Pathological and physiological studies on some disease root disease of soybean

Ahmed Mohamed Hassanain

In recent years, soybeanhas been considered one of the most important leguminouscrops in many countries • Damping-off and root-rot diseasesare considered among the most serious diseases which attacksoybean plants , causing large amounts of losses in standand total yield of seedStudies were carried out in the laboratory ,and undergreenhouse , and field conditions to determine factors andagricultural practices which affect incidence of root-rotand charcoal rot diseases and methods of control •Results obtained from this investigation can be summarizedas follows1- Seven genera of fungi i.e., Macrophomina phaseolina(Tassi) Goid., Rhizoctonia solani (kuhn) , Sclerotiumrolfsii Saccc » Cephalosporium gregatum (Allington andChamberL.).,Pythium~. , Fusarium solani (Martius) , andFusarium oxysporum (Schlecht) , were isolated from diseasedsoybe~~ plants of several varieties ,obtained from differentareas .2- Macrophomina phaseolina , the most predominant funguswas obtained from all inspected localities and could beisolated from roots • hypocotyls r stem base , stem anddry seeds • at various growth stages and differentsoybean varieties •3- All isolated fungi were pathogenic and caused pre- andpost-emergence damping-off and root-rot diseases ,butwith different degrees •4- Interaction between the different pathogens in potsshowed that the highest percentage of pre-emergencedamping-off was noted in the treatment of combinedinocula of c.E.. solani +~. rolfsii) (~. rolfsii +F. solani) and all tested fungi. However. in combinationsincluding M. phaseolina , percentages of preemergencedamping-off decreased which may be due to theantagonistic effect of M. phaseolina specially withF. solani .5- The highest percentage of post-emergence damping-offwas noted when M. phaseolina was combined with F.solani . However, the highest percentage of healthysurvival plants and lowest percentage of root rot infectionoccurred when the two previous fungi were combined6- Study of inoculum potential of some pathogenic fungion soybean diseases incidence , showed that percentagesof infection increased by increasing inoculumpotential •7- All thirteen isolates of Macrophomina phaseolina werepathogenic to soybean plants • However , these isolatesvaried in their virulence. In general , the mostvirulent isolates were 3 , 7 • 9c • 10 and 11 • Whileisolates 5 and 6 were the least virulent ,and other isolateswere intermediate in their virulence .8- The thirteen isolates of..!:!.p:haseolina can be dividedto three groups i.e., fast growers, moderate growersand slow growers . Isolates differed in nature of growthon solid media , some isolates formed fluffy growth asolates5 , 7 and 9 B), partially fulffy growth (isolates 2 and 10)or appressed growth (other isolates). Color of the mycelium variedfrom white, creamy white ,light brown , faintgray , dark gray , dark olive or greenish black andblack. Isolates 4 , 9A and 9c produced the highestnumbers of sclerotia , while isolates 2 • 5 and 9B gavethe lowest numbers. Isolate 7 produced sclerotia likebodies and sclerotia were clumbed Sclerotial shapeand size differed from one isolate to another. Thelargest sclerotia were 154 X 133.M for isolate 2 ,whileisolate 9c produced the smallest sclerotia (91 X 72 M).Shape of sclerotia varied from sub-globose to irregular.9- Pycnidia were not formed on PDA or water agar medium when incubatedat different temperatures and exposed to light or darkness10- All isolates of M. phaseolina produced pycnidia on cornor soybean leaves in Petri dishes and exposed to continuousillumination (2000 - 3000 Lux) for 4-8 daysand incubated at 30°C , except isolate The number of pycnidia produced differed from one isolateto another. The highest numbers of pycnidia wereobtained for isolates 2 , 9c and 11 I while ,the lowestnumbers were obtained for isolates 5 , 8 , 9A and 9B

•Pycnidia differed in their shape , size and number ofostioles per pycnidium. In general I size of pycnidiaproduced by all isolates ranged from 151 X 106 to 336.5X 256~. Also, in spite of great variation in lengthand width of pycnidiospores I the ratio between lengthto width remains a 3 : 1 approximately.12- No relationship was noted between formation of pycnidiaand virulence •13- Dry weight of the mycelium was positively correlatedwith virulence. The most virulent isolates yieldedthe highest amounts of dry weight of mycelium , whilethe least virulent isolates yielded the least amount ofdry weight •14- The minimum temperature for growth of M. phaseolina waslower than 15°C, while the optimum was at 30 °C ,andmaximum temperature lied between 35 and 40 °e. Nosclerotia were formed for all isolates at 15 °e andfew at 20 °e .15- The activity of PG , ex and PME enzymes differed fromone isolate to another. However, the highest activityof PG and PME enzymes was for the most virulentisolates 3 , 7 , 9c and 10 ,while the lowest activityof PG and PME enzymes was noted for the least virulentisolates 5 and 6 •16- All fungicides tested, slightly affected the PG andex acti vi ties in fu ngal fi1ra te. However, Benla teand Tecto 60 % depressed PG and ex enzymes activty infungal filtrate of both isolates more than Vitavax/captan when compared with control treatment ,after60 min. incubation.17- In Vivo PG and PME activity was correlated with virulenceof the isolates as the most virulent isolate9c produced the highest enzymes activity, while theleast virulent isolate 5 produced the lowest enzymesactivity. PG , PME and ex enzymes activity increasedin seedling inoculated with either of the three isolateswhen compared with uninoculated seedlingsTheir activity increased with increasing period afterinoculation •18- The activity of peroxidase, polyphenol oxidase ,andcatalase in fungal filtrate and mycelium extracts ofM. phaseolina differed for the different isolates •The activity of peroxidase and polyphenoloxidase washigher in the mycelium extract than the fungal filtrates.Catalase activity was higher. for the virulentisolates than the moderate and least virulentisolates19- Culture filtrates of the different isolates reducedseed germination and length of radical roots whencompared with control On the other hand , placingcut ends of seedlings in autoclaved and non-autoclavedundiluted crude culture filtrates of different isolatesshowed curling of the leaves , browning of theviens , necrosis of stem and leaf tissues followed bydehydration and defoliation and softening of the cut endwith unautoclaved culture filtrate only Severityof these symptoms depended on isolate and immersionperiod in the culture filtrate20- There was positive correlation between pathogenicityof the isolates and toxin (s) production • However,isolate 9c , the highly pathogenic , was highly productiveof the toxin (s) , while isolate 5 , the leastpathogenic , was the least productive 0 fthe toxin (s) •21- The phytotoxin (s) from both culture filtrate and tissuesof soybean seedlings inoculated with the pathogenwas dialysable , soluble in water, insoluble in organicsolvents and heat stable. They moved systemically insoybean seedlings immersed in the solution , producingnecrotic spots in leaf tissues , first appeared in lower-most leaves then in upper leaves Symptoms severityon leaves increased with increasing the concentrationof toxin (s) and time of immersion.22-a) Macrophomina phaseolina exhibited higher competitivesaprophytic ability (CSA) Colonization ratingsgradually increased with the increase of the amountof inoculant of the isolate in the inoculum-soilmixture • Also ,CSA increased with the increase invirulence of the isolate .b) M. phaselina was apparently sensitive to antibioticsproduced by soil micro-organisms. This sensitivityincreased when soil micro-organisms were allowed togrow on the agar plates for 24 hrs. prior to placingthe inoculum of M. phaseolina •- 185-23- The twenty tested soybean varieties could be classifiedinto five groups based on their relative resistance(R.R.) as follows:a) Highly resistant group (H.R.) : showing over 80 %R.R. this group comprised Gammes,Gail and Lee varieties(late maturity group)b) Resistant group (R) showing 70 to 80 R.R. ; thisgroup involved Crawford , Dare and Forrest varieties(intermediate and late maturity groups) •c) Moderately resistant group (M.R.) : shOWing 50 to7a % R. R • this gr0 up inc 1ude d Col es , Des 0 to, Essexand Youn yourk varieties •d) Susceptible group (s) : exhibiting 40 to 50 % R.R. ;this group comprised , Harkor , Cumberland ,and Williamsvarieties (early and intermediate groups) .e) Highly susceptible group (H.S.) : included all thevarieties showing less than 40 % R .R. ; these varietieswere , Mc call , Evans , Calland , ~oodworthCarlin , Clark and Columbus (early and itermediategroups) •24- Seed treatment with fungicides increased percentage ofseed germination excep t Rizolex and Ben la te- 50 whic hcaused significant reduction in seed germination. Also,the tested fungicides were effective in reducing numbersof seed borne fungi and

their frequency , when comparedwith the untreated seed .25- Benlate 50 and Topsin M. ,were uptaken by germinatingseeds during 8 hr On the other hand , the effectof the two fungicides increased with increasing concentrationand exposure period to fungicides. Also,Benlate-50 and Topsin M were uptaken by roots and translocatedto the hypocotyl" cotyledons and leaves.While, Tecto 60 % was concentrated in the roots andonly the high conc~ntrations were translocated to thehypocotyl (200 and 500 ppm) and to the leaves (500 ppm).26- Seed treatment with different fungicides , under greenhouseconditions , indicated that all tested fungicidesimproved percentages of seedling stand as comparedwith the untreated control. The most effective fungicideswere Tecto 60 %, R H 50-50 I Quinolate CTS andBenlate 50 , which gave significant reduction in diseaseincidence ,and increased percentage of healthysurvivals. The least effective fungicides were Merban,Rizolex and Triple Necotin27- Under natural infection in the field ,all fungicidesincreased survival plants in the two seasons , exceptRizolex , Quinolate 151M and Benlate 50 when compared- 187-with the the control. The most effective among thesefungicides t in reducing pre- and post-emergence damping-off and resulted in the highest survival plants andhighest yield t in the two seasons, were Vitavax/capton,Quinolate CTS , RH 50-50 ,and Tecto 60 % •28- Under field conditions • increasing nitrogen level tendedto increase disease incidence and decreased survivalplants. On the other hand , increasing amounts of phosphorusfrom Po to PI or P2 (200 or 300 Kg./fed.) , increa sed number of survival plan ts . Ni trogen and phosphorusincreased significantly number of pods per plant tweight of 100 seed and yield per plot •SUMMARYIn recent years, soybean [Glycine max (1.) Merrill]has been considered one of the most important leguminouscrops in many countries • Damping-off and root-rot diseasesare considered among the most serious diseases which attacksoybean plants , causing large amounts of losses in standand total yield of seedStudies were carried out in the laboratory ,and undergreenhouse , and field conditions to determine factors andagricultural practices which affect incidence of root-rotand charcoal rot diseases and methods of control •Results obtained from this investigation can be summarizedas follows1- Seven genera of fungi i.e., Macrophomina phaseolina(Tassi) Goid., Rhizoctonia solani (kuhn) , Sclerotiumrolfsii Saccc » Cephalosporium gregatum (Allington andChamberL.).,Pythium~. , Fusarium solani (Martius) , andFusarium oxysporum (Schlecht) , were isolated from diseasedsoybe~~ plants of several varieties ,obtained from differentareas .2- Macrophomina phaseolina , the most predominant funguswas obtained from all inspected localities and could beisolated from roots • hypocotyls r stem base , stem anddry seeds • at various growth stages and differentsoybean varieties •3- All isolated fungi were pathogenic and caused pre- andpost-emergence damping-off and root-rot diseases ,butwith different degrees •4- Interaction between the different pathogens in potsshowed that the highest percentage of pre-emergencedamping-off was noted in the treatment of combinedinocula of c.E.. solani +~. rolfsii) (~. rolfsii +F. solani) and all tested fungi. However. in combinationsincluding M. phaseolina , percentages of preemergencedamping-off decreased which may be due to theantagonistic effect of M. phaseolina specially withF. solani .5- The highest percentage of post-emergence damping-offwas noted when M. phaseolina was combined with F.solani . However, the highest percentage of healthysurvival plants and lowest percentage of root rot infectionoccurred when the two previous fungi were combined6- Study of inoculum potential of some pathogenic fungion soybean diseases incidence , showed that percentagesof infection increased by increasing inoculumpotential •7- All thirteen isolates of Macrophomina phaseolina were- 180 -pathogenic to soybean plants • However , these isolatesvaried in their virulence. In general , the mostvirulent isolates were 3 , 7 • 9c • 10 and 11 • Whileisolates 5 and 6 were the least virulent ,and other isolateswere intermediate in their virulence .8- The thirteen isolates of..!:!.p:haseolina can be dividedto three groups i.e., fast growers, moderate growersand slow growers . Isolates differed in nature of growthon solid media , some isolates formed fluffy growth asolates5 , 7 and 9 B), partially fulffy growth (isolates 2 and 10)or appressed growth (other isolates). Color of the mycelium variedfrom white, creamy white ,light brown , faintgray , dark gray , dark olive or greenish black andblack. Isolates 4 , 9A and 9c produced the highestnumbers of sclerotia , while isolates 2 • 5 and 9B gavethe lowest numbers. Isolate 7 produced sclerotia likebodies and sclerotia were clumbed Sclerotial shapeand size differed from one isolate to another. Thelargest sclerotia were 154 X 133.M for isolate 2 ,whileisolate 9c produced the

smallest sclerotia (91 X 72 M).Shape of sclerotia varied from sub-globose to irregular.9- Pycnidia were not formed on PDA or water agar medium when incubatedat different temperatures and exposed to light or darkness10- All isolates of M. phaseolina produced pycnidia on cornor soybean leaves in Petri dishes and exposed to continuousillumination (2000 - 3000 Lux) for 4-8 daysand incubated at 30°C , except isolate 1 •11- The number of pycnidia produced differed from one isolateto another. The highest numbers of pycnidia wereobtained for isolates 2 , 9c and 11 I while ,the lowestnumbers were obtained for isolates 5 , 8 , 9A and 9B •Pycnidia differed in their shape , size and number ofostioles per pycnidium. In general I size of pycnidiaproduced by all isolates ranged from 151 X 106 to 336.5X 256~. Also, in spite of great variation in lengthand width of pycnidiospores I the ratio between lengthto width remains a 3 : 1 approximately.12- No relationship was noted between formation of pycnidiaand virulence •13- Dry weight of the mycelium was positively correlatedwith virulence. The most virulent isolates yieldedthe highest amounts of dry weight of mycelium , whilethe least virulent isolates yielded the least amount ofdry weight •14- The minimum temperature for growth of M. phaseolina waslower than 15°C, while the optimum was at 30 °C ,andmaximum temperature lied between 35 and 40 °e. Nosclerotia were formed for all isolates at 15 °e andfew at 20 °e .15- The activity of PG , ex and PME enzymes differed fromone isolate to another. However, the highest activityof PG and PME enzymes was for the most virulentisolates 3 , 7 , 9c and 10 ,while the lowest activityof PG and PME enzymes was noted for the least virulentisolates 5 and 6 •16- All fungicides tested, slightly affected the PG andex acti vi ties in fu ngal fi1ra te. However, Benla teand Tecto 60 % depressed PG and ex enzymes activty infungal filtrate of both isolates more than Vitavax/captan when compared with control treatment ,after60 min. incubation.17- In Vivo PG and PME activity was correlated with virulenceof the isolates as the most virulent isolate9c produced the highest enzymes activity, while theleast virulent isolate 5 produced the lowest enzymesactivity. PG , PME and ex enzymes activity increasedin seedling inoculated with either of the three isolateswhen compared with uninoculated seedlingsTheir activity increased with increasing period afterinoculation •18- The activity of peroxidase, polyphenol oxidase ,andcatalase in fungal filtrate and mycelium extracts ofM. phaseolina differed for the different isolates •The activity of peroxidase and polyphenoloxidase washigher in the mycelium extract than the fungal filtrates.Catalase activity was higher. for the virulentisolates than the moderate and least virulentisolates19- Culture filtrates of the different isolates reducedseed germination and length of radical roots whencompared with control On the other hand , placingcut ends of seedlings in autoclaved and non-autoclavedundiluted crude culture filtrates of different isolatesshowed curling of the leaves , browning of theviens , necrosis of stem and leaf tissues followed bydehydration and defoliation and softening of the cut endwith unautoclaved culture filtrate only Severityof these symptoms depended on isolate and immersionperiod in the culture filtrate20- There was positive correlation between pathogenicityof the isolates and toxin (s) production • However,isolate 9c , the highly pathogenic , was highly productiveof the toxin (s) , while isolate 5 , the leastpathogenic , was the least productive 0 fthe toxin (s) •21- The phytotoxin (s) from both culture filtrate and tissuesof soybean seedlings inoculated with the pathogenwas dialysable , soluble in water, insoluble in organicsolvents and heat stable. They moved systemically insoybean seedlings immersed in the solution , producingnecrotic spots in leaf tissues , first appeared in lower-most leaves then in upper leaves Symptoms severityon leaves increased with increasing the concentrationof toxin (s) and time of immersion.22-a) Macrophomina phaseolina exhibited higher competitivesaprophytic ability (CSA) Colonization ratingsgradually increased with the increase of the amountof inoculant of the isolate in the inoculum-soilmixture • Also ,CSA increased with the increase invirulence of the isolate .b) M. phaselina was apparently sensitive to antibioticsproduced by soil micro-organisms. This sensitivityincreased when soil micro-organisms were allowed togrow on the agar plates for 24 hrs. prior to placingthe inoculum of M. phaseolina •23- The twenty tested soybean varieties could be classifiedinto five groups based on their relative resistance(R.R.) as follows:a) Highly resistant group (H.R.) : showing over 80 %R.R. this group comprised Gammes,Gail and Lee varieties(late maturity group)b) Resistant group (R) showing 70 to 80 R.R. ; thisgroup involved Crawford , Dare and Forrest varieties(intermediate and late maturity groups) •c) Moderately resistant group (M.R.) : shOWing 50 to7a % R. R • this gr0 up inc 1ude d Col es , Des 0 to, Essexand Youn yourk varieties

•d) Susceptible group (s) : exhibiting 40 to 50 % R.R. ;this group comprised , Harkor , Cumberland ,and Williamsvarieties (early and intermediate groups) .e) Highly susceptible group (H.S.) : included all thevarieties showing less than 40 % R .R. ; these varietieswere , Mc call , Evans , Calland , ~oodworthCarlin , Clark and Columbus (early and itermediategroups) •24- Seed treatment with fungicides increased percentage ofseed germination excep t Rizolex and Ben la te- 50 whic hcaused significant reduction in seed germination. Also,the tested fungicides were effective in reducing numbersof seed borne fungi and their frequency , when comparedwith the untreated seed .25- Benlate 50 and Topsin M. ,were uptaken by germinatingseeds during 8 hr On the other hand , the effectof the two fungicides increased with increasing concentrationand exposure period to fungicides. Also,Benlate-50 and Topsin M were uptaken by roots and translocatedto the hypocotyl" cotyledons and leaves.While, Tecto 60 % was concentrated in the roots andonly the high conc~ntrations were translocated to thehypocotyl (200 and 500 ppm) and to the leaves (500 ppm).26- Seed treatment with different fungicides , under greenhouseconditions , indicated that all tested fungicidesimproved percentages of seedling stand as comparedwith the untreated control. The most effective fungicideswere Tecto 60 %, R H 50-50 I Quinolate CTS andBenlate 50 , which gave significant reduction in diseaseincidence ,and increased percentage of healthysurvivals. The least effective fungicides were Merban,Rizolex and Triple Necotin27- Under natural infection in the field ,all fungicidesincreased survival plants in the two seasons , exceptRizolex , Quinolate 151M and Benlate 50 when comparedwith the the control. The most effective among thesefungicides t in reducing pre- and post-emergence damping-off and resulted in the highest survival plants andhighest yield t in the two seasons, were Vitavax/capton,Quinolate CTS , RH 50-50 ,and Tecto 60 % •28- Under field conditions • increasing nitrogen level tendedto increase disease incidence and decreased survivalplants. On the other hand , increasing amounts of phosphorusfrom Po to PI or P2 (200 or 300 Kg./fed.) , increa sed number of survival plan ts . Ni trogen and phosphorusincreased significantly number of pods per plant tweight of 100 seed and yield per plot •