Efficiency of some fungicides, plant extracts, chemical inducers and plant hormones on the management of damping-off and root rot diseases of *Khaya senegalensis* under greenhouse conditions

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ABSTRACT--This study was conducted at Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt and El-Orman potency, Min. of Agric. at Giza, Egypt during the two successive seasons of 2008 and 2009. The experiments were carried out under greenhouse conditions to investigate the effect of soil types and some seed treatments on Khaya senegalensis root rot disease, their interactions on the growth and chemical constitute of K. senegalensis seedlings. Seedlings of K. senegaleinsis showing root rot symptoms were collected from nurseries at Giza, Qalubyia and Ismailia governorates.

Fusarium solani and Rhizoctonia solani were the most frequently isolated fungi under natural infection at Giza, Qalubyia and Ismailia governorates .

Peat moss or peat moss + sand as planting medium for K.senegalensis in the presence of F. solani or R. solani were generally effective soil for increasing the survived seedlings under all the tested treatments.

Soaking seeds of K. senegalensis in Vitavax-Thirum was the most effective measure in decreasing pre- and post- emergence damping-off and increasing the survived seedlings, grown in soil infested with any of both fungi. Azedarachta indica and Thymus vulgaris extract decreased significantly pre- and post- emergence damping-off as well as improving seedling survivals. Salicylic acid as chemical inducer was generally the best in reducing the post-emergence damping-off compared to oxalic acid with the two fungi. Regarding to plant hormones, Gibberellic acid was the most effective in reducing the post-emergence damping-off disease compared to 1H-Indole-3-butanoic acid (IBA). On the other hand, Khaya seeds treated with A. indica and T.vulgaris, salicylic acid and Gibberellic acid were more effective and showed the highest amount of phenols content, chitinase, polyphenoloxidase enzymes in soil infested with any of the tested fungi.

Root anatomy of K. senegalensis seedling infected with root- rot caused by R. solani showed more damage symptoms than roots infected with F. solani. However, the necrotic tissues and plasmolysis cells were recorded at for both fungi. Treating seeds of K. senegalensis with any of Vitavax-Thiram, A. indica and T. vulgaris extracts, oxalic acid, salicylic acid and Gibberellic acid before planting in soil infested with any of R. solani and F. solani improved some histological characteristics of the root anatomy of the tested plant. In this respect, epidermal cells, cortical regions, phloem and xylem tissues, which were unaffected as result of treating K. senegalensis seeds with Vitavax-Thiram. On the other hand, epidermal cells and cortical regions shown moderate affect by treating seeds with any of these treatments before planting in soil infested with any of the two tested fungi comparing with untreated seeds.

Key words: Soaking seeds, Khaya senegalensis, damping-off, root rot, plant extracts, Vitavax-Thiram , phenols, enzymes, root anatomy.

1 INTRODUCTION

Trees are an important part of the terrestrial ecosystem (Lowman 2009). Trees stabilize the soil, prevent rapid run-off of rain water, help to prevent desertification, have a role in climate control and help in the maintenance of biodiversity and ecosystem balance (Bellefontaine *et al*., 2002).

Damping-off disease on tree seedlings including woody trees caused by *Fusarium* spp., *Macrophomina phaseoli*, *Phytophthora* spp., *Pythium* spp. and *Rhizoctonia solani* were reported worldwide by several investigators (Butin, 1995 a and b). Whereas, the genera of *Alternaria, Fusarium, Rhizoctonia, Penicillium* and *Trichothecium* are among the more common seed borne fungi (Mittal *et al*., 1990; Habashy, 2006 and Halawa, 2012).

The present investigation was carried on *K. senegalensis* seedlings and aimed to study:

1- The effect of pathogenic fungi grown in different soils on incidence of damping-off.

2- The efficiency of several control measures (fungicides, plant extracts, chemical inducer and plant hormones) on the management of damping-off of *K. senegalensis* seedlings under greenhouse conditions.

3- Also, some chemical constitutes and root anatomy of healthy and diseased plants of *K. senegalensis* seedlings were carried out.

2 MATERIALS AND METHODS

This study was conducted at Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt and El-Orman potency, Ministry of Agric. at Giza, Egypt during the two successive seasons of 2008 and 2009. The experiment was carried out under greenhouse conditions to investigate the effect of Disease assessments, Pathogenicity tests, soil types and some seed treatments on *Khaya senegalensis* naturally infected with root rot and their interactions on some growth measurements and chemical composition of *K. senegalensis* seedlings and their effect on root anatomy of healthy and diseased seedlings.

1. Disease survey and assessment:

1.1. Disease assessment:

Naturally, infected seedlings (showing symptoms of damping-off) of *K. senegalensis* were collected from nurseries of Giza, Qalubyia and Ismailia governorates, during years of 2008 and 2009 . In addition, inspection was carried out twice a month in the nurseries examined in order to collect samples of damped-off seedlings in different ages. Samples of the diseased seedlings were immediately used to isolate the casual fungi in the lab. On the other hand, samples of seeds (showing full ripening) of each woody tree were collected from immediately fallen fruits.

1.2. Isolation, purification and identification of the casual fungal organisms:

The collected infected roots *K. senegalensis* were thoroughly washed with tap water. Pieces contained brown lesions with uncolored tissues on the root and basal-stem of *K. senegalensis* plants were cut into small parts (0.5 x 1.0 cm). The pieces of necrotic tissues were taken off and surface sterilized in 2% sodium hypochlorite for 3 min. followed by several rinses in sterilized water before being transferred onto potato dextrose agar medium (PDA) plats. Cultures were purified by hyphal tip technique or single spore method .

The purified fungi were indentified according to their morphological features depending on the description of Gilman (1954), Barnett (1960), Booth (1971), Domsch *et al.* (1980) and Plaats-Niterink and Vandler (1981).

1.3. Pathogenicity test:

Pathogenicity test was carried out using the purified and indentified fungi that were isolated from the seedlings of *K. senegalensis* under investigation. Experiments, however, were carried out in greenhouse at Orman garden, Giza. Isolated fungi were grown on autoclaved cornmeal sandy medium consisted of 100g cornmeal, 50g sand and 100ml distilled water in 500ml glass bottles. The inoculation was carried out with 3mm fungal discs taken from the margin of 7 days old cultures of each fungus. The inoculated glass bottles were incubated at 27 ±1 °C for 15 days. Formalin-sterilized plastic pots or bags (15 cm diam.) were packed with formalin-sterilized soil mixture (peat moss, clay and sand, 1:1:1, v/v). The upper layer of soil in each pot was infested with 3% (w/w) soil weight of the fungal propagates inoculums, thoroughly mixed and slightly watered every day for a week before planting seeds. For control treatments, soil in pots provided with the same amount of autoclaved cornmeal sandy medium only without fungi. They were irrigated regularly for 7 days before planting to insure good distribution and growth of the tested fungi. The tested woody trees seeds were soaked in tap water for 24 hrs, then sterilized with 3% sodium hypochlorite for 3min, washed several times with sterilized water and planted at the rate of 5 seeds/pot, then covered with a thin layer of the soil and watered. Five pots were used for each particular treatment and the control. Percentages of pre- and post-emergence damping-off as well as survived seedling were estimated after 15, 45 and 60 days from planting, respectively. Also, all infected seedling were collected in order to re-isolate the causal pathogens.

2. Effect of inoculums potential:

Various inoculums concentrations of each pathogen were used to determine the effect of inoculums potential on disease incidence. This trial was conducted by adding different amounts of fungal growth to the soil before sowing. The inoculums were added at the rate of 1, 2, 3 and 4% w/w inoculums /soil for both pathogens, *i.e. F. solani* and *R. solani*. The infestation soil was distributed into 15 cm in diameter pots. Apparently five healthy seeds were sown in the infested soil and untreated autoclaved soil (control). Five replicates (pots) were used for each particular treatment . The percentages of pre- and post-emergence damping-off as well as survived seedlings were estimated 15, 45 and 60 days after sowing, respectively.

3. Effect of different soil types:

Five types of soil, *i.e.* clay, sandy, peat moss, mixture of clay: sand (1:1 v/v) and peat moss: sand (2:1v/v) were used in this experiment. The soils were autoclaved and separately infested with any of the tested pathogens (*F. solani* and *R. solani*). The inoculum was added to the soil at the rate of 3% (w/w) for each pathogen. Four replicates (pots) each with 6 seeds were used for each treatment. The percentages of pre- and post-emergence damping-off were estimated 15 and 45 days after sowing, respectively. Also, dead seedlings due to the infection by root-rot were counted 45 days after sowing then the survived seedlings were calculated and recorded.

4. Disease control:

Seeds of *K. senegalensis* were soaked for 24 hrs in tap water before treatment of the transaction used in the experiment then soaking transactions were as follows:

1. Control seeds were soaked for 30 min in tap water.
2. The seeds were soaked in the fungicides Carbendazim, Rizolex-T and Vitavax-Thiram at the rate of 3g/liter water for 30 min just before sowing.
3. Hot water plant extracts of *Cymbopogon citrates*, *Azedarachta indica* and *Thymus vulgaris* were prepared according to the method used by Agha (1977) .The seeds were soaked for 30 min. in 75% of any of the tested plant extracts for 30 min just before sowing.
4. Chemical inducers , *i.e.* oxalic acid, ascorbic acid and salicylic acid at the rate of 300 ppm were prepared then the

seeds were soaked for 30 min just before sowing according to Halawa (2012).

5. Plant hormones of Gibberellic acid and IBA at the rate of 500 ppm were prepared then the seeds were soaked for 24 hrs before sowing according to Abd-El-Daym (1982).

Six seeds for each treatment were planted in polyethylene bag. For each particular treatment, 4 polyethylene bags (15cm diam.) were packed with formalin-sterilized soil constituted from equal volumes of peat moss: sand (2:1 v/v). Soil infestation was carried out with 14-days-old of cornmeal sand medium inocula of *F. solani* and *R. solani* at the rate 3% (30g inoculums/kg soil). The inoculums of each fungus was thoroughly mixed with the surface layer of the potted soil and regularly watered every other day for a week before planting.

5. Morphological parameters:

Stem length (cm), root length (cm), leaves number, roots number, fresh stem weight (g) and fresh root weight (g) were estimated 60 days after sowing.

6. Biochemical changes associated with the infection by the two pathogenic fungi:

Reducing and total sugars were determined using spectropholometer at wavelength 540 nm according to method described by Thomas (1966). Phenolic compounds were determined using colourimetric of analysis described by Snell and Snell and Snell (1953). Oxidative enzymes analysis was carried out according to method described by Tuzun *et al.* (1989), polyphenoloxidase activity assay was expressed as the increase in absorbance at 495 nm/g fresh weight/30 minutes (Matta and Dimond, 1963). The substrate colloidal chitin was prepared from chitin powder according to the method described by (Ried and Ogryd-Ziak, 1981) and determination of chitinase enzyme was carried out according to the method of Monreal and Reese (1969).

5. Histopathological studies:

Samples of *K. senegalensis* seedlings of infected by the two pathogenic fungi and uninfected roots were taken 60 days after sowing. The infected and uninfected small pieces of the roots were embedded in paraffin wax (58-60 °C) according to the method of Johansen (1940). Section cross 15µ thick were carried out by the rotary microtome. Finally, the sections were examined microscopically according to method described by Ann and Dean (2014).

2 RESULTS AND DISCUSSION

1. Diseases survey and assessment:

The occurrence of damping-off on seedlings of *K. senegalensis* trees was evident during a survey performed in the nurseries of Giza, Qalubyia and Ismailia governorates during 2008 and 2009 seasons (Table 1). The incidence of damping-off on the seedlings of *K. senegalensis* at the three localities during both seasons was ranged between 16.8 - 32.2%.

K. senegalensis seedlings grown in nurseries of Qalubyia governorate showed the highest incidence during both seasons, followed by that grown at Ismailia and Giza nurseries, respectively during 2008 season. However, data showed the highest disease incidence was occurred during 2009 season in Qalubyia nurseries followed by that grown at Giza and Ismailia nurseries, respectively. In this respect, Manion (1981) reported that damping-off is a problem that may regularly produce losses of 15% or more to tree seedlings in nurseries. Moreover, once cells of the succulent root and stem tissues have stopped expanding and their walls become lignified, they become resistant to infection by damping-off fungi. Also, Butin (1995 b) mentioned that tree seedlings rots are among the most common and most feared diseases in nurseries and they occur both on germinating seeds and on first year seedlings. Moreover, Hridha *et al.* (1986) stated that fungal diseases incidence on tree seedlings of Bangladesh nurseries ranged from 20 to 73%. In Egypt, damping-off (18.5-43.4%) was recorded on *Cassia fistula* seedlings in nurseries of Qalubyia governorate (Radwan *et al.*, 1996).

Table (1) Percentage of naturally infected damping-off seedlings in surveyed nurseries in 3 districts during 2008-2009.

	Governorates						Means
	Giza		Qalubyia		Ismailia		
	Growing seasons						
	2008	2009	2008	2009	2008	2009	
% Damping-off	16.8	25.7	30.9	32.2	19.5	28.3	25.9

2. Isolation, purification and identification of the causal fungi:

Data presented in (Table, 2) proved that, 12 fungal genera were isolated from damping-off seedling of the tested *K. senegalensis* plants.The isolated fungi were purified and identified as:

Alternaria tenuis, Aspergillus sp.**,** *Botryodiplodia* sp.,*Curvularia lunatamm, Fusarium oxysporum, F. semitectum* and *F. solani*, *Macrophomina phaseolina, Penicillium* sp., *Pythium torulosum, Rhizoctonia solani, Rhizopus* sp., *Trichoderma* sp. and *Helminthosporium* sp.The. isolated fungi were previously isolated from the roots of many woody trees (Shukla *et al.* ,1990; Abd El-Latif *et al.* ,1991 ; Radwan *et al.*,1996; Arya and Kaushik 2001 and Khalid *et al.* , 2002).

2.1. Frequencies of fungi isolated from the damped-off seedlings of *K. senegalensis* :

Data presented in (Table, 2) proved that, *Fusarium* sp. (31.21%) was the most frequently isolated fungus followed by *Rhizoctonia solani* (17.38%). While, *Penicillium* sp. (3.28%) was isolated at low frequency.

In this respect, Shukla *et al.* (1990) isolated *F. solani* and *F. equiseti* from damped-off seedlings of *Leucaena leucocephala*, while *F. oxysporum* was isolated from *Delonix regia* damped-off seedlings (Gbadegesin, 1993). In Egypt, Abd El-Latif *et al.* (1991) and Radwan *et al.* (1996) supported our findings concerning the fungi isolated from *C. fistula* damped-off seedling, except *B. theobromae* and *P. torulosum* fungi. On the other hand, *Fusarium* spp. were the most common fungi associating with the damped-off seedlings of all trees tested. They were also isolated more frequently from *Bauhinia variegata*, *D. regia* and *L. leucocephala.* While, *A. tenuis*, *P. torulosum* and *R. solani* were, generally, isolated with moderate frequencies, followed by *B. theobromae* and *M. phaseolina*. Variable occurrence of the present fungi on damped-off tree seedling tested were somewhat, similar to those reported by Schonhar (1965), Filer and Peterson (1975) and Butin (1995 b).

Table (2) Frequency percentages of the fungi isolated from damped-off seedlings of *K. senegalensis*.

The isolated fungi	% Frequency
Alternaria tenuis	5.57
Aspergillus sp.	4.26
Botryodiplodia sp.	5.90
Curvularia lunata	3.94
Fusarium ssp.*	31.21
Macrophomina phaseolina	7.54
Penicillium sp.	3.28
Pythium torulosum	9.27
Rhizoctonia solani	17.38
Rhizopus sp.	3.48
Trichoderma sp.	4.59
Helminthosporium sp.	3.64

**Fusarium oxysporum, F. semitectum* and *F. solani*

3. Pathogenicity test:

Data presented in Table (3) show the percentages of pre- and post-emergence damping-off and survivals of *K. senegalensis* seedlings.

Table (3) reveals that, *R. solani* was the most virulent on *K. senegalensis* seedlings, which caused the highest percentage of pre-emergence damping- off (48%), followed by *F. solani* (40%) and *F. semitectum* then *M. phaseolina* (36%).Whereas, *F. oxysporum* and *R. solani* ware the most virulent at stage of post-emergence damping-off (32%) followed by *F. solani* (28%). In term of survivals, *R. solani* seems to be the most harmful on *K. senegalensis*, since it caused the highest decrease in percentage of survivals (20%), followed by *F. solani* (32%) compared with the control (100%). On the other hand, *B. theobromae* and *A. tenuis* were the lowest virulent as they caused the lowest decrease in the percentages of seedlings survival (72-76%). The present results concerning damping-off caused by pathogenic fungi as well as the virulent ones on *K. senegalensis* were, to somewhat, similar to those previously found in Egypt by Hilal *et al.* (1995). While, the results of damping-off caused by pathogenic fungi on *L. leucocephala* and their virulence are in agreement with those obtained by Hsieh (1982), Perez-Guerrero (1982), Alonso *et al*. (1996), Arya and Kaushik (2001) and Khalid *et al*. (2002).

Table (3) Pathogenecity test of the isolated fungi from *K.senegalensis* expressed as pre- and post-emergence damping-off and survived seedlings.*

The tested fungi	% Damping – off *		Survived seedlings
	Pre-emergence	Post-emergence	
A. tenuis	16	12	72
Botryodiplodia sp.	16	8	76
F. oxysporum	32	32	36
F. semitectum	36	20	44
F. solani	40	28	32
M. phaseolina	36	24	40
P. torulosum	32	20	48
R. solani	48	32	20
Control	--	--	100

*Recorded 15, 45 and 60 days after planting the seeds in the infested soil, respectively.

Table (4). Effect of different soil types infested with *F. solani* and *R. solani* on the percentages of pre- and post-emergence damping-off and survival of *K.senegalensis* seedlings.

Soil types	% Damping-off and survived seedlings caused by					
	F. solani			*R.solani*		
	Pre-emer.	Post- emer.	Survived seedlings	Pre- emer.	Post- emer.	Survived seedlings
Clay	41.7	33.3	25.0	50.0	33.3	16.7
Sandy	50.0	29.2	20.8	45.8	33.3	20.8
Peat moss	33.3	29.2	37.5	33.3	33.3	33.3
Clay + sand	37.5	29.2	33.3	50.0	29.2	20.8
Peat moss + sand	33.3	29.2	37.5	41.7	29.2	29.2

5. Effect of different inoculums on the percentages of pre- and post-emergence damping-off and survived seedlings:

The obtained data (Table 5) show effect of different inoculums on the percentages of pre- and post-emergence damping-off and survived seedlings . In this respect, the incidence of pre-and post-emergence damping-off were gradually increased by increasing the inoculums level. The fungus *R. solani* was the most virulent one than *F. solani* .

The percentages of survived seedlings were increased by decreasing the inoculums level. One % inoculum level showed the highest percentage of seedlings survival with 56 and 44% for *F. solani* and *R. solani*, respectively. These results are agreement with Radwan (1992) reported that increasing inoculums levels of *F. oxysporum* and *R. solani* increased the pre-and post emergence damping-off *Casuarina glauce*. Mostafa (1995) showed that increasing the inoculums potential of *Verticillium albo-atrum* in the soil, positively, intensified apricot seedlings wilt severity. Radwan (1996) mentioned that percentage of root-rot disease on pea was increased by increasing *Fusarium* and *Rhizoctonia* inoculums from 1 to 7% w/w inoculums/soil.

Table (5). Effect of different inoculums on the percentage of pre- and post-emergence damping-off and survival of *K. senegalensis* seedlings.

Inoculums level (%)	% Damping-off and survived seedlings caused by					
	F. solani			*R. solani*		
	Pre-emergence	Post-emergence	Survived seedlings	Pre-emergence	Post-emergence	Survived seedlings
Control	0.0	0.0	100	0.0	0.0	100
1	24	20	56	32	24	44
2	32	28	40	36	32	32
3	36	32	32	44	36	20
4	44	36	20	48	40	12
Mean	27.2	23.2	49.6	32.0	26.4	41.6

6. Effect of the two isolated fungi on the incidence of pre and post-emergence damping-off as well as the survived seedlings of some wood trees genera:

Results presented in Table (6) show that the two pathogenic fungi were able to infect all the tested woody trees seedlings under study. Both fungi caused the highest percentages of pre- and post-emergence damping-off to *C. toona* followed by *A. polystachya* . *M. azedarach* and *A. polystachya* gave the highest percentage of the survived seedlings, being 54.2 and 41.7%, respectively with *F. solani.* While *C. toona* with *R. solani* gave the highest percentage of pre-emergence damping-off, being 58.3%. *M. azedarch* gave the highest percentage of post-emergence damping-off, being 37.5%. *C. toona* gave the lowest percentages of survived seedlings with *F. solani* and *R. solani.*

Table (6) Effect of the two isolated fungi on pre- and post-emergence damping-off and survival of some wood trees genera.

Tree Species	% Damping-off and survived seedlings caused by					
	F. solani			*R. solani*		
	Pre-emergence	Post-emergence	Survived seedlings	Pre-emergence	Post-emergence	Survived seedlings
K. senegalensis	32.0	28.0	40.0	36.0	32.0	32.0
A. polystachya	41.7	16.7	41.7	45.8	29.2	25.0
C. toona	50.0	29.2	20.8	58.3	33.3	8.3
M. azedarach L.	29.8	16.7	54.2	41.7	37.5	20.8

7. Effect of soaking *K. senegalensis* seeds in some treatments and sown in soil infested with *F. solani* and *R. solani* on the incidence of pre- and post-emergence damping-off and survived seedlings under greenhouse conditions:

Percentages of pre-emergence damping-off (Table, 7) were decreased compared with the control (without treatments) in most cases by using each one of the tested treatments. In soil infested with *F. solani*, the lowest effect was occurred by Carbendazim, which gave the highest

percentage of pre-emergence damping-off, being 20.8% compared with the other two fungicides. Also, While Carbendazim and oxalic acid gave the highest percentage of post-emergence damping-off which was 12.5%. Vitavax-Thiram and Salicylic acid gave the highest percentage of survival seedlings 91.7%. While with *R. solani* Oxalic acid and Salicylic acid gave the highest percentage of pre-emergence damping-off which was 29.2%. *C. citratus* and IBA gave the highest percentage of post-emergence damping-off which was 20.8%. Rizolex-T and Salicylic acid gave the highest percentage of survival seedlings which was 66.7%. Hot water extracts of *A. indica*, *C. citrates* and *T. vulgaris* at conc. of 75% inhibited the mycelia growth of *F. solani* and *R. solani* with *K. senegalensis*. Thyme extract however, was the most effective treatment in case *F. solani* but Neem extract was the most effective treatment in case of *R. solani*. While lemongrass extract was the lowest effective treatment in all cases. There was positive effect of the tested water plant extracts on the mycelial growth inhibition of the two pathogenic fungi . These results are similar to those reported by Baiuomy (1997); Ali (1999); El-Habaa *et al*. (2002) and Shafie (2004). Also, the obtained results concerning increase in inhibitory effect for each plant extract by increasing its concentration are coincide with Baiuomy (1997); Ali (1999); El-Habaa *et al*. (2002) and Shafie (2004). On the other hand, aqueous extracts of the plant under study may be contain fungicidal substance (s), causing inhibition to germination of *Fusarium* spp. spores and *M. phaseolina sclerotia* (Afifi and Dowidar, 1977 and Shafie, 2004) and preventing formation of reproductive organs (Abd El-Ghafor, 2000).

Table (7). Effect of soaking *K. senegalensis* seeds in some treatments and planted in soil infested with any of *F. solani* and *R. solani* on pre- and post-emergence damping-off and survived seedlings, under greenhouse conditions.

Treatments	% Damping-off				% Survived seedlings	
	Pre-emergence		Post-emergence			
	F. solani	*R. solani*	*F. solani*	*R. solani*	*F. solani*	*R. solani*
Control	33.3	45.8	25.0	25.0	41.7	29.2
Fungicides:						
Carbendazim	20.8	20.8	12.5	12.5	66.7	66.7
Rizolex-T	16.7	8.3	4.2	4.2	79.1	87.5
Vitavax-Thiram	8.3	16.7	0.0	8.3	91.7	75.0
Plant extracts:						
A. indica	12.5	16.7	8.3	4.2	79.2	79.1
C. citratus	12.5	25.0	4.2	20.8	83.3	54.2
T. vulgaris	8.3	20.8	4.2	8.3	87.5	70.8
Chemical inducers:						
Ascorbic acid	12.5	12.5	4.2	12.5	83.3	66.7
Oxalic acid	16.7	29.2	12.5	8.3	70.8	79.2
Salicylic acid	4.2	29.2	4.2	4.2	91.7	87.5
Plant hormones:						
Gibberellic acid	8.3	12.5	4.2	4.2	87.5	83.3
IBA	12.5	12.5	8.3	20.8	79.2	50.0

8.

Effect of soaking *K. senegalensis* seeds in some treatments and sown in soil infested with *F. solani* or *R. solani* on vegetative growth, under greenhouse conditions:

Data listed in Table (8) indicate that all treatments under study increased vegetative growth of *K. senegalensis* seedlings grown in artificially infested soil with *F. solani* or *R. solani.* In soil infested with *F. solani*, Ascorbic acid and Gbberelic acid increased stem lengths , being 26.3cm. While IBA gave the highest root length, leaves number and roots number, being 19.3 cm, 20.3 leaf and 5.0 root, respectively. Meanwhile, ascorbic acid increased fresh stem and root weight , being 1.54 and 0.74 g, respectively. On the other hand, with *R. solani* Gibberellic acid increased stem length to 23.5cm. As for, *C. citrates* ascorbic acid gave the highest root length (19.5cm.). *A. indica* and *C. citratus* gave the highest leaves number 14.8cm. IBA increased roots number to 2.3 r\ root . While, oxalic acid increased fresh stem weight to 1.73g. As for IBA increased fresh root weight to 0.48g. These results are in accordance with those reported by Sochacki and Chmiel (1994) on *Euphorbia pulcherrima*, observed that the best rooting was obtained by using the Polish rooting formulation Urchrys (containing 0.05% IAA, 0.2% NAA and vitamin B complex) and Ursynroot 3 (containing 0.8% IAA, 1.8%IBA, 0.2% NAA and vitamin B complex). Souidan *et al.* (1995) dipped hardwood cutting of *Ficus elastic* var. *decora* in IAA, NAA or IBA each at 1000, 2000 or 4000 ppm then planted in moist sawdust. Among these results, most treatments increased rooting parameters compared with controls. Mc Cracken *et al.* (1996) treated terminal stem cutting of *Magnolia grandiflora* cv. Brown velvet with 0.3% IBA in talc, or 0.5% NAA quick dip+0.3 IBA in talc, or 0.5% NAA + 1% IBA quick dip. They came to conclusion that higher auxin concentration produced more primary roots but reduced the formation of secondary roots. However, rooting percentage was not affected by auxin treatment. Zhang Yu Song *et al.* (1997) soaked *Jasminum sambac* cutting for 24h in various concentration of IAA, NAA or IBA. They recommended that the best rooting was obtained with 300 mg/L IAA, 50 mg/L NAA and 300 mg/L IBA. Imarah (2000) stated that the combined treatment Rizolex-T or Vitavax /Thiram)+IBA gave the highest number of branches of *Pelargonium graveblens* grown in *Rhizoctonia solani* infested soil. The pre-treatments of cuttings with the fungicides tested, IBA and their

combinations increased the fresh weight of shoot compared to the control.

9. Effect of soaking seeds of *K. senegalensis* in some treatments and sown in soil infested with *F. solani* on sugars, phenols content , chitinase enzyme, polyphenol-oxidase enzyme in the seedlings 60 days after sowing:

In soil infested with the tested pathogen *F. solani* (Table, 9), the obtained results indicate that, all tested treatments (fungicides, plant extracts, chemical inducer and plant hormones) increased reducing, total sugars, phenols, Chitinase enzyme and PPO enzyme in seedling compared with the untreated control. Vitavax-Thiram increased reducing, total sugars, free- and total phenols which were 8.52, 12.69, 14.56 and 21.27mg/g fresh weight respectively.

Salicylic acid increased chitinase enzyme and PPO enzyme which were 12.78 and 1.96 respectively. This effect was reported by Fouad *et al*. (1992). They showed that the highest rooted percentages of eight olive cvs. coincided with low values for phenols and extractable rooting inhibitor contents. Farag (1998) reported that the artificial inoculation of grapevine twigs with *B. theobromae* increased the total and reducing sugars content in the infected tissues than that in the healthy ones. Al-Sayed (2005) who found that *Trichoderma harizianum* increased β-1, 3-glucanases, chitinase and cellulose activities where *T. harzianum* attached to *Sclerotium rolfsii* or *Rhizoctonia solani* in the experiment.

Table (8) .Effect of soaking *K. senegalensis* seeds in some treatments and sown in soil infested with any of *F. solani* and *R. solani* on vegetative growth, under greenhouse conditions.

Treatments	Stem length (cm)		Root length (cm)		No. of leaves		Secondary roots number		Stem fresh weight (g)		Root fresh weight (g)	
	F. solani	*R. solani*	*F. solani*	*R. solani*	*F. solani*	*R. solani*	*F. solani*	*R. solani*	*F. solani*	*R. solani*	*F. solani*	*R. solani*
Control	12.8	13.5	8.8	10.0	7.5	3.8	1.8	1.8	0.05	0.20	0.05	0.12
Fungicides:												
Carbendazim	16.0	13.0	13.3	15.0	11.3	7.8	2.3	1.8	0.24	0.70	0.13	0.30
Rizolex-T	12.0	17.0	12.0	16.5	11.3	11.3	3.3	1.8	0.32	0.99	0.13	0.48
Vitavax-Thiram	16.3	21.5	14.3	14.0	13.0	11.8	5.0	2.3	0.59	1.13	0.23	0.41
Plant extracts:												
A. indica	17.0	19.0	13.3	17.0	12.8	14.8	2.0	1.0	0.30	1.04	0.19	0.33
C. citratus	20.3	21.3	14.8	19.5	15.0	14.8	1.0	1.8	0.66	1.43	0.20	0.42
T. vulgaris	19.0	19.5	14.8	16.0	14.0	14.3	1.0	1.3	0.51	1.22	0.21	0.41
Chemical inducers:												
Ascorbic acid	26.3	16.3	16.0	19.5	21.3	10.3	5.3	2.0	1.54	0.76	0.74	0.24
Oxalic acid	22.0	19.0	18.3	16.3	18.0	12.0	1.3	2.0	1.07	1.73	0.56	0.41
Salicylic acid	23.5	21.0	19.3	16.5	19.3	12.8	4.8	1.8	0.81	1.30	0.44	0.42
Plant hormones:												
Gibberellic acid	26.3	23.5	16.8	15.5	18.3	13.8	4.0	1.8	1.37	1.10	0.59	0.22
IBA	23.3	14.0	19.3	11.5	20.3	9.3	5.0	2.3	1.20	0.34	0.66	0.14

Table (9) .Effect of soaking seeds of *K. senegalensis* in some treatments and swon in soil infested with *F. solani* on sugars, phenols content (mg/g FW) and chitinase (CHT) enzyme, Polyphenoloxidase (PPo) enzyme (mg/g FW) in the seedlings 60 days after planting ,under greenhouse conditions.

Treatments	Sugars (mg/g fresh weight)			Phenols (mg/g fresh weight)			Chitinase enzyme	PPO* enzyme
	Reducing	Non-reducing	Total	Free-	Conjugated	Total		
Control	2.01	3.52	5.53	7.53	3.21	10.74	3.78	1.69
Fungicides:								
Carbendazim	4.63	2.52	7.15	10.15	6.16	16.32	4.78	1.72
Rizolex-T	6.21	2.92	9.13	11.78	7.15	18.93	5.35	1.38
Vitavax-Thiram	8.52	4.17	12.69	14.56	6.70	21.27	7.15	1.21
Plant extracts:								
A. indica	6.21	2.78	8.99	6.74	8.12	14.87	12.53	1.12
C. citratus	7.02	3.60	10.62	6.96	7.97	14.93	10.97	1.88
T. vulgaris	7.11	3.65	10.76	7.38	8.00	15.38	11.33	1.10
Chemical inducers:								
Ascorbic acid	7.15	3.71	10.86	10.58	7.16	17.74	11.25	1.09
Oxalic acid	5.07	2.10	7.17	9.52	5.96	15.48	7.20	1.43
Salicylic acid	8.50	4.15	12.65	14.50	6.70	21.26	12.78	1.96
Plant hormones:								
Gibberellic acid	7.24	2.84	10.08	8.21	7.99	16.20	11.47	1.00
IBA	6.50	2.84	9.34	9.47	5.90	15.37	7.25	1.44

*PPO : Polyphenoloxidase enzyme.

10. Effect of soaking *K. senegalensis* seeds in some treatments and sown in soil infested with *R. solani* on sugars, phenols content and chitinase enzyme and Polyphenoloxidase (PPo) enzyme in the seedling 60 days after planting, under greenhouse conditions.

In soil infested with the tested pathogen *R. solani* (Table, 10), presented data proved that, all tested treatments (fungicides, plant extracts, chemical inducer and plant hormones) increased reducing, total sugars, phenols, chitinase enzyme and PPO enzyme in seedling compared with the untreated control. Salicylic acid increased reducing, total sugars, total phenols, chitinase enzyme and PPO enzyme which were 8.32 and 12.23, 28.73, 12.38 and 1.93 respectively. Vitavax-thiram increased free-phenols which was 17.21mg/g fresh weight. Determination the effects of some fungicides, plant extracts, chemical inducer and Plant hormone as Soaking treatment for *K. senegalensis* before planting in infested soil with *F. solani* and *R. solani*, on total phenol, sugar contents and chitinase (CHT) enzyme, Polyphenoloxidase (PPo) enzyme (mg/g FW) in seedling was achieved. In case of soil infested with *F. solani* the total phenol contents, however, were increased compared with the control and the highest ones were found in case of Vitavax-Thiram, Salicylic acid, Rizolex-T, followed by Ascorbic acid and Gbberelic acid. In this respect, the presence of the pathogenic fungi *F. solani* and *R. solani* in *K. senegalensis* rhizosphere and its permanent trials to infect seedling roots stimulate their tissues to produce and accumulate phenolic compounds similar to those reported by Habashy (2006). Also Halawa (2012) found that the fungicide carbendazim, as the superior treatment in controlling the diseases (infection by *Corynespora cassiicola*) in *Pritchardia filifera* (ornamental palm), recorded the highest increases in root contents from total sugars, phenols and peroxidase activity, followed by rue oil and Salicylic acid treatments.

Table (10). Effect of soaking of *K. senegalensis* in some treatments grown in soil infested with *R. solani* on sugars, phenols content , chitinase enzyme and polyphenoloxidase enzyme in the seedlings 45 days after planting ,under greenhouse conditions.

Treatments	*R solani*							
	Sugars (mg/g fresh weight)			Phenols (mg/g fresh weight)			Chitinase enzyme	PPO enzyme
	Reducing	Non-reducing	Total sugars	Free-	Conjugated	Total		
Control	2.08	2.97	5.05	7.60	3.23	10.84	3.80	1.68
Fungicides:								
Carbendazim	6.23	2.96	9.19	10.09	5.61	15.70	9.32	1.69
Rhizolex-T	8.25	3.86	12.11	14.37	8.37	22.74	12.31	1.93
Vitavax-thiram	6.32	4.58	10.90	17.71	9.82	27.53	10.20	1.79
Plant extracts:								
Azedarachta indica	8.20	3.45	11.65	5.11	17.10	22.21	7.11	0.99
Cymbopogon citratus	5.10	2.25	7.35	8.41	4.61	13.02	7.50	0.96
Thymus vulgaris	7.27	2.96	10.23	8.35	6.35	14.70	7.02	0.97
Chemical inducers:								
Ascorbic acid	6.20	2.95	9.15	11.18	6.61	17.79	9.30	1.69
Oxalic acid	8.28	3.50	11.78	5.16	17.17	22.34	7.06	1.37
Salicylic acid	8.32	3.91	12.23	16.78	11.94	28.73	12.38	1.93
Plant hormones:								
Gibberellic acid	7.54	4.26	11.80	12.33	4.421	16.75	5.96	1.89
IBA	4.42	2.60	7.02	7.95	3.589	11.54	6.81	1.72

11. Root anatomy of healthy and infected *K. senegalensis* seedlings with the tested fungi:

The cross sections of non-infested root of *K. senegalensis* seedlings grown at un-infested soil and without any treatments under study were showed in Fig (1). The Crosse section showed that all investigated parts as periderm, secondary cortex, phloem, cambium, secondary xylem and primary xylem were healthy and without any indication of changing in the colors of cells.

While, the cross section of rotted *K. senegalensis* seedling root grown in infested soil with *R. solani* or *F. solani* are shown in Figs. (2.1 and 2.2), which illustrate that, the outer tissues showed plasmolysis and disorganization with dark brown discoloration of both preiderm and cortical cells in both root infested with *R.solani* or *F. solani*. Root anatomy of *K. senegalensis* seedling infected with rot root caused by *R. solani* showed in Fig. (2.1) more damage symptoms than root infected with *F. solani*. However, the necrotic tissues and plasmolysis cells were recorded at either infested fungi. The cross section of rotted root *K. senegalensis* seedling showed in Fig. (2.2) brownish epidermal cells and thin-walled cells when soil was infested with either *R. solani* or *F. solani*. The cortex consists of thin layer walled of paranchematous cells at the outer part and with a single layer of small size at the inner part. The endodermis was formed of single layer of isodiametric thin walled cells.

Fig (1). Crosse section in healthy root of *K. senegalensis* seedlings showed that all investigated parts as periderm, secondary cortex, phloem, cambium, secondary xylem and primary xylem were healthy and without any indication of changing

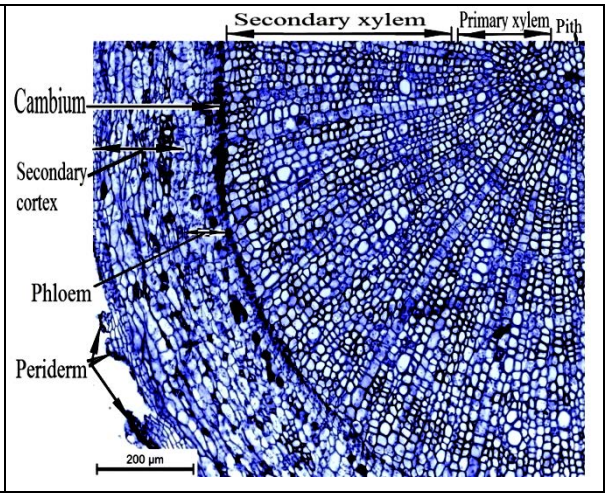


Fig. (2.1) also illustrate some changes in epicycle paranchematous cells layer below the endodermis compared to that in root anatomy of root at un-infested soil. The cross section of root showed that, the cambium was consists of non-continuous ring and reduced in layer of cells with destroyed cells and showed dark and necrotic area in both xylem paranchyma and xylem vessels.

The cambium was not differentiated to secondary phloem on the outer side or secondary xylem on the inner side. The secondary xylem was formed to compacted cylinder and interrupted. This may be due to the soil infested with *R.solani*. Ploetz *et al.* (1996) found that *Lasiodiplodia theobromae* (*Botrydiplodia theobromae*) caused significant necrosis, gummosis, and vascular discoloration on mango trees. Kamhawy (2001) announced that after 21 days of inoculation with *B. theobromae*a necrotic area was noticed in both xylem parenchyma and xylem vessels of grapevine trees colonized by hyphae, and dark inclusions inside the xylem as well as abundant production of tylosis and gummosis. The pathogen spread to all various tissues causing complete breakdown.

12. Root anatomy of *K.senegalensis* seedlings grown from seeds untreated and treated with the tested treatments before planting in soil infested with the tested fungi :

Figs. (3 and 4) indicate that treating seed of *K. senegalensis* with one of control treatments like Vitavax-Thiram, *A. indica* extract, *T. vulgaris*, oxalic acid, salicylic acid and Gibberellic acid before planting in soil infested with *R. solani* or *F. solani* improved some histological characteristics of root anatomy of *K. senegalensis* seedling. In this respect, epidermal cells, cortical regions, phloem and xylem tissues were unaffected as result of treating *K. senegalensis* seeds with Vitavax-Thiram. On the other hand, epidermal cells and cortical regions were shown moderately affect by treating seeds with each one of the five tested control treatments (*A. indica* extract, *T. vulgaris*, Oxalic acid, Salicylic acid and Gibberellic acid) before planting in soil infested with *R. solani* or *F. solani* comparing with cross sections of infected root were taken from untreated seeds. The primary anatomical response of plants to fungal infections is induced structural defenses, such as cell wall thickening that prevent pathogens from penetrating host cells. There is also some evidence suggesting that the development of the vascular structure of plants can be disrupted during pathogen infection, and the cambium layer reduced in infected plants compared to uninfected plants (De Cal *et al*., 2000). During infection, typical *K. senegalensis* anatomy (Lersten and Carlson, 2004) was observed in both infected and non-infected plants. During pathogenic infection, the vascular tissue structure of infected plants differed from the uninfected plants. Plants infected with *F. solani* and *R. solani* had the same anatomical features present, but to different extents. The width of the cylindrical ring of secondary xylem became smaller and less pronounced in infected plants compared to uninfected plants of *R. solani*. A similar but less pronounced trend was observed in *F. solani* (Ann and Dean 2014). The secondary xylem of root plant infected with *R. solani* was similar in plants infected with *F. solani* and different than control (the uninfected plants). *K. senegalensis* plant infected with *R. solani* had less secondary xylem than the uninfected plants. Xylem vessels collapsed, the cambium layer began to breakdown, and vessel occlusions were not observed, but not to a great extent when inoculated with either *F. solani* or *R. solani*. These results also are in line with Nemec *et al.* (1986) stated that *F. solni* principally invaded broken epidermal cells, root hair cell walls and/or direct penetration.

Fig.2.1 Cross sections of infected roots tissue samples of *K. senegalensis* seedling were taken from infested soil with *R. solani* showing. **(A)** Affected outer bark tissues, **(P)** plasmolysis cells and **(D)** disorganization with **(B)** dark brown discoloration of periderm and cortical cells. **(N)** Necrotic tissue. **(T)** Thin layer walled of xylem paranchyma and xylem vessels.

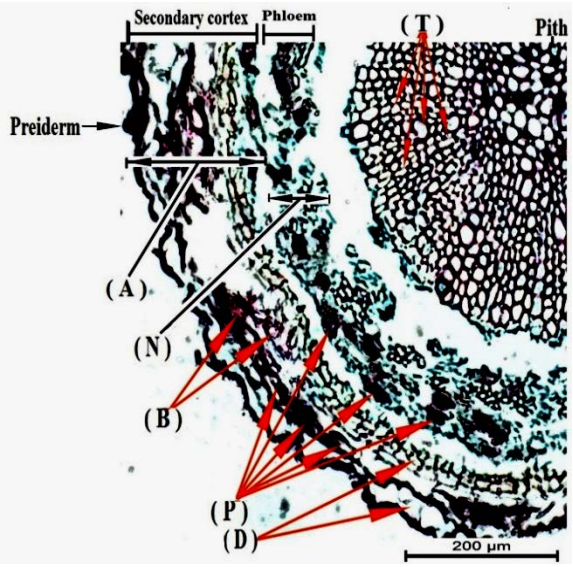
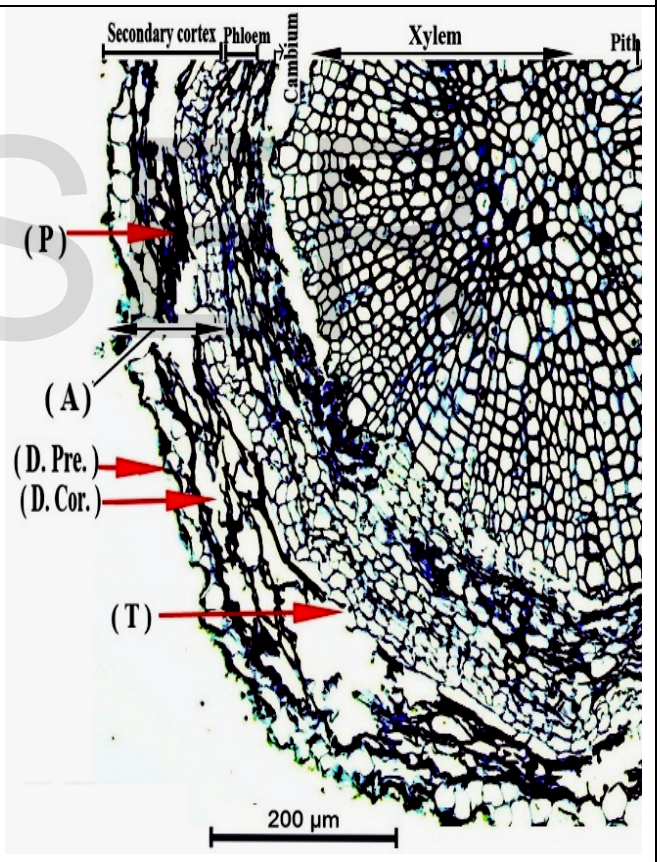


Fig.2.2 Cross sections of infected roots tissue samples of *Khaya senegalensis* seedling were taken from infested soil separately with *F. solani* showing. **(A)** Affected outer bark tissues, **(P)** plasmolysis cells, **(D. Pre)** disorganization periderm and **(D. Cor)** disorganization cortical with dark brown discoloration cells. **(T)** thin layer walled of paranchematous cortex cells



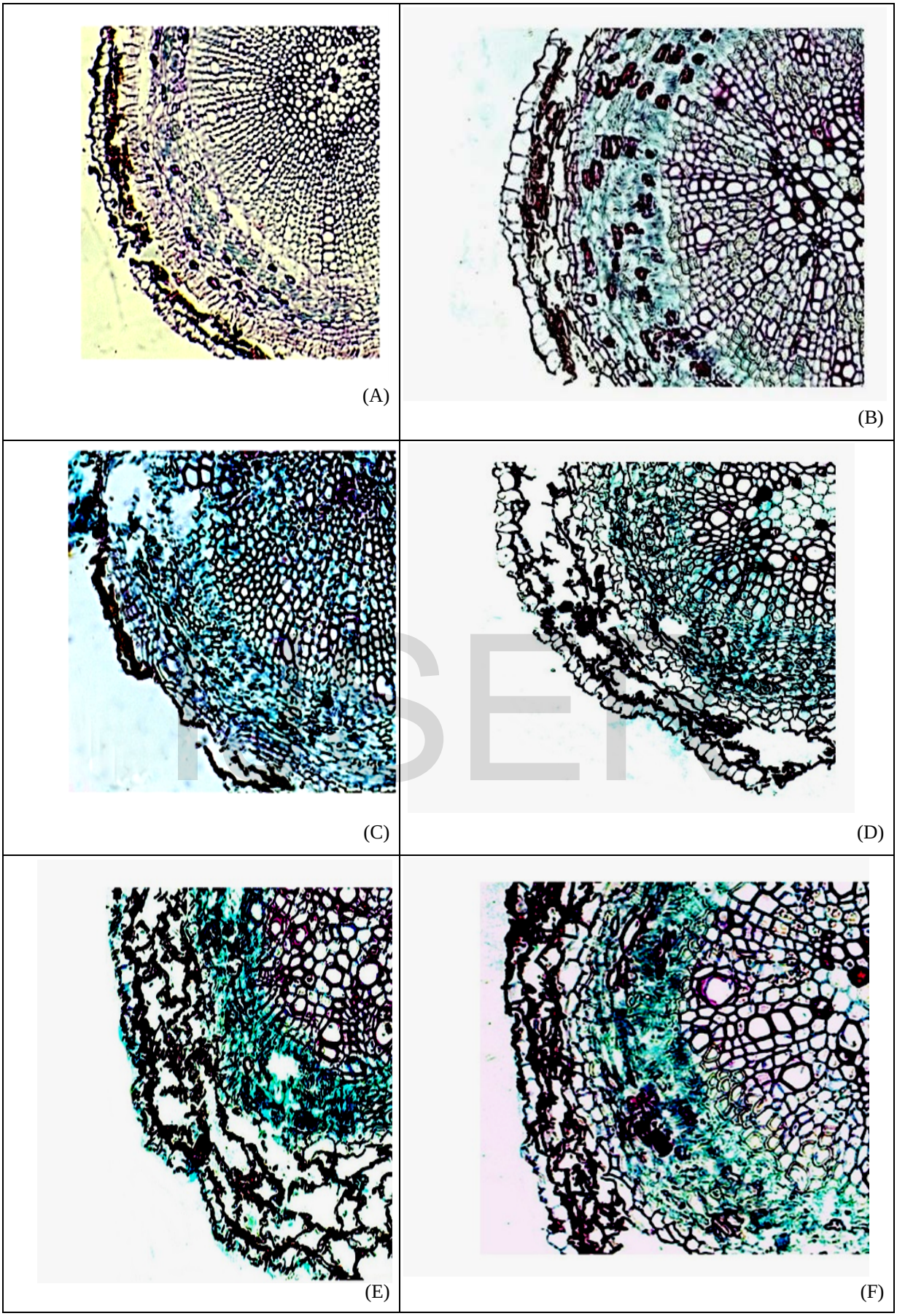


Fig. (3) Root anatomy of *K. senegalensis* seedling were taken after 60 days from planting seeds were treated with A) Vitavax-Thiram; B) *A.indica* extract; C) *T. vulgaris* extract; D) Oxalic acid; E) Salicylic acid and F) Gibberellic acid. in soil infested with *R. solani.*

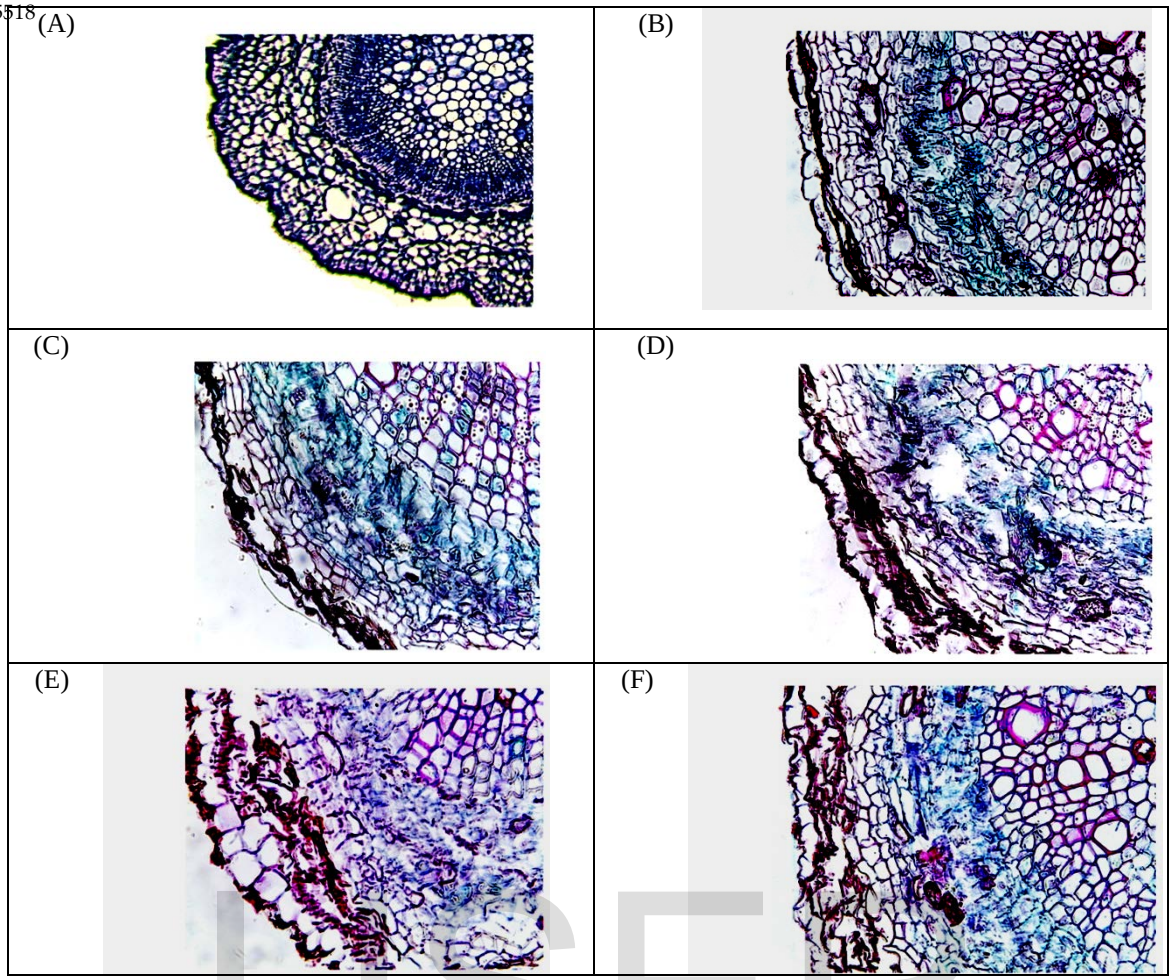


Fig. (4). Root anatomy of *K. senegalensis* seedling were taken after 60 days from planting seeds were treated with A) Vitavax-Thiram; B) *A. indica* extract; C) *T. vulgaris* extract; D) Oxalic acid; E) Salicylic acid and F) Gibberellic acid. in soil infested with *F. solani*.

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