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RESEARCH

STUDY ON POLLEN MORPHOLOGY AND BIOCHEMICAL STUDIES IN HEALTHY AND INFECTED PLANT PARTS OF *VIGNA RADIATA* L.

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Abstract: Pollen morphology has great significance in field of plant sciences especially in field of Taxonomy and helps to classify and understand the evolutionary history of plants. At present, it plays a key role in taxonomy for identification of plant flora and in forensic department. It contains all genetic information for complete plant. Our observation indicates that non-acetolysed pollen grains of *Vigna radiata* show reduction in size as compared to acetolysed pollen grains. In case, the effects of healthy and infected plant parts on the nutrient uptake and distribution, it revealed that total N and P and its distribution in selected plant parts such as in stem, leaf, anther and pollen grains clearly declined in infected plant as compare to healthy (control) plant parts due to fungal infection. Present study also indicates that the development of chlorophyll-a and chlorophyll-b are affected by fungal infection in infected plant leaf disc as compare than healthy plant leaf disc. Thus, a comparison of chl-a and chl –b development indicates that in general chl-a development is more as compare than chl-b in both healthy and infected leaf disc of *Vigna radiata*.

Keywords: *Vigna radiata*, Acetolysis, Fungal infection, Pollen grains, Chlorophyll

INTRODUCTION

Vigna radiata (Green gram) is member of family Fabaceae, which is cultivated in the month of June-July as kharif crop and flowering appear at 80th day. Pollen grains and fungal spores were collected at 60th day. Pollen morphology is of great significance particularly in plant taxonomy. Man has been always interested to find out air quality and microorganisms, pollen grains and fungal spores in air. Pollen is a very minute and microscopic structure encloses all genetic information for a complete plant. The aim of pollen grains is pollination leading to fertilization and seed production. Some contribution has been done in the past on pollen morphology of family Poaceae (Parveen, 2006**).** The morphological work on Pollen flora of Pakistan has been worked out (Parveen and Quiser, 2012). Several studies on pollen morphology and pollen analysis of certain socio-economical important families of Angiosperms such as Liliaceae and Fabaceae (Nair and Sharma, 1965). Studies on Pollen morphology of sub-family Caryophyllaceae and its taxonomic significance has been worked out **(**Ullah *et al*.2018).

Less significant works have been done till date regarding to pollen flora so it was of interest to carry some work on morphological and biochemical studies on pollen and healthy and infected parts of selected cultivated plant of *Vigna radiata*. Nitrogen and Phosphate are a universally occurring element in all living being and major component of protein. For investigations on total N and P uptake and their distribution in different plant parts, the samples of healthy and infected vegetative and floral parts collected from the crop field. The plant was dissected into different parts and air dried to N and P analysis. Along with the plant parts, soil samples were also analyzed. Side by side chlorophyll development studies also done in the leaf disc in healthy and infected plant of *Vigna radiata*.

MATERIALS AND METHODS

Present study was carried out in the research field of M.S. Degree College, Saharanpur. Anther and pollen grains of *Vigna radiata* were collected on glycerine jelly coated microslides during flowering season at 80th day from the experimental crop field. For study of pollen morphology, anther and pollen of plant were collected just before anthesis. The collected anther and pollen were fixed in 70% FAA (Formalin acetic acid) for 24 hours. Certain parameters related to pollen shape were determined on the basis of studies done with micrometry technique by using ocular micrometre and stage micrometre. Pollen grains were studied for their shape and size in healthy and infected plant. Apart from this, pore diameter, annulus diameter and exine thickness also studied. Studies on total N and P uptake and its distribution in healthy and fungal infected plant parts also carried out. For investigation of biochemical analysis of total N and P, plant samples were taken at

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40th day and 80th day of seeding emergence. Stem, leaf, anther and pollen grains were collected from crop field. After weighing, dry samples of experimental plant parts were used for quantitative estimation of total N and P in healthy and infected plant parts. Along with the plant parts, soil samples were also collected with healthy and infected plant for estimation of total N and P.

For quantitative estimation of uptake and distribution of total N in healthy and infected experimental plant parts (Stem, leaf, anther and pollen grains) was done according to (Snell and Snell 1954) method and the total N content in digest was estimated calorimetrically. While in case of P, protocol given by (Allen 1954) was followed and the total P content in digest was estimated calorimetrically using metol reducing agent.

For estimation of chlorophyll development in healthy and infected leaf disc of *Vigna radiata* the amount of chlorophyll- a and chlorophyll-b were estimated according to Arnon formulae which are shown below-

Chl-a mg / l =12.83 A_{645} – 2.58 A_{665}
Chl –b mg / l = 22.87 A_{645} – 4.67 A_{665}
Chl-a + chl-b mg / l = 8.05A_{665} + 20.29 A_{645}

Observations:

Result of all observations are given in table-1,2,3 and 4 and fig-2a, b, c and d & 3a, b ,c and d on gram dry weight basis for revealing the effects of fungal infection on the uptake rates of total nitrogen ,phosphate and its distribution in healthy and infected plant parts.

Table 1. Size of pollen grains in *Vigna radiata* (µm) acetolysed and non-acetolysed, pore diameter, annulus diameter and exine thickness.

Plant Species	Acetolysed Diameter (µm)	Non-Acetolysed Diameter (µm)	Pore diameter (µm)	Annulus diameter (µm)	Exine thickness (µm)
Vigna radiata	32.50 ±1.86	28.48 ±1.30	2.80 ±0.25	7.10 ±0.35	1.50 ±0.26

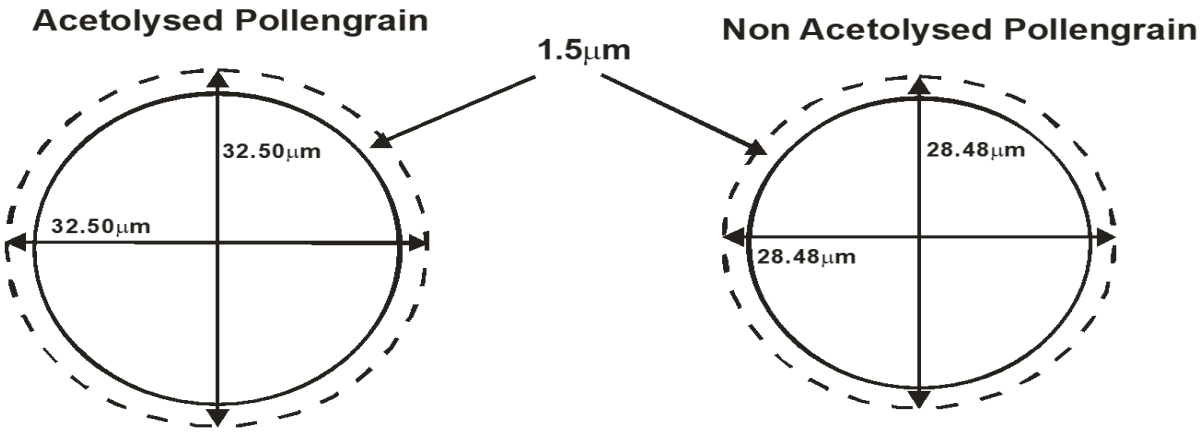

Fig 1. Size of pollen grains in *Vigna radiata*

Table 2. Total Nitrogen (Mg/gm dry weight) uptake and distribution in healthy and infected plant parts of *Vigna radiata*

Days from emergence	Soil without plant (Blank) mg/kg	Soil with plant mg/kg	Total nitrogen level in			
			Stem	Leaf	Anther	Pollen Grains
			mg/gm dry wt.			
Plant without infection (Control)						
0	455.0	455.0	.....	.....	.....	.....
30	450.0	451.0	26.40	20.50	…..	…..
60	440.0	465.0	28.00	21.00	14.55	21.50
Plant with infection						
0	455.0	455.0	.....	.....	.....	.....
30	448.0	460.0	22.00	18.10	.....	.....
60	440.0	464.0	26.00	20.00	12.00	19.10

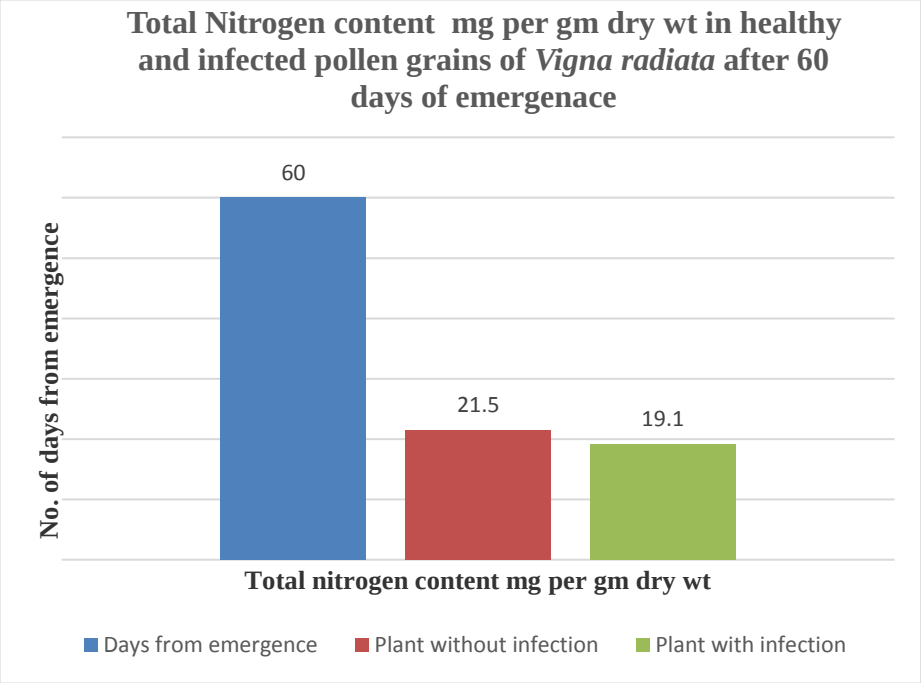
Total Nitrogen content mg per gm dry wt in healthy and infected pollen grains of Vigna radiata after 60 days of emergenace
No. of days from emergence
60
21.5
19.1
Total nitrogen content mg per gm dry wt
Days from emergence
Plant without infection
Plant with infection

Fig.- 2 a

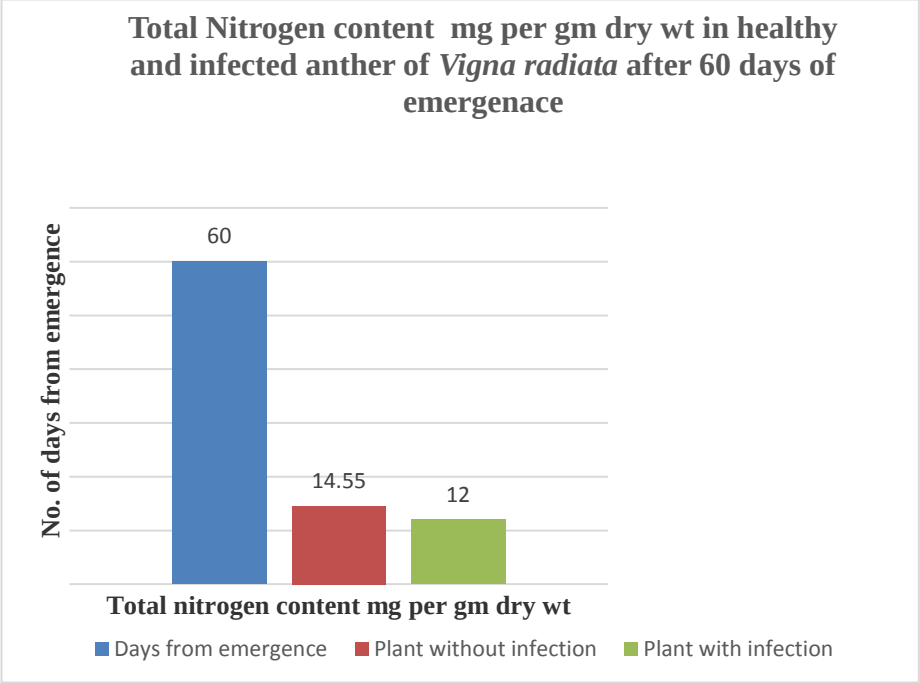
Total Nitrogen content mg per gm dry wt in healthy and infected anther of Vigna radiata after 60 days of emergenace
No. of days from emergence
60
14.55
12
Total nitrogen content mg per gm dry wt
Days from emergence
Plant without infection
Plant with infection

Fig.- 2 b

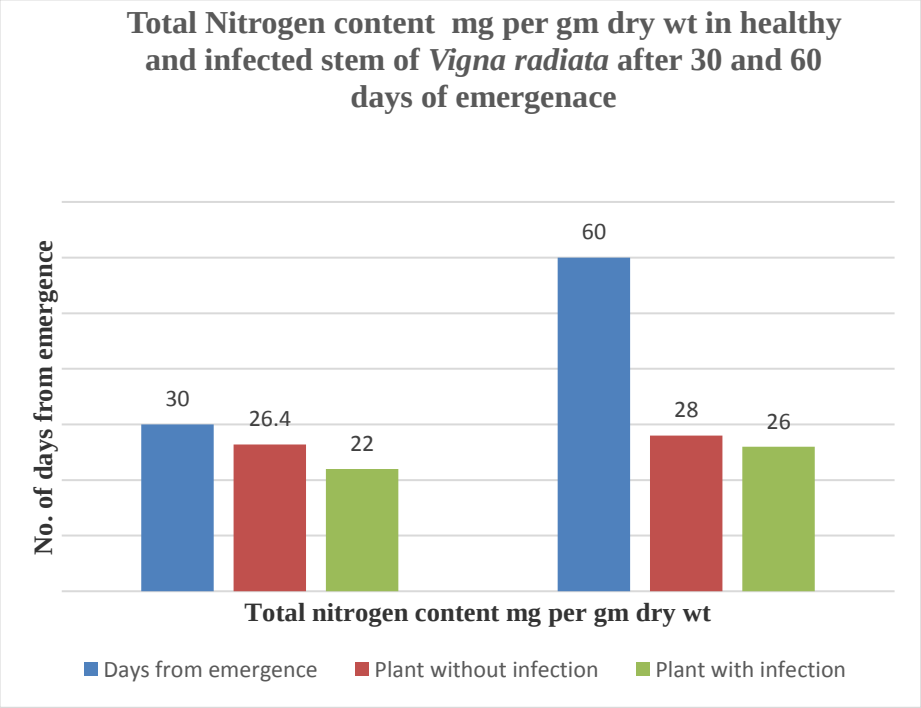

Fig.- 2 c

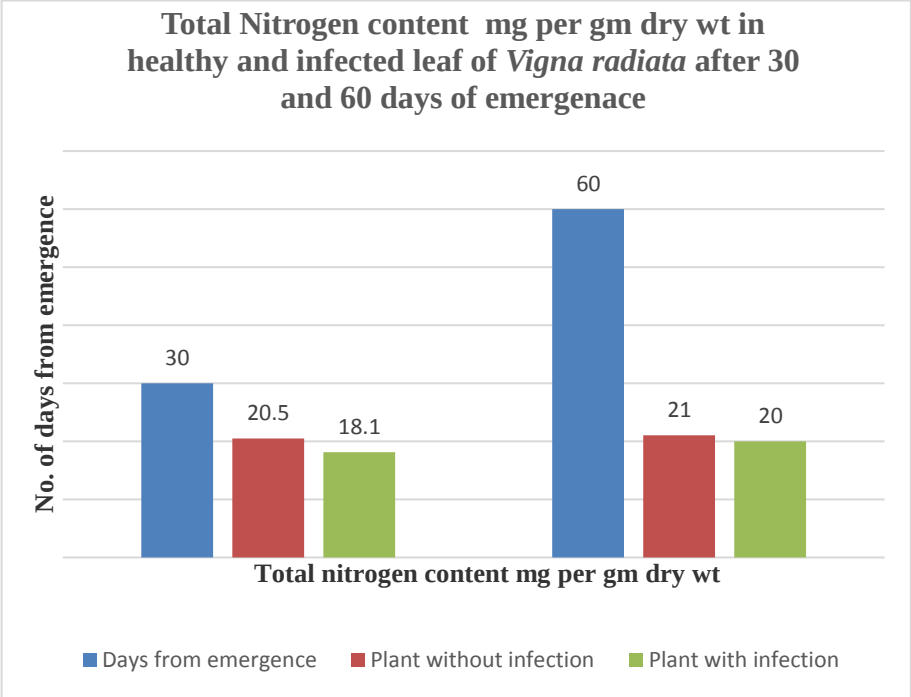

Fig.- 2 d

Table 3. Total Phosphate (Mg/gm dry weight) uptake and distribution in healthy and infected plant parts of *Vigna radiata*

Days from emergence	Soil without plant (Blank) mg/kg	Soil with plant mg/kg	Total phosphate level in			
			Stem	Leaf	Anther	Pollen grains
			mg/gm dry wt.			

Plant without infection (Control)						
0	290.0	290.0	.....	.....	.....	.....
30	288.0	280.0	25.40	19.40	…..	…..
60	280.0	270.0	26.00	20.60	11.50	13.85
Plant with infection						
0	290.0	290.0	....	....	.....	.....
30	286.0	284.0	20.20	18.00	.....	.....
60	280.0	272.0	22.60	19.50	9.65	11.36

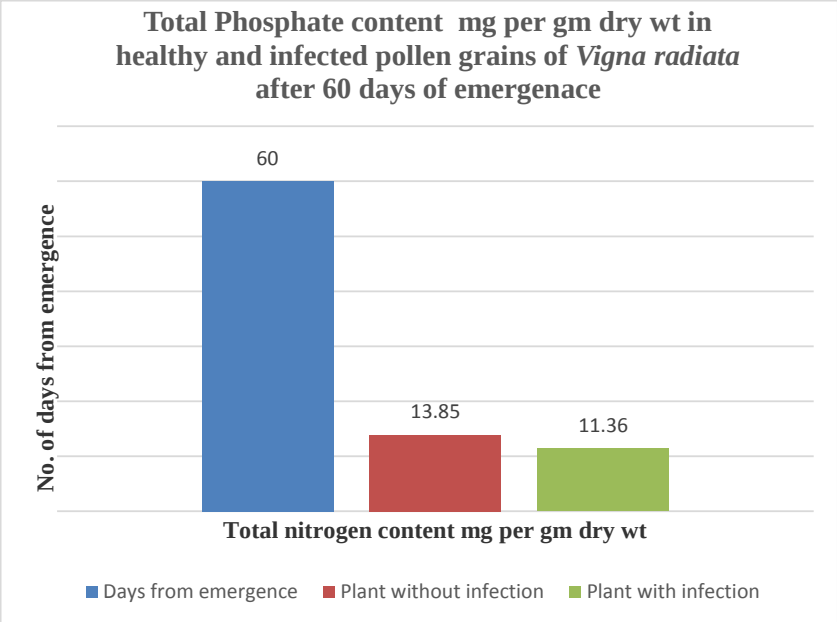

Fig.- 3 a

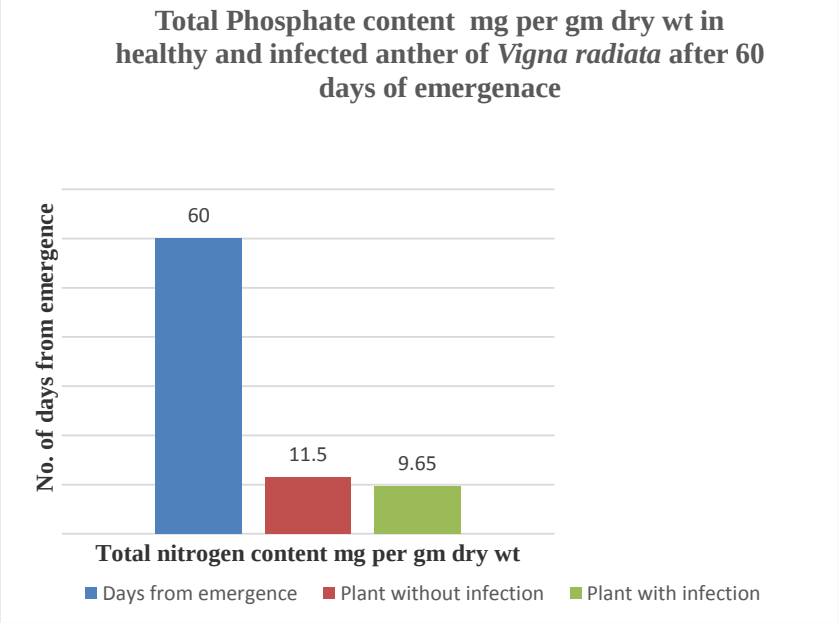

Fig.- 3b

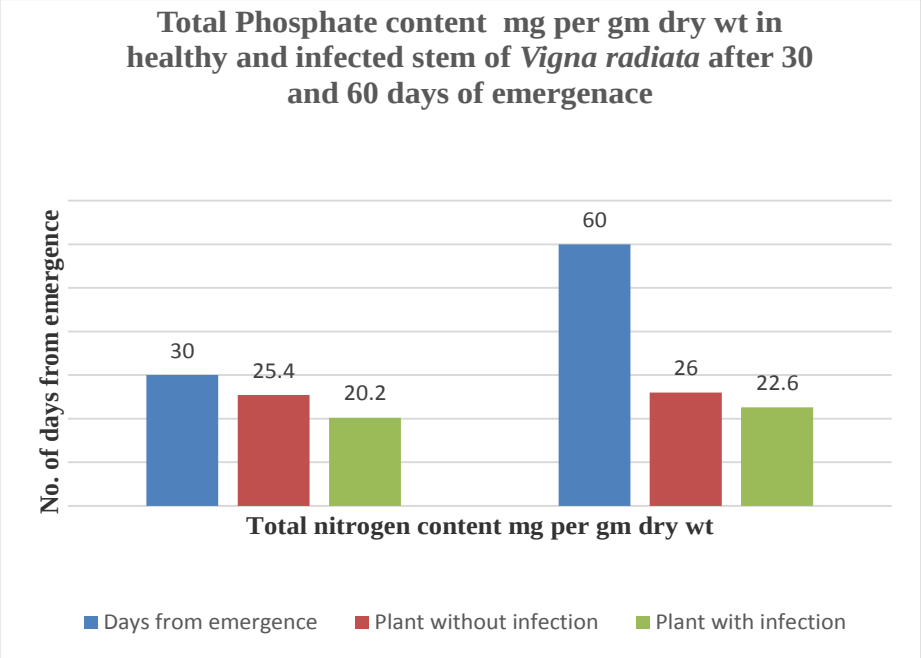

Fig.- 3 c

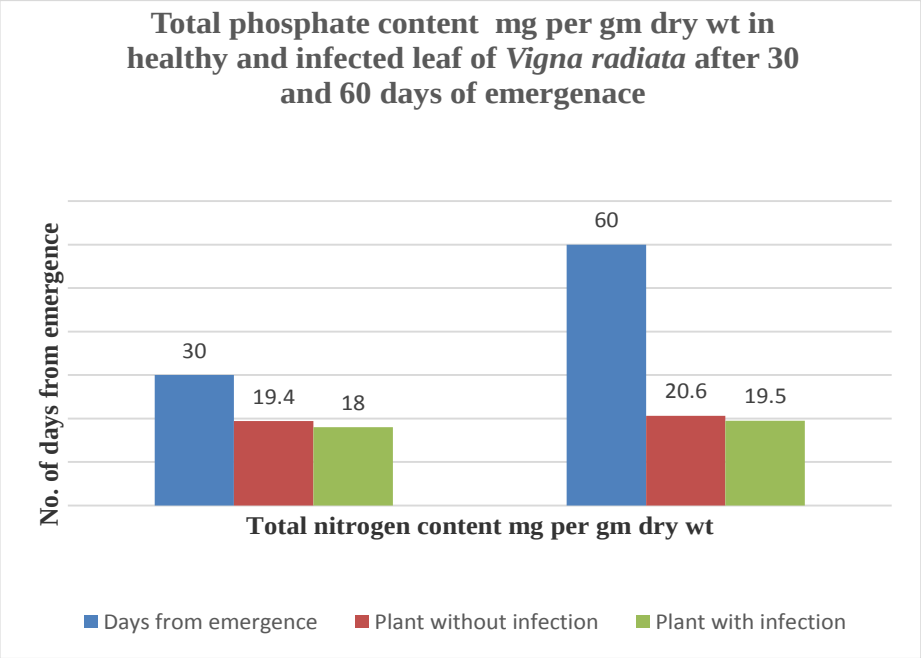

Fig.- 3 d

Table 4. Chlorophyll development in healthy and infected leaf disc in *Vigna radiata*

Treatment	Leaf disc		Chlorophyll content in healthy and infected plant							
	Fresh weight mg, leaf disc^{-1}	Dry weight mg, leaf disc^{-1}	mg/g fw^{-1}				mg/g dw^{-1}			
			chl-a	chl-b	chl-a+b	chl-a/b	chl-a	chl-b	chl-a+b	chl-a/b
Healthy plant	25.00	5.30	0.27	0.19	0.46	1.42	1.10	1.05	2.45	1.04
Infected plant	24.50	5.30	0.230	0.15	0.38	1.53	1.00	0.90	2.20	1.11

RESULTS AND DISCUSSION

Our observation indicates that non-acetolysed pollen grains of *Vigna radiata* show reduction in size. This decrease in size found ca nearly 13 % in non-acetolysed pollen grains of *Vigna radiata* as compared to acetolysed pollen grains. In table -1 & fig. -1, result shows the pore diameter, annulus diameter & exine thickness in pollen grains of *Vigna radiata*. The study with pollen morphology shows the size of pollen grains of *Vigna radiata* increases under acetolysis as compared to non-acetolysed condition of pollen grains. Result is agreement with the result of (Sampat and Ramanathan 1957) and (Sheeba and Vijyavalli 1998).

Table-2, fig-2 a, b, c and d shows the total N content uptake and its distribution in plant parts of *Vigna radiata* decline in infected plant parts as compared to healthy plant parts as in stem, leaf, anther & pollen grains. Table-2 shows that at 60^{th} day anther and pollen grains of infected plant of *Vigna radiata* contain ca 82.4 % and 88.8% of total N as compared to pollen grains of healthy (control) plant. Similarly total N content of infected leaf and stem also decline as compared to healthy plant leaf and stem of *Vigna radiata*. Table-2 also show that total N per plant organ is suppressed in infected plant due to fungal infection. Table-2 also shows that in case of soil without plant the total N content per kg decline from 0- 60^{th} days in both healthy and infected plant. Our finding of total N in various plant parts of healthy and infected plant are agreement with previous work done by (Singh and Pokhriyal 2005), (Divya 2003) and (Bhargava 2006).

Similarly, table-3 and fig-3 a, b, c, and d show total P uptake and its distribution in plant parts of *Vigna radiata* in healthy and infected plant parts. Result shows that there are decreases growth and inhibited total P content of different plant parts due to of fungal infection. Thus, total P content in the infected leaf at 40^{th} and 60^{th} day are 92.7 % and 94.6 % as compared to non- infected (control) leaf. This seems to indicate that in presence of fungal infection increases with age so, uptake and distribution of total P decreases. Table-3 also indicates that translocation of P from vegetative part to pollen grains is much affected in the infected plant. Thus, in anther and pollen grains of infected plant of *Vigna radiata* total P content is 83.9% as compared to healthy (control) anther and pollen grains at 60 days from emergence.

Tablle-3 also shows that total P content decline in stem, leaf in infected plant due to fungal infection. In case of soil without plant the total P content per kg decline from 0-60^{th} days in crop field, however this decline is more in the soil with plant. Our finding with total P in healthy and infected plant parts of experimental plant are agreement with previous work done by (Singh and Pokhriyal 2005), (Divya 2003) and (Bhargava 2006).

Table -4 shows that total chlorophyll development in *Vigna radiata* leaf discs of healthy and infected plant. Result shows that there are increases in chlorophyll development in healthy leaf disc as compared to infected leaf disc. In healthy experimental plant leaf disc total chlorophyll development is promoted by ca 121 % as compare to infected leaf disc in *Vigna radiata*. Total chlorophyll development in infected plant leaf disc is decline as compared to healthy (control) plant. Thus, Total chlorophyll on gm fresh weight basis is ca 82% in infected leaf disc as compared to healthy leaf disc of *Vigna radiata*. In same manner development of chlorophyll-a and chlorophyll-b are also affected by fungal infection in plant. Thus, a comparison of chl-a and chl –b development indicates that in general chl-a development is more as compared chl-b in both healthy and infected leaf disc of *Vigna radiata*.

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