Original Research Article

Influence of different growth supplementson culturalcharacteristics of *Corynesporacassiicola*causing targetleaf spot disease in Tomato

ABSTRACT:

Target leaf spot incited by *Corynesporacassiicola* is considered as an important emerging disease that ~~are responsible forhuge crop losses in tomato production.It~~ causes 25 to 43% crop losses in Tomato. Keeping view, the importance of the destructiveness of the disease, present study was conducted with an objective of influence of different Nutrient media, Carbon,Nitrogen,Temperature and pH on mycelial growth of this phytopathogen fungus~~*C.cassiicola*.which causes Target leaf spot disease~~.Potato Dextrose Agar (PDA) media were suitable for mycelia growth of *C.cassicola* followed by Oat Meal Agar (OMA) and Carrot Meal Agar(CAMA). A significant difference was recorded among the growth of tested fungus for utilization of all carbon and nitrogen sources used. On 9thday of transferring the fungus culture, it was observed that maximum radial colony diameter (8.53 cm) was obtained from Dextrose containing medium and least vegetative growth (4.72 cm) was recorded on Mannitol medium. For the nitrogen source study, Potassium Nitrate projected maximum colony diameter (8.92 cm) and least radial colony diameter (2.33 cm) was obtained from Ammonium sulphate medium. The best mycelial growth of *C. cassiicola* was noticed at 27˚C followed by 30˚C temperature. The most suitable pH for vegetative growth of particular fungus was found at pH 7.0 followed by pH 8.0.¿¿and the quantity of conidiospores formed in the different nutrient media????

***Keywords*:** Tomato, Target leaf spot, *Corynesporacassiicola,* Cultural characteristics.

1. INTRODUCTION:

Tomato (*Solanum lycopersicum* Mill.) is a most popular and commercially important crop grown in worldwide. It is native of Peru in Tropical America and belongs to family Solanaceae [1]. After China, India ranks second in the production and area of tomatoes. The major tomatogrowing countries are China, India, USA, Turkey, and Egypt. Annual Production of tomatoes in India throughout the year 2021-22 was 20331thousand metric ton with an area of about 840 thousand ha[2]. Tomato crop is

highly sensitive to different stresses like high and low temperature, moisture, humidity, drought, salinity, alkalinity etc. It is also affected by large number of diseaseswhich causes considerable losses in tomato production. Among these disease, Target leaf spot incited *C.cassiicola* is considered as an important emerging disease that causes drastic reduction in tomato production in humid climate condition [3]. In Brazil, the disease causes huge losses to cotton and soybean crop, and considered as an emerging disease [4]. The pathogen was reported to cause economic loss of 25 to 43% in tomato [5,6].

Various ecological and physiological factors affect the growth of fungi. Nutrient status of soils, especially Nitrogen and Carbon sources, greatly influence the establishment and growth of fungi in the field and as well as *in vitro* conditions. fungi vary in their ability to utilize various Carbon and Nitrogen sources for mycelial growth and development and it determines their colonization, virulence and pathogenicity within the host plant.The utilization of carbohydrates by fungi are dependent on the amount of nitrogen sources present [7]. For that reason, growth tests among different Carbon and Nitrogen sources are necessaryfor elucidation of the nutritional characteristics of fungi.The mycelial growth of fungus varies not only with different Carbon and Nitrogensources but also the different range of pH and temperature. Considering these background concepts in mind, theeffect of different Nutrient Media,Carbon, Nitrogen, Temperature and pH on growth characteristics of *C. cassiicola* was taken into account for investigation.

2.MATERIAL AND METHODS:

2.1Location: The experiment was conducted at Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal, India during January to May of 2022.(Latitude-22°94'47" N and longitude- 88°53'19" E).

2.2 Collection and isolation of the pathogen: Tomato leaves showing typical target spot symptoms were collected from the farmers field of surveyed plot.The pathogen was isolated and purified by using standard isolation technique.Pure culture of fungus was obtained by following hyphal tip method[8].

2.3 Pathogenicity test: ~~In detached leaf assay, leaves of tomato plants were detached from 30 days old plants in order to get the leaflet of similar uniformity~~. In the detached leaf test, leaves of similar shape and size from 30-day-old tomato plants were used.

Leaflets were placed abaxial side up on moistened filter paper in glass petri plates, and each was inoculated with conidial suspension adjusted to a concentration of 1×10^{-4} conidia/ ml. A single leaflet was used as an experimental unit and 3 replications were replicated per test. Leaflets were incubated at 27±1ºC and examined 24 hrs after inoculation, then every 12 hrs until 4 days post inoculation. After the development of symptoms re-isolation of the fungus was made from the affected portion following the isolation technique and Koch's postulates were proved.

2.4 Effect of Nutrient media: The culture media used in the present investigation were prepared according to the standard formula approved by the source book "Committee of the American Phytopathological Society [9].Following eight media were used for the cultural studies: Potato Dextrose Agar (PDA). Potato Sucrose Agar (PSA), Oat Meal Agar (OMA), Corn Meal Agar (CMA), Carrot Meal Agar (CAMA), Host Extract Agar (HEA), Malt Extract Agar (MEA) and V8 Juice Agar. The20 ml of each medium was poured in each 90 mm petri plates. 5 mm disc of fungus culture was cut with the ~~help of~~sterilized cork borer from the margin of 7 days old pure culture of fungus. One disc of the culture was placed in inverted position in the centre of each petri plates. The petri plates were incubated at 27±1°C. 3 replications were maintained for each medium.

2.5 Effect of Carbon sources: Five different carbon sources were tested by incorporating them in Richard's Agar media (Composition/litre of water was as follows: Potassium Nitrate -10g, Mono potassium di-hydrogen phosphate-5 g, Magnesium sulphate -2.5 g, Ferric Chloride-0.02g, Sucrose-50g, Agar-20g). Media containing without any carbon source i.e., Sucrose were taken as control. The quantity of each carbon sources was adjusted on the basis of their molecular weight so as to provide equivalent amount of carbon present in each medium. The following carbon sources were used for the study such as Dextrose, Sucrose, Maltose, Mannitol and Lactose. The pH of the medium was adjusted to 7.0 by adding 0.1N hydrochloric acid or 0.1N sodium hydroxide. Mycelial growth was recorded on daily basis and continued up to 9^{th} days. There were 5 replications of each carbon sources medium.

2.6 Nitrogen sources: For the nitrogen study, same procedure was followed except changes of different nitrogen sources. Five different nitrogen sources were tested by incorporating them in Richard's Agar media. Without any nitrogen source i.e., Potassium Nitrate in basal medium was kept as control. Potassium Nitrate, Ammonium Nitrate, Urea, Sodium Nitrate and Ammonium Sulphate were used as a different nitrogen sources. The quantity of each nitrogen sources was determined on the basis of their molecular weight so as to provide equivalent amount of nitrogen present in the basal medium. The pH of the medium was adjusted to 7.0 by adding 0.1N hydrochloric acid or 0.1N sodium hydroxide.

2.7 Effect of Temperature:20 mlPDA medium was poured in each petri plates. 5 mm discs of fungus culture was placed in inverted position in the centre of each petri plates. The petri plates were incubated at 21˚C, 23˚C, 25˚C, 27˚C, 30˚C, 32˚C,35˚C, 38˚C. Observation on daily radial growth of mycelium was measured by the help of scale.

2.8 Effect of pH: The pH of the medium was adjusted before sterilization with the help of digital pH meter by addition of 0.1N HCL and 0.1N NaOH. PDA medium was adjusted to different pH levels such as 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 and 10.0. Fungal culture discs were transferred to Petri plates and incubated for daily observation.

2.9 Statistical analysis: The average mycelial growth was calculated from the value of replications of each treatment and analysed statistically by using ANOVA with 5% level of significance. The variations among the data were compared by following Duncan's Multiple Range Test [10].

3. RESULTS AND DISCUSSION:

3.1 Symptoms: From the field survey and combined observation from farmer's field, different types of symptoms were noticed. Numerous small browns to dark brown spots ~~were~~ appeared on the leaf surface surrounded with a yellow zone and it extends to form a dark brown coloured target board type of concentric rings with or without yellowing. Irregular extended necrotic brown spots appeared on the margin or interveinal region of leaf. Severe irregular necrotic lesion starting from the tip and spreading downward through the marginal area of leaf were observed also (Fig 1).Symptoms recorded were matched with the standard descriptions of the disease [3].

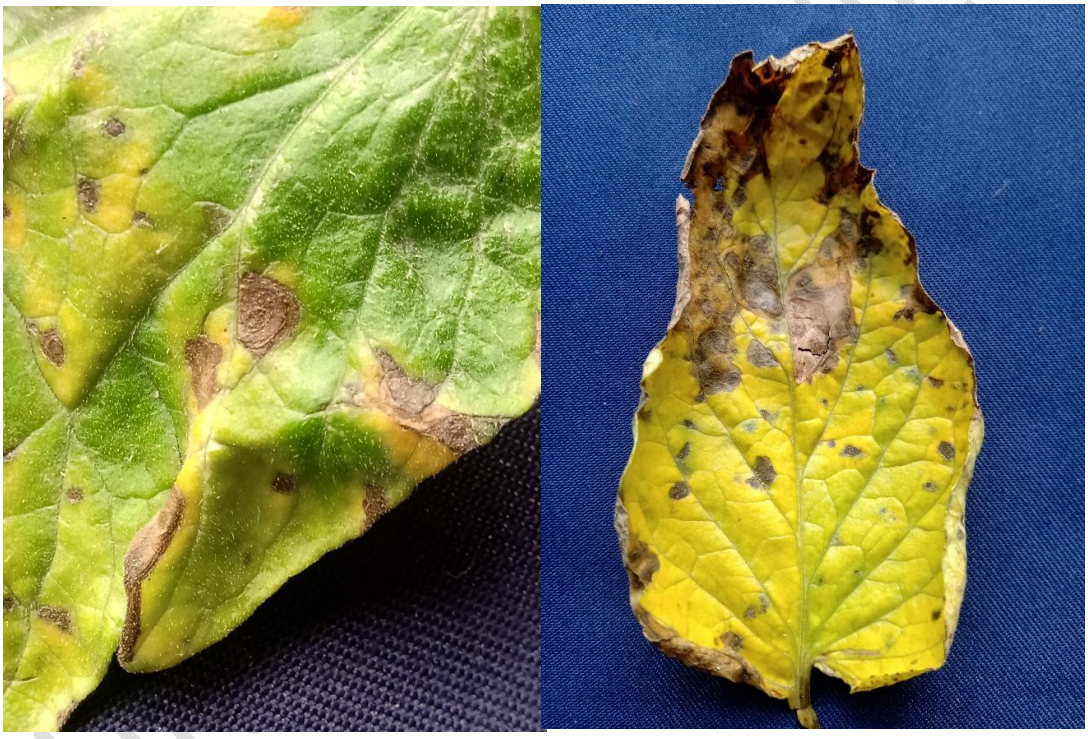

Fig1: Symptoms of Target leaf spot disease of Tomato

3.2 Pathogenicity Test: Leaves inoculated with culture of the test fungus initially produced water-soaked symptoms on the leaf surface after 72 hrs of inoculation. Re-isolation of the pathogen from artificially infected leaves exactly matched with the standard characters of *C.cassicola* was proving the Koch'spostulates.

3.3 Pathogen Description: Mycelium septate, branched, slender, sub hyaline to pale brown, hyphae mostly submerged in the sub stratum, Conidiophores arising singly from the mycelium, 3-7 septate, unbranched, erect, straight to slightly curved, pale brown in colour. Conidia formed singly or in chains of 2 - 3 at the apex of the conidiophore. It's were cylindrical to obclavate, straight to slightly

curved, sub-hyaline to pale olivaceous brown, smooth walled, 0 - 16 pseudosepta, and hilum at the base(Fig 2).Similar type of results wasalso obtained by Sinclair [11] where he studied the microscopic symptoms and characterized the pathogen. Ellis and Holiday described colony morphology, conidiophores and conidia of *C.cassiicola* [12].The present study showed similar result in case of size, shape and septation of conidia of *C. cassiicola*.

Fig 2: Conidia and Conidiophore of *C.cassiicola*

3.4 Cultural growth characteristics on different Nutrient media: Great variation was observed in the colony colour of *C. cassiicola* grown in 8 different nutrient media. At the front part of the Petri plates containing PDA,PSA, CAMA,OMA medium, the mycelial pigmentation showed grey to light grey in colour, while at backside of the dishes, light black to black colour was the predominant (Fig 3). In CMA, HEA, MEA, V8 Juice agar medium showed whitish colour at the front of the dishes and from brown to black at the backside of the dishes. PDA, PSA, and V8 juice agar showed thick mycelia growth and rest medium showed thin mycelia growth. Almost all the culture medium showed the fungal growth in rounded shape except HEA medium. It showed the polygon shape in petri-dish. (Fig 3)

The fungal growth of *C.cassiicola* produced flat mycelial growth in case of PSA, PDA, CAMA and OMA. The characteristics of spreadingtype growth were observed in CMA and rest of the medium produced cottony growth either fluffy, dense fluffy or simple. All media showed circular and smooth characteristics around the margin of colony. CMA, MEA, OMA showed the distinct zonation (Fig 3). The present descriptions were greatly corroborated with the findings of Ahmed *et al*., (2013) who described the colony morphology of *C. cassiicola* on PDA medium and it was appeared as effuse, light olivaceous green at initial stage and turned brown to dark blackish brown at maturity, often hairy or velvety. [13]

Similarities were observed in the characteristics of the mycelial growth of the eight different media by the description of Ellis and Holliday (1971), indicating that they belong to the species *C. cassiicola*. The study of fungi characteristics and morphological variations plays an important role in taxonomy and can assist in the identification of many pathogenic species. Similar growth characteristics was observed by Qi *et al*., (2011)[14] and Sousa *et al*., (2014) [15].They studied *C. cassiicola* isolates from soybean grown in PDA medium and also found variations in texture, ranging from fine to thick, and colony colour, being white to light brown, red-brown, dark brown, dark grey, or black.

Table 1: Growth characteristics of *C. cassiicola*on different Nutrient media:

Media	Pigmentation		Texture	Shape
	Top	Bottom		
Potato Dextrose Agar	Grey	Black	Thick	Round
Potato Sucrose Agar	Grey	Black	Thick	Round
Corn Meal Agar	White	White	Thin	Round
Host Extract Agar	White	Brown	Thin	Round
Malt Extract Agar	White	Black	Thin	Polygon
Oat Meal Agar	Grey	Black	Thick	Round
Carrot Meal Agar	Light Grey	Light Black	Thin	Round
V8 Juice Agar	White	Pinkish Brown	Thick	Round

3.5 Effect of different Nutrientmedia: The mycelial growth of *C. cassiicola* differed significantly with different types of media used. The maximum mycelial growth of *C. cassiicola* (3.10 cm, 5.90 cm and 8.55 cm) was noticed on PDA media at 3rd, 6th and 9th DAI (Days after inoculation)respectively followed by OMA(3.10 cm, 5.80 cm, and 8.50 cm) and CAMA (3.00 cm, 5.70 cm, and 8.50 cm. Minimum mycelial growth (2.37 cm, 4.43 cm and 6.25 cm) were recorded on V8 Juice Agar media at 3rd, 6th and 9th DAI respectively followed by MEA (2.50 cm,5.00 cm, 6.80 cm) (Table 2).The most suitable solid media identified for the mycelial growth was PDA. Sinclair (1982) also observed Potato Dextrose Agar as suitable media for the mycelial growth and sporulation of the fungal pathogen *C.cassicola*.

Table 2: Mycelial growth (cm) of *C. cassiicola* in different Nutrient media

Media	Day 1	Day 2	Day3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Potato Dextrose Agar	0.85^{a}	2.10^{b}	3.10^{a}	3.95^{ab}	4.90^{a}	5.90^{a}	6.75^{ab}	7.75^{ab}	8.55^{a}
Potato Sucrose Agar	0.65^{b}	1.94^{c}	2.85^{b}	3.78^{b}	4.80^{ab}	5.90^{a}	6.60^{b}	7.45^{b}	8.40^{a}
Corn Meal Agar	0.70^{ab}	1.85^{cd}	2.80^{b}	3.50^{c}	4.40^{bc}	5.20^{b}	5.85^{c}	6.60^{c}	7.20^{b}
Host Extract Agar	0.75^{ab}	1.75^{d}	2.50^{c}	3.40^{c}	4.20^{c}	5.10^{b}	5.80^{c}	6.80^{c}	7.25^{b}
Malt Extract Agar	0.65^{b}	1.75^{d}	2.50^{c}	3.10^{d}	4.00^{cd}	5.00^{b}	6.60^{b}	6.50^{c}	6.80^{c}
Oat Meal Agar	0.85^{a}	2.20^{a}	3.10^{a}	4.10^{a}	5.20^{a}	5.80^{a}	7.00^{a}	8.00^{a}	8.50^{a}
Carrot Meal Agar	0.75^{ab}	2.08^{b}	3.00^{ab}	3.90^{b}	4.85^{a}	5.70^{a}	5.80^{c}	7.50^{b}	8.50^{a}
V8 Juice Agar	0.70^{ab}	1.43^{e}	2.37^{c}	3.17^{d}	3.73^{d}	4.43^{c}	5.00^{d}	5.57^{d}	6.25^{d}
C.D. SE(m) SE(d) C.V.	N/A 0.049 0.069 11.495	0.206 0.068 0.097 6.263	0.234 0.077 0.109 4.82	0.192 0.064 0.09 3.049	0.406 0.134 0.19 5.16	0.324 0.107 0.152 3.454	0.304 0.101 0.142 2.82	0.351 0.116 0.164 2.863	0.2 0.066 0.094 1.492

In table 2, indicate, significate: C. D., SE (m), SE (d) and C.V.

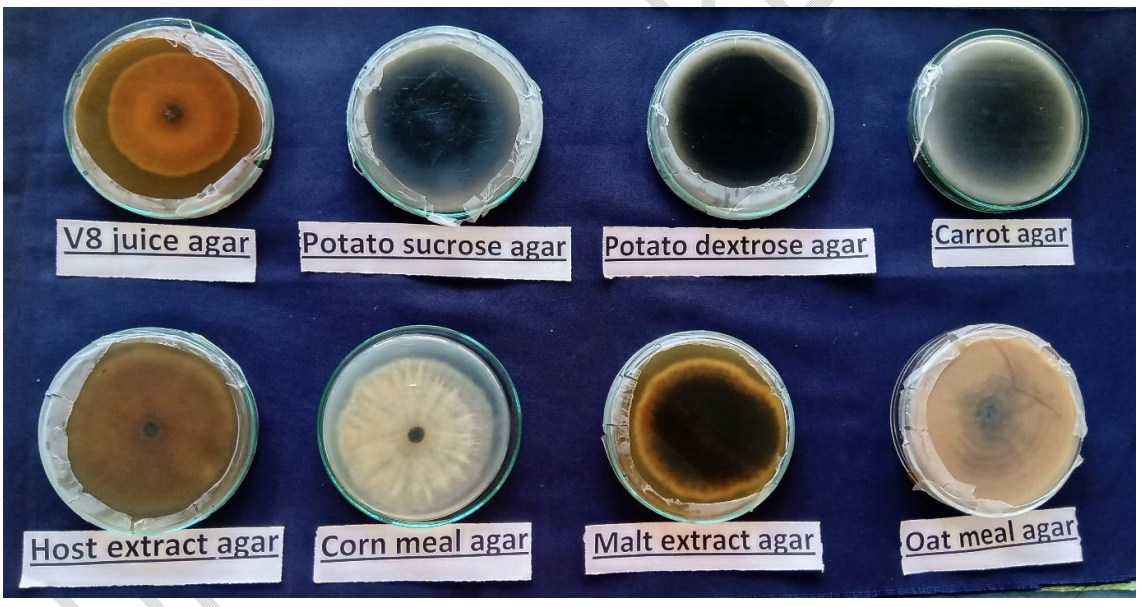

Fig 3: Effect of different Nutrient media on growth of *C. cassiicola*

3.6 Effect of Carbon sources: The fungus could grow well in Dextrose, Maltose, Sucrose, Lactose as compare to control. However, Dextrose became the best carbon source for the fungus. At the final day of incubation of 9th days, maximum radial colony diameter was recorded as 8.53 cm on Dextrose, which was followed by Maltose (7.02 cm). The least vegetative growth (4.72 cm) was noted on Mannitol. It was also noted that, there was a significant difference of radial colony diameter among all the carbon sources used.(Table 3, Fig 4)

In support of our present findings, the highlight of research work done by Chun-xia (2010)~~[15]~~[16]who reported the mycelium of *C. cassiicola* grew well on the media with Maltose as the carbon source. Wang *et al*., (2019) showed the pathogen grew well in the medium with anhydrous Dextrose and D-xylose as the carbon source [17].

Table 3: Mycelial growth (cm) of *C. cassicola* in different carbon sources

Carbon sources	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Dextrose	1.28^{b}	2.63^{a}	3.72^{a}	4.42^{a}	5.48^{a}	6.30^{a}	7.40^{a}	8.28^{a}	8.53^{a}
Sucrose	0.88^{c}	1.25^{c}	1.95^{c}	2.80^{c}	3.73^{c}	4.58^{c}	5.45^{b}	6.15^{b}	6.93^{b}
Maltose	1.41^{a}	2.36^{b}	3.17^{b}	3.65^{b}	4.13^{b}	4.70^{b}	5.43^{b}	6.22^{b}	7.02^{b}
Mannitol	0.55^{ed}	0.71^{e}	1.17^{f}	1.63^{e}	2.18^{e}	2.50^{f}	3.22^{d}	3.75^{d}	4.72^{d}
Lactose	0.63^{d}	0.95^{d}	1.37^{e}	2.40^{d}	3.08^{d}	3.92^{d}	4.57^{c}	4.92^{c}	5.75^{c}
Control	0.72^{d}	1.06^{d}	1.52^{d}	2.43^{d}	3.15^{d}	3.78^{e}	4.58^{c}	5.08^{c}	5.68^{c}
C.D. SE(m) SE(d) C.V.	0.109 0.035 0.05 6.661	0.215 0.069 0.098 8	0.182 0.058 0.083 4.713	0.339 0.109 0.154 6.514	0.186 0.06 0.084 2.851	0.127 0.041 0.058 1.646	0.166 0.053 0.075 1.802	0.247 0.079 0.112 2.395	0.225 0.072 0.102 1.946

3.7 Effect of Nitrogen sources: There was a significant variation in the radial colony diameter among different nitrogen sources used. At the final day of incubation period of 9th day, application of Potassium Nitrate projected maximum colony diameter (8.92 cm)(Table 4). The least radial colony diameter (2.33 cm) was obtained due to Ammonium Sulphate, which was very much lower than the basal media without any nitrogen source i.e., control (6.00 cm). This might be due to the presence of sulphur in Ammonium Sulphate resulting inhibitory effect on growth and development for major plant pathogens including *C. cassicola*. It was also mentioned that, there was a significant difference among the radial colony diameter among all the nitrogen sources tested.(Table 4, Fig 5)

Table 4: Mycelial growth (cm) of *C. cassiicola* in different nitrogen sources

Nitrogen sources	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
Potassium Nitrate	1.27^{a}	1.97^{a}	3.17^{a}	4.50^{a}	5.53^{a}	6.40^{a}	7.58^{a}	8.42^{a}	8.92^{a}
Ammonium Nitrate	1.03^{c}	1.25^{c}	2.00^{c}	2.30^{d}	2.85^{d}	3.17^{d}	3.72e	4.18^{e}	4.62^{e}
Urea	0.88^{d}	1.47^{b}	2.10^{bc}	2.93c	3.50^{c}	3.78^{c}	4.17^{d}	4.75^{d}	5.22^{d}
Sodium Nitrate	1.10^{c}	1.50^{b}	2.30^{b}	3.20^{b}	3.55^{c}	4.40^{b}	5.53^{b}	6.28^{b}	6.85^{b}
Ammonium sulphate	0.88^{d}	1.02^{d}	1.20^{d}	1.50^{e}	1.65^{e}	1.82^{e}	2.05^{f}	2.20^{f}	2.33^{f}

Control	1.17^{ab}	1.50^{b}	2.40^{b}	3.10^{bc}	3.87^{b}	4.40^{b}	5.20^{c}	5.53^{c}	6.00^{c}
C.D.	0.108	0.164	0.203	0.185	0.114	0.232	0.181	0.205	0.227
SE(m)	0.035	0.053	0.065	0.059	0.037	0.075	0.058	0.066	0.073
SE(d)	0.049	0.075	0.092	0.084	0.052	0.105	0.082	0.093	0.103
C.V.	5.693	6.296	5.138	3.516	1.818	3.232	2.139	2.176	2.235

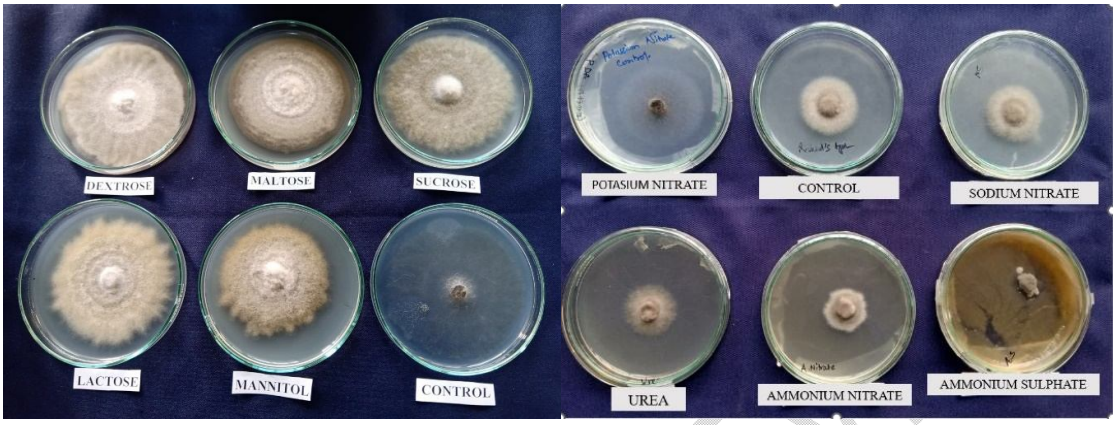

Fig 4:Effect of Carbon (Final Day) and Nitrogen (3rd Day) on radial mycelia growth

3.8 Effect of Temperature: The mycelial growth of *C. cassiicola* varied significantly when it was grown at different temperatures (Fig 5). The mycelial growth was significantly higher at 27°C (2.25 cm, 5.50 cm, 8.67 cm) on 3rd, 6th and 9th DAI respectively followed by 30°C (2.13 cm, 5.20 cm, 8.59 cm). However, minimum growth (0.75 cm, 1.92 cm, and 2.03 cm) was recorded at 38°C on 3rd, 6th and 9th DAI respectively(Table 5). The mycelial growth of *C. cassiicola* was found to be maximum at 27ºC followed by 30ºC.The similar findings were also reported by Sajili*et al*., (2019) [18].They found that temperature ranged from 20°C to 30°C showed the best growth condition for *C. cassiicola.*

Table 5: Effect of temperature on radial mycelia growth (cm)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
21˚C	0.50^{c}	0.72^{cd}	1.25^{d}	1.68^{e}	2.37e	2.85^{f}	3.30^{d}	3.77^{e}	3.60^{d}
23˚C	0.50^{c}	0.72^{cd}	1.45^{c}	2.15^{e}	2.65^{d}	3.07^{e}	3.77^{c}	4.23c	4.62^{c}
25˚C	0.55^{b}	0.92^{b}	1.92^{b}	3.25^{ab}	4.25^{a}	5.33^{ab}	6.50^{a}	7.55^{a}	8.52^{a}
27˚C	0.57^{b}	1.13^{a}	2.25^{a}	3.33^{a}	4.10^{b}	5.50^{a}	6.50^{a}	7.63^{a}	8.67^{a}
30˚C	0.63^{a}	0.93^{b}	2.13^{a}	3.17^{c}	4.10^{b}	5.20^{c}	6.52^{a}	7.52^{a}	8.59^{a}
32˚C	0.50^{c}	0.82^{bc}	1.77^{b}	2.77^{d}	3.80^{c}	4.77^{d}	5.20^{b}	6.10^{b}	6.68^{b}
35˚C	0.50^{c}	0.73^{cd}	1.28^{d}	1.77^{e}	2.48e	3.17^{e}	3.67^{c}	4.07^{d}	4.57^{c}
38˚C	0.50^{c}	0.65^{d}	0.75^{e}	0.92^{f}	1.25^{f}	1.92^{g}	1.65^{e}	1.92^{f}	2.03^{e}
C.D. SE(m)	0.037 0.012	0.121 0.04	0.164 0.054	0.087 0.029	0.122 0.04	0.171 0.057	0.154 0.051	0.157 0.052	0.153 0.051

SE(d)	0.017	0.057	0.077	0.041	0.057	0.08	0.072	0.074	0.072
C.V.	3.928	8.369	5.881	2.101	2.239	2.463	1.906	1.685	1.486

3.8 Effect of pH: The mycelial growth of *C. cassiicola*varied significantly at varying pH range. PDA medium when adjusted to pH 7 recorded significantly maximum mycelial growth (2.17 cm,5.70 cm,8.68 cm) at 3rd, 6th and 9th DAI respectively followed by pH 8 (2.13 cm, 5.63 cm, 8.62 cm) and pH 6 (2.10 cm, 5.65 cm, 8.47 cm). On the other hand, minimum mycelial growth (1.83 cm, 4.70 cm, 6.50 cm) was recorded at pH 4 after 3rd, 6th and 9th DAI respectively (Table 6). An average mycelial growth indicated that *C. cassiicola* can grow better at pH ranged from 6 to 9. The mycelial growth of *C. cassiicola* was found to be maximum at pH 7 and minimum mycelial growth was recorded at pH 4 (Fig 5). Sajili *et al*., (2019) reported pH 7 to pH 8 were the best pH for *C. cassiicola* growth. Similarly, Almeida (1977) also found maximum growth and sporulation of *C. cassiicola* at pH 6.5 to 7.5 [19].

Table6: Effect of pH on radial mycelia growth (cm)

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9
PH 4	0.50^{a}	0.82^{bc}	1.83^{b}	2.67^{c}	3.82^{d}	4.70^{d}	5.20^{d}	5.93^{e}	6.50^{d}
PH 5	0.50^{a}	0.80^{c}	1.97^{ab}	3.10^{b}	4.23^{bc}	5.30^{c}	6.33^{bc}	6.97^{d}	7.73^{c}
PH 6	0.57^{a}	0.90^{b}	2.10^{ab}	3.23^{b}	4.30^{b}	5.65^{ab}	6.50^{b}	7.53^{bc}	8.47^{ab}
PH 7	0.50^{a}	0.90^{b}	2.17^{a}	3.20^{b}	4.57^{a}	5.70^{a}	6.63^{b}	7.73^{ab}	8.68^{a}
PH 8	0.53^{a}	1.00^{a}	2.13^{ab}	3.45^{a}	4.72^{a}	5.63^{ab}	7.10^{a}	7.87^{a}	8.62^{a}
PH 9	0.50^{a}	0.88^{bc}	2.08^{ab}	3.10b	4.20^{bc}	5.37^{bc}	6.47^{b}	7.33^{c}	8.23^{b}
PH 10	0.50^{a}	0.85^{nc}	2.07^{ab}	3.17^{b}	4.00^{d}	5.20^{c}	6.10^{c}	6.90^{d}	7.52^{c}
C.D.	N/A	0.086	N/A	0.138	0.239	0.283	0.341	0.222	0.269
SE(m)	0.028	0.028	0.089	0.045	0.078	0.092	0.111	0.072	0.088
SE(d)	0.04	0.04	0.126	0.064	0.111	0.131	0.157	0.102	0.124
C.D.	9.488	5.554	7.546	2.489	3.177	2.982	3.043	1.746	1.909

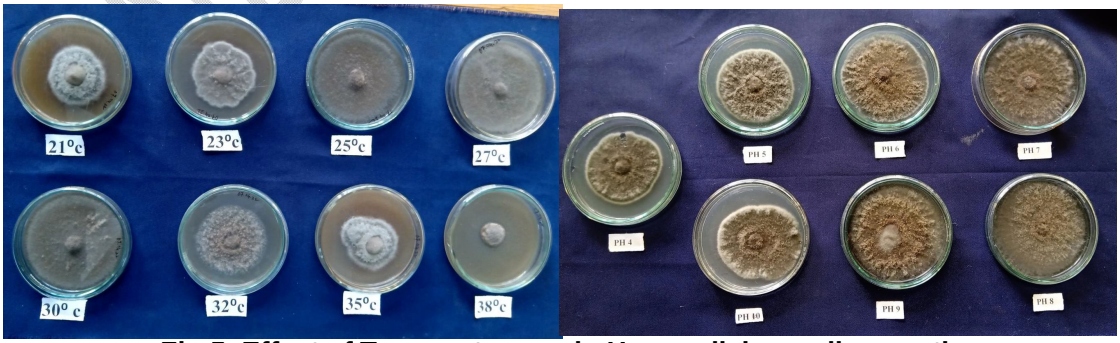

Fig 5: Effect of Temperature and pH on radial mycelia growth

4. CONCLUSION:

The present experiment study showed that Potato Dextrose Agar media were suitable for mycelia growth of *C. cassicola* followed by Oat Meal Agar and Carrot Meal Agar. The effect of different Carbon and Nitrogen sources on mycelial growth of *C. cassicola* was significantly different. Though the irregular growth pattern of mycelial colony was observed. Best carbon source for growth was identified as Dextrose and Potassium Nitrate showed best nitrogen source for the mycelial growth.The best mycelial growth was found at 27˚C followed by 30˚C temperature.The most suitable pH for vegetative growth of *C. cassiicola* was found to be pH 7.0 followed by pH 8.0.

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