Effects of Physical Parameters and Media on Growth of Alternaria alternata causing Alternaria Leaf Spot of Lehsua (Cordia myxa)

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Abstract
Lehsua (Cordia myxa Roxb.) also known as Lasoda, Gunda and Indian cherry, is a major fruit of the family Boraginaceae and grown in Rajasthan and other states of India. Alternaria leaf spot of lehsua, caused by Alternaria alternata is an important disease of this crop and day by day it is becoming severe in Rajasthan. The physiological study i.e. effect of temperature, RH, pH and effect of media on fungus (Alternaria alternata) was studied in vitro. The mycelial growth of Alternaria alternata was studied at different levels of temperature ranging from 15°C to 35°C. Maximum mycelial growth (87.51 mm) was recorded at 25°C, while minimum (55.73 mm) was recorded at 15°C. Maximum (88.71 mm) and minimum (49.00 mm) growth was recorded at 100 and 60 per cent level of RH, respectively; similarly maximum mycelial dry weight (847 mg) was recorded at pH 6.5 and minimum (300 mg) was at pH 8.0. The best growth supportive medium (87.88 mm dia. collopy) for the pathogen was potato dextrose agar medium followed by Czapeck’s Dox agar medium.

Keywords: Lehsua, Cordia myxa, Alternaria leaf spot, Alternaria alternata, temperature, RH and pH, solid growth media

Introduction
Lehsua (Cordia myxa Roxb.), also known as Lasoda, Gunda and Indian cherry, is a major fruit of the family Boraginaceae. It is native of North western India and distributed throughout the country in arid and semi-arid regions especially in warmer regions up to 5,000 ft. It is a perennial, medium size tree with crooked stem attaining a height of nearly 3 to 4 meter and the total height of the tree comes to nearly 10 to 15 meter. Fruits of lehsua contain a good amount of protein (2%), carbohydrate (92%), fat (2%) and also a good source of vitamins, calcium and minerals (Duhan et al., 1992 and Chandra et al., 1994) [6, 3]. The fruit contains about 70 per cent mucilaginous pulp, average moisture (70%), total soluble solids (10%) and alcohol soluble carbohydrate (70%). In India, it is mostly grown in Rajasthan, Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Assam, Maharashtra and Gujarat.

In Rajasthan, lehsua is mainly cultivated in Ajmer (267 ha. area and 390 tonnes production) followed by Bhiwara, Sikar, Kairauli, Dholpur, Jhalawar and Swai Madhapur districts (Anonymous, 2017a) [1]. Lehsua is mainly propagated by seeds. However, to propagation true to type plants, propagation by vegetative methods, particularly by “Patch budding” has been found very successful. lehsua is affected by foliar and post harvest diseases, which are responsible for their qualitative and quantitative deterioration.

The literature is silent about diseases of lehsua. However, Alternaria leaf spot (6-20 PDI), dieback (5-10 PDI), gummosis (33-40 % incidence), fruit rot (5.0%), blue mould rot and Rhizopus rot have been observed from different locations of Jhalawar district of Rajasthan (Anonymous, 2017b) [3]. The intensity of Alternaria leaf spot (Alternaria alternata) has been recorded in the range of 2.00-22.00 per cent in surveyed areas of Rajasthan (Anonymous, 2017b and 2018) [3].

The symptoms of the disease initially were observed as brown or black coloured spots of indefinite size appearing on leaves with light brown or dark brown rings. Affected leaves further blighted and fall down (Maurya et al., 2016) [5]. Alternaria leaf spot of lehsua, caused by Alternaria alternata is an important disease of this crop and day by day it is becoming severe in Rajasthan.
This is the first systemic study carried out in laboratory with the pathogen of leaf spot of lehsua in Rajasthan to see the behavior of the pathogen under changing climatic scenario with some environmental factors (temperature, RH, pH) and with 5 solid growth media for fast multiplication and sporulation.

Materials and Methods

Effect of physical parameters on mycelia growth of the pathogen

Effect of temperature

It is a well-established fact that the temperature yields considerable influence on the biochemical activity of pathogens. Twenty ml of PDA was poured in each of sterilized Petri dish. Each Petri dish was inoculated aseptically by placing in the centre a 5 mm disc from actively growing 7 days old culture on PDA. The inoculated Petri dishes were incubated at 15, 20, 25, 30 and 35°C temperature for 7 days with four replications. Observations on mycelial growth were recorded after 7 days of incubation.

Effect of hydrogen ion concentration (pH)

The study of different pH levels was undertaken with a view to ascertain the effect of different hydrogen ion concentration of the medium on growth of the fungus. The initial pH of the potato dextrose broth, before autoclaving was adjusted from 6.0 to 8.0 with a difference of 0.5 using N/10 NaOH or N/10 HCl. After autoclaving the pH was again tested and potato dextrose broth was filled in flasks (250 ml capacity). The inoculated flasks were incubated at 25±1ºC for 7 days with four replications. Observations on mycelial growth were recorded after 7 days of incubation.

Effect of relative humidity (RH)

To study the effect of relative humidity on mycelial growth of Alternaria alternata, five different levels of relative humidity i.e. 60, 70, 80, 90 and 100 per cent were maintained by using the concentrate sulphuric acid and sterilized distilled water in different proportion in glass desiccators according to the method suggested by Buxton and Mellan by (1934).

Petriplates containing PDA medium were inoculated with 5 mm disc of 7 days old culture of Alternaria alternata, with the help of sterilized cork borer and four replications were maintained. Inoculated Petriplates were immediately accommodated in glass desiccators containing mixture of sulphuric acid and distilled water in required proportion and incubated at 25±1°C. Observations on mycelial growth were recorded after 7 days of incubation.

Effect of media on mycelial growth

Growth on solid media was determined by measuring the colony diameter along with the two diagonals passing through the center of colony by excluding initial diameter (5 mm) bit. Five solid media whose composition is given below were assessed at 25 ± 1°C. To find out the suitable media for growth of the test fungus, following five solid media were assessed at 25 ± 1°C.

| S. No. | Medium                       | Constituents                      | Quantity       |
|-------|------------------------------|-----------------------------------|----------------|
| 1.    | Potato Dextrose Agar         | Agar agar                         | 20.00 g        |
|       |                              | Dextrose                          | 20.00 g        |
|       |                              | Peeled potato                     | 250.00 g       |
|       |                              | Distilled water                   | 1000 ml        |
| 2.    | Czapeck’s Dox Agar medium    | Agar agar                         | 15.00 g        |
|       |                              | Sucrose                           | 30.00 g        |
|       |                              | Distilled water                   | 1000 ml        |
|       |                              | Dipotassium phosphate             | 1.00 g         |
|       |                              | Magnesium                         | 0.50 g         |
|       |                              | Potassium chloride                | 0.50 g         |
|       |                              | Sodium nitrate                    | 2.00 g         |
|       |                              | Ferrus sulphate                   | 0.01 g         |
| 3.    | Malt medium                  | Agar agar                         | 15.00 g        |
|       |                              | Malt extract                      | 30.00 g        |
|       |                              | Distilled water                   | 1000 ml        |
| 4.    | Corn meal medium             | Agar agar                         | 20.00 g        |
|       |                              | Corn meal                         | 20.00 g        |
|       |                              | Glucose                           | 20.00 g        |
|       |                              | Distilled water                   | 1000 ml        |
| 5.    | Yeast Mannitol Agar medium   | Yeast extract                     | 1.00 g         |
|       |                              | Mannitol                          | 10.00 g        |
|       |                              | Distilled water                   | 1000 ml        |
|       |                              | Dipotassium phosphate             | 0.50 g         |
|       |                              | Sodium chloride                   | 0.10 g         |
|       |                              | Magnesium sulphate                | 0.20 g         |
|       |                              | Calcium carbonate                 | 1.0 g          |
|       |                              | Agar agar                         | 15.00 g        |
Experimental Results and Discussion

Effect of temperature

The mycelial growth of Alternaria alternata was studied by incubating Petri dishes at different levels of temperature ranging from 15 °C to 35 °C (Table 1). Maximum mycelial growth (87.51 mm) was recorded at 25 °C. Minimum mycelial growth (55.73 mm) was recorded at 15 °C. The mycelial growth observed at 20 °C (71.00 mm) was found at par with 30 °C (72.58 mm). There was a significant difference among the different temperatures e.i. 15 °C (55.73 mm) followed by 35 °C (63.00 mm) and parallel to Israram et al. (2007) [6] and Singh and Majumdar (2004) [9].

Table 1: Effect of different levels of temperature on mycelial growth of Alternaria alternata at 7th day of incubation (in vitro)

| S. No. | Temperature (°C) | Mycelial growth (mm)* |
|--------|-----------------|-----------------------|
| 1      | 15              | 55.73                 |
| 2      | 20              | 71.00                 |
| 3      | 25              | 87.51                 |
| 4      | 30              | 72.58                 |
| 5      | 35              | 63.00                 |
|       | SEm+            | 0.86                  |
| CD (p=0.05) | -               | 2.66                |

* Average of four replications

Effect of relative humidity

The effect of relative humidity on the mycelial growth was studied at different levels viz., 60, 70, 80, 90 and 100 per cent. Maximum mycelial growth (88.71 mm) was recorded at 100 per cent relative humidity followed by 90 per cent (85.55 mm) relative humidity which was found at par with each other (Table 2). A significantly difference in mycelial growth was recorded at 80, 70 and 60 per cent humidity. Minimum mycelial growth (49.00 mm) was recorded at 60 per cent relative humidity and present findings are in accordance with, Prasad and Ahir (2013) [8].

Table 2: Effect of relative humidity on mycelial growth of Alternaria alternata at 7th day of incubation at 25 ± 1°C

| S. No. | Relative humidity (%) | Mycelial growth (mm)* |
|--------|-----------------------|-----------------------|
| 1      | 60                    | 49.00                 |
| 2      | 70                    | 68.25                 |
| 3      | 80                    | 80.13                 |
| 4      | 90                    | 85.55                 |
| 5      | 100                   | 88.71                 |
|       | SEm+                  | 1.36                  |
| CD (p=0.05) | -               | 4.20                |

*Average of four replications

Effect of pH

Table 3: Effect of pH on mycelial growth of Alternaria alternata on potato dextrose broth at 7th day of incubation at 25 ± 1°C

| S. No. | Ph  | Dry mycelial weight (mg)* |
|--------|-----|---------------------------|
| 1      | 6.0 | 821                       |
| 2      | 6.5 | 847                       |
| 3      | 7.0 | 750                       |
| 4      | 7.5 | 555                       |
| 5      | 8.0 | 300                       |
|       | SEm+| 10.17                     |
| CD (p=0.05) | -       | 31.34                    |

*Average of four replications

Hydrogen ion concentration also affected the growth of Alternaria alternata as evident by growing this pathogen over a wide range of pH (Table 3). Maximum mycelial dry weight (847 mg) was recorded at pH 6.5 which was found at par with 6.0 (821 mg) and minimum mycelial weight (300 mg) was recorded at pH 8.0 which is similar to Gupta et al. (2013) [5].

Effect of media

To find out a suitable medium for the mycelial growth of Alternaria alternata, five different synthetic and semi-synthetic media were tested. On perusal of data (Table 4) revealed that among the five differrentsolid media, the Potato Dextrose Agar medium was significantly superior in supporting maximum mycelial growth (87.88 mm) at 7th day of incubation followed by Czapek’s Dox agar medium (70.00 mm), Malt medium (67.00 mm) and Corn meal medium (56.10 mm). Among these media, Czapek’s Dox agar medium and Malt medium, statistically were found at par to each other. Minimum growth of the fungus was recorded on Yeast mannitol agar medium (40.00 mm). The PDA medium was selected for further studies, as it supported maximum growth, and parallel to Singh et al. (2013) [10].

Table 4: Effect of media on mycelial growth of Alternaria alternata at 7th day of incubation at 25 ± 1°C
Table 4: Effect of solid media on mycelial growth of *Alternaria alternata* after 7 days of incubation at 25±1 °C

| S. No. | Solid medium                        | Mycelial growth (mm)* |
|--------|-------------------------------------|----------------------|
| 1.     | Potato dextrose agar medium          | 87.88                |
| 2.     | Czapeck’s Dox agar medium            | 70.00                |
| 3.     | Malt medium                         | 67.00                |
| 4.     | Corn meal medium                    | 56.10                |
| 5.     | Yeast mannitol agar medium           | 40.00                |

**SEm** + 1.14  
**CD (p=0.05)** 3.52

* Average of four replication

**Conclusion**

Out of above five levels of temperature, maximum mycelial growth of *A. alternata* was recorded at 25 °C. Good mycelial growth of *Alternaria alternata* was observed at 90-100 per cent RH followed by 90 per cent. Similarly, optimum pH for maximum growth of *Alternaria alternata* was pH 6.5. Out of five different synthetic and semi-synthetic media tested, Potato Dextrose Agar (PDA) proved to be the best for mycelial growth of the fungus, followed by Czapeck’s Dox medium. Minimum mycelial growth was observed on Yeast mannitol agar medium.

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