Studies on fungi associated with soybean seeds in A.R.E

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Results obtained from this investigation could besummarized as follows :1. Seed health testing of soybean seed showed thepresence of : Alternaria altern~ta, !. tenuissima,Alternar~ spp., Aspergillus flavus, A. niger, A.tamarii,Aspergi~~~ spp., Botryodiplodia theobrom~~, Botr~~sciner~, Calonectria crotalar~, Cercospora kikuchii,C. sojina, Chaetomium ~~preum, ~. globosum, C. indicum,Chaetomiwn spp., Cladosporium spp., Corynespora csssiicola,Curvularia spp ,, ~echsle~ hawaiiensis, D. h~lodes, Q.specifer, Drechslera spp., Epicoccum Eurpuresc ens,Fusariumequiseli, F. monilif~, F. semitectum, !. ~ani,Fusarium spp., Geotricum ap., Gliocladium roseum, Macrophominsphaseolina, Monilia ap., Myrothecium verrucaria,Nigrospora oryzae, Penicillium spp., Fhialop~or~ gregata,Phoma ap ,, Rhizoctonia aolani. !i..hizopus nigricanB, ~-£hylium botryoswn, s. vesicari~ and Trichothecium rosewn.The following fungi are isolated far the firsttime from soybean seeds in Egypt : Botryodiplodia theobromae,Botrytls cinerea. Phoma ap., Calonectri.!,crotalariae, Cercospora kikuch11, C. sojina. Chaetomiumcupreum, ~. glob~~. Corynespora casslicola,Drechslera hawaiiensis, D. holades, Geotrichum sp.,and Gllocladium roseum.2. Ungerminated soybean seed samples were dividedaccording to external seed appearances to two maingroupS : the first group consisted of heathy lookingseed which are arranged as follows; Clark (80.0 %),Williams (76.75 %), Columbus (68.75 %), and Calland(60.25 %). The second group consisted of non-healthyseed which may be due to pathogenic or non-pathogenicagents.3. Ten species of fungi were isolated from the foursoybean cultivars by washing sediment. The frequenciesof these fungi on the four samples in a decreasingorder, were; 9haet9~~~ spp. and Fusarium semitec~~(100 %), Alterna.ti.!a!l,ternata, Trichothecium roseum,Stemphylium vescarium and Kacrophom..!!:!p!h!aseollna (5° %),Alternaria spp., Drechslera specifer, !. solani andFusarium spp. (25 %). Number of isolated fungi fromeach sample -in an decreasing order- were; Calland (7fungi), Clark (6 fungi), VJilliwns (4 fungi), and Colwnbus(3 fungi). Viability of sedimented fungi were between75 and 100 s,4. Total count of fungi recovered from the four seedsamples (cultivars) were slightly higher in agar-platetest for Columbus seeds and much higher for Galland andClark seeds. while total count of fungi recovered fromWilliams seed were higher in blotter test.5. Total cOWlt of bacteria recovered from the samplesof c~ltivars were much higher in blotter test. Thismay be attributed to the presence of free water whichencouraged growth of the bacteria.6. Blotter and agar-plate tests could not be usedsingly for detection of seedborne fungi and both must betried together.7. The blotter test is superior for detection of Q1!Qcladlumroseum in soybean seeds but it is not preferablefor detection of Alternaria spp •• Aspergil~ niger,Calonectria crotalariae, ~rcospora sojina, Chaetomiumspp., Fusarium eguiseti, Rhizoctonia solani, and Rhizopusnigricans.8. Botryodiplodia theobromae, Botrytis cinerea andPhoma sp. were only isolated by seedling symptoms test.This test allowed better development of seedlings andsymptoms than the blotter and agar- plate tests.- 178 -. Blot~er, agar-plate and seedling symptom tests couldnot be used singly for detection of all seedborne fungiand the three tests must be tried together.10. Location of fungi in different parts of soybeanseeds showed that seed coat and cotyledons containedmost of seed-borne fungi , while the radicle and the plumulecontained less of associated seedborne fungi.Fusariumsemitectum and E. solani were isolated from the radicleof Williams and Columbus cultivars, Phialophora gregatawas isolated from the plumule of Calland and ColumbUScultivars.11.

Histopathological studies to detect location ofseedborne fungi in the different parts of soybean seedrevealed the following results; group 1 : fungi on theoQter surface of the seed coat, represented by Ghaetomiumcupreum, Chaetomium globusum, Calonectria crotalariae,Aspergillus flavus, Aspergillus spp.; group 2 : fungi onthe seed coat or inside the seed coat as Macr2~pminaphaseolina or inside the seed coat as Cercosporakikuchii;group 3 : fungi present inside the seed coat and cotyledonsas Cercospora sojina, Alternaria alternate, Alternariaspp., Drechslera hawaiiensis, D. halodes, Fusariwm equiseti,Fusarium spp. and Trichothecium roseum; group 4 : fungipresent in the cotyledons only and represented byFusarium solani, Gliocladium roseum, Myrothecium verrucaria,and Rhizoctonia solani; group 5 : fungi in thecotyledons, radicle and the plumule, represented by thefungus Phialopho~ gregata; and group 6 : fungi in theseed coat, cotyledons and radicle and is represented by=Fus=ar=iu=m -semi tectum and -F. moniliforme.12. Fungi on soybean seed produced different syptoms,each symptom was induced by one or more pathogenic mainfungi with or without other associated fungi. Seedcracks and wrinkles were caused by many fungi, reducedseed size and discoloration were caused by Phialophoragregata and Macrophomina phaseolina with four associatedfungi. Brown seed rot was caused by Fusarium ~~it~ctumwith four associated fungi, and gray seed rot was causedby Alternaria alternata with six associated fungi. Darkbrown seed necrosis was caused by Corynespora cassiicolawith three associated fungi and gray seed necrosis wascaused by Alternari~ alternata with seven associated fungi.Black~ots or blemishes were caused by Macrophaminaphaseolina with two associated fungi, pale gray discolorationwas caused by Chaetomium species, reddish brown orbrown discoloration was caused by Cercospora sojina withfive associated fungi, purple stain was caUSed by Cercosporakikuchii only, and rose discoloration was caused byTrichothecium roseum only.1]. Studies of seed-plant transmission of fungi showedthat some fungi were transmitted to some or all partsof the seedlings and the plants at 14, 35, 10, 91 and112-days-old. These fungi were Alternaria alt~rnata,~e~c2spora kikuchii, ~. sojina, Coryn~sQora cassiicola,and Fusarium semi tectum ,while Macrophomina phaseolinaand Phialophor§ gregata were transmitted to some partsof the seedlings at 14-days-old, then to the seed at10, 91 and 112-days-old.14. Seedborne fungi cycles and infection courses wereclas~ified into four types :a) Intraembryal infection followed by local infectionrepresented by Fusarium semitectum.b) Extraembryal infection followed by local infectionrepresented by Alternaria alternata, CercosEorakikuchii, Q. sojina, and Corynespora cassi1cola.c) Seed contamination followed by systemic infectionrepresented by Phialophora gregata.d) Seed contamination followed by extramatical saprophytismand then by systemic infection, represented byMacrophomina phaseolina.15. Studies of factors affecting frequency of seedbornefungi showed that :a) Localities (7 governorates) affected the presence andfrequency of seedborne fungi. The governorates werearranged according to the numbers of isolated fungifrom each governorate as follows; Kafr El-Sheikh (11fungi), Beni-Suef (7 fungi), both Giza and Alexandria(6 fungi), Dakahlia (5 fungi), EI-Menia (4 fungi),and Kalubia (3 fungi). Macrophomina phaseolina wasisolated from all the governorates.b) Soybean cultivars also affected presence and frequencyof seedborne fungi; average percentage of isolatedfu.ngi frequency increased with increasing maturitygroups except V and VI maturity groups. Cultivarscould be arranged according to percentage of fungiisolated from seed samples as follows; DaviS, Clark-63, Bethel, Forrest, Beeson, Cumberland, Williams-79and Crawford, Coles, Lee, Bonus, Centennial, Wareand Gammas, and McCall.c) Storage of soybean seed in their pods reduced the totalnumbers of seedborne fungi and reduced the longevityof some fungi such as Cercospora sojina, Fusariumsemi tectum, and Kacrophamina pbaseolina, but increasedseed viability. The total count of field fungidecreased by increasing period of storage, while thetotal of storage fungi increased by increasing periodof storage.16. Longevity of field fungi in or on seeds stored withtheir pods was one year except Macrophomina phaseolinawhich was two years and Chaetomium cupreum which wasthree years. On seed stored without pods, fungi remainedviable for one year were Alternaria. alternata, Alternariaspp., Calonectria crotalari~~, Phialophora gregata,Chaetomi~ cupreum, Chaetomium spp., Dr~chslera hawai!-ensis, Q. halodes, Fusarium equiseti, F. solani, Fusariumspp., Rhizoctonia solani, Stemphylium vesicarium and~richothecium roseum. In the meantime, Cercospora. sojina-Fu-sarium. moniliforme, and -F. semitectum remained viablefor two years while

Macrophamina pha~eolina was viablefor three years.17. Seedborne fungi affected soybean seed germinationand seedling survival.18. FWlgicides used as seed-dressing affected the presenceand frequency of seedborne fungi plated on PDAmediwn in tb.elaboratory. The fungicides could be arrangedaccording to their efficien~iri reducing numbersand frequencies of seedborne fungi as follows; QuinolateV4X, Tecto, Vitavax + Benlate and Vitavax!Captan, Merban- 184 -with extracts of sprayed seeds. Higher concentrationsof residual fungicides was in the embryocompared with the seed coat. Generally, presenceof fungicides increased by one application and twoapplications at one week interval.c) Seeds sprayed before harvest showed reduction in preandpost-emergence damping-off. Treatments werearranged according to their effects considering theeconomic side as follows; Vitavax + Benlate once,twice, and three times, at one week interval, Vitavax/Captan, also, once, twice, and three times at oneweek interval.d) Foliar and pods spraying before harvest caused significantincrease in (RGR) at 14 and 35-days-old seedlingsand plants from sprayed seeds as oompared withthe untreated control. Vitavax + Benlate three times,at one week interval was the best treatment in reducingpre- and post-emergence damp1ng.-off.20. Biological control of seedborne fungi of soybeanusing Chaetomium species as antagonist showed that :a) Four Chaetomium species, C. cupreum, c. globosum,C. indicum and Chaetomiwn sp. t antagonized Alternariaalternata and lusarium semi tectum. c. cupreum was thebest antagonist.b) The percentage of inhibition of radial growth (PIRG)of 29 fungi caused by Chaetomium cupreum indicatedthat (PIRG) was clear on fungi of family Dematiaceae,followed by fungi of family Tubercul ariaceae, fungiof family Mon1liaceae and finally, fungi belongingto order Agronomycetales (Mycelia Sterilia). PIRGof fungi of family Shaeropsidaceae was inconsistentas it was high for some fungi and low for some others.c) Antagonism between Chaetomium cupreum and seven seedbornefungi and their effects on pre- and post-emergencedamping-off showed that ~. cupreum, as an antagonisticfungus to the seven seed borne fungi, significantlydecreased pre- and post-emergence damping-off whichcould be arranged according to differences betweentreatments with and without the antagonist as follows;.Macro phomina phaseolina and Phialophora gregata,FusarilUIlsemi tectum, Alternaria alternata, andFusarium solani, while Cercospora kikuchii was notaffected.21. Biological control of seedborne fungi of soybeanusing filtrate of Chaetomium cupreum indicated that Ia) The tested ether extract (partially purified toxin)concentrations 100, 400, 700 and ·lOOOpg/ml , weregenerally effective in reducing numbers of seedbornefungi which showed more reduction with increasing theconcentrations. The most effective conc en’trat Lonswere 700, 400, 1000 and 100)lg/ml. All tested etherextract concentrations significantiy increased percentageof seed germination as compared with thecontrol.b) Comparing effect of culture filtrate and ether extractconcentrations on seven seedbornefungi showed that:growth inhibition of all the fungi increased withhigher concentrations except Cercospora sojina andFusarium solani with culture filtrate which increasedsignificantly in growth at all the concentrations.Number reproductive propagules of all fungi increasedsignificantly with increasing concentration exceptat 100 and 400)lg/ml of ether extract with £eroosporakikuchii and £. sojina at which number of spores didnot increase.c) Seed treatment with ether extract concentrationsdecreased pre- and post-emergence damping-off significantlywith increasing the concentrations except2500 ~g/ml concentration whioh may be attributedto its toxicity on the soybean seed and seedlings.d) Seed treatment with ether extract· concentrationsdecreased the relative growth rate (RGR) withincreasing concentration of ether extract of ~.cupreum at 14-days-01d. Also, at J5-days-old. Thesame trend of results was obtained, as 14-days-old,except at 700 and 2000 pg/ml when (RGR) increasedbut the increase was not significant.unileaflets ~ While unileaflets of seedlings L~nersed inthe other concentrations curled and w~lted followed bycOffipletedesiccation within the 16 hr. which also occurredin the two previous concentrations within 48 hr.b)Chaetomium cupreum. when grown in one liter of oCzapek’s broth medium and inoculated .at 27.0 for twoweeks produced 0.6263 g Iliter dry weight of partiallypurified toxin.c)The toxin was dialytic, insoluble in n-propanol,n-butanol, ethyl acet~te, acetone, methanol, and.chloroform, but soluble in water.d)ThiS toxin may be composed of more than one compoundbecause three different types of crystals were observed.The maximum absorbance of the partially purified extractin water was detected at 245, 275 and 290 nm in ultravioletlight and 505 nm in the visible light.e)Bioassay for the toxin efficiency was measured usingdifferent toxin

concentrations, which affected Penicilliumexpansum; the equivalent three fungicides concentration(~g/ml) were obtained until 2500 ~g/ml concentrationof toxin.