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OPTIMIZATION OF CARBON AND NITROGEN SOURCES ON MYCELIAL GROWTH AND SPORULATION OF *ALTERNARIA BRASSICAE* CAUSING ALTERNARIA BLIGHT

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ABSTRACT

Among the tested carbon sources, the maximum mycelial growth of *Alternaria brassicae* was observed on glucose (88.17 mm), followed by sucrose (84.67 mm), maltose (82.33 mm), mannitol (82.17 mm), and citric acid (73.83 mm). The minimum radial mycelial growth was recorded in the control (70.00 mm). Excellent sporulation was recorded in glucose and maltose, where more than 30 conidia were recorded per microscopic field. Citric acid showed fair sporulation, with 10-20 conidia per microscopic field. Five nitrogen sources were evaluated for their effect on the growth and sporulation of *Alternaria brassicae*. The maximum mycelial growth was observed on potassium nitrate (74.80 mm), followed by ammonium sulfate (73.00 mm), ammonium nitrate (70.00 mm), silver nitrate (69.67 mm), and the control (68.50 mm). The minimum radial mycelial growth was observed on ammonium chloride (63.83 mm). Excellent sporulation, with more than 30 conidia per microscopic field, was recorded on ammonium sulfate and potassium nitrate, while silver nitrate exhibited fair sporulation with 10-20 conidia per microscopic field.
***Keywords*:** Carbon and Nitrogen Sources, Mycelial Growth, Sporulation, *Alternaria brassicae*, Alternaria Blight

Introduction

Alternaria blight of mustard caused by *Alternaria brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw.), is a very important disease as it has been reported from all the continents of the world, causing considerable losses in yield (Meena *et al.*, 2010). The disease appears regularly in moderate to severe form and its infection generally occurs on leaves, stem and siliqua resulting in reduction of yield and poor seed quality brassica crops (Saharan, 1992).

According to the brown coloured conidiosphores and conidia of *Alternaria brassicae* (Berk.) Sacc. with long beaks can easily be identified and distinguished from *Alternaria brassicicola* which has conidia with very short or no beaks.

Alternaria blight disease occurs on the stems and leaves of seedlings and mature plants, as well as in siliquae at the ripening stage. Both vegetable and oleaceous brassicas produce fewer high-quality seeds due to dark stains on the leaves and siliquae that inhibit photosynthetic ability and stimulate early ripening (Kumar *et al.*, 2014).

General characteristics of *Alternaria brassicae* the mycelium of *Alternaria. brassicae* is septate and it becomes brown to brownish grey in colour. The conidiophores are dark septate, measuring 14-74-*4-8um. The structure of Conidia is brownish black, singly borne or sparingly in chains with 2-4, Muriform along with beak. This species Generally represents the slow and rudimentary growth in media and it forms

Chlamydospores in less frequency given by the scientist (Kolte, 1985). Sporulation occurs between the temp 21- 28°c.When the temperature decreases the time period than it takes for germination increases (Degenhardt *et al.*, 1982) and (Sharma *et al.*, 2013) Found that the 32 Indian isolates of *Alternaria brassicae* and colony of the isolates on PDA varied between olivaceous black to light olive grey whereas mycelia colour varied between brown to golden colour. Most of the Conidia were long and it is pyriform in shape with long beak and colour was found golden or brown with mostly smooth surface. A temperature ranges for mycelial growth and the sporulation of Alternaria brassicae is 25-30°C and 15-35°C was found respectively. Mycelial growth was favoured by the 100 percent relative humidity. The pathogen the genus of *Alternaria brassica* belongs to the phylum Ascomycota which consist of both saprophytic and pathogenic species. Alternaria belongs to the class Dothideomycetes, order Pleosporales and the family Pleosporaceae. The *Alternaria spp* is generally characterized by the formation of transverse septa with long and short beaks and having a polymorphous type of conidia in the form of single or the short long chains. Taxonomy of Alternaria leaf blight is acted upon the morphology and development of Conidia and Conidiophore with a lesser degree on the host plant and along with the Colony morphology given by the scientist (Elliot 1917, Wiltshire 1933, Simmons 1967.

Material and Methods

Effect of different Carbon and Nitrogen sources on the growth and sporulation of *Alternaria brassicae*

The effect of ten nitrogen and carbon sources on the growth of *Alternaria brassicae* was studied. Richard's agar medium was supplemented with different carbon sources, namely Maltose, Sucrose, Mannitol, Glucose and Citric acid, as well as nitrogen sources including Ammonium nitrate, silver nitrate, Ammonium sulphate, Potassium nitrate, and Ammonium chloride. The culture media were prepared using a standardized method and autoclaved at 121.6 °C and 15 psi pressure for 20 minutes. Uniform quantities (20 ml) of each medium were poured into 90 mm Petri plates.

Each Petri plate was inoculated with uniform mycelial culture bits (5 mm) obtained from a vigorously growing 5-day-old culture using a cork borer. These bits were placed in the center of each pre-poured medium and incubated at 25±1°C. Each treatment was replicated three times. The diameter of fungal growth was measured after 3, 5, and 7 days of inoculation.

To measure sporulation on different media, a single 5 mm diameter block was cut from the fungal colony near the margin using a sterilized cork borer and transferred to 5 ml of sterile distilled water in a test tube. The block was thoroughly mixed to create a uniform spore suspension. A small drop of the spore suspension was placed on a slide, and the average spore count across three microscopic fields was recorded using the low power (10X) objective of the microscope.

Table 1: Categorization of conidial density per microscopic field

No. of Conidia per Microscopic Field	Designation
0	Nil (-)
10-20	Fair (+)
20-30	Good (++)
>30	Excellent (+++)

Results and Discussion

Impact of Carbon Sources on the Growth and Sporulation of *Alternaria brassicae*

In the studies, the maximum mycelial growth of *Alternaria brassicae* was recorded in glucose, followed by sucrose, maltose, mannitol, and citric acid compared to the control. Excellent sporulation (>30 conidia) was observed in the glucose and maltose. Sporulation was moderate (20-30 conidia per microscopic field) in sucrose and mannitol. Control plates exhibited good sporulation, while citric acid showed 10-20 conidia, placing it in the fair sporulation category.

The investigation into the growth and sporulation of *Alternaria brassicae* under different nitrogen sources revealed that the highest mycelial growth occurred with Potassium Nitrate, followed by Ammonium Sulphate, Ammonium Nitrate, Silver Nitrate, and the control. The minimum growth was recorded with Ammonium Chloride. Both Ammonium Sulphate and Potassium Nitrate sources showed excellent sporulation in the microscopic field, while good sporulation (more than 20-30 conidia per microscopic field) was observed with Ammonium

Chloride, Ammonium Nitrate, and the control. Fair sporulation (10-20 conidia per microscopic field) was recorded in Silver Nitrate.

These findings are supported by Gholve *et al.* (2015), who reported the highest radial mycelial growth (86.00 mm) and excellent (++++) sporulation on glucose. Additionally, Nair (1997) indicated that ammonium nitrate promotes the growth of Alternaria blight. Similarly, Shinde (1991) found that ammonium nitrate, followed by sodium nitrate and potassium nitrate, provided optimal growing conditions for *Alternaria alternata* marigold isolate. Goyal (1977) noted that *A. alternata* thrived best on sodium nitrate, ammonium nitrate, and ammonium sulfate. These results align with those of Ingawale (1996), who found excellent sporulation of *A. alternata* in ammonium nitrate and good sporulation in sodium nitrate and ammonium sulfate. However, Goyal (1977) and Nair (1997) observed excellent sporulation of *A. alternata* in potassium nitrate.

Table 2: Role of carbon sources on growth and sporulation of *Alternaria brassicae*

Treatment	Mycelial Growth (mm)*			Conidia/ Microscopicfield	Sporulation Category
	3 DAI	5 DAI	7 DAI		
Maltose	29.83	53.83	82.33	>30	Excellent
Sucrose	31.33	55.33	84.67	20-30	Good
Mannitol	28.50	52.66	82.17	20-30	Good
Glucose	39.50	63.83	88.17	>30	Excellent
Citric acid	22.70	46.33	73.83	10-20	Fair
Control	24.33	51.00	70.00	20-30	Good
SE. m±	1.32	1.26	0.97		
C.D at 5%	4.12	3.94	3.04		

Mean of Three Replication, *DAI: Days after inoculation

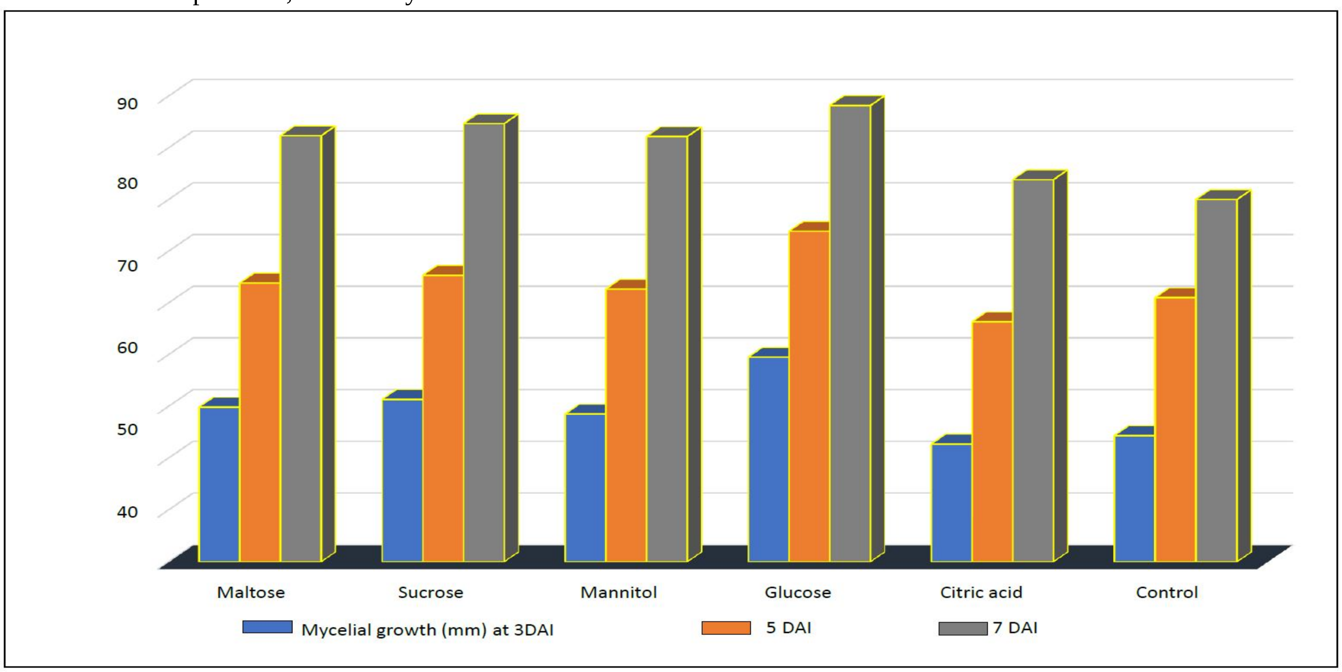


Fig. 1 : Growth of *Alternaria brassicae* under different carbon sources

Effect of Nitrogen Sources on the Growth and Sporulation of *Alternaria brassicae*

The data summarized in Table 3 indicate that among the five nitrogen sources tested for the growth and sporulation of *Alternaria brassicae* namely, ammonium nitrate, silver nitrate, ammonium sulfate, potassium nitrate, and ammonium chloride the highest mycelial growth at 3 days after inoculation (DAI) was recorded in ammonium nitrate (24.33 mm), followed by silver nitrate (23.17 mm) and potassium nitrate (22.50 mm). In contrast, the minimum growth was observed in ammonium chloride (16.33 mm) and the control (17.67 mm).

At five days after inoculation, the maximum mycelial growth was again recorded in ammonium nitrate (51.00 mm), followed by silver nitrate (46.17

mm), potassium nitrate (45.83 mm), ammonium sulfate (43.67 mm), and the control (42.50 mm). The least mycelial growth was recorded in ammonium chloride (38.33 mm).

At seven days after inoculation, significantly higher mycelial growth (74.80 mm) was observed in potassium nitrate, followed by ammonium sulfate (73.00 mm). Other nitrogen sources showed moderate growth, with ammonium nitrate at 70.00 mm and silver nitrate at 69.67 mm, compared to the control (68.50 mm). The minimum mycelial growth was noted in ammonium chloride (63.83 mm) (see Figure 4.4 and Plate 5).

Sporulation of *Alternaria brassicae*

Table 3 also shows that fair to excellent sporulation was recorded in the tested nitrogen sources. Among all sources, excellent sporulation (>30 conidia/microscopic field) was observed in ammonium sulfate and potassium nitrate, while good sporulation (20-30 conidia/microscopic field) was found in ammonium chloride, ammonium nitrate, and the control. In contrast, fair sporulation (10-20 conidia/ microscopic field) was recorded in silver nitrate.

These findings are supported by Gholve *et al.* (2015), who reported the highest radial mycelial growth (86.00 mm) and excellent sporulation (+++++) on glucose. Nair (1997) also reported that ammonium nitrate promotes the growth of Alternaria blight.

Similarly, Shinde (1991) found that ammonium nitrate, followed by sodium nitrate and potassium nitrate, provided the best growing conditions for the *Alternaria alternata* marigold isolate. Goyal (1977) indicated that *A. alternata* grew best on sodium nitrate, ammonium nitrate, and ammonium sulfate. These findings align with those of Ingawale (1996), who found excellent sporulation of *A. alternata* in ammonium nitrate and good sporulation in sodium nitrate and ammonium sulfate. However, both Goyal (1977) and Nair (1997) noted that sporulation of *A. alternata* in potassium nitrate was excellent.

Table 3: In vitro *evaluation of different nitrogen sources on growth and sporulation of Alternaria blight*

Treatment	Mycelial Growth (mm)*				Sporulation Category
	3 DAI	5 DAI	7 DAI	Conidia/ Microscopicfield	
Ammonium nitrate	24.33	51.00	70.00	20-30	Good
Silver nitrate	23.17	46.17	69.67	10-20	Fair
Ammonium sulphate	20.67	43.67	73.00	>30	Excellent
Potassium nitrate	22.50	45.83	74.80	>30	Excellent
Ammonium chloride	16.33	38.33	63.83	20-30	Good
Control	17.67	42.50	68.50	20-30	Good
SE. m±	0.86	1.16	0.85		
C.D at 5%	2.69	3.64	2.65		

Mean of Three Replication *DAI: Days after inoculation

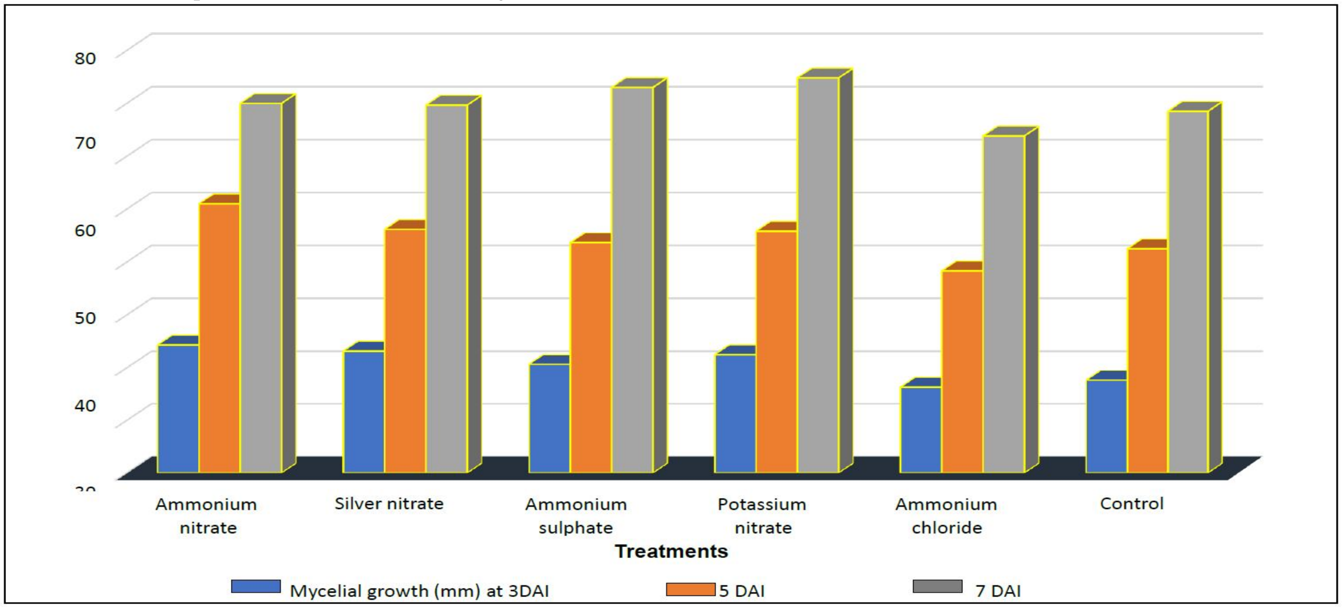
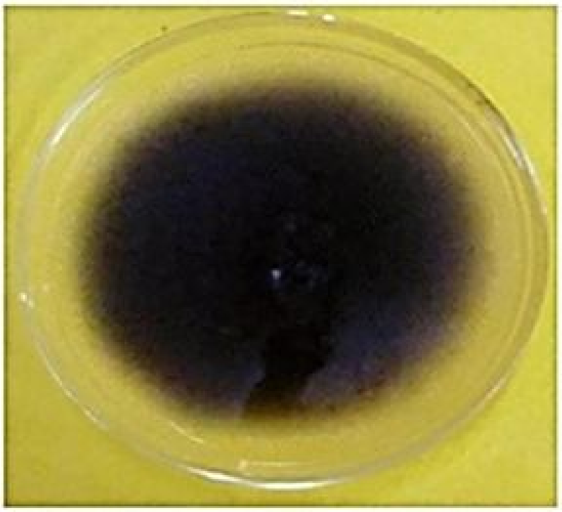
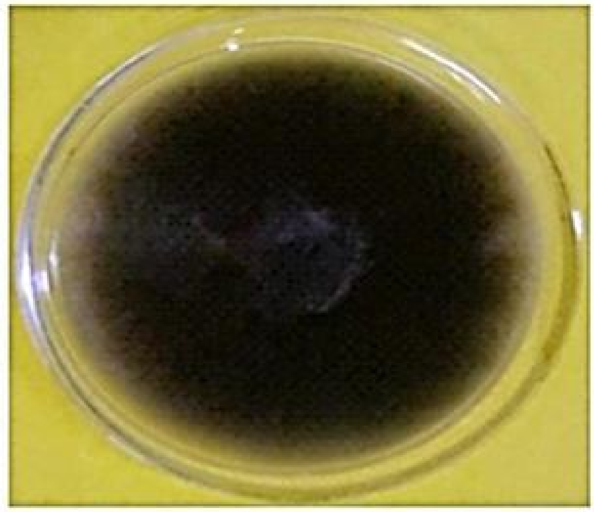


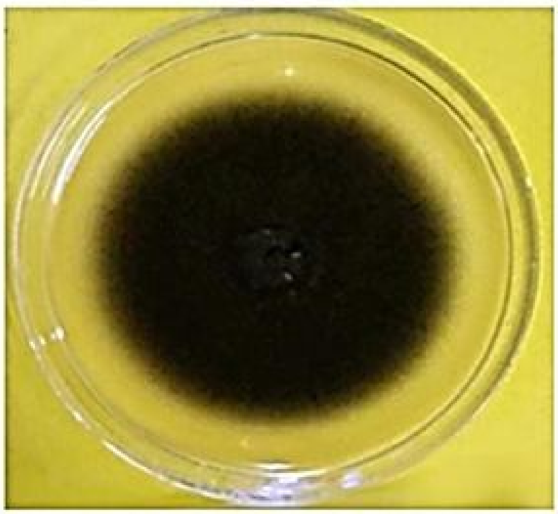
Fig. 2: Influence of Nitrogen sources on growth of *Alternaria brassicae*



T1- Maltose

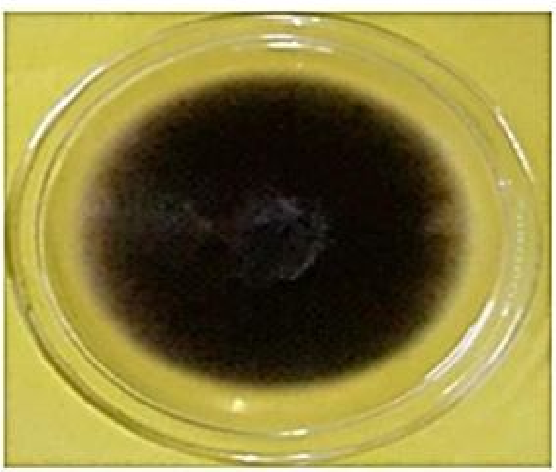


T2- Sucrose

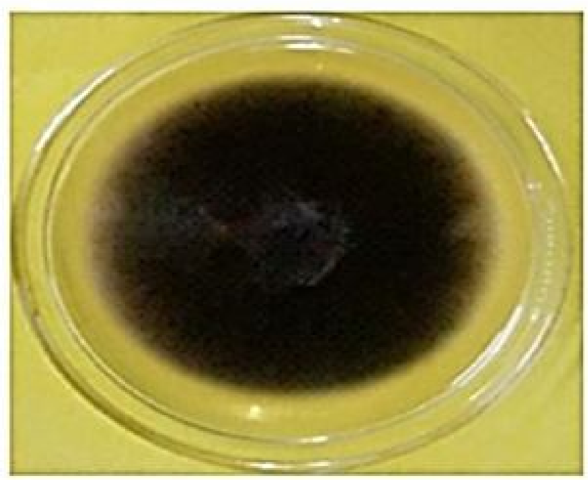


T3-Mannitol

T4- Glucose



T5- Citric acid



T6- Control

Plate 1: Growth of *Alternaria brassicae* under different Carbon sources

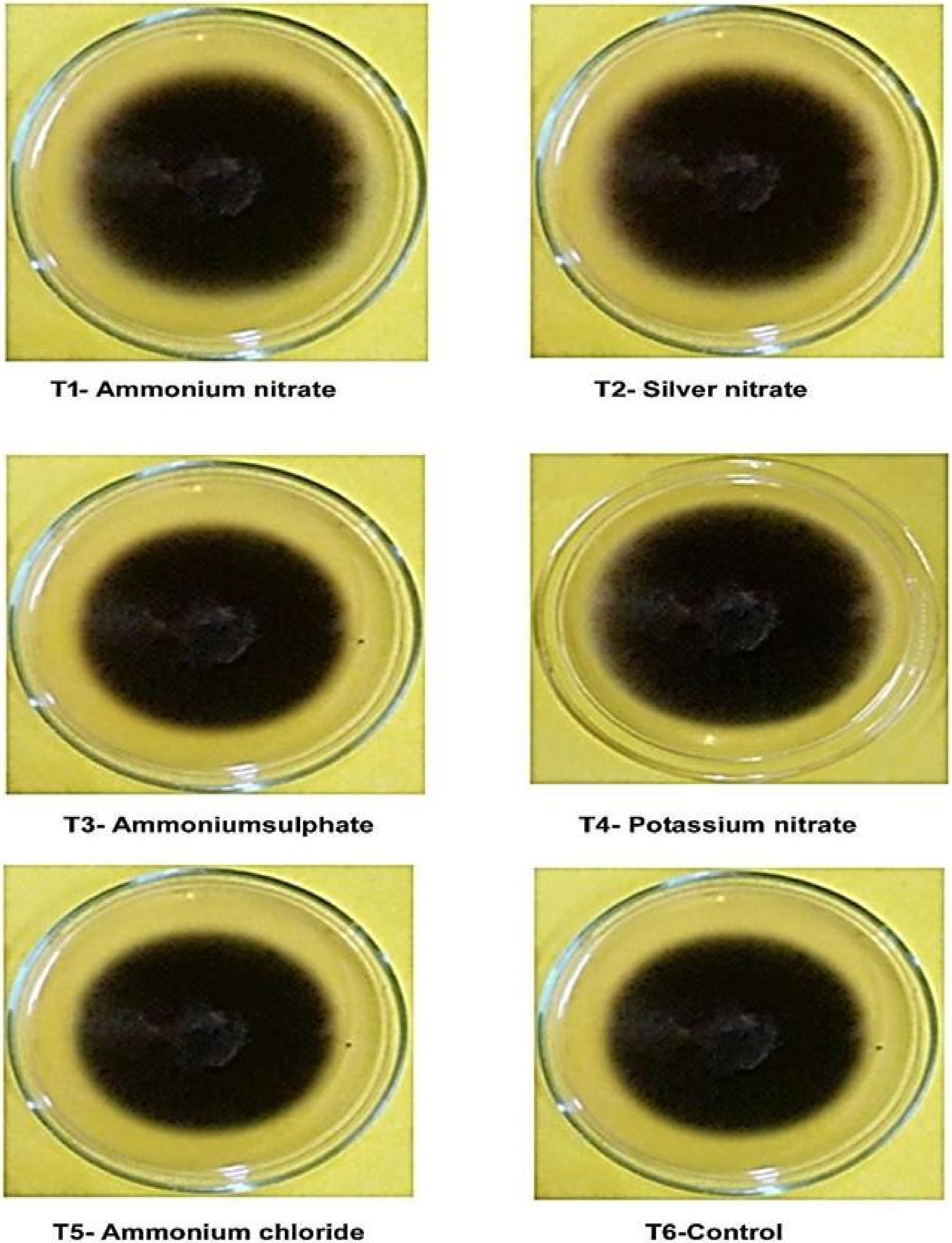


Plate 2: Growth of *Alternaria brassicae* under different nitrogen sources

Conclusion

Among the evaluated carbon sources, the highest mycelial growth was observed in Glucose (88.17 mm), followed closely by Sucrose (84.67 mm), Maltose (82.33 mm), Mannitol (82.17 mm), and Citric acid (73.83 mm). The control exhibited the least radial mycelial growth at 70.00 mm. Notably, excellent sporulation was recorded in Glucose and Maltose, with more than 30 conidia observed per microscopic field. In contrast, Citric acid displayed fair sporulation, yielding 10-20 conidia per microscopic field.

Five nitrogen sources were evaluated for their effects on the growth and sporulation of *Alternaria brassicae*. The maximum mycelial growth was recorded in Potassium nitrate (74.80 mm), followed by Ammonium sulfate (73.00 mm), Ammonium nitrate (70.00 mm), Silver nitrate (69.67 mm), and the control group (68.50 mm). The minimum radial mycelial growth was observed in Ammonium chloride (63.83 mm). More than 30 conidia per microscopic field were recorded in both Ammonium sulfate and Potassium nitrate, while silver nitrate showed fair sporulation, with 10-20 conidia per microscopic field.

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