Nitrogen Metabolism of Groundnut (*Arachis hypogeae* L.)Plants Inoculated with *Sclerotium rolfsii*

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Abstract— *Sclerotium rolfsii* is a ubiquitous soil-borne fungal pathogen known to cause disease on worldwide range of agricultural and horticultural crops. In spite of economic loss caused by this pathogen, very few reports were available on this aspect; hence the present study was under taken to study the nitrogen metabolism in *Sclerotium rolfsii* inoculated groundnut plants. Inoculation of *Sclerotium rolfsii* to groundnut plants influenced the various nitrogen fractions (total, protein, soluble and amino nitrogen) at different stages of disease development. All the nitrogen fractions were increased with the progression of the disease development and age of the plant.
Index Terms: *Sclerotium rolfsii*, groundnut, nitrogen fractions, stem rot

1 INTRODUCTION

Sclerotium rolfsii is a ubiquitous soil-borne fungal pathogen known to cause disease on worldwide range of agricultural and horticultural crops [3]. It infects more than 500 plant species in 100 families throughout the world [1, 6]. Most *S.rolfsii* diseases have been reported on dicotyledonous hosts and monocotyledonous species are also being infected, indicating the wide host range of parasitism of *S.rolfsii.*

It is a facultative parasite and survives in the soil mainly as sclerotia as a protective structure, which represents the main source of inoculums and remain viable for several years [4, 10]. *S. rolfsii* causes severe damage during any stage of crop growth [6] and attacks all parts of the plant but stem infection is the most common and serious. The first symptom is sudden wilting of the branch with the stem near the soil level is the most point of attack and a white coating of mycelium appears. Sclerotia of mustard seed size appear on the infected area at later stages. About 85% of yield loss due to the *S. rolfsii* has been reported from India. Keeping in view the losses caused by this fungus, the aim of the present investigation therefore was to evaluate the biochemical characterization of the *S.rolfsii.*

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2 MATERIALS AND METHODS

2.1 Isolation of pathogen:

The culture of *Sclerotium rolfsii* was isolated from the plants showing stem rot or southern blight symptoms of groundnut from the area of Sadhanavaripalem, Chittoor District in Kharif season on PDA. The pure culture of the fungus was obtained and maintained on PDA for further study. The stock culture was maintained on PDA slants in a refrigerator and subcultured every two months.

2.2 Disease development:

a) Method of raising plants: High quality seed material (average 95% germination) of groundnut variety TMV-2 was obtained from the local Agricultural Research. Sound seeds were surface sterilized with 0.1% mercuric chloride for 2-3 min followed by repeated washings with sterile water and sown in sterilized soil contained in seed pans. The seeds germinated and emerge in 4-5 days. One week old seedlings were used for inoculation purposes.

b) Inoculation:

Ten days old oatmeal-sand culture of *S. rolfsii* was thoroughly mixed with sterilized soil at 10%. This inoculum-soil mixture was then distributed in 12" diameter earthenware pots and left undistributed for two days. After this period, one week old seedlings grown in seed pans were lifted carefully without causing much damage to the root system and transplanted into the pots. They were watered on alternate days and kept in an open atmosphere.

c) Disease indexing: The plants were periodically examined for the progress of the disease. Samples were collected at random from four pots each time at '0' hours, 2

days, 5 days, 9 days and 11 days after inoculation. Almost all the seedlings collapse by 11 days after transplantation. The progress of disease in the hypocotyls of the seedlings could be differentiated into the following five fairly distinct stages, on the basis of lesion development.

Stage 1 : (0 hours, i.e. immediately after inoculation): Healthy seedlings

Stage 2 : (2 days after inoculation): The early or young phase, characterized by water-soaked appearance of invaded portions of hypocotyls which remained almost colourless or were light brown in colour.

Stage 3 : (5 days after inoculation): The intermediate stage, in which the lesion surface become brown to dark brown in colour.

Stage 4 : (9 days after inoculation): Well developed, dark necrotic lesions often girdling hypocotyls. This marks the final stage in lesion maturation. The lesions also show sunken appearance.

Stage 5 : (11 days after inoculation): Characterized by dry appearance of the lesion surface. Downward destruction or rotting of the tap root occurs and then the seedlings wilt and die.

Symptoms characteristic of each of the above stages of lesion maturation are shown in Figure 1.

Fig 1: Groundnut seedlings inoculated with *S. rolfsii*. Stage-1 (immediately after inoculation), 2, 3, 4 & 5 of disease development on stems until the maturation of lesions.

1 2 3 4 5

Stages

Estimation of Nitrogen fractions:

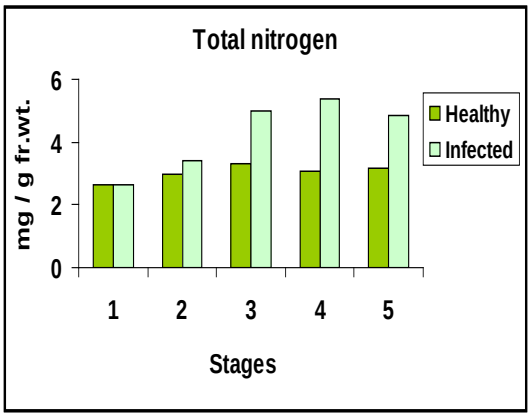
About one gram of fresh plant material both healthy and infected were chopped into small pieces, extracted with 80% boiling alcohol, ground in a porcelain mortar and reextracted. The extracts were pooled, centrifuged and the supernatant was used for the estimation of nitrogen fractions.

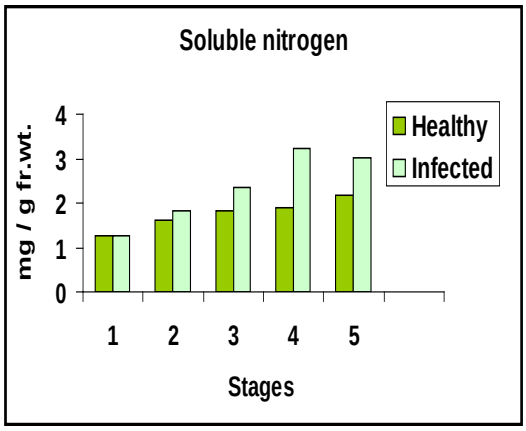
Total nitrogen was estimated according to the method of Markham [8] and the protein nitrogen by that of Thimann and Loos [16]. Soluble nitrogen fraction was then calculated by subtracting protein fraction from total nitrogen. Amino nitrogen in the ethanol extract was determined by the ninhydrin method of Moore and Stein [9].

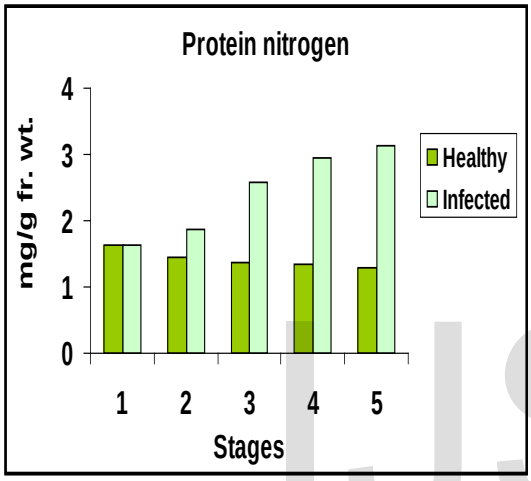
3 Results and Discussion

Various nitrogen fractions (total, protein, soluble and amino nitrogen) in both healthy and *S. rolfsii* infected groundnut hypocotyls were estimated at different stages of disease development and the results are summarized in Fig. 2.

In healthy plants, the total nitrogen gradually increased to a small extent during the sampling period. The hypocotyls of inoculated plants contained higher concentrations of total nitrogen than the healthy plants and the rate of increase was also higher. There was a decrease in the quantity of protein nitrogen in healthy hypocotyls with advancing age. The hypocotyls of infected plants, on the other hand, showed a significant increase in the quantity of protein nitrogen.







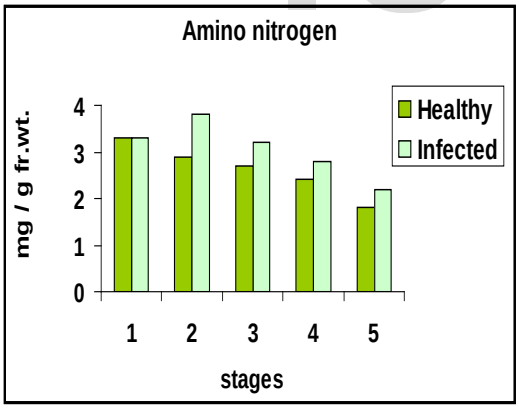


Fig.2: Effect of *S. rolfsii* infection on nitrogen fractions (total, soluble, protein & amino nitrogen) of groundnut hypocotyls at different stage of disease development.

The soluble nitrogen contents of the hypocotyls of healthy as well as infected plants gradually increased during the experimental period. There was no significant alteration in the soluble nitrogen content of the hypocotyls as a consequence of infection. In healthy hypocotyls the amino nitrogen decreased gradually with advancing age. On the other hand, infection resulted in higher quantities of amino nitrogen at all stages of disease development.

The nitrogen compounds received attention in host-pathogen interaction. In the present investigation infected tissues showed increased nitrogen content during the course of disease development. Several workers have noticed an accumulation of nitrogen in infected tissues in different host-pathogen interactions [14, 2, 11, 7].

Enhanced nitrogen content may reduce the toxicity of phenolic compounds [5]. The accumulation of amino nitrogen in wheat may be due to release of free amino acids from proteins of plant cells by the action of proteolytic enzymes of the pathogen.

S. rolfsii infection results in a marked influence on the free amino acid pool of groundnut hypocotyls. It appears that free amino acids are being utilized by the pathogen because of the quantity of most of them decreased with the progress of the disease. With the consumption of most of the carbohydrates the parasite may use amino acids as respiratory substrates. The concentration of a number of amino acids was either reduced in response to infection or remained unchanged. The increase in amino acids or the synthesis of new amino acids may be due to an interference of host metabolism by the pathogen or due to host-pathogen interaction. Qualitative increase in certain amino acids in the host tissues by infection may either be due to de novo synthesis by the host [12] or proteolysis of certain host tissue proteins, since decreased tissues often show higher proteolytic activity than healthy tissues [13]. The effect of infection on protein amino acid pool has been studied by several workers [15, 17] and reported notable increase of protein amino acids in infected tissues. This may be due to the translocation of amino acids from other parts of the host to the infected tissues (15, 13] or due to increase in amino acid synthesis as well as protein synthesis [15].

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