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RESEARCH ARTICLE

THE STUDY OF NITROGEN CONCENTRATION IN SOIL OF *Azadirachta Indica* AND ITS EFFECT ON GROWTH OF *Fusarium Oxysporum*

[1]Jyoti Sharma, [2]Meenakshi Yadav, [*,3]Gaurav Kumar, [4]Maneesh Gupta, [5]Jayanand and [6]Rai, D.V.

[1.2.4.5]Centre for Biotechnology, Faculty of Biological Engineering, Shobhit University, Meerut
[3.6]Department of Agriculture Science and Engineering, Shobhit University, Gangoh, Saharanpur, UP

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ABSTRACT

An evaluation of nitrogen in soil can have a profound impact on nutrient availability as well as physical and chemical properties of soil. Although chemicals in the form of pesticide and fertilizers may have undesirable impact on the quality of plants as well as micro-organism but the effect of nitrogen concentration is poorly known of *Fusarium oxysporum*. The objective of present study was to explore the effect of nitrogen concentration in soil of medicinal plants (*Azadirachta indica)* on soil borne *in vitro Fusarium oxysporum*. The present work was performed under the Soil sample which was taken to a depth of 20 cm at different sites of *Azadirachta indica*. Snell & Snell methodology was used for determination of Nitrogen concentration. Result has showed the concentration of nitrogen present was 0.34mg per gram soil sample. We conclude that soil of *Azadirachta indica* contain low nitrogen concentration which directly related to the growth of *Fusarium oxysporum*. Higher nitrogen concentration favours profuse proliferation in case of *Fusarium oxysporum*.

INTRODUCTION

The plant rhizosphere is an important ecological environment in soil for plant microbe interactions. These inter-actions with plants could be beneficial, neutral or with detrimental effects resulting in plant diseases[14,1,15]. The pathogenic microorganisms cause various plant diseases that usually weaken or destroy plant tissues and reduce crop yields varying from 25% to 100%. Root diseases are estimated to cause 10-15% yield losses annually in the world. These plant diseases are mostly controlled by application of chemical pesticides. However, the widespread use of chemical pesticides has been a subject of public concern due to potential harmful effects on the environment, their undesirable effect on non-target organisms and the possible carcinogenicity effect of some chemicals. Pathogenic strains of *Fusarium oxysporum* have been studied for more than 100 years. It have a broad host range, individual isolates usually cause disease only on a narrow range of plant species. Fusarium wilt is an important disease provoked by Fusarium oxysporum schlecht emends, synd & Hans. f.sp. ciceri causing considerable damage to the crop. The wilt pathogen is soil-borne and survives in infected seedling and dead plant debris in soil[7]. Species, as defined by Snyder and Hansen[3], has been widely accepted for more than 50 years[3,11]. It is a saprophytic fungi and it degrade lignin[13,18] & complex carbohydrates[6,17], associated with soil debris. *Fusarium oxysporum* is primarily spread short distances by irrigation water and contaminated farm equipments. More than 50% of isolates of the known Fusarium species are toxicogenic and produce deleterious secondary metabolites[10]. They damage host plants through penetration of hyphae into the host vascular tissues, secretion of hydrolytic enzymes related to pathogenesis, mycotoxin production and cellular apoptosis of host plant cells in the progression of the infection[3,12]. *Fusarium oxysporum* had been destroyed, resulting in the control of disease caused by pathogen but disease control without a reduction in pathogen have been studied by several researchers. Organic amendments may influence disease because of their effect on the availability of nutrients to the pathogen, but an effect on the host should be considered. Nitrogen has a greater effect than any other element on individual soil fungi and, in general, disease severity increase as rate of nitrogen increases. The present investigation was conducted to study the effect of soil nitrogen of *Azadirachta indica* on mycelial growth of *Fusarium oxysporum.*

***Corresponding author: Gaurav Kumar,** Department of Agriculture Science and Engineering, Shobhit University, Gangoh, Saharanpur, UP.*

MATERIAL AND METHODS

Soil sample collection and preparation

The soil sample collected should be representative of the area sampled. The soil sample was collected, taken to a depth of 20 cm of *Azadirachta indica* at the region of Dulehra nearby Meerut. A sample brought to laboratory, was spread out on an aluminium tray, plastic or thick brown paper. Coarse concentrations, stones and pieces of roots, leaves and other undecomposed organic residues are removed. Large lumps of moist soils are broken by hand. After air drying soil samples are crushed gently in pestle and mortar and sieved through a 2 mm sieve. Crushing was continued until the soil retained on the sieve contains no aggregates. The material larger than 2 mm is discarded.

Estimation of Nitrogen

To determine Nitrogen concentration in soil of *Azadirachta indica* Snell & Snell methodology (1949)[16] was used. In this method, the soil was homogenized with Hydrogen Peroxide and Sulphuric acids making use of heat of dilution of Sulphuric acid. Total nitrogen

analysis in soil sample was performed by using UV-VIS Spectro-photometer. One gm of soil sample was taken in 250 ml conical flask. Homogenized the soil sample in 5ml conc. Sulphuric acid (H_2SO_4) and 2ml of 30 % Hydrogen Peroxide (H_2O_2). For digestion of the soil sample, placed it in sand bath for 30 min, cool it and then again add 3ml of 30% Hydrogen Peroxide. Placed the sample again in sand bath till the digest material was clear (1hr.). Cool the solution and make up the final volume upto 10ml by distilled water. For sample analysis take 1ml of digested part, 3ml of Nessler's reagent and 1ml of distilled water in a test tube. The reading of samples, standard and blank was noted at 424nm. Nitrogen concentration were evaluated with respect to standard solution of 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 ml concentration from sucrose standard stock solution. The concentration of nitrogen were calculated by equation obtained from the standard curve (R^2=0.98).

Growth characters on solid media

The growth characters of *Fusarium oxysporum* were studied on three different solid media *viz.*, potato dextrose agar, Richards's agar, Sabouraud's agar. All the media were sterilized at 1.1 kg/cm^2 pressure for 15 min. To carryout the study, 20 ml of each of the medium was poured in 90 mm Petriplates. Such Petriplates were inoculated with 5 mm disc cut from periphery of actively growing culture and incubated at 27±1ºC. Each treatment was performed ten times. Observations were taken when the fungus covered complete Petriplate in any one of the media. The colony diameter was recorded. The fungus colony colour, margin and Sporulation were also recorded.

Effect of Nitrogen on Growth of *Fusarium oxysporum*

The nitrogen requirement of the fungi was studied by replacing potassium nitrate of Richard's medium for *Fusarium oxysporum* with different nitrogen sources[4]. The nitrogen sources used in the present study were *viz.*, ammonium sulphate, and ammonium nitrate. The quantity of the nitrogen sources was determined on the basis of molecular weight so as to provide equivalent amount of nitrogen as was as potassium nitrate present in the basal medium as described by Lily and Barnet (1951)[9]. Further they were than sterilized at 1.1 Kg/cm^2 pressure for 15 minutes. From three days old culture of the fungus, discs were cut and inoculated and incubated at 27±1ºC for seven days. Dry mycelial weight of the fungus was measused to check the growth of *Fusarium oxysporum.*

RESULT AND DISCUSSION

Effect of Solid Media on Growth of *Fusarium oxysporum*

The effect of 3 different media on the growth of the fungus was significant. The radial growth of the fungi was measured when the maximum growth was attained in any of the media tested. The maximum radial growth was observed in potato dextrose agar (86.00 mm) after seven days of incubation which was significantly superior over other media. This was followed by Richard's agar (84.0 mm). Minimum radial growth was observed in Sabouraud's dextrose agar (71.0 mm) (Table 1).

Table 1. Growth of *Fusarium oxysporum* on different solid media

Medium	Mean Colony diameter (mm) (SD< +_ 0.01)
PDA	86.0
Richard's agar	84.0
SDA	71.0

Growth characters of *F. oxysporum* studied in different solid media indicated that potato dextrose agar, Richards's agar supported maximum growth of fungal colony. Jamaria *et al.*, in 1972 describe the nutritional requirement of *Fusarium oxysporum.* They also reported the maximum growth of *F. oxysporum* on PDA[8]. Anjaneya Reddy (2002) observed maximum growth of *F. udum* on Richard's agar and potato dextrose agar. Margin of *Fuzarium* colony was irregular in potato dextrose agar and Richards's agar[2]. Mycelium was whitish in all the media except in case of potato dextrose agar and Sabouraud's agar where mycelium was pink. Sporulation was abundant in all the respective media (Table 2).

Table 2. Cultural characters of *F. oxysporum* on different solid media after seven days of incubation

S.No	Media	Growth characters	Sporulation
1.	PDA	Pink cottony and pluffy growth, irregular margin	+++
2.	Richard's agar	white cottony and pluffy growth, irregular margin	+++
3.	SDA	pink cottony growth of mycelium.	++

+++: Good sporulation (more than fifty spores per microscopic field)
++ : Moderate sporulation (30-50 spores per microscopic field)

Estimation of Nitrogen concentration & its Utilization

Nitrogen concentration in soil of *Azadirachta indica* was determined by Shell & Snell method. 0.34 mg nitrogen was present in per gram soil of *Azadirachta indica*. The effect of different nitrogen compounds on the growth of the fungus was significant. Maximum growth of 212 mg was recorded when ammonium nitrate was used as a source of nitrogen. Least mean dry mycelial weight 172mg was observed in case of ammonium sulphate (Table 3).

Table 3. Effect of Nitrogen source on growth of *Fusarium oxysporum*

Nitrogen source	Mean Dry Mycelial weight (mg)
Ammonium nitrate	212
Ammonium sulphate	172

Conclusion

The study demonstrates the maximum growth of *Fusarium oxysporum* on PDA (86 mm) followed by Richard's medium (84 mm). Among the nitrogen sources tested, ammonium nitrate was found to be the best (212mg) suitable for fungal growth. It was observed that lowering the concentration of nitrogen suppressed the growth of *Fusarium oxysporum*. We conclude that soil of *Azadirachta indica* contain low nitrogen concentration (0.34mg per gram soil) which is directly related to the growth of *Fusarium oxysporum.* Our study suggested that *Azadirachta indica* soil inhibit the growth of *Fusarium oxysporum* so it is fungi toxic and it can be implemented as a part of a sustainable integrated nutrient and pest management strategy in future.

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