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Anthracnose disease caused by *Colletotrichum dematium* affected CO_2 assimilation, thylakoid electron transport and other related photosynthetic traits in groundnut (*Arachis hypogaea*)

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Abstract
Colletotrichum is one of the most important plant pathogen, causing economically important disease anthracnose in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits. Anthracnose disease (caused by *Colletotrichum dematium*) of groundnut (*Arachis hypogaea*) has become a serious problem in recent years in central India. The disease is characterized by leaf spotting resulting in 'shot hole' symptoms and finally defoliation, which affects the yield greatly. Infection of leaves directly hampered the photosynthetic machinery and intercellular mechanisms of the plant. Therefore, it is necessary to understand the severity of the disease and factors affecting under diseased conditions, which will help in devising suitable and effective management practices for proper yield. In India, research work on important aspects of the anthracnose disease of groundnut has not been done systematically. Information on crop photosynthetic traits under diseased conditions and related strategies to overcome yield loss is lacking. The purpose of this research was to understand the effects of anthracnose disease on some important photosynthetic traits such as rate of CO_2 assimilation, thylakoid electron transport, water use efficiency and stomatal conductance etc.

Keywords: anthracnose, CO_2 assimilation, grain yield, stomatal conductance, water use efficiency

Introduction
Groundnut (*Arachis hypogaea*) is one of the most important oil yielding crop as well as used as dry fruits, grown mainly in the Asian countries. India is the largest producer of groundnut in the world. The average yield of groundnut is approximately 745 kg/hac[1]. One of the important factors contributing to low yield is disease attack. More than 55 pathogens including viruses, fungus and nematodes have been reported to affect groundnut production. Anthracnose disease is a pre harvest disease caused by the fungus *Colletotrichum dematium* which develops under humid condition. The disease has been reported from all major bean crops growing regions of India in mild to severe form and in tropical and subtropical areas [1]; it causes considerable damage by reducing seed quality and yield. The disease causes qualitative as well as quantitative losses.

The disease process that is a result of the interaction between a pathogen and its host leads to changes in several physiological processes of the host plant, including dark respiration, photosynthesis, translocation of water and nutrients, transpiration, and regulation of growth [2]. Fungal infection generally reduces the photosynthetic rate by decreasing functional leaf area and reducing the photosynthetic efficiency of the remaining green leaf area [3, 4]. Dark respiration of leaves usually rises after infection in consequence of increased metabolic activity of cells of the diseased leaf [5, 6]. The transpiration rate can go up or down during the infection process, depending on the pathosystem[7]. Water loss from an infected leaf can increase due to destruction of part of the leaf cuticle, increased permeability of leaf cell membranes, or inhibition of stomatal closure. Reduction of transpiration may result from stomatal closure, reduction of air space by hyphae or hypertrophy of mesophyll cells, obstruction of conducting tissue and stomata, and defoliation. Although it is well known that plant pathogens alter the physiological processes of their hosts, comparative studies involving

biotrophic, hemibiotrophic and necrotrophic pathogens and their changes in the host physiological processes are not very common and are made at a speciec stage of disease development [7]. The relative photosynthetic rate per unit of wheat leaf area were smaller for rust and powdery mildew, caused biotrophic pathogens, than for Septoria leaf blotch, caused by a necrotrophic pathogen. The greatest impact on photosynthesis of the necrotrophic pathogens could be explained by the secretion of enzymes or phytotoxic compounds that diffuse to uncolonized portions of the leaf [7]. Necrotrophic pathogens that cause vein infection can induce a systemic stomatal closure in green areas of the leaf [8], reducing the photosynthetic rate of the whole leaf considerably even when disease severity is low. For biotrophic pathogens, however, the greatest decline in photosynthesis occurs in fungal invaded regions of the leaf or in the areas adjacent to the fungal mycelium [9]. The reduction in the rate of transpiration of plants infected by necrotrophic pathogens was proportional to the diseased area [7]. The destruction of cells was directly related to the presence of the fungus. Plants with rust, however, had a decrease in transpiration that was proportionally smaller than the corresponding reduction of healthy leaf area due to disease. At low levels of rust infection, the transpiration rate exceeded that of healthy leaves. This has been attributed to the epidermal rupture after pustule formation.

The methods commonly used for in vivo measurements of photosynthesis are gas exchange and chlorophyll fluorescence emission [10]. Variables usually obtained by gas exchange systems are CO_2 assimilation, transpiration rate, intercellular concentration of CO_2, and stomatal conductance. Measurements of chlorophyll fluorescence provide information about photosystem II (PSII) activity and changes in photosynthetic metabolism of diseased leaves [11-13]. The aim of this study was to compare the alterations in photosynthetic mechanism especially in CO_2 assimilation, thylakoid electron transport, water use efficiency, transpiration and other related photosynthetic traits in leaves infected with *Colletotrichum dematium* pathogens.

Materials and Methods

Plants location and inoculation: Groundnut (*Arachis hypogeae*) plants were grown at Central Agroforestry Research Institute Jhansi, India (25° 27/ N latitude and 78° 35/ E longitude, 271 m above MSL) during kharif (rainy) season in 2012-13 from seed in pots in natural environment. Soil used in the experiment was black having a mean pH 7.02. Air temperature was 32 ± 4 °C. Irradiance (PAR) at the top of the plants was 1250– 1400 μ mol m^{-2} s^{-1} during the 14 h photoperiod. All measurements were performed on the central leaflet of the first trifoliate leaves. Two-week-old seedlings were spray-inoculated with spore suspensions of inoculum of *Colletotrichum dematium* according to standard refereed techniques. After inoculation, plants were covered with transparent plastic bags for 24 h. First symptoms became visible 2 days after inoculation, and then the disease developed over a period of 20 days. Spraying with water and covering with bags for 24 h after spraying did not significantly affect photosynthesis in control plants. The crop was grown taking all the standard agronomic practices.

Assessment of gas exchange: The assessments of net photosynthetic rate, dark respiration, stomatal conductance, transpiration rate and intercellular concentration of CO_2 were made with a Portable Photosynthesis System (LI-6400XT; Licor, USA) on an area of 2 cm^2. The conditions during the assessments were: leaf temperature at 25-30 °C; CO_2 concentration in the air coming into the ranged from 380-390; relative humidity in the ranged from 30 to 50%. The central leaflet was kept under light until the stabilization of P_N, E, gs, and Ci values and then the central leaflet was kept in the dark for 15 min. to measure dark respiration. Assessment of variables of chlorophyll fluorescence the minimal fluorescence (Fo), maximal fluorescence (Fm) and optimal quantum yield of PSII (Fv/Fm) were assessed in the leaflets of non-inoculated plants, and in two regions of inoculated leaves: lesioned and apparently healthy areas. These fluorescence variables were assessed only on plants kept at room temperature (25-30 °C) in the experiment.

The Fo was assessed after the emission of a modulated red light beam of 600 Hz and very low intensity. The Fm was obtained with a saturating light pulse for 0.6 s. The value of Fv/Fm was calculated using Fv/Fm = (Fm-Fo)/Fm. The efficiency of PSII photochemistry was calculated as: ΦPSII = (Fm'-Ft)/Fm'. Since ΦPSII is the quantum yield of PSII photochemistry, it can be used to determine linear electron transport rate (ETR) as described by Genty *et al*., (1989): ETR = ΦPSIIxPPFDx0.5x0.84. NPQ is linearly related to heat dissipation and varies on a scale from 0 until infinity even if in a typical plants value ranges between 0.5 and 3.5 at light saturation level, calculated as: NPQ = (Fm-Fm')/Fm'.

Assessment of disease severity: The trial consisted of two treatments viz., *Colletotrichum dematium* inoculated groundnut plants (infected) and un-inoculated or healthy plants (control), and each treatment was replicated six times (total 12 pots). Seedlings were harvested after 12 weeks growth (one per replicate), and observations on shoot length (cm) and fresh weight (gm/plant) were taken by standard methods.

The area assessed for gas exchange on the central leaflet was marked so that the next assessments could be made in the same area. The necrotic area as well as the yellowish halo and the soaked, faded or dried areas that surrounded the lesions were all included in the assessment of disease severity. The disease severity of the leaf region where the variables of chlorophyll fluorescence were assessed was estimated by counting the number of lesions and multiplying this number by a visual estimate of average lesion size with the aid of standard area diagrams, then dividing by assessed leaf area.

To study the anthracnose disease severity index in Groundnut (*Arachis hypogeae*) plants, similar set of experiments were carried out with aforesaid treatments (i.e. four) in two successive years i.e. 2012 and 2013. All the treatments were replicated three times where one plant was maintained per pot, and observations were taken every 10 days interval after inoculation up to complete wilting of the seedlings. The severity of disease on individual plants were rated (infected stages) on a scale from 0 to 4 point scale; where 0 = 0% (no wilting), 1 = 1–30%, 2 = 31–60%, 3 = 61–90% and 4 = 91% - 100% wilting or dead plant[22]. The disease severity index at the time of harvesting is being presented here.

Chlorophyll estimation: Six leaves from each treatment were collected for chlorophyll estimation. Chlorophyll (Chl) was extracted from the leaves with acetone and DMSO (Dimethyl sulphoxide) solvents [17].

Data from the two repetitions of experiments for disease were analyzed together. Data were analyzed through standard deviation (SD) and standard error (SE) calculation followed

by t-test through SYSTET-11 software. All the graphs were generated through Microsoft Excel-97-2003.

Results and Discussions

In the present experiment it has been observed through P_{Nmax} versus PPFD curve that the rate of CO_2 assimilation (P_{Nmax}) reduced under anthracnose disease infection (Table-1). The value of P_{Nmax} under non infected condition was approximately two fold than the infected condition. In non-diseased (healthy) groundnut plants the rate of CO_2 assimilation was 24.37 µ mol m^{-2} s^{-1}; whereas it reduced upto 13.70 µ mol m^{-2} s^{-1} under disease infection. Similar to CO_2 assimilation thylakoid electron transport rate (ETR) was also reduced under infected condition. Under infected condition the ETR was 88.40 µ mol m^{-2} s^{-1}, whereas under non infected condition the ETR was 119.08 µ mol m^{-2} s^{-1}(Table-1). Net photochemical quenching (NPQ) was also reduced from 3.24 to 2.79 under infection (Table-1). Water use efficiency reduced under infection significantly from 14.78 to 8.87 (Table-1). The efficiency of PSII photochemistry (ΦPSII) was also reduced under infection. Minimal fluorescence (Fo) increased under infected condition from 461.32 to 543.44, whereas maximal fluorescence (Fm) decreased in groundnut leaves from 2311.55 to 1982.83 under infection (Table-1). Optimal quantum yield of PSII (Fv/Fm) considerably reduced from 0.80 to 0.72 under infected condition (Table-1). Stomatal conductance and rate of transpiration reduced under infection.

First symptoms became visible 2 days after inoculation, and then the disease developed over a period of 20 days. The severity of disease was gradually increased after inoculation. After 20 days of inoculation the disease severity index was about 22%, whereas after 40 days of inoculation disease severity index reached up to 54%, while after 60 days of inoculation disease severity index was 73%. Chlorophyll a, chlorophyll b and total chlorophyll decreased under infection significantly (Figure-2).

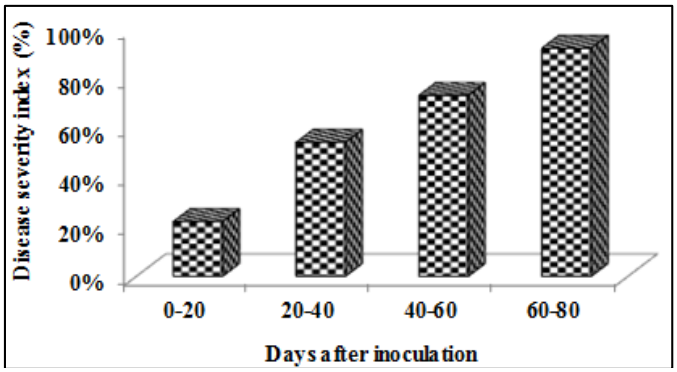
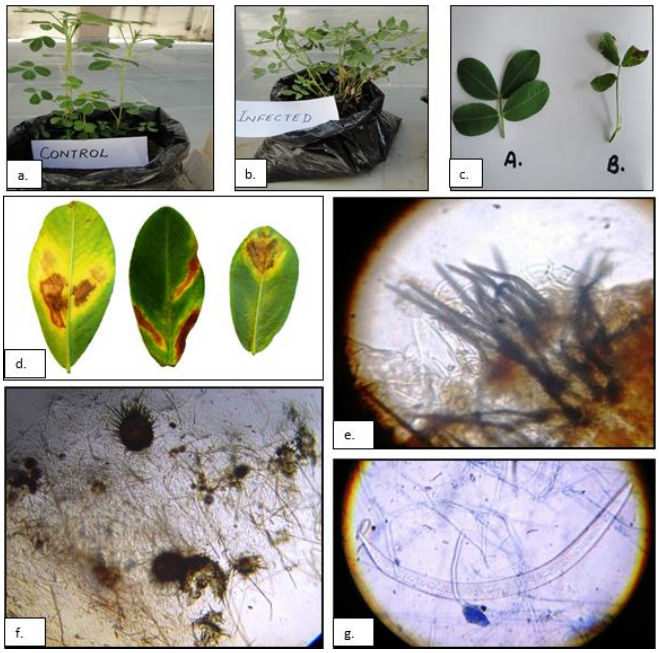


Fig 1: Disease Severity Index (DSI) of groundnut plants infected by *Colletotrichum dematium*.



a. Control or non-infected groundnut plants **b.** Infected plants of groundnut, **c.** Comparison in non-infected and infected plant leaves, **d.** Close view of infected leaves **e.** Conidia and setae from infected leaves, **f.** Acervuli fruiting body, **g.** Falcate shape conidia

Table 1: Comparative photosynthetic traits of groundnut indicating limitations of CO_2 assimilatory functions under three different stages of *Anthracnose* disease infection. $P_{N\ max}$= rate of CO_2 assimilation at maximum PPFD (2000 µmol $m^{-2}s^{-1}$) (µmol $m^{-2}s^{-1}$); gs= stomatal conductance (m mol $m^{-2}s^{-1}$); Fo= minimal fluorescence; Fm= maximal fluorescence; Fv/Fm= dark adapted value of variable fluorescence/maximal fluorescence; Φ_{PSII}= effective PSII quantum yield; NPQ= net photochemical quenching; ETR = rate of thylakoid electron transport (µ mol $m^{-2}s^{-1}$); E= rate of transpiration (m mol) ; WUE = water use efficiency (µ mol CO_2 $mmol^{-1}$ water). Mean ± S.E, (n = 6)'

Treatment	$P_{N\ max}$	gs	Fo	Fm	Fv/Fm	ΦPSII	NPQ	ETR	E	WUE
Non infected Plants	24.371 ±0.518	0.055 ±0.003	461.325 ±5.393	2311.553 ±3.563	0.800 ±0.002	0.136 ±0.001	2.791 ±0.007	119.082 ±1.292	1.652 ±0.033	14.787 ±0.525
Infected Plants (stage-1)	19.584 ±0.521	0.048 ±0.000	500.584 ±1.914	2099.215 ±4.936	0.772 ±0.003	0.121 ±0.002	3.014 ±0.024	101.226 ±0.878	1.624 ±0.005	11.558 ±0.333
Infected Plants (stage-2)	13.705 ±0.783	0.040 ±0.001	543.442 ±3.709	1982.837 ±3.283	0.725 ±0.001	0.101 ±0.001	3.241 ±0.008	88.400 ±1.388	1.595 ±0.103	8.871 ±1.061
Infected Plants (stage-3)	8.861 ±0.514	0.032 ±0.000	575.891 ±1.047	1754.081 ±14.080	0.701 ±0.006	0.093 ±0.002	3.326 ±0.010	63.558 ±1.513	1.238 ±0.009	6.881 ±0.195

LSD (at 0.05)	1.604	0.012	9.291	21.120	0.0120	0.003	0.038	3.475	0.147	1.677

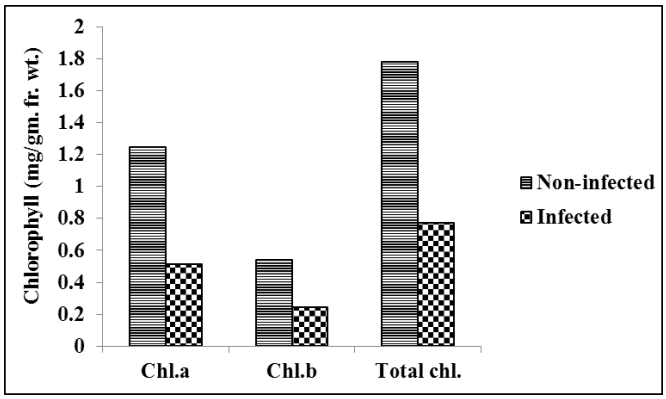


Fig 2: Pigment profile of infected and non-infected leaves of groundnut.

With the development of integrated pest management work, knowledge of the effect of pests and disease on the host physiological processes is must, because there are several microorganisms which act as photosynthetic rate reducer[18]. Since photosynthesis is the biological process that leads to yield accumulation and because the water status of the crop markedly affects photosynthesis, carbon metabolism and water relations are the critical areas of concern in understanding the effects of disease on plants [19]. Cells of leaves penetrated by the fungal structure or killed by the secretion of some toxic biochemical lose their chlorophyll and probably their capacity to photosynthesize and transpire. In present case study the destruction of cells is directly related to the presence of the fungus, and uncolonized portion of the leaves remain normally active. The effect of infection on the physiological processes completely accounted for by the loss of leaf area to the pathogen.

In present study the rate of CO_2 assimilation (P_{Nmax}) observed in P_N versus PPFD curve under diseased and healthy (non-infected) groundnut plants clearly demonstrated the status of photosynthetic efficiency of groundnut (Table-1). It has been observed through P_N versus PPFD curve that the reduction in P_{Nmax} under infection was mainly due to decrease in photosynthetic leaf area under diseased condition. A similar study in maize and banana showed a net decrease in photosynthetic efficiency due to fungal infection [20]. Low absorption of photosynthetic active radiation diminished thylakoid electron transport rate, which ultimately decreased net rate of CO_2 assimilation [16]. From above results, it is clear that *Colletotrichum dematium* infection poses a great limitation to ETR (Table-1). ETR is extremely essential for photosynthetic reactions. This ultimately led to reduced photosynthetic process due to lack of sufficient photosynthetic electrons under infected conditions [21]. This has been supported by relatively enhanced net photochemical quenching in infected plants (Table-1). These facts are well corroborating with some earlier reports. The dark adapted Fv/Fm values around 0.80 in non-infected plants indicate that the plants were in good condition especially free from any cellular damage (Table-1). Under infected condition decrease in Φ_{PSII} and increase in NPQ were related with the potential efficiency of PSII photochemistry. Further, results indicated that gs gradually decreased under infected condition (Table-1). Reduction in gs under infection in addition to the decrease in PSII activity especially P_{Nmax} and ETR clearly demonstrated the reduced functioning of photosynthetic machinery under infection. The water use efficiency (WUE) decreased under infected condition (Table-1). Water loss from an infected leaf can increase due to destruction of part of the leaf cuticle, increased permeability of leaf cell membranes or inhibition of stomatal closure. Reduction of transpiration may result from stomatal closure, reduction of air space by hyphae or hypertrophy of mesophyll cells, obstruction of conducting tissue and stomata and defoliation etc.

Under infected condition leaves of groundnut plants could not use CO_2 assimilation as proficiently as the plants did in case of non-infected condition (Table 1). This indicated that anthracnose disease infection has conspicuously affected the whole photosynthetic processes at cellular and functional level and finally affected yield and productivity of crop as it has also been noted in other fungal diseases [7-9]. Under infected condition chlorophyll a, chlorophyll b and total chlorophyll decreased significantly because of reduction in green leaf area under infection (Figure-2). After a brief study we have concluded that under anthracnose disease infection, production of infected plants diminished due to loss in photosynthetic pigments of leaves. Loss in pigments like chlorophyll a and chlorophyll b and green photosynthetic surface of laves ultimately reduced the rate of CO_2 assimilation and electron transport rate as well.

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