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In vitro effect of nitrogen sources on growth of *Alternaria solani* and *Rhizoctonia solani*, causing diseases of tomato

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Abstract
Present *In Vitro* investigation was conducted to study of different nitrogen sources effect on growth (dry weight) of *Alternaria solani* and *Rhizoctonia solani* causing early blight and root rot of tomato at Department of Botany, Dr. Rafiq Zakaria College for Women, Aurangabad, Maharashtra, during experiment were used different nitrogen sources (0.25% Conc.) viz, Ammonium sulphate, Sodium Nitrite, Calcium Nitrate, Sodium Nitrate, Ammonium Chloride (NH_4Cl), Potassium Nitrate (KNO_3), Magnesium Nitrate $(NO_3)_2$. The effect nitrogen sources on growth of fungi were estimated in the term of biomass production. In Ammonium nitrate with 0.25% concentration showed that suitable source for the utilization nitrogen for growth of both *Alternaria solani* and *Rhizoctonia solani* mycelium with maximum dry weight 117 and 122 mg respectively on 10^{th} inoculation period. These two nitrogen sources may be involved in vegetative and reproductive growth of *Alternaria solani* and *Rhizoctonia solani*.

Keywords: *In vitro*, *Alternaria solani*, *Rhizoctonia solani*, dry weight in mg, nitrogen sources, tomato

1. Introduction
Tomato (*Lycopersicon esculentum* Mill) belongs to the family *Solanaceae* that originated in the Andian region of South America. It is one of the most popular and widely grown vegetable crops, in India and throughout the world. Among the vegetables tomato ranks next to potato in world acreage and ranks first among the processing crops. Tomato is grown for its edible fruits, which can be consumed either fresh or it can be processed to several products like puree, paste, soup, juices, ketchup, whole canned fruits etc.
Tomato plants interact with biotic and abiotic factors which have direct impact on production and productivity. Among different biotic factors diseases, the fungal disease early blight of tomato incited by *Alternaria Solani* (Ellis and Martin). Bose *et al* (1882) first time recorded Early blight of tomato caused by *Alternaria Solani* is of the most destructive disease incurring huge loss both at pre and post-harvest stages in tomato growing tracks in india (Prasad, 2004., Munde *et al*., 2013., Sahu *et a*l., 2013) [11, 10, 13]. C. D. Mayee *et al*. (1986) [9] reported that Symptoms of the disease are characterized by brown to dark brown colored necrotic spots. Severe infection of the early blight fungus leads to defoliation, drying off of twigs and premature fruit drop causing 50 to 86% losses in fruit yield (K.P. Akhtar *et al.* 2004) [1].
Alternaria Solani necessary different nutrient for their growth and development. In *In vitro* study, *Alternaria Solani* was isolate as pure culture in media containing different nitrogen sources with specific concentration for studies on dry weight growth. Different nitrogen sources containing media was fevor the dry weight growth and sporulation. It has shown that *Altenaria Solani* variation respond in the utilization of nitrogen from different nitrogen sources for dry weight growth. Hence, thorough knowledge on the influence of culture media containing nitrogen sources on growth of the fungus as well as sporulation of the fungus isolated from early blight and Root Rot infected tomato leaves is needed to be developed for suitable management strategies of the disease and may help in taxonomical and physiological study of the fungus.

2. Material and Methods
2.1 Collection of material
The present experiment conducted *In Vitro* at Department of Botany, Dr. Rafiq Zakaria College for Women, Aurangabad.

During this experiment, plant sample were collected from Tomato infected by early blight and root rot disease in growing track of Marathwada region.

2.2 Isolation of *Alternaria solani* and *Rhizoctonia solani* method followed by C.V. Chudhary in 2006 and Blancard, D. *et al*., 2012.

Pathogen was isolated from infected plant parts by tissue isolation technique on Potato Dextrose Agar (PDA) medium. Diseased parts were cut into small pieces with the help of sterilized blade. Pieces were washed with sterilized distilled water and disinfected with 1 per cent $HgCl_2$ solution for 10 seconds. Thus, obtained disinfected tissues were immediately washed thrice with sterilized distilled water and aseptically transferred on PDA plates. Inoculated Petri plates were incubated at room temperature (27±2 °C). The obtained culture was purified by using hyphal tip culture method, and maintained on same medium for the further investigations.

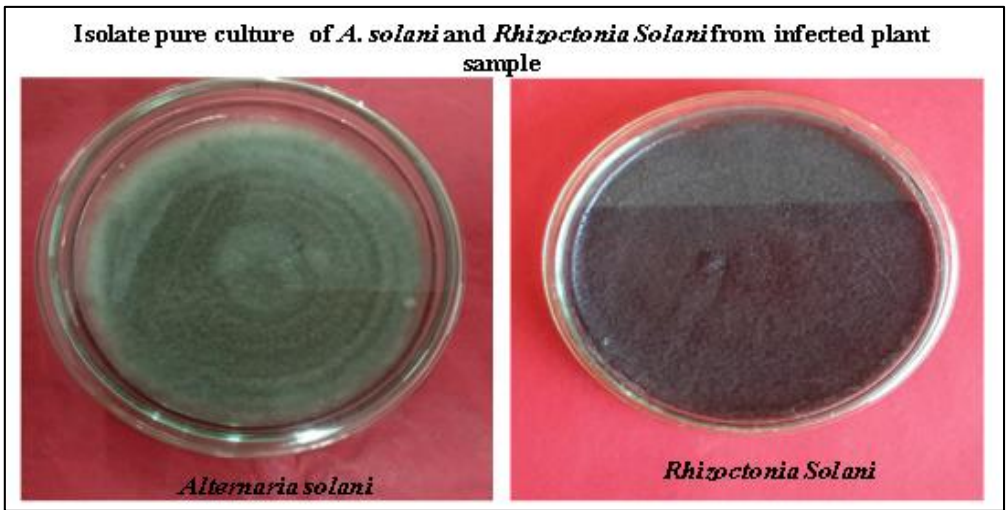
2.3 Inoculation of *Alternaria solani* and *Rhizoctonia solani* on media containing different nitrogen sources method followed by Arunakumara *et al*., 2015

Inoculation of Pathogen on Various nitrogen sources were incorporated molecular weight in Potato Dextrose liquid medium. The quantity of nitrogen required in each case was determined on the basis of their so as to provide equivalent amount of nitrogen as that of potassium nitrate present in the basal medium. The nitrogen sources were Ammonium sulphate, Sodium Nitrite, Calcium Nitrate, Sodium Nitrate, Ammonium Chloride (NH_4CL), Potassium Nitrate (KNO_3), Magnesium Nitrate $(NO_3)_2$. All the above nitrogen sources were mixed thoroughly and the pH of medium was adjusted to seven by using 0.1 N sodium hydroxideor 0.1 N hydrochloric acid. 50 ml of each of the medium was taken in 100 ml flasks, sterilized and then inoculated with 4 mm discs borer taken from 10 days old culture of *A. solani and R.Solani* and incubated at 27±1°C for 10 days. Three replications were maintained for each treatment. According to H. S. Nagaraj Rao *et al*., 1964 [7] to Dry weights of the mycelium were estimated after filtering, washing and drying of the harvested mats.

3. Results and Discussion

This experiment was conducted to study the effect of nitrogen sources on the growth of *A. solani* and *Rhictonia Solani.*

3.1 Isolate of *A. solani* and *Rhictonia Solani* from infected plant sample

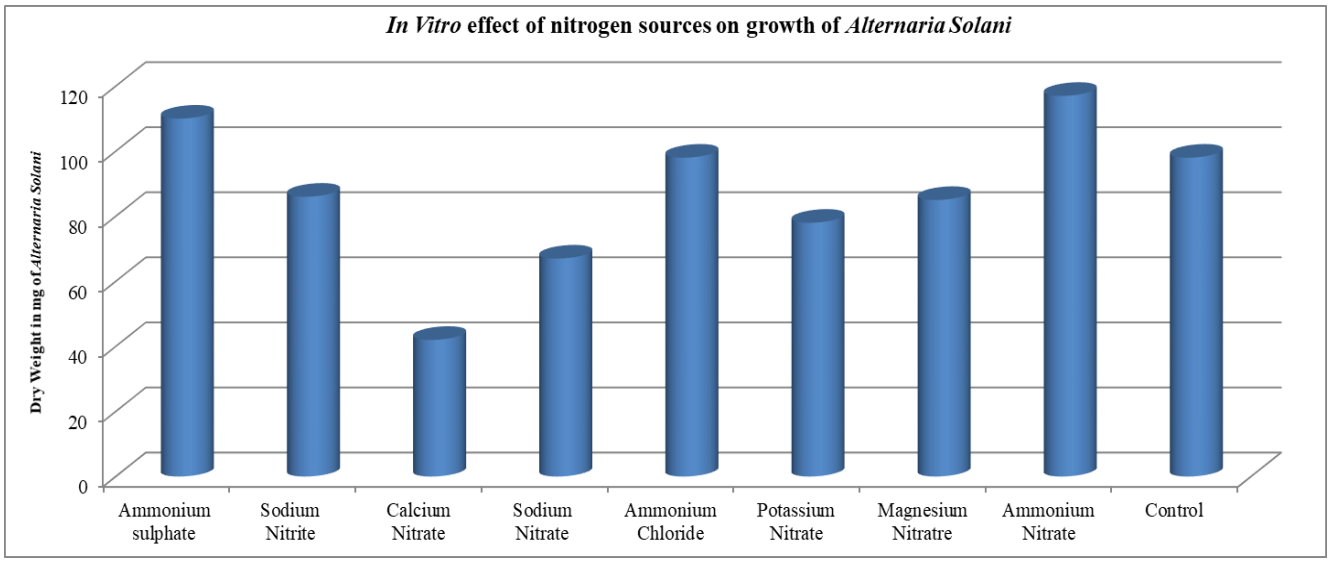


3.2 *In vitro* effect of nitrogen sources on the growth of *Alternaria Solani*

Utilization of eight different nitrogen sources by *Alternaria Solani* was studies in this experiment. The mycelium dry weight was recorded after 10th day's inoculation of the *Alternaria Solani* (Table. 1). Among the nitrogen sources, ammonium nitrate utilized by *Alternaria Solani* for producing maximum dry weight of mycelium (117 mg) and followed by Ammonium Sulphate (110 mg), Ammonium Chloride (98 mg), Control (98 mg), Sodium nitrate (86 mg), Magnesium Nitrate and least utilized nitrogen sources was Potassium Nitrate (78 Mg) Calcium Nitrate (42 mg). this results revealed that all nitrogen sources are not equally good for the growth of *Alternaria Solani* (K. T. Arunakumara *et al*., 2015). Also among all nitrogen sources tested, Ammonium Nitrate and Ammonium Sulphate supported the maximum growth of *Alternaria solani* and also found to be best source of nitrogen.

Table 1: *In Vitro* effect of nitrogen sources on growth of *Alternaria solani*

Sr No.	Nitrogen sources/0.25% Conc.	Dry weight in mg
1	Ammonium sulphate	110
2	Sodium Nitrite	86
3	Calcium Nitrate	42
4	Sodium Nitrate	67
5	Ammonium Chloride	98
6	Potassium Nitrate	78
7	Magnesium Nitratre	85
8	Ammonium Nitrate	117
9	Control	98

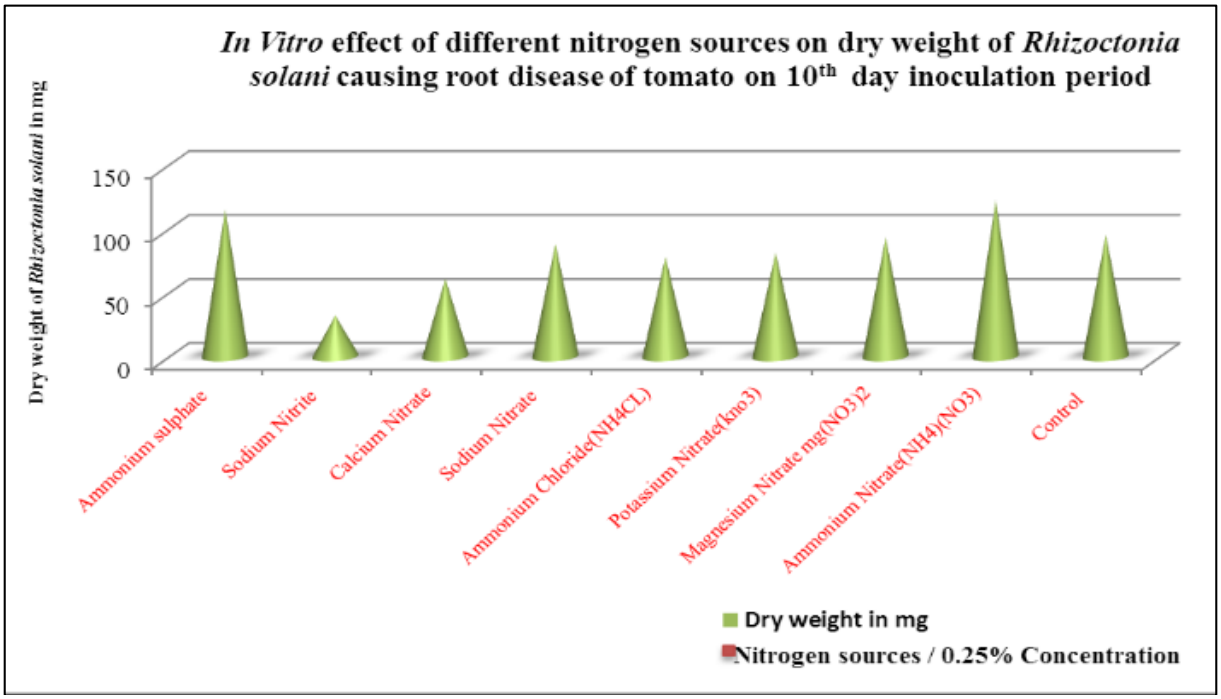


3.3 *In vitro* effect of different nitrogen sources on dry weight o*f Rhizoctonia solani* - causing root rot diseases of tomato on 10th day of inoculation period

Utilization of nitrogen by *Rhizoctonia Solani* fungus was studied with different eight nitrogen sources Ammonium sulphate, Sodium Nitrite, Calcium Nitrate, Sodium Nitrate, Ammonium Chloride(NH_4CL), Potassium Nitrate (KNO_3), Magnesium Nitrate $(NO_3)_2$. The effect on growth of fungus was estimated in the term of biomass production. Results showed in that ammonium Nitrate with 0.25% concentration was the best source of nitrogen for growth of *Rhizoctonia Solani* mycelium and found maximum dry weight (122mg) of *Rhizoctonia Solani* fungus on the 10th days of inoculation period followed by ammonium sulphate (155 mg), control (95 mg), magnesium nitrate (93), sodium Nitrate (88) and sodium nitrate was poor source of nitrogen for growth of *Rhizoctonia Solani* mycelium (Table. 2). Vibha and Sinha (2005) also reported that the potassium nitrate as nitrogen source is most appropriate for growth and sporulation of the different cellulolytic fungi, *C. lunata*, *Trichoderma harzianum*, *Penicillium citrinum*, *Aspergillus flavus*. Tolba and salama (1960), Beever and Bollard (1970) [12], Midgley *et.al* (2006) [5] observed that fungus can grow in vitro on PDA broth and it supports the good growth of fungus. The factor influence the growth of fungus that have been studied here were utilization of carbon and nitrogen sources which was in in confirmly with earlier report (Israel & Ali, 1964; Bakshi, 1974; Smith and Read, 1997; Midgley *et al*., 2006) [5].

Table 2: *In Vitro* effect of different nitrogen sources on dry weight o*f Rhizoctonia solani* - causing root rot diseases of tomato on 10th day of inoculation period

Sr No.	Nitrogen sources/0.25%concentrtion	Dry weight in mg
1	Ammonium sulphate	115
2	Sodium Nitrite	32
3	Calcium Nitrate	61
4	Sodium Nitrate	88
5	Ammonium Chloride(NH4CL)	78
6	Potassium Nitrate(kno3)	81
7	Magnesium Nitrate mg(NO3)2	93
8	Ammonium Nitrate(NH4)(NO3)	122
9	Control	95



Rhizoctonia Solani Inoculation on Potato Dextrose medium containing Nitrogen Sources

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