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# Cultural, morphological, physiological and pathogenic diversity among the isolates of *Alternaria* spp., incitant of Blight disease of sesame

**SAVITHA A.S., M.K. NAIK AND K. AJITHKUMAR**
*Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Raichur 584 104, Karnataka, India*

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Fourteen isolates of *Alternaria* spp. obtained from Raichur, Gulbarga, Dharwad, Bidar, Bangalore, Hyderabad and Coimbatore districts comprising of eight isolates *Alternaria alternata* and six isolates of *Alternaria sesami* were studied for cultural, morphological, physiological, pathogenic and genetic variability. The conidiophores of *Alternaria* spp. bearing conidia were solitary, straight, muriform with yellowish brown in colour. The isolates $ALT_1$ and $ALT_2$ showed maximum conidial size of 37.77 – 44.40 X 6.66 – 9.62 µm and 37.77 – 44.40 X 6.66 – 9.62 µm respectively. The isolate $ALT_4$ showed maximum beak length of 14.80 – 19.24 µm. The colony colour of isolates varied from light grey to dark brown colour. Six isolates showed irregular margins whereas another set of eight isolates showed raised growth on the culture. The maximum dry mycelial weight and moderate to excellent sporulation was noticed at 25°C in 14 isolates (262.62 mg) over other temperature regimes. The maximum radial growth was noticed with 100 per cent RH (84.12 mm) when incubated in alternated dark and light cycles of 12 h each (270.59 mg). The pathogenic reaction was indicated $G_1$ as more virulent than others.

**Key words:** *Alternaria*, cultural, physiological, pathogenic, sesame

## INTRODUCTION

India is the largest producer of sesame in the world covering an area of 1.84 m.ha producing 893 thousand tonnes annually recording productivity of 485 kg/ha (FAOSTAT 2010). In Karnataka it occupies an area of 56.3 thousand ha with the production of 31.67 thousand tonnes and productivity of 593 kg/ha (Directorate of Economics and Statistics 2009). Many factors operate in low and unreliable yield. Among the limiting factors, diseases rank first as the forming community fail to diagnose the disease in time and follow any plant protection measures (Reddy 2001; Naik *et al.* 2003). Sesame crop suffers from many diseases, among them *Alternaria* leaf blight caused by *Alternaria sesami* is one of the most catastrophic disease incurring loss in India and also in north-eastern dry zone of Karnataka recording up to 43 per cent loss (Dolle 1981).

*Alternaria sesami*, the incitant of blight leaf disease of sesame is a highly variable pathogen. Based on the differential reaction of *A. sesami* on five test sesame genotypes revealed that a significant difference exists in their ability to infect the sesame genotypes. The variation in pathogenicity as well as genetic variability in *A. solani* was established by many workers (Tong Yunshi *et al.* 1999; Naik *et al.* 2010). The more virulent pathotypes within population may affect the rate of disease development and induce infection in most host lines. The availability of variable isolates virulent on different resistant cultivars are useful in determining the resistant loci in the host.

To develop an effective programme of breeding for disease resistance, a comprehensive understanding of causal organism with reference to cultural, morphological, physiological and pathogenic variability are essential. This research is focussing in that direction.

## MATERIALS AND METHODS

### *Collection of isolates of Alternaria* spp.

The isolates of *Alternaria* spp. were obtained from the infected leaves by using standard tissue isolation technique. The pure culture of the fungus was obtained by hyphal tip isolation. Further the identity of the fungus was confirmed by reporting the culture at National Bureau of Agriculturally Important Microorganisms (NBIAM), IARI, New Delhi with ITCC No.170.83 to 180.83. Fourteen isolates of *Alternaria* spp. were obtained from sesame, including eight of *A. alternata* and six of *A. sesami,* were maintained by incubating at 28±1° C (Table 1).

### *Cultural and morphological variability*

The variability for cultural characters of 14 isolates of *Alternaria* spp. such as colony diameter, colony colour, type of margin and sporulation were recorded on different media *viz.*, potato dextrose agar (PDA), PDA with $CaCO_3$, host extract agar, oat meal agar, Czapeck's agar, Sabourd's agar and Richard's agar.

Morphological character of 14 isolates of *Alternaria* spp. including size of the conidia, length of beak and hyphal width was measured under high power objective (40X) using calibrated filar micrometer and microscope from 10 days old culture on PDA supplemented with $CaCO_3$. Number of transverse and longitudinal septa was also recorded.

### *Physiological variability*

### *Effect of temperature on the growth and sporulation*

The growth of 14 isolates *A. sesami* was tested at 15°, 20°, 25°, 30° and 35° C. Twenty ml of potato dextrose broth was dispensed and sterilized in 100 ml flasks. Each flask was inoculated with and incubated for eleven days in incubators adjusted to required temperature levels. Each treatment was replicated thrice. After incubation period, the dry mycelial weight and sporulation were recorded by passing culture through tared Whatman No. 42 filter paper. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to get rid off the salts likely to be associated with the mycelial mass. The filter paper along with the mycelial mat were dried at 60° C and weighed immediately.

### *Effect of light on growth and sporulation*

The effect of light on growth and sporulation of 14 isolates of *A. sesami* and *A. alternata* was studied by using potato dextrose broth (PDB) by exposing the inoculated flasks to continuous light, continuous dark and alternate cycles of 12 h complete light and 12 h of complete darkness. After incubation dry mycelial weight and sporulation were recorded.

### *Effect of RH on growth and sporulation*

Five mm discs of 10 days old culture of 14 isolates of *A. sesami* and *A. alternata* were placed in the Petridish containing PDA medium under aseptic condition and Petridish was exposed to 65, 75, 95 and 100 per cent relative humidity levels maintained in desiccators. Different levels of relative humidity were created by using different solutions of $H_2SO_4$. The desiccators were kept at 27 ± 1° C with three replications. Observations on colony diameter and sporulation were recorded at 11 days after incubation.

### *Pathogenic variability*

Pathogenic variability was carried out by grouping the 14 isolates of *A. sesami* and *A. alternata* into five groups based on the cultural characteristics and inoculated on to the resistant (RT-273), moderately resistant (Tharikere local), moderately susceptible (NIC-17986), susceptible (NIC-7866) and highly susceptible genotype (DS-1) by detached leaf technique. Detached leaflets obtained from 30 days old seedling of sesame varieties, were inoculated with spore suspension ($1X10^6$ spore / ml) obtained from 11 days old culture of each of five groups of 14 isolates of *Alternaria* spp. separately and replicated thrice. After inoculation the leaflets were kept in sand culture in laboratory to ensure high humidity. Observations on disease intensity were scored using 0 - 5 scale after incubation for a week (Mayee and Datar 1986) and per cent disease index was calculated using formula given by Wheeler (1969).

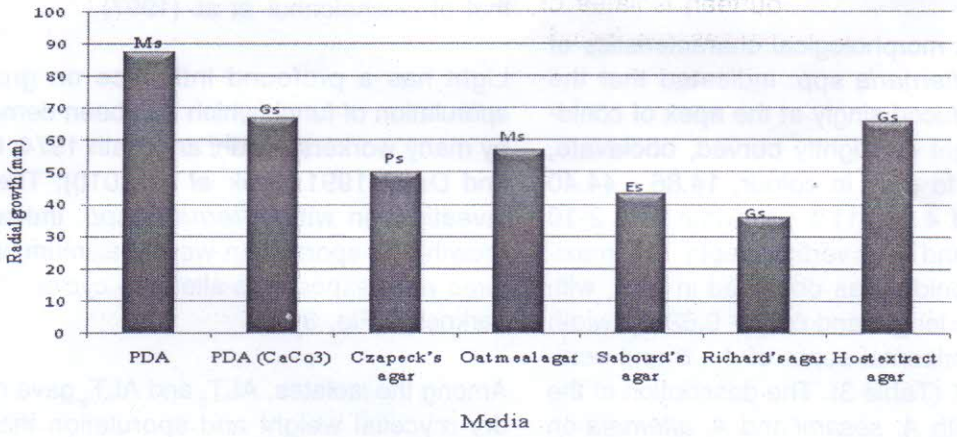
## RESULTS AND DISCUSSION

### *Cultural and morphological variability*

The best media for growth and sporulation of *A. sesami* on both synthetic and non synthetic media

were tried in the present investigation. It was found that potato dextrose agar (87.33 mm) supported good growth and sporulation of *A. sesami* followed by PDA supplemented with $CaCO_3$ is attributed to inherent complex nature of natural media supporting good fungal growth owing to provision of some additional nutrients (Fig. 1). These observations are in conformity with the findings of earlier worker (Moretto and Baretto 1995; Benlioglu and Delen 1996).

Diversity in cultural characters such as colony colour, its margin and topography were noticed among the isolates of *Alternaria* spp. Three isolates $ALT_1$, $ALT_2$ and $ALT_3$ (G1) showed dark brown colour and other five isolates $ALT_2$, $ALT_7$, $ALT_8$, $ALT_{12}$ and $ALT_{14}$ (G2) exhibited brown colour. Whereas $ALT_4$, $ALT_6$, $ALT_9$ and $ALT_{10}$ (G3) showed light grey colour and $ALT_5$ (G4) and $ALT_{11}$ (G5) showed grey and light brown colour respectively. The type of margin is irregular in $ALT_1$, $ALT_3$, $ALT_7$,




Es = Excellent sporulation; Gs = Good sporulation; Ms = Moderate sporulation; Ps = Poor sporulation;

**Fig. 1 :** Growth and sporulation of *Alternaria sesami* on different solid media

**Table 1:** Source of 14 isolates of *Alternaria* spp. with their identity

| Districts | Locality | Isolates | Identity of *Alternaria* spp. |
|---|---|---|---|
| Raichur | Agricultural College Farm (Plant Pathology field) | $ALT_1$ | *A. sesami* |
| | Neermanvi | $ALT_2$ | *A. alternata* |
| | Agricultural College (GPB field) | $ALT_3$ | *A. alternata* |
| Gulbarga | Gulbarga (farmers field) | $ALT_4$ | *A. alternata* |
| | Aland (farmers field) | $ALT_5$ | *A .sesami* |
| Bidar | Bidar (farmers field) | $ALT_6$ | *A. alternata* |
| | Balki (farmers field) | $ALT_7$ | *A. alternata* |
| | Humnabad (farmers field) | $ALT_8$ | *A. sesami* |
| Dharwad | Agronomy field, MRS, UAS | $ALT_9$ | *A. sesami* |
| | GPB Field, MRS, UAS | $ALT_{10}$ | *A. sesami* |
| Hyderbad | Narkod (farmers field) | $ALT_{11}$ | *A. sesami* |
| | ANGRAU | $ALT_{12}$ | *A. alternata* |
| Coimbatore | TNAU | $ALT_{13}$ | *A. alternata* |
| Bangalore | Kanakapura (farmers field) | $ALT_{14}$ | *A. alternata* |

GPB – Genetics and Plant Breeding, MRS – Main Research Station, ANGRAU – Acharya NG Ranga Agricultural University, TNAU – Tamilnadu Agricultural University

*A. sesami* – $ALT_1$, $ALT_5$, $ALT_8$, $ALT_9$, $ALT_{10}$, $ALT_{11}$ *A. alternata*– $ALT_2$, $ALT_3$, $ALT_4$, $ALT_6$, $ALT_7$, $ALT_{12}$, $ALT_{13}$, $ALT_{14}$

$ALT_8$, $ALT_{13}$ and $ALT_{14}$ isolates whereas the isolates $ALT_2$, $ALT_4$, $ALT_5$, $ALT_6$, $ALT_9$, $ALT_{10}$, $ALT_{11}$, $ALT_{12}$ exihibited smooth margin. Based on cultural characteristics isolates were grouped in to five and designated as $G_1$, $G_2$, $G_3$, $G_4$ and $G_5$. Several workers (Kual and Saxena 1988; Perez and Martinez 1996; Naik *et al.* 2006) also observed diversity in cultural characteristics such as growth rate, type of growth, colony colour and sporulation among different isolates of *A. macrospora*, *A. sesami* and *A. solani* (Table 2).

The study on the morphological characteristics of 14 isolates of *Alternaria* spp. indicated that the conidia were produced singly at the apex of conidiophore as straight or slightly curved, obclavate, yellowish brown to dark in colour, 14.86 - 44.40 µm in length and 4.7 – 11.1 µm width with 2-10 horizontal septa and 0 - 2 vertical septa. The maximum length of conidia was observed in $ALT_1$ with 37.74 – 44.40 µm length and 6.66 – 9.62 µm width and maximum horizontal septa of 4 - 6 and vertical septa of 0 to 1 (Table 3). The description of the fungus agreed with *A. sesami* and *A. alternata* on sesame as reported by several workers (Dolle 1981; Shekarappa 1999; Ramegowda and Naik, 2008).

out as 25$^0$ C (Kual and Saxena 1988; Stevensona and Packer 1988). Among 14 isolates of *Alternaria* spp., $ALT_7$ and $ALT_2$ recorded significantly higher dry mycelial weight than rest of the isolates tested at different temperature (Fig. 2). Maximum sporulation of the fungus was observed at 25$^0$ C followed by 30$^0$C, whereas the isolates $ALT_5$, $ALT_4$ and $ALT_8$ recorded moderate sporulation even at extreme temperature indicating variation among isolates in sporulation behaviour which is an important pathogenic character. The result was in accordance with that of Liucheicnhui *et al.* (1997).

Light has a profound influence on growth and sporulation of fungi, which has been demonstrated by many workers (Padhi and Rath 1974; Choulwar and Datar 1991; Naik *et al.* 2010). The present investigation with *Alternaria* spp. indicated that growth and sporulation were maximum, when cultures were exposed to alternate cycles of light and darkness (Fig. 3).

Among the isolates, $ALT_3$ and $ALT_9$ gave maximum dry mycelial weight and sporulation than rest of isolates when exposed to alternate dark and light treatments. This may be due to the induction of certain metabolic processes necessary for growth

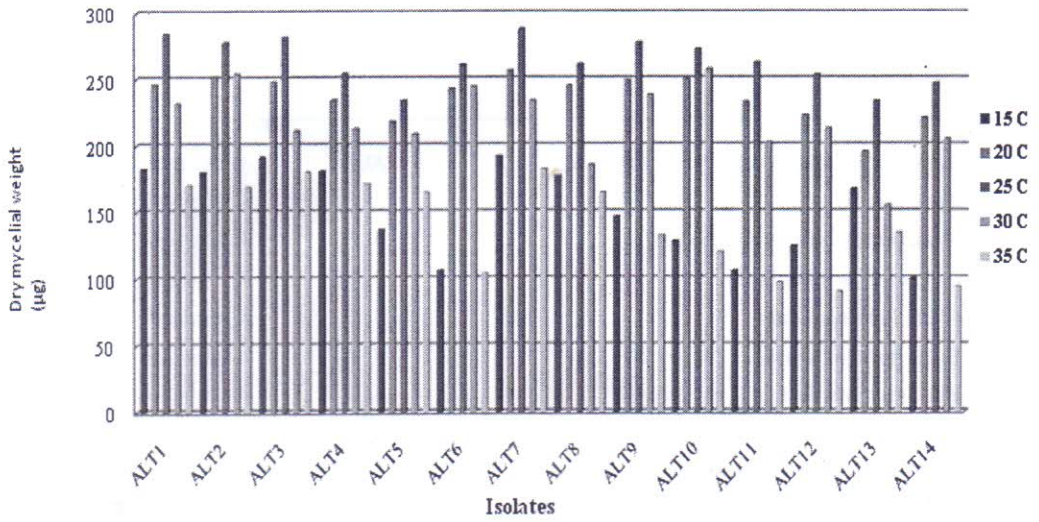



**Fig. 2 :** Effect of temperature on dry mycelial weight (µg) of fourteen isolates of *Alternaria* spp

### *Physiological variability*

A temperature of 25 - 30$^0$ C was found optimum for the growth and sporulation of isolates of *Alternaria* spp. The optimum temperature required for the growth of *Alternaria* spp. had been earlier worked and sporulation of the fungus, which usually does not occur in continuous light. Similar type of observation has been supported by Singh *et al.* (2001).

The maximum growth and sporulation of *Alternaria* spp. occurred when there was high humidity

**Table 2.** Morphological and cultural diversity of 14 isolates of *Alternaria* spp., infecting sesame

| Isolates | Colony colour | Mycelial growth | Type of margin | Mycelial width (µm) | Sporu-lation | Septaion vertical | Horizontal | Size of conidia (µm) (length X breadth) | Beak length (µm) |
|---|---|---|---|---|---|---|---|---|---|
| $ALT_1$ | Dark brown | Raised fluffy growth | Irregular margin | 3.70 | Ms | 0 - 1 | 4 | 37.74 - 44.40 X6.62 - 9.62 | 10.90 |
| $ALT_2$ | Brown | Fluffy growth | Smooth | 8.63 | - | 1 | 3 | 31.82 - 35.52 X 6.66 - 8.88 | 11.10 |
| $ALT_3$ | Dark brown | Raised fluffy growth | Irregular | 4.20 | Ps | 0 - 1 | 4 | 21.46 - 22.94 X 5.18 - 6.66 | 5.43 |
| $ALT_4$ | Light grey | Flat growth | Smooth | 7.90 | Gs | 1 - 2 | 4 | 14.80 - 20.72 X 8.14 - 11.10 | 3.21 |
| $ALT_5$ | Grey | Raised growth | Smooth | 6.17 | Es | 0 - 1 | 6 | 23.68 - 27.38 X 6.66 - 10.36 | 17.02 |
| $ALT_6$ | Light grey | Raised growth | Smooth | 6.66 | Ms | 1 | 6 | 18.50 - 25.90 X 3.70 - 7.40 | 7.40 |
| $ALT_7$ | Brown | Raised fluffy growth | Irregular | 5.70 | Ps | 0 - 1 | 2 | 14.80 - 18.50 X 14.80 - 18.50 | 6.66 |
| $ALT_8$ | Brown | Raised fluffy growth | Irregular | 4.31 | Gs | 1 - 2 | 3 | 18.50 - 22.10 X18.50 - 22.10 | 10.36 |
| $ALT_9$ | Light grey | Flat growth | Smooth | 3.95 | Ps | 0 - 1 | 4 | 14.06 - 15.54 X14.06 - 15.54 | 3.50 |
| $ALT_{10}$ | Light grey | Raised growth | Smooth | 8.40 | Ps | 1 - 2 | 4 | 15.54 - 19.24 X15.54 - 19.24 | 9.40 |
| $ALT_{11}$ | Light brown | Fluffy growth | Smooth | 7.15 | Ms | 1 - 2 | 6 | 20.72 - 23.68 X20.72 - 23.68 | 6.17 |
| $ALT_{12}$ | Brown | Raised fluffy growth | Smooth | 7.40 | Ps | 1 - 2 | 4 | 21.40 - 25.90 X 21.40 - 25.90 | 8.88 |
| $ALT_{13}$ | Dark brown | Flat growth | Irregular | 5.43 | - | 1 - 2 | 5 | 18.50 - 22.94 X18.50 - 22.94 | 2.92 |
| $ALT_{14}$ | Brown | Raised fluffy growth | Irregular | 5.70 | - | 1 - 2 | 4 | 24.42 - 27.38 X24.42 - 27.38 | 17.02 |

Es = Excellent sporulation; Gs = Good sporulation; Ms = Moderate sporulation; Ps = Poor sporulation; - = No sporulation

(95 - 100%). Among the isolates $ALT_6$ produced significantly higher growth and sporulation at 100 per cent RH, while isolate $ALT_5$ and $ALT_6$ had the ability to produce sporulation even under low RH of 65 per cent, when no sporulation was found in other isolates (Fig. 4). Dickinson and Bottomley (1980) and Naik *et al.* (2010) also reported that 100 per cent RH to be the best for germination, growth and sporulation of *A. alternata*.

### *Pathogenic variability*

The five groups of isolates of *Alternaria* spp. were grouped based on the cultural characteristics and used for pathogenicity study on five test genotypes of sesame revealed that a significant difference existed in their ability to infect sesame genotypes. The five group of isolates used in these tests were pathogenic and produced disease reaction when inoculated on susceptible genotypes. However group 1 ($G_1$) isolates induced higher per cent disease index on all the genotypes tested followed by the isolates under group 2 ($G_2$). Irrespective of the genotypes tested all group of isolates were virulent. Least virulent nature was

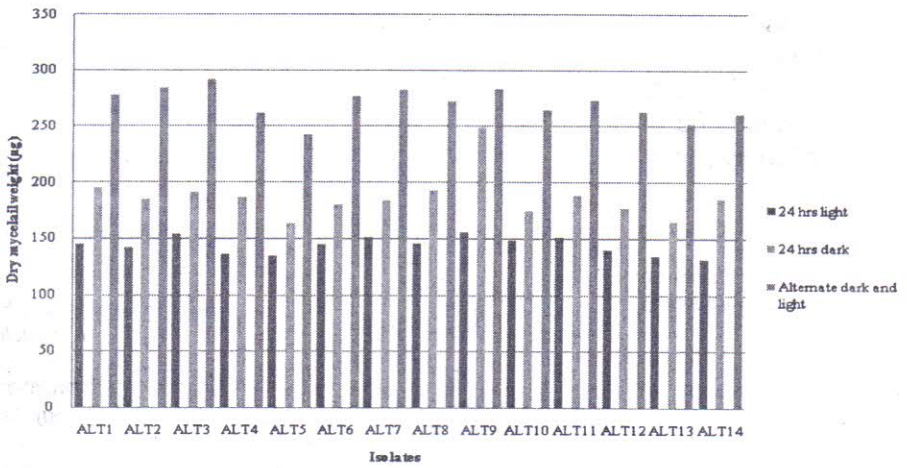



**Fig. 3** : Effect of light on dry mycelial weight of fourteen isolates of *Alternaria* spp

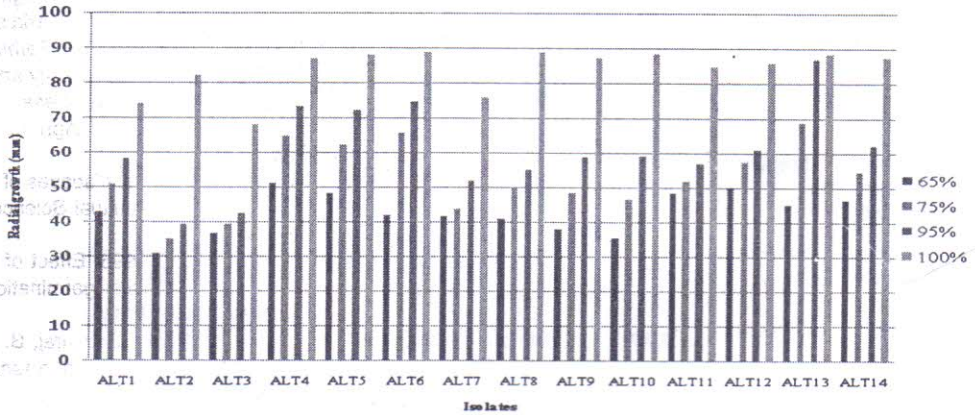



**Fig. 4** : Effect of Relative humidity on radial growth of fourteen isolates of *A. sesami* and *A. alternata*

**Table 3** : Pathogenic variation of five groups of isolates of *Alternaria* spp. on five sesame genotypes

| Isolates Groups | Per cent disease index and reaction of genotypes | | | | | Mean |
|---|---|---|---|---|---|---|
| | R | MR | MS | S | HS | |
| $G_1(ALT_1)$ | @10.67 R | 22.67 MR | 30.67 MS | 41.33 MS | 57.33 S | 32.53 |
| | #(19.09) | (28.45) | (33.65) | (39.99) | (49.20) | (34.82) |
| $G_2(ALT_2)$ | 10.67 R | 17.33 MR | 32.00 MS | 42.67 MS | 52.00 S | 30.93 |
| | (19.09) | (24.58) | (34.45) | (40.80) | (46.15) | (33.83) |
| $G_3(ALT_4)$ | 9.33 R | 18.67 MR | 24.00 MS | 36.00 MS | 45.33 MS | 26.67 |
| | (17.76) | (25.62) | (29.33) | (36.87) | (42.30) | (31.11) |
| $G_4(ALT_5)$ | 9.33 R | 16.00 MR | 28.00 MS | 33.33 MS | 38.67 MS | 25.07 |
| | (17.76) | (23.58) | (31.95) | (35.24) | (38.47) | (40.28) |
| $G_5(ALT_{11})$ | 10.67 R | 17.33 MR | 26.67 MS | 32.00 MS | 33.33 MS | 24.00 |
| | (19.09) | (24.58) | (31.11) | (34.45) | (35.29) | (27.97) |
| Mean | 10.13 | 18.40 | 28.27 | 37.07 | 45.33 | 29.30 |
| | (18.53) | (25.40) | (32.14) | (37.47) | (42.30) | (32.77) |

| | Isolates (I) | Genotypes (G) | Interaction |
|---|---|---|---|
| C.D. at 1% | 1.923 | 2.158 | 3.985 |

@ : Original values; # : Arc sine transformed values; R : Resistant; MR : Moderately Resistant; MS : Moderately Susceptible; S : Susceptible

shown by isolates of group 5 ($G_5$) (Table 3). The variability in the pathogenicity among the isolates of *A. solani* was also established by Tong-Yunshi *et al.* (1994), they demonstrated the variability of 14 isolates of *A. solani* on five tomato genotypes.

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