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STUDIES ON *Macrophomina phaseoli* (Maub.) ASHBY GROWTH AND SOME PHYSIOLOGICAL ASPECT OF GROUNDNUT (*Arachis hypogaea L*) PLANT INFECTED WITH THE FUNGUS

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ABSTRACT

Some synthetic media used to investigate the best media to support growth of *Macrophomina phaseoli*. The fungus grew in both solid and liquid synthetic media, but best growth was recorded in peanut leaflet Oat meal (POMA) and potato dextrose agar (PDA). There was no absolute nutrient requirement for its growth. The best inoculation technique for disease symptom development was a juxtaposition of 48 - hour old mycelial mat on wounded roots of 10-day old groundnut plants. Infection of the groundnut plants increased with higher inoculum potential in the soil. Dry weight of diseased plants was drastically reduced as infection advanced, possibly due to decreased anabolic activities of the plants. In addition, the reduction in the leaf area equally led to reduction of photosynthesis. Transpiration in inoculated plants decreased and remained lower than that of the uninoculated plants. The disease may have caused plugging of the xylem vessels, hence reduction in the plants' water absorption capacity.

KEY WORDS: Groundnut, *Macrophomina phaseoli*, physiology, juxtaposition, inoculum potential, dry weight, leaf area.

INTRODUCTION

Macrophomina phaseoli has a very wide hose range, infecting over 300 different species of wild and cultivated plants (Smith, 1969). The organism has been grown successfully on Czapek's Dox Agar medium and Richard's solution (Raj and Prasad, 1957; Thirumalachar 1955, Moniz *et al;* 1956; Kulkami *et* al; 1962; Mayer *et* al 1983 and Kirkpatrick, 1973). Thirumalacher (1955), showed that the fungus grows very rapidly on Potato Dextrose Agar (PDA) forming fluffy mycelium which is progeotropic in growth response at 30°C a. d turns black with time due to formation of sclerotia. Different inoculation techniques have been employed to inoculate different soil pathogens to the roots of different plant species. Johnson and Greaney (1942) inoculated plants by mixing inoculum of pathogens with the soil before sowing seeds of wheat after a lapse of 3 days. Moniz *et al*; (1956) carefully scooped out mycelial mat of the fungus using sterilized spatula and placed it in close juxtaposition with the roots by lifting little soil near the roots. The fungus remained undisturbed in situ covered with light soil and maintained at 28°C. Threlfall (1959), poured the inoculum of *Verticillium sp.* around the roots of tomato plants on transplanting. This produced better infection results than hypodermic injection of spore suspension or dipping the roots of the in mycelial suspension before transplanting. Armstrong and Armstrong (1960), inoculated the plants after they had germinated by cutting the roots on one side by pressing the moist sand to a depth of 3.5cm either on inverted Buchner funnel placed in the centre of the pot or a large test-tube placed close to the stalks of the plant before the inoculum was poured into the depression. The roots were covered with sand and water sprinkled over the surface to settle the sand. Stover (1962) obtained infection on the host by injecting the spore suspension of the pathogen into the vascular system of the host. Kulkami *et al;* (1962) obtained wilting on the 4th day after placing fungal mycelium scooped with a portion of the growth medium on the surface of the soil in close contact with the collar of cotton seedling and covering it with sterile soil. Ilyas and Sinclair (1974) placed mycelia of the fungus into wounds cut on the plants and the wounds covered with petroleum jelly to prevent them from drying up. Raj and Prasad (1951) buried groundnut seeds infected with *M. phaseoli* near the hypocotyl region of 15-day old groundnut seedling at the depth of 1.5cm and maintained the soil moisture content at 50% level. The plants showed disease symptoms 12 days after inoculation. Gerwitz and Durbin (1965) found that infection of bean plant by rust decreased the daily water loss by the diseased leaves until the time of epidermal rupture during which period the loss from infected plants increased and remained greater than that of the healthy leaves. Duniway (1971), reported that *Fusarium* wilt affected the transpiration of tomato plants, making it significantly less than the rate in

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healthy plants at all stages of development of thee plants and concluded that it was quite unlikely that excessive transpiration was the cause of wilting in the diseased plants

The present work was intended to investigate the growth medium which best supported the growth of *Macrophomina phaseoli*, the best inoculation technique and inoculum strength for disease symptom development on groundnut plants. It was also intended to investigate the inflence of inoculum potential and disease incidence on dry transpiration and dry weight of groundnut plants.

MATERIALS AND METHODS

1. **Groundnut Seeds:** Groundnut (*Arachis hypogaea* L.) of the erect Spanish Valencia cultivar was grown from seeds obtained from Department of crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria, Nigeria. This cultivar is susceptible to *Macrophomina phaseoli* (Narayana and Seshadri 1954, Feakin 1967). The experimental design was a randomized block design with six or four replications as the case may be. The seeds were sown in 15cm plastic pots containing sterilized soil and left in the field.

2. **Inoculum Test:** The inoculum employed in the study was obtained courtesy of Malam Gumel of the Deportment of Crop Protection, Faculty of Agriculture, Ahmadu Bello University, Zaria. The culture (number 1363), was sub-cultured on slopes of potato dextrose agar (PDA), and peanut Oat meal agar (POMA) in Mc Carteny bottles and in 15.2cm test- tubes and incubated at 32^{0}C for 10 days after which the bottles were tightly capped and stored at about -10^{0}C in a deep freezer. From this stock culture, sub-cultured were made when necessary.

3. **Selection of Medium:** To determine the best medium / media for growth of the culture, Potato dextrose Agar (PDA), Peanut leaflet – oatmeal Agar (POMA) (Smith, 1971), Czapek – Dox Agar (CDA) and Corn Meal Agar (CMA) were tested. The study was based on the radial growth of *Macrophomina phaseoli* mycelium on the media. The measurement of the redial growth was made by drawing two lines which intercepted at the underside of the 13cm petridishes containing 20ml of the sterilized media poured into them. The petridishes were inoculated with uniform amount of hyphal bits at the point of interception of the lines on the underside of the petridishes using sterilized forceps, and the dishes incubated at 32^{0}C; readings were taken at 12 hourly intervals by measuring the diameter of the culture along the lines. The average of 6 replicates were taken for each medium on each occasion.

4. **Inoculation Technique:** to establish the best inoculation technique that would produce the best infection, the following experiments were conducted with pure cultures of the fungus. (a) The contents of one petridish each was mixed with equal amount of distilled water and was poured onto the surface of each of the 4 sets of plant pots containing sterilized soil and four groundnut seeds dressed with ALDREX 'T'. (b) Four seeds of groundnut each were sown in each pot and the seedlings inoculated 10 days after germination, by making a hole at the same point in the pots and pourring in the contents of each prtridishe. (c) 10 days old seedlings of groundnut were inoculated with ¼ portions of mycelial mat grown in 9cm petridishes containing 20ml of media. Each division of the mycelial mat was carefully scooped out using a sterilized spatula taking care that no mycelia strand was damaged and placed carefully in close juxtaposition with the roots of 10 day old plants which were wounded by pricking with a sterile needle by lifting little soil near the roots of plants. The fungus was allowed to remain undisturbed *in situ* by covering with light sterile soil layer. (d) Method (c) was repeated with 20 -day old plants. The control plants were pricked with needle but no inoculum was introduced.

5. **Inoculum Potential:** To find out the inoculum strength that would give the best infection result, inoculum strength of 20%, 10% and 5% were used to inoculate the plants. The inoculation of petridishes containing 20ml of POMA with uniform amount of inoculum using a sterile number two cork- borer. The dishes were incubated at 320C for 48 hours. The contents of each petridish were then divided into 5, 10 and 20 portions representing 20%, 10% and 5%, respectively. These were used to inoculate 10 day old groundnut plant in a set of 6 plants randomly selected from a group of 36 plants in each case,. To study the effects of infection and disease incidence on the dry weight of the groundnut plants were harvested 40 days after planting by carefully removing them from the pots and washing the roots carefully to remove adhering soil. The harvested plants were divided into stems, leaves and roots and these were dried at 60 ^{0}c for 48 hours and their weights determined.

6. Transpiration Studies

The rate of water loss by both healthy and

Table I: Inoculation technique and disease incidence.

Treatment	Age of Plants (days)	No of plants Control.	No of plants Inoculated	No. of infected Plants control	No. of infected Plants inoculated	Incubation period
Mycelial mat of one petridish each +distilled water poured on sterile soil of pot with 4 ground seeds	0	36	36	0	5	40days (15^{th} may to 23rd June)
Pouring the contents of petridish in Holes made in pots Containing 10 day Old seedlings	0	36	36	0	9	39days, one (25^{th} May to 3^{rd} June
Juxtaposition of Mycelial mat with wounded roots 10 days after sowing	10	36	36	0	25	39 days 25^{th} may to 3rd July
20 days after sowing	20	36	36	0	13	39 days (5^{th} July to Aug).

Table 2, Inoculum potential and Disease symptom development.

Treatment	No. of plants inoculated	No of Plants showing symptom in indicated days after inoculation					
		10	15	20	25	30	35days
20% inoculum	6	3	3	4	5	5	6
Control	0	0	0	0	0	0	0
10% inoculum	6	0	1	1	2	3	3
Control	0	0	0	0	0	0	0
5% inoculum	6	0	0	0	1	2	2
Control	0	0	0	0	0	0	0

infected groundnut plants was determined by gravimetric (weighing method following the procedure of Gerivitz and Durbin (1965). The plants were placed in 125ml Erlenmeyer flasks containing distilled water and washed sand. The flasks were wrapped with black polyethylene bags and the stems of the plants were sealed at the top with petroleum jelly to ensure that no water was lost through the area. The water loss through transpiration was then determined by weighing the flasks every two hours for 8 hours after exposure to the sun. Six replicates of both health and inoculated plants were used in each treatment.

Determination of leaf Area

The leaf area of both inoculated and uninoculated groundnut plants was determined using a portable leaf area meter (Model Li – 3000) attached to a transparent belt conveyor accessory (Model Li - 3050 A/3) manufactured by Lambda instrument Corporation Limited USA. (Khan, 1981). In case of very fragile or folded leaves, the leaves were spread out and traced on a sheet of paper. The paper was carefully cut out and the area of the representative sheet of paper measured to obtain an area equivalent to the area of the leaves (Sestak, et al.1971). Analysis of variance (Anova) was used to determine significant differences between the variables. Duncan's Multiple Range Test (DMRT) and Least significant Difference (LSD) were used to determine test of significance between pairs of variables. The test values were compared at 0.01 and 0.05 levels of significance.

RESULTS

As shown in fig 1. *Macrophomina phaseoli* grew best in peanut leaflet Oat- meal Agar

(POMA) and potato Dextrose Agar (PDA). Significantly poor growth was observed in corn meal Agar (CMA) and Czapek- Dox Agar CDA). Growth in POMA was very luxuriant and fluffy, and the individual hyphae appeared more distinct than those of the other media. In 72 hours the linear growth in POMA was 5.5cm, that in PDA was 5cm while in the Growth in CMA and CDA were 3cm and 2.9cm respectively, while in 84 hours the liner growth in POMA and PDA had gone up to 6cm and 5.5cm respectively, and that is CMA and CDA remained 3.5cm and 3.8 cm respectively

The inoculation technique involving juxtaposition of mycelia mat with wounded root of the seedlings 10 days after sowing resulted to effective infection of the groundnut plants. With this technique 25 out of a total of 36 plants inoculated on 15th May, (i.e. 10 days after sowing), wilted on 3rd July (i.e. 39 days after inoculation). The other two methods involving the pouring of the contents of one pepridish each mixed with distilled water on the surface of each of 4 sets of plant, pots containing sterile soil and four groundnut seeds dressed with ALDREX- T, and inoculation by pouring the contents of one petridish each into holes made at am equivalent point in the pots containing four seedlings each, did not give encouraging infection. Only 5 and 9 plant out of the 36, wilted in the same period respectively. Juxaposing mycelial mat on wounded plants 20days after planting showed 13 infected plants. The affected plants in all the methods wilted permanently. Juxtaposaing mycelial mat on wounded plant 10 days after planting was a significantly better method of inoculation than the other tested methods (Table. I)

Symptoms of infection on the hypocotyl and cotyleddons were first visible on the plants inoculated with 20% inoculum concentration 10 days after inoculation. At this stage, 3 out of plants showed symptoms in each case and by the 35th day, all the 6 plants were visibly infected. Signs of infection in 10% and 5% inoculum concentrations appeared much later and by the 35th day after inoculation on an average of 3 and 2 plants out of 6 were infected, respectively. The 20% inoculum potential therefore showed the highest disease incidence (Table 2).

The result of the influence of disease incidence and inoculum potential on dry weight of 35day old plants is presented in Fig.2. With 20% inoculum concentration, the mean dry weight of the shoot of the infected plants was 1.11g that of the root was 0.52g. With 10% inoculum concentration, the dry with of the shoot and roots were 2.02 and 0.91 respectively, while the values of the shoot and root of the plants were 2.32 and 0. 86g respectively with 5% concentration. With 0%

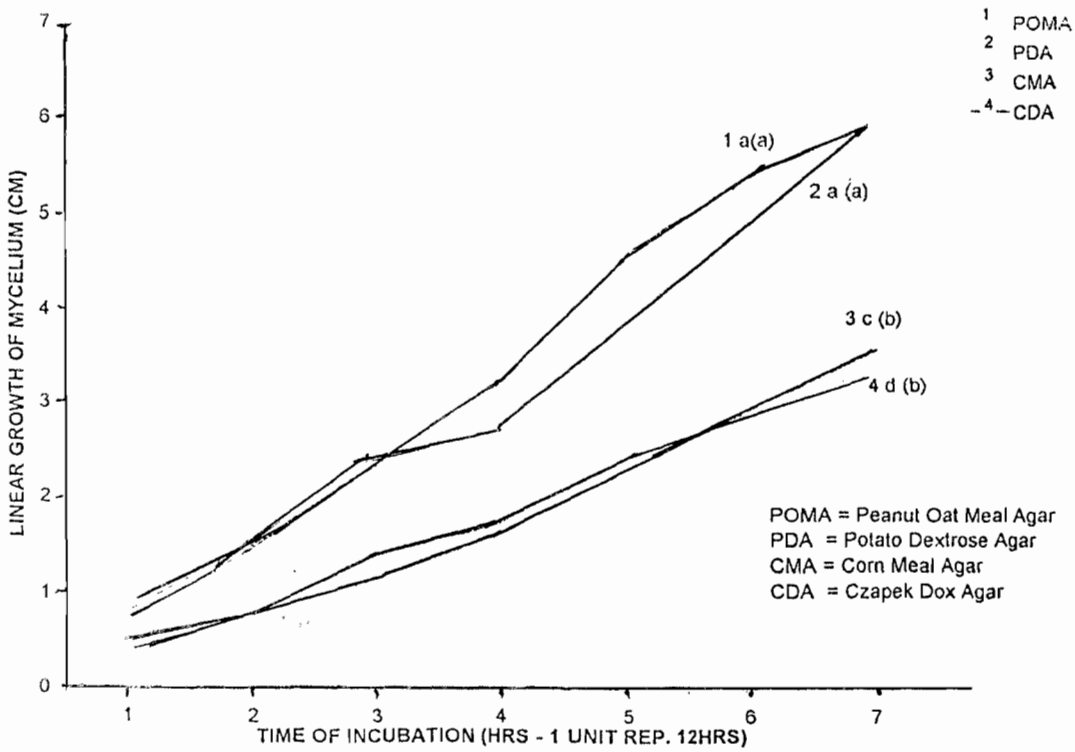

fIG. 1 - LINEAR GROWTH OF MYCELIUM IN THE TEST MEDIA (values are means of 6 replicates, and any two curves bearing the same letter are not significantly different, P = 0.05)

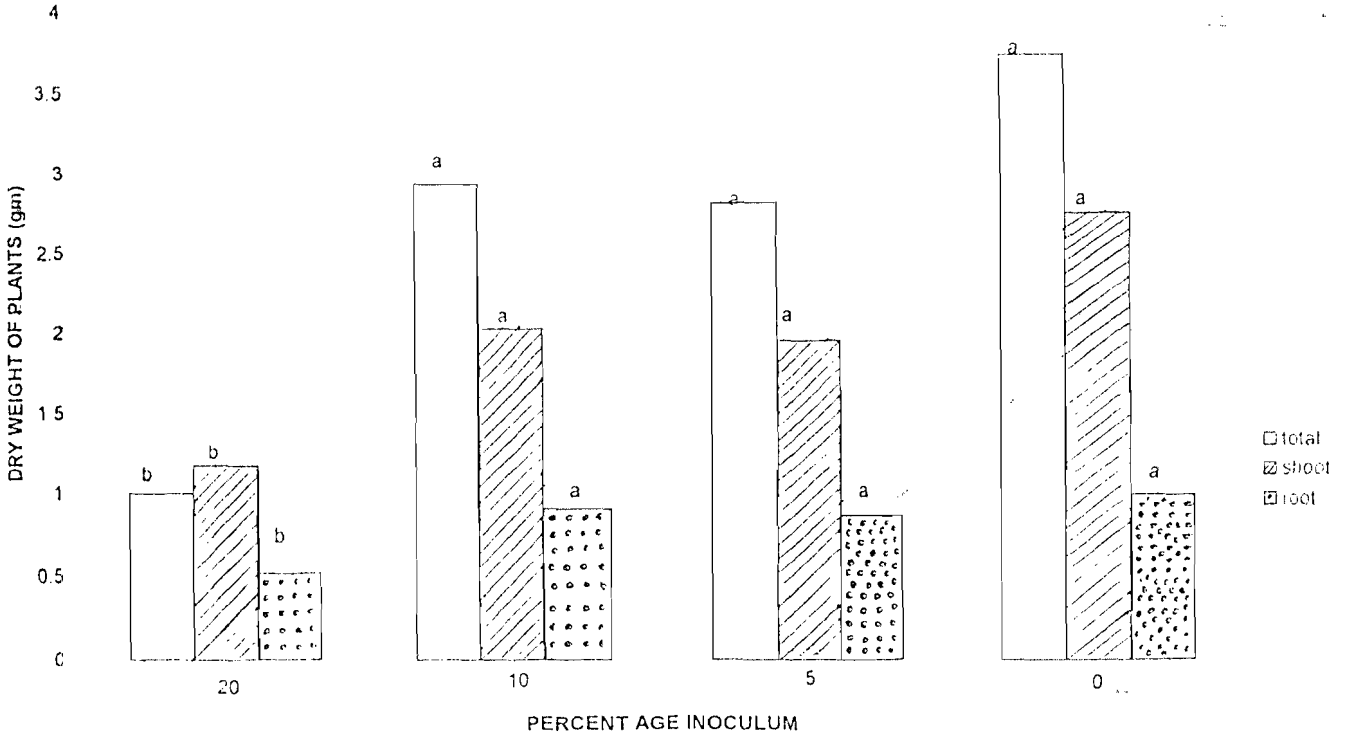

FIG 2: INLUENCE OF INCULUM POTENTAL AND DISEASE INCIDENCE ON DRY WEIGHT OF PLANT (values are means of 6 replicates and any similar blocks bearing the same letter are not significantly different p=0.05)

concentration, the shoot and root were 2.75g and 0.98g respectively. The dry weight values showed significant difference ($P < 0.05$). Test of significant between individual means showed significant difference between dry weights of shoots and roots of the plants inoculated with 20% inoculum concentration and the mean dry weight of the plants inoculated with 10%, 5% and 0% inoculum concentrations. With 20% inoculum concentration, the dry weight was significantly less than those of the other percentages. (Fig.2). There was no significant different between the dry weight values of the plants inoculated with 10%, 5%, and 0%. This showed that infection was more severe at higher inoculum load. The result of the influence of infection on the transpiration of the groundnut plants is shown in fig3. The rate of transpiration of the inoculated and uninoculated plants were 2.5mg/min/dm^2 and 2.69mg/min/dm^2, respectively for the first day after inoculated. From the 10th day the rate in inoculated became faster reaching up to 3.25mg/min/dm^2 while that of the inoculated plant rose slightly went down to 1.3mg/min/dm^2 on the 40th day after inoculated. The values for the mean leaf area of inoculated and uninoculated plants at the different stages of infection is summarized in table 3. There were no significant difference in the leaf area at the first three stages after inoculated. At the last two stages, the values for the uninoculated plants became significantly greater than those of the inoculated plants ($P = 0.05$).

Table 3, mean leaf area of inoculated and uninoculated plants.

	Leaf area (cm2)	
Days after inoculation	inoculated	uninoculated
1	1.78	1.88
5	2.00	2.22
10	1.52	2.03
20	1.58	2.67
40	1.66	2.57
S.E.	0.085	0.152

DISCUSSION

Many workers have reported that *Macrophomina phaseoli* grows well in varieties of media such as 2% malt extract agar, water agar and peanut shell agar (Jackson and Bell, 1969), Czapek Dox agar and Richard's solution (Raj and Prasad, 1957), and Potato dextrose agar (Small, 1973 and Kickpatrcck, 1973). The present study

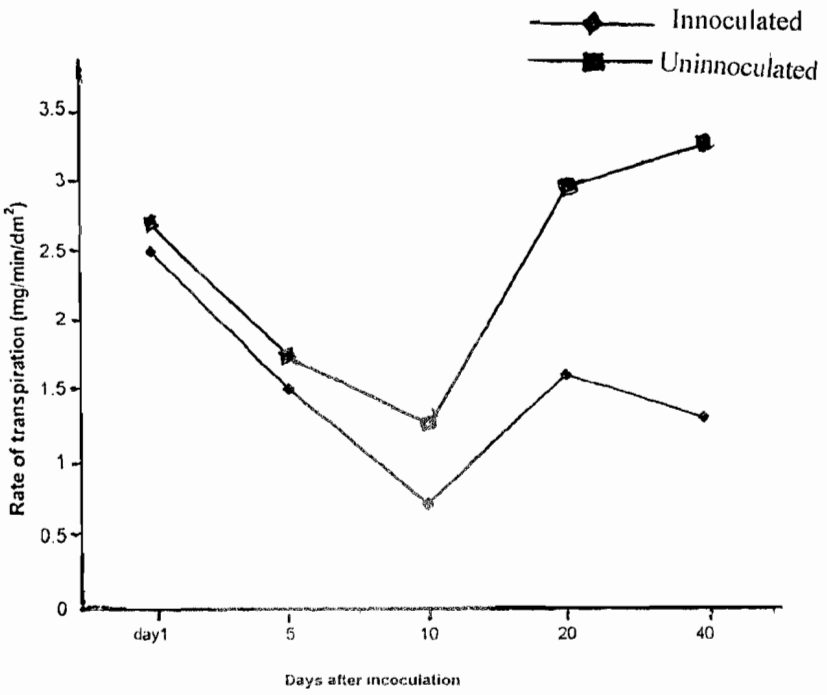

Fig 3 Influence of *Macrophomina phaseoli* inoculation on the rate of transpiration of groundnut plant. (values are means of six replicates).

showed that *M. phasęoli* in addition to growing well in Czapek Dox agar and potato dextrose agar equally grows well in peanut oat meal agar and Corn meal agar. POMA supported more growth than any of the other media tested (Fig.1). This confirms that the organism can ably utilize different carbon sources for growth as supplied by synthetic media. (Smith, 1969). In other words the organism had no absolute nutrient requirement for growth, but showed more affinity to materials containing groundnut in it as indicated by the fluffy growth in peanut leaflet oatmeal agar (POMA).

Juxtaposition of mycelial mat of *M.* phaseoli with wounded roots of groundnut plants was most effective in inducing infection of the groundnut plants, and 10 day old plants were most susceptible to this disease. The delay or absence of disease symptoms in plants more than 10 days old might be due to resistance caused by increased vigour or some physiological barriers (Talboys, 1957); and the absence of symptoms in the plants inoculated at the day of sowing might be due to the breaking down of the mycelia and the formation of sclerotial bodies on account of its evanescent nature before the seeds germinate (Kulkarni et al 1962). El Nur and Fattah, (1970), reported high infection following high inoculum polential and higest infection during early stages of plant development at intermediate levels. Momin and Main (1937) reported 100 percent pre-emergence mortality at 0.2 percent inoculum potential and lower mortality at concentrations lower than 0.2 percent.

The present study showed that high inoculum concentration (20 percent) of *M. phaseoli* resulted in increased infection of groundnut plants. The severity of infection under high inoculum potential might be the result of increased competition for food by fungal hyphae. The dry weights of the inoculated plants were altered considerably. Infection reduced the dry weights of the plants, especially during the dry late stages of infection. The dry weights of the uninoculated plants were significantly greater than those of the inoculated plant (P= 0.01) Similar results have been reported in bean plants infected with obligate parasites (Gerwitz And Durbin, 1965; Zaki and Durbin; 1965). The reduction in growth as explained by the decreased dry weight of the inoculated plants, might be due to decreased anabolic activities of the plants; since the disease equally caused reduction in the expansion of the leaves.

Transpiration studies indicated that there was reduced transpiration throughout the late stages of infection, (i.e. from day 10 after inoculated.) This result was similar to the result reported by. Duniway (1971). He reported that *Fusarium* wilt of tomato plants affected the transpiration rate of inoculated plants, making it significantly less than the rate in healthy plants at all stages of development of the plants. He

therefore concluded that it was quite unlikely that it was excessive transpiration that caused wilting in the diseased plants. The result was different from the observations of Subramaniam and Saraswathi-Devi (1959) and Threlfall 1959), who reported that decreased transpiration rate in inoculated plants followed an initial increase which occurred before the onset of wilting of the plant caused by *Fusarium* spp. They noted that the rate of transpiration decreased rapidly below that of uninoculated plants after the initial increase. Blackhaurst (1963) suggested the involvement of translatable factor in the initial increase. Alteration in metabolic pathway of the inoculated plants could also account for the increase since interaction between host and pathogen resulted in abnormal physiology of the host, (Horsfall and Dimond, 1960; sempio 1959). The decreased transpiration rate which occurred in the inoculated groundnut plants at the later stages of infection might be as a result of the inability of the infected plants to obtain enough water or sap to maintain turgidity due to lack of loss of control over transpiration (Subramanian and Seraswathi- Devi, 1959). Reduced water supply to the leaves due to the reduced absorption by roots of the inoculated plants might equally contribute to the decreased transpiration (Threlfall 1959). The blocking effect of metabolic substances on the xylem vessels might also contribute to the low transpiration rate.

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