Morpho-physiological characters of *Alternaria tenuissima* (Fr.) Keissl causing Leaf blight in Kodo millet

K. HARIPRASAD, A. NAGARAJA* AND SURESH PATIL

J. Mycopathol, Res, 56(1) : 51-55, 2018;
ISSN 0971-3719

Department of Botany,
University of Calcutta,
Kolkata 700 019, India

J. Mycopathol. Res. ***56(1)*** *: 51-55, 2018;* (ISSN 0971-3719)

Morpho-physiological characters of*Alternaria tenuissima*(Fr.) Keissl causing Leaf blight in Kodo millet

K. HARIPRASAD, A. NAGARAJA* AND SURESH PATIL[1]
Department of Plant Pathology, University of Agricultural Sciences, GKVK Bengaluru 560065, Karnataka
[1]Project Coordinating Unit (Small Millets), ICAR, GKVK, Bengaluru 560065, Karnataka

Received : 16.02.2018 Accepted : 19.02.2018 Published : 30.04.2018

Leaf blight is a recent disease in kodo millet and is caused by deuteromycete *Alternaria tenuissima*. Investigations carried out to determine the best media for growth and sporulation of the organism *vis-a-vis* the ideal temperature, light, carbon and nitrogen requirement of *A. tenuissima* revealed that, Czapek's Dox agar was the best medium that recorded maximum growth in terms of colony diameter (8.50 cm). Among the 13 carbon and eight nitrogen sources studied , mannitol and sodium nitrate respectively supported maximum growth (9.00 cm) besides higher sporulation. Of the different temperature regimes 25 ºC recorded maximum mycelial dry weight. However, the growth of the fungus was unaltered by the presence or absence of light during incubation.

Key words: Kodo millet, *Alternaria tenuissima*, morphology, physiological studies

INTRODUCTION

Kodo millet (*Paspalum scrobiculatum* L.) is one of the oldest cultivated cereal of the genus *Paspalum* and family Poaceace.It is a good substitute to rice or wheat and has been domesticated in India 3000 years ago. Of the few diseases afflicting the crop leaf blight is one of the most destructive after head smut, which if occurs at early stages of growth reduces the crop yield drastically (Nagaraja *et al.* 2016).Considering the prevalence and significance of the disease to the crop, the present investigation was undertaken to determine the best medium and optimal growth conditions of the pathogen *in vitro* that may help to correlate its field occurrence and to devise management strategies.

MATERIALS AND METHODS

Cultural studies

The experiment was aimed at knowing the cultural characters such as color, colony texture, surface topography, consistency, margin and luster of the leaf blight pathogen on 16 different solid media. Twenty ml of each solid media was poured into 90 mm diameter Petri plates and 7mm culture discs of the pathogen was inoculated separately and incubated @ 27 ± 1 °C for 10 days. Three replications were maintained; the cultural characteristics and the colony diameter (mm) on each of the medium were recorded and the data so obtained was statistically analyzed. The composition and preparation of synthetic and non-synthetic media was as per Hawksworth *et al.*(1983).

Physiological studies Effect of different carbon sources

In PDA medium dextrose was replaced by different carbon sources *viz.,*glucose, galactose, maltose, sucrose, fructose, lactose, pectin, mannitol, cellulose, starch (Lilly and Barnett,1951) and PDA devoid of dextrose served as control. The amount of each carbon compound replaced was determined based on their molecular weight to provide an equivalent amount of carbon as that of dextrose present in PDA. Dextrose was also taken for comparison. The fungus was inoculated to Petri plates containing different carbon sources and incubated at 27 ± 1 °C. Three replications were maintained per treatment. After 12 days of incubation the radial growth of the fungus and sporulation was recorded.

Effect of different nitrogen sources

In Czapek's Dox medium sodium nitrate was re-

*Corresponding author : anagaraja60@gmail.com

placed with, potassium nitrate, ammonium chloride, ammonium nitrate, ammonium oxalate, ammonium phosphate, ammonium sulphate. Medium without sodium nitrate served as control. The amount of each nitrogen compound replaced was determined based on their molecular weight to provide an equivalent amount of nitrogen as that of sodium nitrate present in the Czapek's Dox medium, sodium nitrate was also taken for comparison. Radial growth (mm) of the fungus was measured after 12 days of incubating the fungus in different media.

Effect of temperature

The growth of the fungal isolate was studied at different temperature regimes *viz*., 15, 20, 25, 30, 35 °C. To 250 ml conical flask containing 100 ml of sterilized Czapek's Dox broth, one 7 mm fungal disc was inoculated and kept for incubation at 27 ± 1 °C for 10 days. Each treatment was replicated thrice; the ideal temperature for growth of the fungus was determined by harvesting mycelial mat filtered through What man filter paper and recording the dry mycelial weight (mg).

Effect of hydrogen ion concentration

The growth of *A. tenuissima* was tested at six pH levels *viz.,* 5, 6, 7, 8, 9 and 10. Hydrogen ion (pH) concentration of the Czapek's Dox broth was determined by using pH meter. Adjustment of pH was done using 0.1 N alkali (Sodium hydroxide) and 0.1 N acid (Hydrochloric acid) and was sterilized in autoclave at 121 °C for 15 minutes. To 250 ml flasks containing 100 ml of sterilized medium, one 7 mm fungal disc was inoculated and kept for incubation at 27±1 °C for 10 days. Each treatment was replicated thrice, the ideal pH for growth of the fungus was determined by harvesting mycelial mat that was filtered through Whatman filter paper and dry mycelial weight (mg) was recorded.

Effect of light

The effect of light on growth and sporulation of *A. tenuissima* was studied by using Czapek'sDox medium. Seven mm culture discs were inoculated into the flasks and were incubated at 27 ± 1 °C for three weeks. The flasks were exposed to different light treatments *viz*., continuous (24 h), light and dark as well as alternate cycles of light and dark (12 h each). Observations on the mycelial growth and sporulation were recorded. The data obtained was analyzed statistically.

RESULTS AND DISCUSSION

The pathogen showed significantly varied radial growth ranging from 42.7 to 83.3 mm on different media with significantly higher radial growth (83.3 mm) on Saboraud's agar that was on par with peptone dextrose agar (83.0 mm); whereas least growth (42.7 mm) was obtained in water agar medium (Table 1). This is quite obvious as water agar do not contain any nutrient to support the growth of the organism. Though the radial growth of the fungus was the highest on Saboraud's agar, profuse sporulation was observed in Czapek'sDox agar and PDA+$CaCO_3$withmore than 20 conidia per microscopic field. The other media that supported profuse sporulation are kodo host meal agar, kodo sucrose host extract agar and kodo host extract agar that is attributed to the presence of required nutrients in the host extracts. However, as profuse sporulation was observed on Czapek's Dox agar which also had good radial growth, this medium was selected for the physiological studies of *A.tenuissima* (Fig.1).

Observations on color, colony texture, surface and topography, consistency, margin, luster of the colonies and sporulation are listed in Table 2.The color of the fungus culture on different media varied from light grey to dark grayish and creamy white. Prabhu and Prasada (1970) reported the colonies of *A.tenuissima* as deep olive grey to dark olive gray. Other characters such as colony texture, surface and topography, consistency, margin and luster were found to differ with the composition of media and might have been influenced by the media composition.

Effect of different carbon sources

The carbon sources showed significant variation in growth and sporulation of the pathogen. Radial growth ranging from 45.0 to 71.3 mm was observed (Table 3). However, mannitol (71.3 mm) supported excellent mycelial growth, but profuse sporulation was evident in fructose, glucose and sucrose besides mannitol. Carbon is the most essential element required by the fungi and it comprises of about 50 per cent of total mycelial weight as a component of both structural and functional constituent (Bilgrami and Verma, 1978). Similarly,

Gupta *et al*. (1979) recorded the maximum growth of *A. alternata* on mannitol but the maximum sporulation of the fungus was observed in sucrose, but Arunakumara *et al*. (2008) recorded the maximum mycelia growth of *A. solani* on glucose.

Table 1: Growth and sporulation of *A. tenuissima* on different solid media

Medium	Radial mycelial growth(mm)	Sporulation
Saboraud's dextrose agar	83.3	++
Dextrose nitrate agar	49.0	++
Kodo host meal agar	65.0	+++++
Rose bengal agar	48.3	++++
Water agar	42.7	+++
Mathur's agar	51.3	++++
Oat meal agar	81.7	++
Czapek's dox agar	64.7	+++++
Peptone dextrose agar	83.0	++
Potato dextrose agar	57.7	++++
Richard's agar	79.3	++++
Kodo sucrose host extract agar	70.7	+++++
Malt extract agar	72.3	++
Potato dextrose agar+ $CaCO_3$	53.3	+++++
V8 Juice agar	44.3	+
Kodo host extract agar	80.0	+++++
S. Em ±	0.37	-
CD (P 0.01)	1.47	-

where, +++++ = Excellent sporulation (> 20 conidia per microscopic field), ++++ = Very good sporulation (15-20 conidia per microscopic field), +++ = Good sporulation (10-15 conidia per microscopic field), ++ = Better sporulation (5-10 conidia per microscopic field), + = Poor (< 5 spores per microscopic field).

Effect of different nitrogen sources

The highest mycelial growth of 68.3 mm was recorded in sodium nitrate(Table 4). But profuse sporulation was evident in both sodium nitrate and potassium nitrate. Proteins are the building blocks of any living organism and nitrogen is essential for protein synthesis. Similarly Patil and Suryawanshi (2015) also found that sodium nitrate followed by potassium nitrate and calcium nitrate were good sources for the growth of *A. alternata.*

Effect of temperature

Growth of *A. tenuissima* in terms of dry mycelial weight at different temperature levels is presented in Table 5. Highest growth of *A. tenuissima* was recorded ata temperature of 25ºC (123.4 mg) and on either side of which the growth declined.Temperature is one of the most crucial physical factor regulating vegetative growth as well as reproductive activity of any living organism and *A. tenuissima* is no exception. Kumar and Arya (1978) also reported that 25 °C as best for the growth of *A. triticina* in wheat. Similarly Kumar *et al.* (2015) also found 25 °C as the most favorable temperature for growth of the *Alternaria* spp. How-

Table 2: Cultural characteristics of *A. tenuissima* on different media

Media	Color	Colony texture	Surface and Topography	Consistency	Margin	Luster
Saboraud's agar	Light grey	Flat	Flat, surface and radial towards margin	Flat	Regular	Shiny
Dextrose nitrate agar	Light grey	Raised	Fluffy and filamentous towards margin	Fluffy	Regular	Dull
Kodo host meal agar	Light grey	Raised	Wavy margin with sectoring	Fluffy	Slightly wavy	Dull
Rose Bengal agar	Dark grey	Raised	Fluffy and raised at centre and irregular towards margin	Fluffy	Irregular	Shiny
Water agar	Creamy	Flat	Flat, scanty growth.	Flat	Irregular	Dull
Mathur's agar	Dark grey	Flat	Light grayish at centre	Smooth	Irregular	Shiny
Oat meal agar	Dark grey	Raised	Smooth colony with regular towards margin	Fluffy	Wavy	Shiny
Czapek's Dox agar	Light grey	Flat	Smooth colony with regular towards margin	Fluffy	Wavy	Dull
Peptone dextrose agar	Light grey	Flat	Smooth surface and regular margin	Fluffy	Regular	Shiny
Potato dextrose agar	Light grey	Raised	Smooth with irregular whitish margin	Fluffy	Irregular	Shiny
Richard's agar	Dark grey	Flat	Flat, sectoring and regular margin	Fluffy	Regular	Dull
Kodo sucrose host extract agar	Dark grey	Flat	White, centre with regular margin	Fluffy	Regular	Shiny
Malt extract agar	Dark grey	Raised	Grayish centre ,Fluffy, sectoring	Fluffy	Irregular	Shiny
Potato dextrose agar+ $CaCO_3$	Dark grey	Flat	Raised, Fluffy, sectoring	Fluffy	Irregular	Shiny
V8 Juice agar	Dark grey	Flat	Sectoring	Fluffy	Irregular	Dull
Kodo host extract agar	Dark grey	Flat	Regular margin, Grayish	Fluffy	Regular	Shiny

ever, Alhussaen (2012) reported 25-30 °C asoptimum temperature for the growth of the *A. solani*.

Table 3 : Growth and sporulation of *A.tenuissima* in different carbon sources

Carbon source	Radial mycelial growth (mm)	Sporulation
Fructose	65.7	+++++
Mannitol	71.3	+++++
Maltose	61.0	++++
Glucose	70.0	+++++
Lactose	45.0	++++
Dextrose	63.3	++++
Pectin	65.3	+++
Control	60.0	+++
Cellulose	56.3	+++
Starch	52.0	+++
Sucrose	60.0	+++++
S. Em ±	0.18	
CD (P 0.01)	0.74	

Effect of hydrogen ion (pH) concentration

A. tenuissima showed variation in its growth with respect to different pH levels(Table 6).Maximum mycelial dry weight of 161.2 mg was recorded at a pH of 6.0 with the next best of 142.4 mg at 7.0 pH.

Table 4 : Growth and sporulation of *A. tenuissima* in different nitrogen sources

Nitrogen source	Radial mycelial growth (mm)	Sporulation
Potassium nitrate	67.7	+++++
Sodium nitrate	68.3	+++++
Ammonium oxalate	59.7	++++
Ammonium nitrate	19.3	+++
Ammonium chloride	13.0	++
Ammonium phosphate	21.0	++++
Ammonium sulphate	13.5	+++
Control	17.3	++
S.Em±	0.14	
CD (P 0.01)	0.57	

Thus, *A. tenuissima* prefers pH around neutrality for profuse growth(6.0 to 7.0).There is an interrelationship between the growth of the organism and the hydrogen ion concentration of the medium.Probably, there may be high metabolic activity of *A. tenuissima* at this pH range. Similarly, Kumar and Arya (1978) reported maximum growth of *Alternaria triticina* at the pH of 6, so also Maheshwari *et al*. (2000) reported that a pH of 6.00 was ideal for maximum growth of *A. alternata*.

Table 5 : Growth of *A. tenuissima* at different temperatures

Temperature (ºC)	Dry mycelial weight (mg)
15	116.0
20	118.2
25	123.4
30	122.4
35	119.9
S.Em±	0.17
CD (P 0.01)	0.70

Effect of light

Though, light has a profound influence on growth and sporulation of organisms, in the present study the growth in terms of dry mycelial weight did not differ with exposure of *A. tenuissima* to various light conditions(Table7).Probably, *A. tenuissima* is insensitive to the presence or absence of light for growth and sporulation, but Manjunath *et al.* (2010) observed that the exposure of *A.alternata* to alternate cycle of 12h light and 12 h darkness for 10 days resulted in the maximum mycelial growth of the fungus.

Table 6: Growth of *A. tenuissima* at different pH

pH	Dry mycelial weight (mg)
5.0	50.1
6.0	161.2
7.0	142.4
8.0	44.0
9.0	21.5
10.0	21.1
S.Em ±	0.97
CD (P 0.01)	4.22

Table 7: Growth of *A. tenuissima* as influenced by different light conditions

Source	Dry mycelial weight(mg)
Light	11.7
Dark	11.6
Light+ Dark	11.8

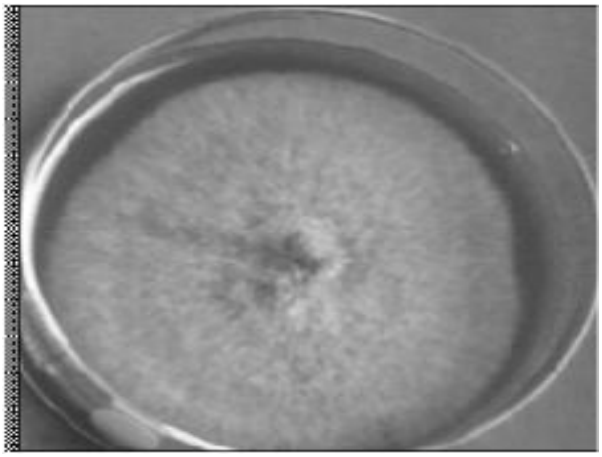

Fig. 1 : Growth and sporulation of *A. tenuissima*

REFERENCES

Alhussaen, K. M. 2012.Morphological and physiological characterization of *Alternaria solani* isolated from tomato in Jordan valley. *Res. J. Biol. Sci*. **7**: 316-319.

Arunakumara, K. T., Kulkarni, M. S., Thammaiah, N. and Yashoda Hegde. 2008.Nutritional and physiological studies on *Alternaria solani* causing early blight of tomato. *Asian J. Hort.***3** : 58-60.

Bilgrami, K. S. and Verma, R. N.1978.*Physiology of Fungi.* Vikas Publications, New Delhi.

Gupta, Y. K., Gupta, M. N. and Seth, A. K. 1979. Effect of carbon compounds and sporulation of *Alternaria alternata* (Fr.) Keissler, causing leaf blight of sunflower. *Proc*. *Indian Nat. Sci. Acad*. **45:** 190-192.

Hawksworth, D. L., Sutton, B. S. and Ainsworth, G. C.1983. *Ainsworth and Bisbys Dictionary of the fungi*, VIII Edition, Commonwealth Mycological Institute, London, p. 445.

Kumar, V. R. and Arya, H. C. 1978. Nutritional requirements of *Alternaria triticina* causing leaf blight of wheat. *Geobios.* **5** : 12-15.

Kumar, V., Pandey, K. K. and Mishra, K. K. 2015. Study of variability and sporulation by isolates of *Alternaria solani* of *Lycopersicon esculentum* (Mill.).*Asian J. Sci. Tech.***6**: 1264-1270.

Lilly, V. G. and Barnett, R. L. 1951.*Physiology of Fungi*. McGraw Hill Book Co. Inc., London, pp 164.

Maheshwari, S. K., Singh, D. V. and Singh, S. B. 2000. Effect of temperature and pH on growth and sporulation of *Alternaria alternata* causing *Alternaria* leaf spot of dolichos bean. *Annu. Pl. Prot. Sci.***8** : 33-35.

Manjunath, H., Sevugapperumal, N., Thiruvengadam, R., Theerthagiri, A. and Ramaswamy, S. 2010.Effect of environmental conditions on growth of *Alternaria alternata* causing leaf blight of noni. *World J. Agril Sci.***6**: 171-177.

Nagaraja, A., Bijendra Kumar, Jain, A. K. andSabalpura, A. N.2016. Emerging diseases: Need for focused research in small millets. *J. Mycopathol. Res.* **54:**:1-9.

Patil, J. S. and Suryawanshi, N. S. 2015. Effect of nutritional and physiological characters of *Alternaria alternata* causing fruit rot of strawberry. *Bionano frontier*, **8** : 57-64.

Prabhu, A. S. and Prasada, R. 1970. *Investigations on the leaf blight disease of wheat caused by Alternaria triticina*, In: Plant Disease Problems, Proc. First Int. Symp. Pl. Pathology. New Delhi, India, pp. 9-27.