spots per leaf recorded maximum on *Brassica juncea* (14.89 mm) followed by *B. campestris* var. yellow *sarson* (12.48 mm), *B. nigra* (7.89 mm), *B. napus* (6.96 mm), while minimum in *B. carinata* (5.12 mm) at 80 DAS. The number of spots on pods recorded maximum on *Brassica juncea* (14.66) followed by *B. campestris* var. yellow *sarson* (12.66), *B. nigra* (10.66), *B. napus* (7.66), and *B. carinata* (6.33) at 105 DAS. The results revealed that maximum PDI was noted on *Brassica juncea* (66.70) followed by *B. campestris* var. yellow *sarson* (62.99), *B. nigra* (60.25), *B. napus* (29.85), while minimum in *B. carinata* (24.42). On the basis of pathogenic variability, these *A. brassicae* isolates may be useful in developing integrated disease management strategies and breeding programs for the mustard crop.

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**Table 3: Incubation period of different isolates of *A. brassicae* on *Brassica* spp.**

| Isolates | Incubation period (days) | | | | |
|---|---|---|---|---|---|
| | ***B. juncea*** | ***B. carinata*** | ***B. napus*** | ***B. nigra*** | ***B. campestris*** |
| $Abr_1$ | 5.41 | 8.42 | 8.20 | 7.26 | 5.46 |
| $Abr_2$ | 5.48 | 8.36 | 8.16 | 7.18 | 5.52 |
| $Abr_3$ | 4.00 | 7.76 | 7.72 | 6.48 | 4.48 |
| $Abr_4$ | 5.36 | 8.52 | 8.28 | 7.38 | 5.30 |
| $Abr_5$ | 5.61 | 8.64 | 8.59 | 7.43 | 5.36 |
| $Abr_6$ | 5.30 | 8.28 | 8.10 | 7.10 | 5.26 |
| $Abr_7$ | 5.21 | 8.00 | 8.00 | 6.89 | 5.20 |
| $Abr_8$ | 5.82 | 8.73 | 8.34 | 7.86 | 5.68 |
| $Abr_9$ | 5.83 | 8.81 | 8.45 | 7.56 | 5.83 |
| $Abr_{10}$ | 6.00 | 8.82 | 8.70 | 7.68 | 5.80 |
| **Average** | **5.40** | **8.43** | **8.25** | **7.28** | **5.38** |
| **SEm±** | **0.22** | **0.34** | **0.33** | **0.29** | **0.22** |
| **CD at 5 %** | **0.66** | **1.01** | **0.99** | **0.87** | **0.65** |
| **CV (%)** | **7.18** | **7.03** | **7.04** | **7.03** | **7.10** |

**Table 4: Number of spots of different isolates of *A. brassicae* on *Brassica* spp. at 80 days after sowing**

| Isolates | Number of spots on leaves at 80 days after sowing | | | | |
|---|---|---|---|---|---|
| | ***B. juncea*** | ***B. carinata*** | ***B. napus*** | ***B. nigra*** | ***B. campestris*** |
| $Abr_1$ | 12.66 | 6.00 | 11.66 | 8.66 | 6.66 |
| $Abr_2$ | 11.66 | 4.33 | 11.33 | 8.00 | 5.66 |
| $Abr_3$ | 17.66 | 4.66 | 12.66 | 9.66 | 9.00 |
| $Abr_4$ | 13.66 | 7.66 | 10.66 | 5.00 | 7.66 |
| $Abr_5$ | 15.66 | 6.66 | 10.33 | 5.66 | 5.33 |
| $Abr_6$ | 16.66 | 4.00 | 9.33 | 7.66 | 8.66 |

| | | | | | |
|---|---|---|---|---|---|
| $Abr_7$ | 16.33 | 5.66 | 9.66 | 9.00 | 8.33 |
| $Abr_8$ | 12.33 | 5.33 | 9.00 | 8.33 | 6.00 |
| $Abr_9$ | 14.66 | 5.00 | 8.66 | 6.66 | 8.00 |
| $Abr_{10}$ | 14.33 | 3.66 | 7.66 | 5.33 | 6.33 |
| **Average** | **14.56** | **5.29** | **10.09** | **7.39** | **7.16** |
| **SEm±** | **0.58** | **0.19** | **0.39** | **0.29** | **0.27** |
| **CD at 5 %** | **1.72** | **0.58** | **1.17** | **0.86** | **0.81** |
| **CV (%)** | **6.94** | **6.46** | **6.83** | **6.82** | **6.64** |

**Table 5: Size of spots of different isolates of *A. brassicae* on *Brassica* spp. at 80 days after sowing**

| **Isolates** | **Size of spots on leaves (mm) at 80 days after sowing** | | | | |
|---|---|---|---|---|---|
| | ***B. juncea*** | ***B. carinata*** | ***B. napus*** | ***B. nigra*** | ***B. campestris*** |
| $Abr_1$ | 12.52 | 4.83 | 5.38 | 5.92 | 7.52 |
| $Abr_2$ | 9.63 | 4.00 | 6.54 | 6.73 | 7.46 |
| $Abr_3$ | 14.89 | 5.12 | 6.96 | 7.89 | 12.34 |
| $Abr_4$ | 8.34 | 3.54 | 5.54 | 6.68 | 8.37 |
| $Abr_5$ | 10.82 | 3.12 | 6.38 | 6.89 | 8.25 |
| $Abr_6$ | 13.68 | 4.00 | 6.71 | 7.68 | 12.48 |
| $Abr_7$ | 13.34 | 5.42 | 6.12 | 7.76 | 10.76 |
| $Abr_8$ | 11.62 | 3.61 | 6.25 | 6.54 | 11.68 |
| $Abr_9$ | 8.14 | 3.78 | 5.27 | 7.34 | 6.12 |
| $Abr_{10}$ | 7.18 | 3.89 | 5.12 | 4.83 | 5.12 |
| **Average** | **11.01** | **4.13** | **6.02** | **6.82** | **9.01** |
| **SEm±** | **0.43** | **0.16** | **0.24** | **0.26** | **0.35** |
| **CD at 5 %** | **1.28** | **0.47** | **0.72** | **0.79** | **1.05** |
| **CV (%)** | **6.82** | **6.75** | **7.07** | **6.80** | **6.84** |

**Table 6: Number of spots of different isolates of *A. brassicae* on *Brassica* spp. on pods at 105 days after sowing**

| **Isolates** | **Number of spots on pods at 105 days after sowing (DAS)** | | | | |
|---|---|---|---|---|---|
| | ***B. juncea*** | ***B. carinata*** | ***B. napus*** | ***B. nigra*** | ***B. campestris*** |
| $Abr_1$ | 10.66 | 5.00 | 5.66 | 9.00 | 10.66 |
| $Abr_2$ | 10.33 | 4.66 | 6.33 | 8.66 | 7.66 |
| $Abr_3$ | 14.66 | 6.33 | 7.33 | 10.66 | 12.66 |
| $Abr_4$ | 11.66 | 4.00 | 6.00 | 9.66 | 11.66 |
| $Abr_5$ | 11.33 | 5.33 | 6.66 | 9.33 | 11.00 |

| | | | | | |
|---|---|---|---|---|---|
| $Abr_6$ | 12.66 | 5.66 | 7.00 | 8.33 | 12.00 |
| $Abr_7$ | 13.66 | 6.00 | 7.66 | 10.33 | 12.33 |
| $Abr_8$ | 9.66 | 4.33 | 5.00 | 8.00 | 7.33 |
| $Abr_9$ | 9.33 | 3.66 | 4.66 | 7.66 | 7.00 |
| $Abr_{10}$ | 7.66 | 3.00 | 4.33 | 5.33 | 6.66 |
| **Average** | **11.16** | **4.79** | **6.06** | **8.69** | **9.89** |
| **SEm±** | **0.43** | **0.19** | **0.24** | **0.33** | **0.38** |
| **CD at 5 %** | **1.27** | **0.56** | **0.72** | **0.98** | **1.12** |
| **CV (%)** | **6.71** | **6.91** | **6.97** | **6.62** | **6.68** |

**Table 7: Per cent disease intensity of different isolates of *A. brassicae* on *Brassica* spp.**

| **Isolates** | **Per cent Disease Intensity (PDI)** | | | | |
|---|---|---|---|---|---|
| | ***B. juncea*** | ***B. carinata*** | ***B. napus*** | ***B. nigra*** | ***B. campestris*** |
| $Abr_1$ | 45.75 (42.56) | 12.74 (20.91) | 20.29 (26.77) | 45.25 (42.27) | 46.95 (43.25) |
| $Abr_2$ | 44.73 (41.97) | 20.51 (26.91) | 22.26 (28.13) | 43.74 (41.39) | 44.95 (42.09) |
| $Abr_3$ | 66.70 (54.77) | 24.42 (29.61) | 29.85 (33.11) | 56.85 (48.94) | 62.99 (52.54) |
| $Abr_4$ | 53.59 (47.06) | 22.00 (27.97) | 18.00 (25.10) | 50.85 (45.49) | 56.99 (49.02) |
| $Abr_5$ | 54.80 (47.76) | 22.88 (28.56) | 22.54 (28.33) | 52.22 (46.27) | 51.66 (45.95) |
| $Abr_6$ | 63.13 (52.65) | 24.00 (29.32) | 24.40 (29.59) | 58.25 (49.77) | 60.82 (51.28) |
| $Abr_7$ | 61.29 (51.53) | 23.28 (28.84) | 25.76 (30.50) | 60.25 (50.92) | 61.96 (51.93) |
| $Abr_8$ | 47.00 (43.28) | 15.80 (23.42) | 23.14 (28.75) | 48.56 (44.17) | 50.77 (45.44) |
| $Abr_9$ | 55.96 (48.64) | 19.28 (26.13) | 17.98 (25.17) | 47.77 (43.90) | 58.29 (50.00) |
| $Abr_{10}$ | 48.74 (44.27) | 20.20 (26.69) | 16.77 (24.16) | 49.73 (44.84) | 48.99 (44.42) |
| **Average** | **54.16** | **20.51** | **22.09** | **51.34** | **54.43** |
| **SEm±** | **1.25** | **0.59** | **0.61** | **1.18** | **1.23** |
| **CD at 5 %** | **3.68** | **1.76** | **1.80** | **3.49** | **3.63** |
| **CV (%)** | **4.56** | **3.85** | **3.78** | **4.48** | **4.48** |

Figure in parenthesis are angular transformed value.