



## Cultural and morphological characterization of *Curvularia geniculata* causing leaf spot of Orchid in Indo-gangetic plain of West Bengal



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### ABSTRACT

Orchids are one of the most diverse and extensively evolved groups of angiosperms with 25,000 to 35,000 species belonging to 600-800 genera, that is why Orchids occupy the largest family of flowering plants. In India, orchids comprise 158 genera and 1331 species. Asia now becoming the primary source of orchids for the entire world market. Orchids, like any other crop, are exposed to various biotic and abiotic stresses. Very recently more than 130 diseases are reported in orchids caused by fungi, bacteria, nematodes and viruses. In the Indo-gangetic plain of West Bengal, the orchid growers/farmers are facing problems mainly due to the leaf spots like diseases of orchids. Among which *Curvularia geniculata* produce necrotic leaf spot symptoms. But very less information is available in the literature on these new diseases of orchids and their favourable conditions as well as on management strategies. So this study is based on pathogen isolation, identification and morphological characterization of pathogen. Through morphological (*i.e.*, fruit body, mycelial properties, conidiogenous cell, conidial morphology) it was confirmed that the pathogens were similar to *Curvularia geniculata*. *Curvularia geniculata* produced maximum growth on Potato dextrose agar medium and Oat meal agar medium, whereas it least grew on Host extract agar medium. It produced black to brownish colour powdery texture colony. Zonation is present on the colony of Potato sucrose agar medium, Oat meal agar medium, whereas no zonation present on the colony of Potato dextrose agar medium. The growth rate of *Curvularia geniculata* showed that maximum 7 days after inoculation at Potato dextrose agar (26.33 mm/day) followed by Carrot agar media (21.67 mm/day) and Potato sucrose agar (19.67 mm/day).

**KEY WORDS:** Orchid; West Bengal; Leaf spot; *Curvularia geniculata*

### 1. Introduction

Orchids are one of the most diverse and extensively evolved groups of angiosperms. With 25,000 to 35,000 species belonging to 600-800 genera, orchids occupy the largest family of flowering plants, covering 6.8% of the total flowering plants in India (Yonzon and Kamran, 2008). They are treasured for their diverse sizes, shapes, forms and colours (Pant *et al.*, 2012). The

majority of them are from Central and South America's tropical wet forests, as well as India, Sri Lanka, Burma, China, Thailand, Malaysia, the Philippines, New Guinea, and Australia (Karthigeyan *et al.*, 2014).

In India, Orchids comprise with 158 genera and 1331 species, which grow up to an elevation of

5000 m. In North-Western India, orchids are commonly found under tree shades of humas, rich moist soil and Western-Ghats harbor the small flowered orchids (Hegde, 2014). In India, native genera such as *Cymbidium*, *Paphiopedilum*, *Vanda*, *Arachnis*, and *Dendrobium* are cultivated for cut flower production on a huge scale (Pant *et al.*, 2013).

Orchids, like any other crop, are impacted by a variety of biotic and abiotic variables. More than 130 plant diseases caused by fungi, bacteria, nematodes, and viruses have been identified in different genera of orchids (Pant *et al.*, 2012). Among fungal diseases, black rot (*Phytophthora palmivora*, *P. parasitica*, *Pythium ultimum* and *P. splendens*), anthracnose (*Colletotrichum gloeosporioides*), Orchid wilt (*Sclerotium rolfsii*), petal blight (*Botrytis cinerea*), rust (*Uredo* sp.), leaf blight (*Fusarium oxysporum*), *Sclerotinia* white rot (*Sclerotinia sclerotium*) and leaf spot (caused by species of *Fusarium*, *Cercospora*, *Alternaria*, *Pestalotia* and *Haplosporella*) are most common (Pant *et al.*, 2013).

*Curvularia geniculata* is the most important leaf spot-causing fungi. *Curvularia geniculata* (*Botryosphaeriaceae*, *Dothideomycetes*) is a fast-growing anamorphic fungus. *Curvularia* sp. is a soil-born, seed-borne as well as airborne fungus that's mostly found in tropical areas and has been reported in different hosts such as rice (Kusai *et al.*, 2016), maize (Manzar *et al.*, 2021), tomato (Rao *et al.*, 2020), pearl millet (Khatal *et al.*, 2019) etc. But in orchids pathogens belong to *Curvularia* sp. are either endophytes or saprophyte in nature. The presence of a necrotic lesion on the leaves is one of the most typical indications of leaf spot (Kusai *et al.*, 2016).

Based on the abovementioned information, it can be concluded that the orchid is one of the most important popular high-value flower crops contributing in different regions of India and exploring new areas for cultivation. But due to the climate changing scenario, the orchid growers are facing problems which may cause mild to severe losses if not reported at the right time. But very less information is available in the literatures on these diseases of orchid, keeping this research gap on mind, the present investigation was carried out with the objective of isolation, identification, morphological and cultural characterization of Orchid leaf spot disease causing pathogen *Curvularia geniculata*.

## 2. Material and Methods

The laboratory experiments were carried out in the “Survey Selection and Mass Production (SSMP) of Nodule Bacteria” laboratory at Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal.

The orchid leaf showing typical leaf spot symptoms were first scrapped with sterilized teasing needle. The scrapped bits were placed on clean glass slide in a drop of Lactophenol, covered with cover slip and examined under microscope for the presence of mycelium/spores if any, for identification of pathogen associated with the disease symptoms.

Fungal mycelia and spores were observed under a Light microscope and photographed. Conidia were measured using a light microscope with a micrometer at 40X magnification. The isolates were identified initially by comparing morphological and cultural characteristics (*i.e.*, size of conidia, color, number of cells and number of apical appendages, formation of pycnidia etc.).

## 2.1 Culture media preparation

Several types of culture media were prepared and used for the isolation, maintenance, and experimental evaluation of fungal growth and antagonism studies.

*Potato Dextrose Agar (PDA)*: was used as a general-purpose medium for growth studies, inhibition assays, and culture maintenance. To prepare PDA, 200 g of peeled potatoes were boiled in 500 ml of distilled water to obtain an extract. After filtration, 20 g of dextrose was added to the potato extract. Separately, 20 g of agar was dissolved in 500 ml of distilled water by heating. Both solutions were combined, and the final volume was adjusted to 1000 ml with distilled water. The medium was sterilized by autoclaving at 15 psi for 15–20 minutes.

*Corn Meal Agar (CMA)*: was prepared by boiling 40 g of corn meal in 600 ml of distilled water. Separately, 20 g of agar was dissolved in 400 ml of distilled water. After complete dissolution, both mixtures were combined, and the final volume was adjusted to 1000 ml. The medium was autoclaved at 15 psi for 15 minutes to ensure sterility.

*Oat Meal Agar (OMA)*: was prepared by suspending 60 g of oatmeal and 12 g of agar in distilled water, and the final volume was adjusted to 1000 ml. The mixture was heated until fully dissolved and then autoclaved under standard conditions at 15 psi for 15–20 minutes.

*Potato Sucrose Agar (PSA)*: 200 g of potatoes were boiled in distilled water, and the extract was mixed with 20 g of sucrose and 20 g of agar. The final volume was brought up to 1000 ml using

distilled water. This medium was also sterilized by autoclaving at 15 psi for 15–20 minutes.

*Carrot Agar Medium*: was formulated by extracting juice from 200 g of fresh carrots boiled in distilled water. After filtration, 10 g of agar was added, and the volume was made up to 1000 ml with distilled water. The medium was then autoclaved at 15 psi for 15 minutes.

*Host Leaf Extract Agar*: was prepared using 200 g of host leaves extracted in 500 ml of distilled water. To this extract, 20 g of dextrose was added, and the solution was gently heated to ensure dissolution. Separately, 20 g of agar was melted in 500 ml of distilled water and combined with the leaf extract. The final volume was adjusted to 1000 ml, and the medium was sterilized at 15 psi for 15–20 minutes.

## 2.2 Experimental design and statistical analysis

All laboratory experiments were conducted following a Completely Randomized Design (CRD). The data recorded from various experiments were subjected to statistical analysis using SPSS software (version 20.0; SPSS Inc., Chicago, IL, USA). One-way Analysis of Variance (ANOVA) was employed to assess the significance of the treatment effects. Where significant differences were detected, means were separated using Tukey's Honest Significant Difference (HSD) test at a 5% level of significance ( $P = 0.05$ ).

## 3. Results and Discussion

*Curvularia geniculata* is one of the most important leaf spot-causing pathogen in tropical region. In recent years these pathogen become an emerging threat in Indo-gangetic plain of West

Bengal. Initially, symptoms arise as light brown to yellow spots from the tip of leaves or terminal portion of leaf blades. Spots were surrounded by a light green chlorotic margin. With advances of disease dark brown necrotic spots developed in the infected region. At severe condition, entire lesions were turn into turn into necrotic, gradually dark brown with a yellow halo and leaves become fall off (Fig. 1).



**Fig. 1:** Typical *Curvularia geniculata* symptom produce in leaf

### 3.1 Isolation of pathogens

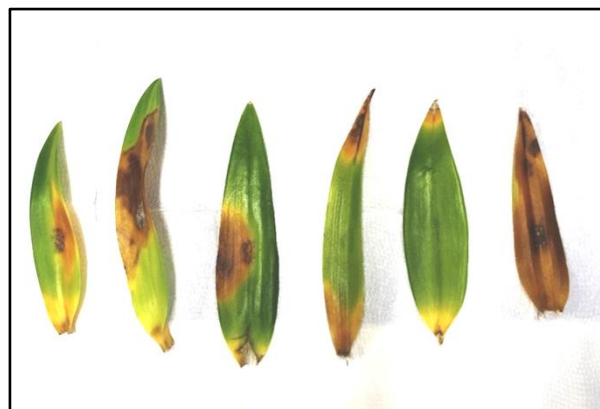
To isolate present microorganisms from the collected samples, different parts of the infected plant leaves were used. Diseased samples were cut into small specimens, washed of impurities dirt under running water before disinfecting with a solution of with 0.1%  $\text{HgCl}_2$  solution for 20 seconds and then rinsed 5 times with sterile distilled water. The sterilized specimens were cut into small pieces of  $0.5 \times 0.5$  cm (for the leaves). Diseased portion contain with a small healthy portion of initial nutrient source. These pieces were cultured on the plates of PDA and incubated at  $27^\circ$ . When mycelial growth and spores were observed, further isolation was carried out into

PDA plate for preparing pure culture. The microbial isolates were identified by morphological structures observed under a microscope with a magnification of x10 and x40.

### 3.2 Pathogenicity test

In case of *Curvularia geniculata* symptom developed five days after inoculation. After symptom expression, isolation was carried out to confirm the genus identification with subsequent pathogen re-isolation in PDA medium to fulfil Koch's postulates and to identify the species morphologically.

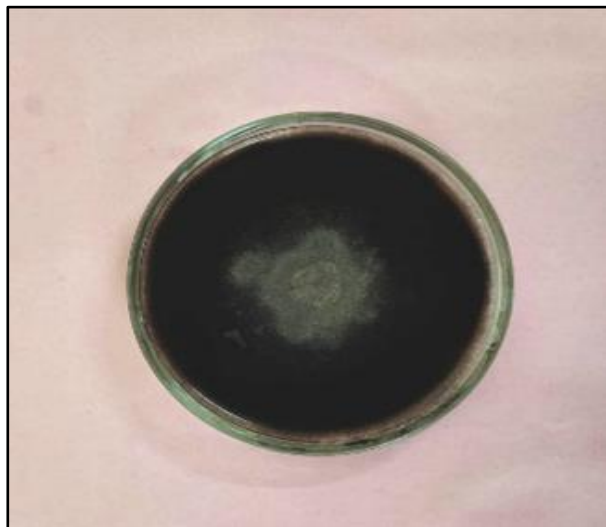
To comply with Koch's postulates, lesions were resembling with the initial symptoms observed on leaf five days after inoculation of *Curvularia geniculata* in all the orchid leaves. No symptoms were observed in control leaves. *Curvularia geniculata* first isolated from field after its natural occurrence and re isolated from artificially inoculated leaves were identical (Fig. 2).



**Fig. 2:** Pathogenicity test of *Curvularia geniculata* on orchid leaves

### 3.3 Morphological identification of pathogen

In *Curvularia geniculata*, fungal colonies are dark brown colour, zonation present (Fig. 3). Hyphae also brown to black colour, brown conidiophore. The conidia are geniculata to almost straight and boat shaped with disproportional enlargement at the third cell from the base. They are straw coloured to dark brown and sometimes paler coloured at both ends. Conidia contain with 4 distosepta with geniculated or inflated at the middle part. Length of conidia varies from 9.56 to 11.79  $\mu\text{m}$  ( $x = 10.84 \mu\text{m}$ ; S.D.= 1.01), Width of conidia varies from 4.32 to 5.38  $\mu\text{m}$  ( $x = 4.76 \mu\text{m}$ ; S.D.=0.40), Area ranges from 41.29 to 58.19  $\mu\text{m}^2$  ( $x = 51.67 \mu\text{m}^2$ ; S.D.= 6.56) (Fig. 4).



**Fig. 3:** Cultural character of *Curvularia geniculata* on petri plate

Qi *et al.*, (2022) observed fluffy greyish green fungal colonies with white aerial mycelium. Manzar *et al.*, (2021) observed similar dark greyish to black fungal colonies. Spindle to elliptical in shape and light brown conidia, with 3 to 4 septa with an enlarged central cell. Conidial size ranged from 10.0 to 14.1  $\mu\text{m}$  wide and 19.3 to

26.2  $\mu\text{m}$  long. Sumangala *et al.*, (2010) observed large colonies that had a very dark, blue-black appearance on the reverse and produced brown, floccose aerial mycelium on the obverse. Hyphae brown to black with curved shape, smooth wall, brown colour conidia.



**Fig. 4:** Conidia of *Curvularia geniculata*

### 3.4 Cultural characteristic of pathogen

*Curvularia geniculata* when cultured on six different media it showed a variation in their colony characteristic (Table 1; Fig. 5). The colony shape was varied round in Potato dextrose agar media, filamentous in Potato sucrose agar media and Carrot extract agar media, circular in Host extract agar media, Irregular in Oat meal agar media and Corn meal agar media. The surface colour of the colony was mostly black in Potato dextrose agar media, Potato sucrose agar media, Oat meal agar media, Carrot extract agar media whereas light black in Host extract agar media and Corn meal agar media. The elevation showed umbonate for Potato sucrose agar media, Potato dextrose agar media and Carrot extract agar media whereas flat on Host extract agar media, Oat meal agar media and Corn meal agar media. The margin of culture plate was entire on Potato



**Table 1:** Colony morphology of *Curvularia geniculata* on different medium

| Media | Colony Shape/Form | Surface Colour | Elevation | Margin      | Reverse Plate Colour | Growth    | Texture | Special Character | Time Taken for Full Plate |
|-------|-------------------|----------------|-----------|-------------|----------------------|-----------|---------|-------------------|---------------------------|
| PDA   | Round             | Black          | Umbonate  | Entire      | Black                | Rapid     | Powdery | No                | 7 days                    |
| PSA   | Filamentous       | Black          | Umbonate  | Undulate    | Black                | Rapid     | Powdery | Zonation Present  | 8 days                    |
| HEA   | Circular          | Light Black    | Flat      | Filiform    | Brown                | Very Slow | Powdery | No                | 12 days                   |
| OMA   | Irregular         | Black          | Flat      | Filamentous | Black                | Moderate  | Powdery | Zonation Present  | 9 days                    |
| CMA   | Irregular         | Light Black    | Flat      | Undulate    | White                | Slow      | Powdery | Zonation Present  | 10 days                   |
| CEA   | Filamentous       | Black          | Umbonate  | Filiform    | Black                | Slow      | Powdery | Zonation Present  | 10 days                   |

Note: PDA – Potato Dextrose Agar, PSA – Potato Sucrose Agar, HEA – Host Extract Agar, OMA – Oat Meal Agar, CMA – Corn Meal Agar, CEA – Carrot Extract Agar, Umbonate - Elevated at the center flattened at the margin

dextrose agar media; filiform on Host extract agar media and Carrot extract agar media; undulate on Potato sucrose agar media and Corn meal agar media whereas filamentous on Oat meal agar media. Reverse plate colour showed black on Potato dextrose agar media, Potato sucrose agar media, Oat meal agar media and Carrot extract agar media; brown on Host extract agar media and white on Corn meal agar media. Rapid growth rate was observed on Potato dextrose agar media and

Potato sucrose agar media; moderate growth rate was observed on Oat meal agar media; slow growth rate was observed on Corn meal agar media and Carrot extract agar media; whereas very slow growth rate was observed on Host extract agar media. The textures of all the culture media were powdery. Zonation as a special characteristic was present in Potato sucrose agar media, Oat meal agar media, Carrot extract agar media, Corn meal agar media whereas zonation absent in Potato dextrose agar media and Host extract agar media.

**Fig. 5:** Growth of *Curvularia geniculata* on different growth medium

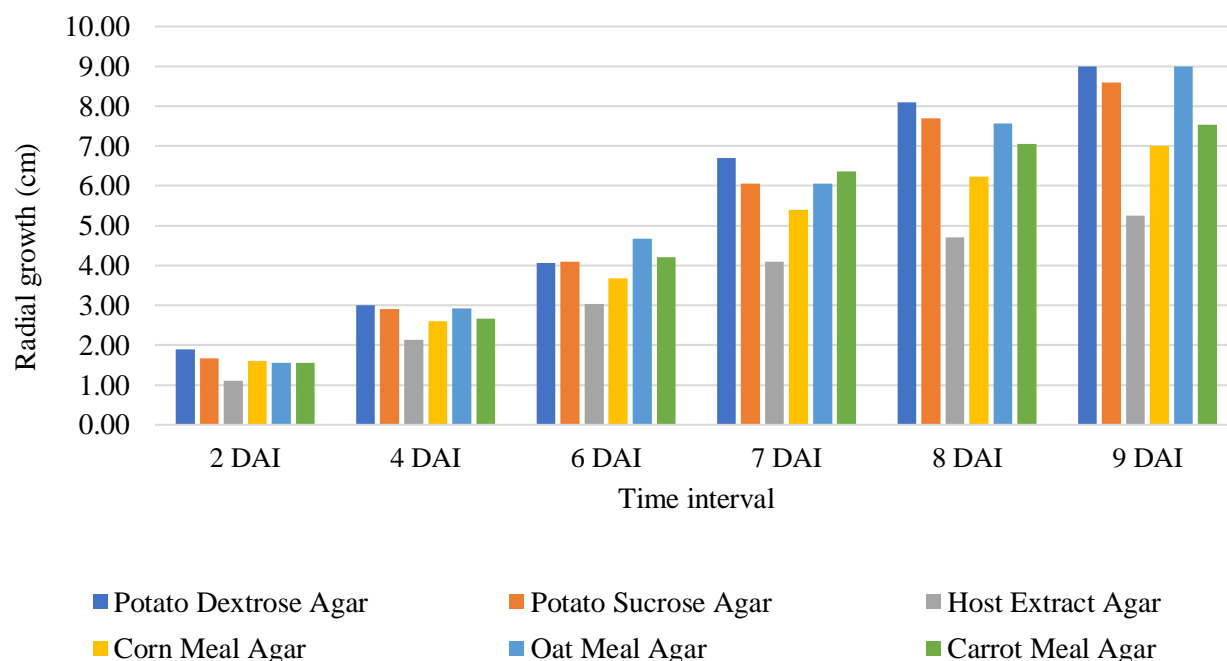
*Curvularia geniculata* growth on 6 different media i.e. Potato dextrose agar (PDA), Potato sucrose agar (PSA), Host extract agar (HEA), Oat meal agar (OMA), Corn meal agar, Carrot extract agar media showed differential result on different days after inoculation from 2 days after inoculation to 9 days after inoculation and differences were statistically significant (Table 2; Fig. 6). The data indicated that all the media supported the growth of *Curvularia geniculata*

with statistically significant variation in radial growth. At 2 days after inoculation, it was resulted that maximum growth was obtained in Potato dextrose agar (1.90 cm) followed by Potato sucrose agar (1.67 cm) statistically at par with

Corn meal agar (1.60 cm), Oat meal agar (1.56 cm) and Carrot extract agar media (1.56 cm). At 4 days after inoculation the growth was increased and maximum was noticed in Potato dextrose agar (3.00 cm) statistically at par with Potato sucrose

**Table 2:** Radial growth (cm) of *Curvularia geniculata* on different medium

| Different Medium     | Radial growth (cm) |                   |                   |                   |                   |                   |
|----------------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                      | 2 DAI              | 4 DAI             | 6 DAI             | 7 DAI             | 8 DAI             | 9 DAI             |
| Potato Dextrose Agar | 1.90 <sup>a</sup>  | 3.00 <sup>a</sup> | 4.06 <sup>b</sup> | 6.70 <sup>a</sup> | 8.10 <sup>a</sup> | 9.00 <sup>a</sup> |
| Potato Sucrose Agar  | 1.67 <sup>b</sup>  | 2.90 <sup>a</sup> | 4.10 <sup>b</sup> | 6.06 <sup>b</sup> | 7.70 <sup>a</sup> | 8.60 <sup>a</sup> |
| Host Extract Agar    | 1.10 <sup>e</sup>  | 2.13 <sup>e</sup> | 3.03 <sup>c</sup> | 4.10 <sup>e</sup> | 4.70 <sup>e</sup> | 5.26 <sup>e</sup> |
| Corn Meal Agar       | 1.60 <sup>b</sup>  | 2.60 <sup>b</sup> | 3.67 <sup>c</sup> | 5.40 <sup>c</sup> | 6.23 <sup>c</sup> | 7.00 <sup>c</sup> |
| Oat Meal Agar        | 1.56 <sup>b</sup>  | 2.93 <sup>a</sup> | 4.67 <sup>a</sup> | 6.06 <sup>b</sup> | 7.56 <sup>a</sup> | 9.00 <sup>a</sup> |
| Carrot Extract Agar  | 1.56 <sup>b</sup>  | 2.67 <sup>b</sup> | 4.20 <sup>b</sup> | 6.36 <sup>a</sup> | 7.06 <sup>b</sup> | 7.53 <sup>b</sup> |
| S.E.(m)±             | 0.06               | 0.08              | 0.12              | 0.21              | 0.28              | 0.32              |
| C.D. at 5%           | 0.18               | 0.21              | 0.35              | 0.58              | 0.79              | 0.91              |



**Fig. 6:** Graphical representation of Radial growth (cm) of *Curvularia geniculata* at different medium

agar (2.90 cm) and Oat meal agar (2.93 cm). Whereas minimum was observed in Host extract agar (2.13 cm) followed by Corn meal agar (2.60 cm) and their difference were not statistically at par. Similarly at 6 days after inoculation maximum growth was notice at Oat meal agar (4.67 cm) followed by Potato sucrose agar (4.10 cm) and Potato dextrose agar (4.06 cm) and minimum in Host extract agar (3.03 cm) followed by Corn meal agar (3.67 cm) and their difference was not statistically at par. At 7 days after inoculation similar type of observation was noticed and maximum was observed on Potato dextrose agar (6.70 cm) statistically at par with

Carrot extract agar media (6.36 cm) and minimum in Host extract agar (4.10 cm) followed by Corn meal agar (5.40 cm) and their differences were statistically at par. At 8 days after inoculation maximum radial growth was noticed in Potato dextrose agar (8.10 cm) statistically at par with Potato sucrose agar (7.70 cm) and Oat meal agar (7.56 cm), whereas lowest in Host extract agar (4.10 cm) followed by Corn meal agar (6.23 cm) and their differences were not statistically at par. At 9 days after inoculation *i.e.* at final day maximum growth was noted at Potato dextrose agar (9.00 cm) stastically at par with Oat meal agar (9.00 cm) and Potato sucrose agar (8.60 cm)

whereas minimum in Host extract agar (5.26 cm) followed by Corn meal agar (7.00 cm) and their differences were not statistically at par.

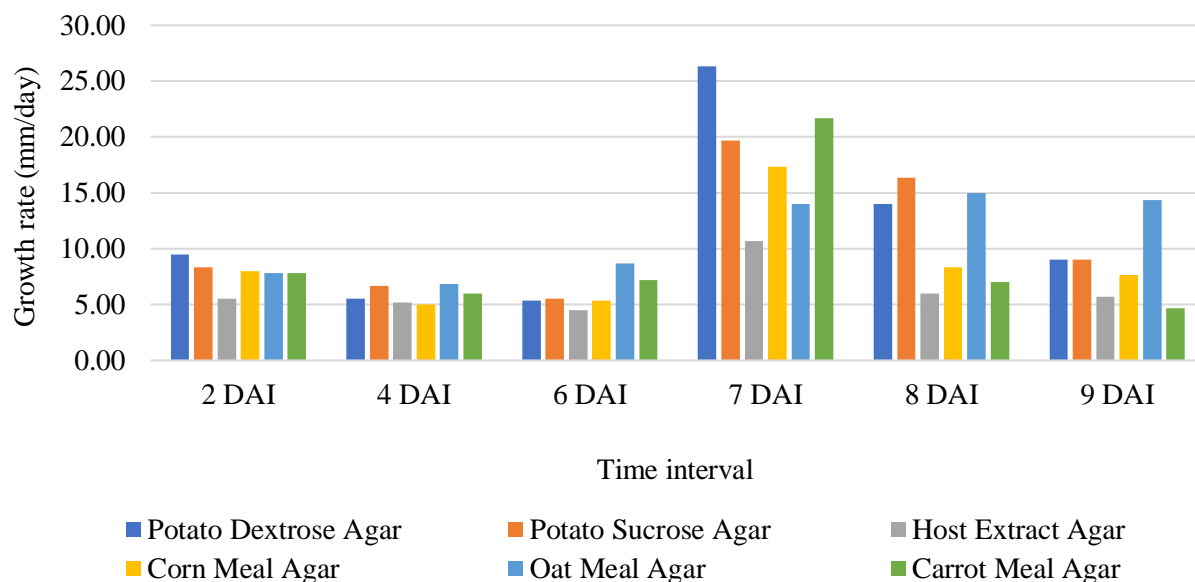
This result therefore indicated that Potato dextrose agar media has maximum induce the growth of *Curvularia geniculata* followed by Oat meal agar and Potato sucrose agar. Although they were statistically at par with each other. Similar type of observation was also noticed by Sumangala and Patil (2010). They reported that *Curvularia geniculata* produce maximum growth and sporulation on Potato dextrose agar and followed by Oat meal agar media. Majumdar and Mondal (2019) also reported that *Curvularia geniculata* produce highest growth on Potato dextrose agar media after 7 days of inoculation. Bhatt and Kumar (2018) reported that *Curvularia geniculata* produce maximum radial growth on Potato dextrose agar media followed by Oat meal agar and Czapeck's dox agar and minimum on Corn meal agar media at 30 °C temperature.

The growth rate of *Curvularia geniculata* showed that maximum 7 days after inoculation at Potato dextrose agar (26.33 mm/day) followed by Carrot agar media (21.67 mm/day) and Potato sucrose agar (19.67 mm/day) though Carrot extract agar media and Potato sucrose agar media were

**Table 3:** Growth rate (mm/day) of *Curvularia geniculata* on different media

| Different Medium     | Growth rate (mm/day) |                   |                   |                    |                    |                    |
|----------------------|----------------------|-------------------|-------------------|--------------------|--------------------|--------------------|
|                      | 2 DAI                | 4 DAI             | 6 DAI             | 7 DAI              | 8 DAI              | 9 DAI              |
| Potato Dextrose Agar | 9.50 <sup>a</sup>    | 5.50 <sup>c</sup> | 5.33 <sup>d</sup> | 26.33 <sup>a</sup> | 14.00 <sup>b</sup> | 9.00 <sup>b</sup>  |
| Potato Sucrose Agar  | 8.33 <sup>b</sup>    | 6.67 <sup>a</sup> | 5.50 <sup>d</sup> | 19.67 <sup>b</sup> | 16.33 <sup>a</sup> | 9.00 <sup>b</sup>  |
| Host Extract Agar    | 5.50 <sup>e</sup>    | 5.17 <sup>c</sup> | 4.50 <sup>e</sup> | 10.67 <sup>e</sup> | 6.00 <sup>d</sup>  | 5.67 <sup>d</sup>  |
| Corn Meal Agar       | 8.00 <sup>b</sup>    | 5.00 <sup>d</sup> | 5.33 <sup>d</sup> | 17.33 <sup>c</sup> | 8.33 <sup>c</sup>  | 7.67 <sup>c</sup>  |
| Oat Meal Agar        | 7.80 <sup>b</sup>    | 6.83 <sup>a</sup> | 8.67 <sup>a</sup> | 14.00 <sup>d</sup> | 15.00 <sup>a</sup> | 14.33 <sup>a</sup> |
| Carrot Extract Agar  | 7.83 <sup>b</sup>    | 6.00 <sup>b</sup> | 7.17 <sup>b</sup> | 21.67 <sup>b</sup> | 7.00 <sup>d</sup>  | 4.67 <sup>e</sup>  |
| S.E.(m)±             | 0.31                 | 0.21              | 0.362             | 1.267              | 1.025              | 0.796              |
| C.D. at 5%           | 0.88                 | 0.59              | 1.02              | 3.58               | 2.90               | 2.25               |





**Fig. 7:** Graphical representation of Growth rate (mm/day) of *Curvularia geniculata* at different media

statistically at par with each other with regards to growth rate of *Curvularia geniculata*. Whereas minimum growth rate was noticed 9 days after inoculation in every growth media and here also maximum was noticed in Oat meal agar media (14.33 mm/day) and minimum in Carrot agar media (4.67 mm/day). It was noticed that 4 days after inoculation and 6 days after inoculation the growth rate was reduced in comparison to 2 days after inoculation and it was observed in all growth media (Table-3; Fig. 7).

Whereas this result contradict with the result of Majumdar and Mondal (2019), There *Curvularia lunata* shows maximum growth rate on 6 days after inoculation. This result may be due to induction of enzyme for utilization of substrate. Zhao and Shamoun (2006) reported that the type of culture media and their chemical composition significantly affect the mycelial growth, growth

rate and conidial production of other pathogen like *Phoma exigua*.

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