**Estimation of different carbon and nitrogen sources against *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) causing wilt disease of cotton**

**Abstract**

Cotton (*Gossypium hirsutum* L.) is a major fiber crop that contributes significantly to global economic and social development. It is often referred to as "The White Gold" or "The King of Fibers". Cotton is a major cash crop in our country and belongs to the family Malvaceae. Cotton is a historically important commercial commodity, next to food grains, and serves as the primary raw material for the thriving textile industry. Cotton production, processing, textiles, and allied industries employ approximately 42 million people and sustain their livelihoods. In this present study, seven different carbon and nitrogen sources were tested for their suitability for the growth and sporulation of *F*.*oxysporum* f. sp. *vasinfectum* of cotton. Fusarium wilt caused by *F. oxysporum* f. sp. *vasinfectum* (FOV) is one of the most important and serious diseases. This disease is capable to cause tremendous loss to this crop in no time. Among the different carbon source, the maximum growth was observed in glucose (345.20mg), which was followed by galactose (306.15mg), mannitol (281.10mg), fructose (270.34mg), sucrose (268.16mg), starch (257.91mg) and lactose (172.10mg).While, the poor growth was observed in control (85.38mg). For sporulation category, it was found that the fungus showed abundant (++++) sporulation in glucose, galactose and mannitol. Among the different nitrogen source, the maximum growth was observed in potassium nitrate (310.13mg)which was followed by calcium nitrate (302.30mg), urea (298.37mg), sodium nitrate (288.58mg), ammonium nitrate (272.57mg), ammonium chloride (183.45mg), ammonium oxalate (162.30mg). While, the poor growth was observed in control (59.11mg). For sporulation category, it was found that the fungus showed abundant (++++) sporulation in potassium nitrate and sodium nitrate.

**Keywords**: Cotton, *Fusarium,* Carbon, Nitrogen, Growth, Sporulation.

**Introduction**

“Cotton is a globally important crop used for both its [natural fiber](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/natural-fibre) and seed. [Fusarium wilt](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fusarium-wilt), caused by the fungus [*Fusarium oxysporum*](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fusarium-oxysporum) f. sp. *vasinfectum*, is a major disease of cotton capable of causing significant economic losses. The fungus persists in soil as [chlamydospores](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/chlamydospore) and in association with the roots of susceptible, resistant and non-cotton hosts as well as in seed. Management of [Fusarium wilt](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fusarium-wilt) is difficult and most successfully achieved through the use of resistant cultivars and pathogen-free [cotton seed](https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/cottonseed)” (Cianchetta and Davis, 2015).

“In India, the productivity of cotton is very low due to many constraints including diseases. Diseases are inherent compounds of the agroecosystem that must be dealt with continuously and on a knowledge basis. Cotton is affected by various diseases caused by fungi, bacteria and viruses. The most common cotton diseases reported in India are wilt (*Fusarium oxysporum* f. sp. *vasinfectum*(G.F. Atk.) W.C. Snyder & H.N. Hansen), root rots (*Rhizoctonia bataticola* Taubenh.), verticillium wilt (*Verticilliumdahliae*Kleb.), anthracnose (*Colletotrichum gossypii* Southworth. or *C. capsici* (Syd.) Butler &Bisby), grey mildew (*Ramularia areola* G.F. Atk.), blackarm (*Xanthomonas campestris*pv. *malvacearum*(Pammel) Dowson), leaf blight (*Alternaria macrospora*Zimm), leaf curl (Cotton leaf curl virus), corynespora leaf blight caused by *Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei, boll rot and physiological disorders as para wilt, leaf reddening and sometimes leaf elongation etc. The bacterial blight is the widest spread and destructive disease reported to cause yield losses of about 10 to 30 per cent” (Kalpana *et al*., 2004 and Sandipan *et al*., 2022). “Losses due to alternaria leaf spot (26.6%), grey mildew (29.2%) and myrothecium leaf spot (29.1%) have been reported. Moreover, sometimes myrothecium leaf spot, caused by the fungus *Myrothecium roridum* Tode, was responsible for losses of 50 per cent in the town of Balsas in Maranhão and also been reported in the state of Mato Grosso. The symptoms of the disease can appear on the leaves and cotton bolls” (Suassuna *et al*., 2006). “Cotton boll rot can cause 20-30 per cent losses in productivity” (Iamamoto, 2007) and “a large number of pathogens were associated to cause the boll rot infection including external as well as internal pathogens” (Sandipan *et al*., 2022).

“Among them wilt, root rot and verticillium wilt are soil borne diseases of cotton and anthracnose, grey mildew, black arm and leaf blight are foliar diseases of cotton” (Ramod, 2016). Among these diseases, Fusarium wilt caused by *F. oxysporum* f. sp. *vasinfectum* (FOV) is one of the most important and serious diseases. It was the first vascular wilt described by Atkison in (1892) and “this disease is still causing enormous yield losses in several parts of the world and remains a threat to cotton production” (Feng *et al*., 2000). “The disease is responsible for serious losses to the crop in the central and western India on a large scale and on almost all the cultivated varieties of both *G. arboreum* and *G. herbaceum*, the two indigenous species, especially in black cotton soils of Maharashtra, Madhya Pradesh, Karnataka and Gujarat. At present, the most of cultivated cultivars are susceptible to wilt disease (*F. oxysporum* f. sp. *vasinfectum*) and caused 54-60 per cent yield loss” (Anonymous, 2003) “because of reduced stand, stunted growth, small balls and poor lint quality. Fusarium wilt of cotton (*Gossypium hirsutum* L. and *Gossypium barbadense* L.) caused by *Fusarium oxysporum* f. sp. *vasinfectum* W.C. Snyder & H.N. Hansen (FOV), is an important and widespread disease affecting nearly all cotton growing regions of the world. FOV is both seedborne and soilborne, and colonizes the roots and vascular system of susceptible cotton cultivars, causing root and vascular discoloration vascular wilt” (Chen *et al*., 1985, Hillocks, 1992, Patel *et al*., 2021a and Davis *et al*., 1996). “Discrete symptoms vary with pathogen genotype, inoculum density, cotton cultivar and plant age” (Hao *et al*., 2009).

“Symptoms of this wilt may appear at any stage of crop development (generally from 30 to 120 DAS), depending on inoculum density, temperature and host susceptibility. At very high inoculum density or the very beginning of infection, plants may be killed at the seedling stage itself. Usually, the first symptoms in the field appear 30-60 days after planting generally, on the onset of flowering. The pathogen colonizes in plant roots and grows systemically into the vascular tissues and proliferates within the xylem vessels eventually spreading throughout the plant. It grows out of the vascular tissues and after the death of the host, sporulates on crop residues. The disease can be recognized at the seedling stage with symptoms first appearing on the cotyledons as the darkening of veins, followed by peripheral chlorosis and necrosis before they drop. In older plants, the infection occurs as yellowing at the margin of one or more of the lower leaves. As the disease progresses within the plant more leaves develop chlorosis, which characteristically appears in patches between the main veins, the rest of the leaf remains green. Under optimal conditions, all the leaves of affected plants succumb and shed before the stem dries out” (Patel *et al*., 2021 and Sain *et al*., 2023). “There are six nominal races of FOV, 1, 2, 3, 4, 6 and 8 known to affect cotton worldwide” (Holmes*et al.*, 2009). Depending upon the serious losses to the cotton crop and therefore the nature of this disease is soil borne, which finds difficult to eliminate or will take time to abolish. And hence this aspect has been studied with the following aim.

**Material and Methods**

**SOURCE ON THE GROWTH OF *Fusarium oxysporum* f*.* sp. *vasinfectum***

All the glassware used in laboratory studies *viz*., test tubes, Petri plates and flasks were immersed in chromic acid solution (Potassium dichromate 80g, sulphuric acid 400ml and water 300ml) for 24hours, then thoroughly cleaned with tap water and finally rinsed with sterile water.

. The plasticwares were airdried and the glassware was sterilized in hot air oven at 160-180ºC for about 90min.

Isolations and purification were conduct under aseptic condition of laminar air flow cabinet. Before working under the hood, the working surface was uniformly sterilized using 70 per cent alcohol. The blades, forceps, inoculation loop *etc*. was sterilized by incineration on the flame of the spirit lamp.

Seven different carbon and nitrogen source were used to find out the most suitable one for the growth and sporulation of *F*. *oxysporum* f. sp. *vasinfectum.*

To study the effect of different carbon sources on the growth and sporulation of *F. oxysporum* f. sp. *vasinfectum*, seven different carbon sources were tested. Richard's medium (Potassium nitrate (KNO3): 10.0g, Magnesium sulphate (MgSO4.7H2O): 2.50g, Sucrose: 50.0g, Agar: 15.0g and Distilled water: 1000ml) with five per cent carbon source was used as basal medium. The effect of each carbon source was studied by replacing the sucrose from different carbon sources in Richard's medium. The quantity of carbon compounds was determined on the basis of their molecular weight so, as to provide equivalent amount of carbon (21.05g) as was present in 50.00g of in a litre of Richard's medium. The identical control was kept with a basal medium devoid of any carbon source after that 20ml of the medium was poured in each conical flask of 100ml and autoclaved at 121.6ºC at 15 psi for 20min. These were cooled to 45ºC. Then 5mm disc of the test fungus was cut with the help of sterilized cork borer from the margin of seven days old culture grown on PDA Petri plate (90mm). One disc was placed in the centre of each conical flask. Three repetitions of each medium were maintained at 27±2°C for seven days. After seven days of incubation period, dry mycelial weight and sporulation was recorded. The result was tabulated and the data were analysed statistically using complete randomized design (Singh *et al*., 2023).

To study the effect of various nitrogen sources on the growth and sporulation of the fungus, seven different nitrogen sources were tested in the manner similar to that of carbon sources. In this case, potassium nitrate of the Richard's medium was replaced by different nitrogen sources to provide an equivalent quantity of nitrogen (1.39g) as was present in 10.00g of potassium nitrate in a litre of Richard's medium after that 20ml of the media was poured in each conical flask of 100ml and autoclaved at 121.6ºC at 15 psi for 20min. These were cooled to 45ºC. Then 5mm disc of the test fungus was cut with the help of sterilized cork borer from the margin of seven days old culture grown on PDA Petri plate. One disc was placed in the centre of each conical flask. Three repetitions of each medium were maintained. After seven days of incubation period, dry mycelial weight and sporulation was recorded. The result was tabulated and the data were analysed statistically using complete randomized design.

**Table: 1 Carbon source tested for growth and sporulation of *F*. *oxysporum* f. sp. *vasinfectum***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr. No.** | **Carbon source** | **Empirical formulae** | **Molecular wt.** | **Per cent carbon content** | **Wt. added in one litre medium (g)** |
| **MONOSACCHARIDES** | | | | | |
| T1 | Fructose | C6H12O6 | 180.16 | 40 | 52.633 |
| T2 | Galactose | C6H12O6 | 180.16 | 40 | 52.633 |
| T3 | Glucose | C6H12O6 | 180.16 | 40 | 52.633 |
| **DISACCHARIDES** | | | | | |
| T4 | Lactose | C12H22O11.H2O | 360.32 | 40 | 50.00 |
| T5 | Sucrose | C12H22O11 | 342.30 | 42.11 | 50.00 |
| **SUGAR ALCOHOL** | | | | | |
| T6 | Mannitol | C6H14O6 | 182.18 | 39.56 | 52.633 |
| T7 | Starch | C6H10O5 | 162.14 | 51.81 | 47.369 |

**Design:** Completely Randomized Design

**Treatments:** 7

**Repetitions:** 3

**Location:** Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari, Gujarat.

**Table: 2 Nitrogen source tested for growth and sporulation of *F. oxysporum* f.sp. *vasinfectum***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr.**  **No.** | **Nitrogen sources** | **Empirical formulae** | **Molecular wt.** | **Percent nitrogen content** | **Wt. added in one litre medium(g)** |
| **ORGANIC** | | | | | |
| T1 | Urea | NH2CONH2 | 60.06 | 46.82 | 2.984 |
| **INORGANIC** | | | | | |
| 1.Nitrate nitrogen | | | | | |
| T2 | Calcium nitrate | Ca(NO3)2.4H2O | 236.16 | 11.86 | 8.1 |
| T3 | Potassium nitrate | KNO3 | 101.10 | 13.85 | 10.00 |
| T4 | Sodium nitrate | NaNO3 | 84.99 | 16.47 | 8.430 |
| 2.Ammonical nitrogen | | | | | |
| T5 | Ammonium chloride | NH4Cl | 53.49 | 26.17 | 5.310 |
| T6 | Ammonium nitrate | NH4NO3 | 80.04 | 35.00 | 3.950 |
| **ORGANIC AMMONICAL NITROGEN** | | | | | |
| T7 | Ammonium oxalate | (COONH4)H2O | 142.11 | 19.70 | 7.055 |

**Design:** Completely Randomized Design

**Treatments:** 7

**Repetitions:** 3

**Location:** Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari,Gujarat.

**Result and Discussion**

**SOURCE ON THE GROWTH OF *Fusarium oxysporum* f*.* sp. *vasinfectum***

In the present investigation different carbon and nitrogen sources were utilized to find out which are the best source for the growth of *F. oxysporum* f*.* sp. *vasinfectum*. For this purpose, seven different carbon sources from various groups of carbohydrates were investigated to determined that which carbon source is optimal for the growth of fungus.

For development and sporulation, the fungus used every carbon source examined however, the amount of use varied depending on the kind of carbon source. Maximum dry mycelium weight was observed in glucose with 345.20mg which, was followed by galactose with 306.15mg, mannitol with 281.10mg and fructose with 270.34mg, sucrose with 268.16mg. While, the least dry mycelium weight was observed in the control with 85.38mg. (Table: 3, Photograph: 1, Fig.: 1).

For sporulation category, it was found that the *F. oxysporum* f. sp. *vasinfectum* showed abundant sporulation in glucose, galactose and mannitol. Fungus produced good sporulation in fructose and sucrose. In starch, it produced moderate sporulation while, in lactose and control it produced scanty sporulation.

From this present investigation it can be concluded that glucose and galactose were the best carbon sources, where mannitol, fructose and sucrose were good carbon source for the growth of *F. oxysporum* f. sp. *vasinfectum*.

To know the best nitrogen source for the growth and sporulation of *F. oxysporum* f. sp. *vasinfectum,* seven different nitrogen sources were tested in this study. The fungus utilized every nitrogen source examined however, the amount of use varied depending on the kind of nitrogen source. Maximum dry mycelium weight was observed in potassium nitrate with310.13mg, which was followed by calcium nitrate with 302.30mg, urea with 298.37mg, sodium nitrate with 288.58mg and ammonium nitrate with 272.57mg while, the least dry mycelium observed in control with 59.11mg, which was followed by ammonium oxalate 162.30mg and ammonium chloride with 183.45mg(Table: 4, Photograph: 2, Fig.: 2).

It was found that the *F. oxysporum* f. sp. *vasinfectum* produced abundant sporulation in potassium nitrate and sodium nitrate. Fungus produced good sporulation in calcium nitrate and ammonium nitrate. In ammonium oxalate and urea, it produced moderate sporulation while, in control it produced scanty sporulation.

From these findings it can be concluded that potassium nitrate, calcium nitrate and urea were the best nitrogen source, where sodium nitrate and ammonium nitrate were good for growth of *F*. *oxysporum* f. sp. *vasinfectum.*

Khilare and Rafi (2011) revealed that nitrogen in the form of calcium nitrate, potassium nitrate and urea showed the maximum mycelium growth of *Fusarium oxysporum* f. sp. *ciceri* (>87.5 mm).

Malathi and Mohan (2012) conducted an experiment on the physiological and cultural variabilities among *Fusarium oxysporum* f. sp. *cepae* an isolate of onion. They found that the fungus grew the best on glucose among the different carbon sources.

Maitlo *et al.* (2017) studied that the sucrose and dextrose were the most suitable carbon sources for the growth of *Fusarium oxysporum* f. sp. *ciceris.* potassium nitrate and peptone are the best nitrogen sources for the growth of fungus.

Vasumathi and Devi (2020) reported that the glucose was the best carbon source for the growth of *Fusarium oxysporum* of groundnut crop. The fungal growth recorded with a mean mycelium diameter of 9.00cm and mycelium dry weight of 453.40mg, in the solid and liquid media, respectively.

Menge *et al*. (2021) conducted “an experiment on *Fusarium oxysporum* f. sp. *capsici* of chilli crop. The nutritional requirement of the test pathogen was carried by using the various carbon and nitrogen sources. All the seven carbon sources were significantly utilized by the pathogen. Among that, glucose and dextrose showed the maximum growth followed by lactose. From the seven nitrogen sources, potassium nitrate, calcium nitrate and urea were best for the growth of the test fungus”.

Kashif *et al*. (2022) observed “a physicochemical characterization of *Fusarium moniliforme* of maize crop. Fungus was tested on different carbon and nitrogen source for the growth and sporulation. Among them, glucose as a carbon source showed the highest fungal growth (11.62mm)”. Vikram *et al*. (2022) reported “among six carbon sources maximum mycelial growth was recorded in sucrose, starch, glucose, mannitol, maltose, and control and minimum was in lactose”.

Singh *et al*. (2023) studied “*in vitro* evaluation to determine the nutritional requirements of the linseed wilt fungus *Fusarium oxysporum* f. sp. *lini*. Maximum mycelial growth was found in fructose to the influence of different carbon sources on the development of the test pathogen. Fructose followed by D-glactose, Mannital and Glucose. All of the carbon sources used was superior to control. Potassium nitrate was determined to be the most suitable nitrogen source for growth of *F. oxysporum* f. sp. *lini* followed by ammonium acetate”.

**Table: 3 Growth and sporulation of *Fusarium oxysporum* f*.* sp. *vasinfectum* in Richard's medium supplemented with different**

**carbon sources after fifteen days of incubation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr.**  **No.** | **Carbon source** | **pH after autoclaving** | **pH of filtrate** | **Dry mycelial weight (mg)** | **Sporulation#** |
| 1 | Fructose | 5.6 | 6.6 | 270.34 | +++ |
| 2 | Galactose | 5.7 | 7.2 | 306.15 | ++++ |
| 3 | Glucose | 5.9 | 7.5 | 345.20 | ++++ |
| 4 | Lactose | 5.6 | 6.1 | 172.10 | + |
| 5 | Sucrose | 5.5 | 6.9 | 268.16 | +++ |
| 6 | Mannitol | 5.7 | 7.5 | 281.10 | ++++ |
| 7 | Starch | 5.7 | 7.2 | 257.91 | ++ |
| 8 | Control | 5.5 | 5.3 | 85.38 | + |
| SEm± | | | | 4.70 | - |
| CD at 5% | | | | 14.21 | - |
| CV% | | | | 3.27 | - |

#Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good and ++++ Abundant

**Table: 4 Growth and sporulation of *Fusarium oxysporum* f*.* sp. *vasinfectum* in Richard's medium supplemented with different**

**nitrogen sources after fifteen days of incubation**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Sr.**  **No.** | **Nitrogen source** | **pH after autoclaving** | **pH of filtrate** | **Dry mycelial weight (mg)** | **Sporulation**# |
| 1 | Urea | 6.3 | 6.9 | 298.37 | ++ |
| 2 | Calcium nitrate | 5.7 | 6.1 | 302.30 | +++ |
| 3 | Potassium nitrate | 5.6 | 7.4 | 310.13 | ++++ |
| 4 | Sodium nitrate | 5.7 | 7.2 | 288.58 | ++++ |
| 5 | Ammonium chloride | 5.6 | 5.7 | 183.45 | ++ |
| 6 | Ammonium nitrate | 5.4 | 6.1 | 272.57 | +++ |
| 7 | Ammonium oxalate | 5.8 | 6.0 | 162.30 | ++ |
| 8 | Control | 5.6 | 5.5 | 59.11 | + |
|  | SEm± | | | 3.19 | - |
|  | CD at 5% | | | 9.64 | - |
|  | CV% | | | 2.35 | - |

#Sporulation category: - Absent, + Scanty, ++ Moderate, +++ Good and ++++ Abundant

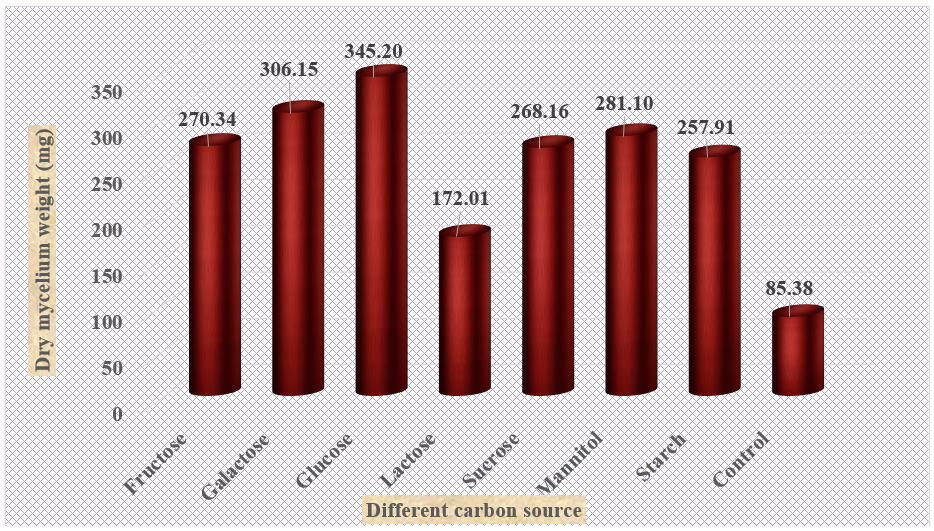
**Fig.: 2 Effect of different nitrogen source on the growthof *Fusarium oxysporum* f. sp*. vasinfectum***

**Photograph: 1 Effect of different carban source on the growth of *Fusarium oxysporum* f. sp*. vasinfectum***

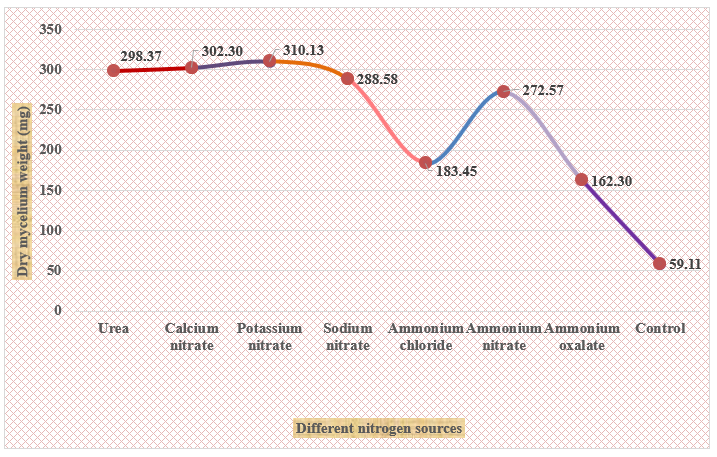


**Photograph: 2 Effect of different nitrogen source on the growth of *Fusarium oxysporum* f. sp*. vasinfectum***





**Fig.:1 Effect of different carbon source on the growth of *Fusarium oxysporum*f. sp*. Vasinfectum***



**Fig.: 2 Effect of different nitrogen source on the growth of *Fusarium oxysporum* f. sp*. vasinfectum***

**Conclusion**

In present experiment, under *in vitro* evaluation of different carbon and nitrogen source studied, In carbon source, the maximum growth was observed in glucose, which was followed by galactose, mannitol, fructose, sucrose, starch and lactose while, the poor growth was observed in control. For sporulation category, it was found that the fungus showed abundant sporulation in glucose, galactose and mannitol. In nitrogen source, the maximum growth was observed in potassium nitrate which was followed by calcium nitrate, urea, sodium nitrate, ammonium nitrate, ammonium chloride and ammonium oxalate while, the poor growth was observed in control. For sporulation category, it was found that the fungus showed abundant sporulation in potassium nitrate and sodium nitrate.

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2.

3.

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