

Pathological studies on charcoal rot disease of sesame

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1-*Macrophomina phaseolina* shows, in general, the highest frequency in most inspected locations followed by *Fusarium* spp., then if present, *R. solani* and *S. rolfsii*. *M. phaseolina* and *Fusarium* spp. were isolated, while, *R. solani* was not isolated from plant materials that collected from the six locations i.e., Menia "Samalot, Abo-Qemas and Maghagha", - Qalubia "Shebien El-Qanter", Sharkia "Belbeis" and Behira "El-Tahrir". *Sclerotium rolfsii* was isolated from the seven locations, Giza "El-Saff and Giza", Beni-Suif "Beba", Asuit "Asuit", Qalubia "Qaha" and Ismaelia "Ezz-El-Din and Qassasin". 2-The isolates of *M. phaseolina* were significantly differed in their growth nature, mycelial color, linear growth and sclerotial production. Number of sclerotia for different isolates was ranged between 0.3 to 127.0 sclerotia per microscopic field "X10"). The color of mycelial growth of a given isolate of *M. phaseolina* seems to be correlated with density of sclerotial formation. The color of mycelial growth of isolate M13, which produced the highest number of sclerotia, was black meanwhile it was white in isolates M14, 16, and 23 which produced the lowest numbers of sclerotia. 3- Germination of sesame seeds, shoot and root lengths of sesame seedlings were significantly reduced to different extents by the autoclaved and non-autoclaved culture filtrates of the tested *M. phaseolina* isolates. Degree of reduction was depended on the pathogen isolate, the bioassay test (seed germination, shoot or root length) and kind of cultural filtrates. 4-The virulence of tested isolates was significantly varied in inducing disease incidence. Isolates M26, M13, M24, and M9 were the most pathogenic as they produced the lowest % survived seedlings, meanwhile isolates M17 and 2 exhibited no significant effect in this respect compared with control. As for charcoal rot, isolate M9 was the most virulent followed by M3, M1, M11 and M12. The isolates M8, M18, M21, and M22 were the least pathogenic meanwhile, isolate M23 was not pathogenic. Finally, the highest significant decrease in % healthy standing plants was induced by isolate M9 followed by isolate M24. While the lowest significant decreases were produced by isolates M8 and M22 followed by isolates M18 and M23. 5-Wide variations was found among isolates of *M. phaseolina* during the different stages of disease development based on an innovated procedure suggested to facilitate quantitative comparisons between the present 27 isolates. These isolates could be grouped as follow: 1-Very weakly pathogenic isolates (M8 and M22). 2 -Weakly pathogenic isolates (M18, M23, M5, M6, M16 and M21). 3-Moderately pathogenic isolates (M10, M27, M15, and M2). 4-highly pathogenic isolates (M19, M17, M20, M7 and M3). 5-Severe isolates (M4, M1M25, M11, M13, M26 and M24). 6-Destructive isolates (M9). 6- By using the stern pricking technique, the isolates M9 and M4 were the most pathogenic. Meanwhile isolates M1, M2, M5, M8, M10, M13, M22, M24 and M25 seemed to be less pathogenic when tested by this technique. The length of diseased stem portion infected by some isolates (12 isolates) was increased proportionally and significantly with increase in plant, aging from 30 to 90 days. Thus, the older plants seemed to be more susceptible to these isolates than the younger one. The isolates M5 and M25 were more pathogenic on the youngest plant (30 days old) while isolates M1, M2, M15, M17, M18, and M27 were more pathogenic on plants of 60 days old. 7- Based on the protein patterns of different selected 11 isolates of *M. phaseolina*, the similarity was higher between isolates obtained from host plants grown in the warmer soils than those isolated from host plants grown in less warmed soils. 8- Sesame was more susceptible to sesame isolate (M9), followed by soybean isolate (M13) and sunflower isolate (M17). However,

soybean was more susceptible to sunflower isolate M17 followed by isolates M13, M9, M27, and M8 which isolated from soybean, sesame, cotton, and peanut, respectively. Sunflower was more affected by soybean-isolate M13 followed by M17 "from sunflower", M9 "from sesame", M13 "from peanut" and M17 "from cotton", respectively. While, cotton was affected by M9 "from sesame" and M17 "from sunflower" more than M27 "from cotton". The harmful effect of sesame-isolate was greater and similar on sesame, sunflower, and cotton plants. Soybean-isolate was more severe on sunflower and sesame plants than soybean plants. Among all tested host plants, peanut only seems to be not significantly affected by cotton-isolate. Effect of cotton-isolate on sesame, soybean, sunflower, and cotton plants was equal.

9- The highly pathogenic isolate M9 shows the highest PME, PG and Cx enzymes activities in vitro and in vivo followed by the moderate isolate M15 and the weakly pathogenic isolate M22. The activity of these enzymes was increased in vitro by culture aging from 7 to 30 days and in vivo increasing time elapsed after inoculation from 15 to 60 days.

10- Growth of isolates M9, M15, and M22 was completely stopped by Maxim at 1.0, 0.5 and 5.0 ppm; Vitavax-T at 100.0, 50.0 and 100.0 ppm; Rizolex-T at 200.0, 25.0 and 100.0 ppm; and Benlate at 400.0, 200.0 and 400.0 ppm, respectively. Maxim and Benlate caused complete inhibition of sclerotial production of isolate M15 at 1.0 and 100.0 ppm., respectively. However, Amconil prevented sclerotial formation at 200.0, 800.0, and 1600.0 ppm. for the 3 isolates M9, M15, and M22, respectively.

11- Growth of isolate M9 was resist the effects of the antagonistic fungi and bacteria, followed by the isolate M15 whereas, the isolate M22 was more affected by different antagonistic fungi. *Trichoderma viride* and *Trichoderma* sp. (No. 5) were the most antagonistic fungi followed by *Trichoderma* spp. No. 9, 6, 2, and *T. hamatum*, *T. harzianum* and *Trichoderma* sp. No. 8. While, *Gliocladium penicilloides* and *C. bostrycoides* were the lowest effective.

12- *Trichoderma viride*, *Trichoderma* spp. No. 5, 2, 6 were the best for suppressing growth of isolate M9. *Trichoderma viride*, *Trichoderma* spp. No. 9, *T. hamatum*, *Trichoderma* spp. No. 10, 8, 2, 5, *Trichoderma harzianum*, and *Trichoderma* sp. No. 3 exhibited similar effect on *M. phaseolina* isolate M15. However *Trichoderma* spp. No. 5, 6 and *T. viride* were the best antagonistic for growth reduction of *M. phaseolina* isolate M22.

13- *Bacillus subtilis* and *B. megitla* were the best antagonistic bacteria for limiting growth of *Macrophomina* isolates followed by *Bacillus* spp. No. 3 and 2 whereas, *Bacillus* sp No. 1 had the lowest effective.

14- The filtered garlic extracts caused complete inhibition of growth and sclerotial production of all isolates at concentration (10%). While, those of rhubarb could prevent growth of isolate M9 and caused the highest inhibition in growth of isolates M15 and M22 at concentration (50%). Anise filtered extract was more effective against isolate M22 than isolates M9 and M15 at the highest concentration. Sclerotial production in isolates M9 and M22 was mostly decreased with increasing concentrations of all extracts except extract of cumin in isolate M22. Contrary, in the moderately isolate M15, production of sclerotia was increased by increasing concentrations of most tested filtered extracts compared with control treatment. The filtered extracts of clove and ginger caused the highest increase, while, extract of garlic, rhubarb, thyme, marjoram and cumin in listed order decreased the sclerotial formation of the this isolate.

15- The autoclaved extracts of rosselle, clove and rhubarb caused the highest suppression effect on growth of the 3 isolates of *M. phaseolina*. In isolate M9 the autoclaved extracts produces the lowest numbers of sclerotia, followed and cumin. In isolate M22, the autoclaved extracts was the best for reducing sclerotial formation, fs of fennel, y rhubarb, of rhubarb lollowed by Fennel, clove, and rosselle. Increasing concentrations of all autoclaved extracts caused significant decrease in sclerotial productiOn by isolates M9 and M22. While, in isolate M15, increasing concentrations of some autoclaved extracts i.e., eucalytus, azedarach, anise and ginger from 10% to 50% increased the number of sclerotia.

16- The autoclaved extracts of garlic, anise and thyme (in M9), fennel and garlic (in M15) never affected the growth when compared with control. The filtered extracts of these plants especially garlic and anise were the most toxic against growth of all isolates. Also, toxicity of rhubarb extracts (against all isolates),, anise (against isolates M15 and M22) and garlic (against isolate M22) was partially decreased by autoclaving if compared with filtered same extracts.

17- IBA and IAA caused the highest decrease in growth meanwhile, KCl and H₂O₂ were the least effective. However, salicylic acid, Bion and tanic acid caused intermediate decrease in growth compared with control. IAA and IBA, caused no growth of isolates M9 and M15 at 800 ppm and isolate M22 at 400

ppm.. Meanwhile, salicylic acid caused no growth of the 3 isolates at 1600 ppm: 18-IBA and IAA were the best chemical inducers for decreasing sclerotial formation followed by SA, Bion, tannic acid, whereas KC1 and H2O2 were the least effective in this respect. The produced number of sclerotia was inversely correlated with concentrations of any chemical inducer. 19-Flax, onion, and garlic induced the best residual effect on controlling infection with *M. phaseolina*. They increased survived seedlings (85.0%) and healthy plants (70.0-72.5%) and decreased pre- and post-emergence damping off (5.0-10.0%) and charcoal rot (12.5-15.0%). Barley and clover came next followed by safflower, lupine and rapeseed, respectively. 20-Soils amendment with vegetative growths of faba bean and clover significantly decreased pre- emergence to 20.0 & 21.67%, respectively meanwhile amendment with lentil and chickpea increased it significantly to 39.17 & 37.5%, respectively compared with 27.5% in control treatment. However, post-emergence damping-off was increased 25.2% significantly in soils amended with lentil (34.2%) and chickpea (37.5%) compared with control (27.5%). Soil amendment with clover only caused slight increase in survived seedlings (54.2%) and healthy standing (45.8%) compared with 45.0% and 36.7%, respectively in control treatment. The incidence of charcoal rot was not affected significantly by different soil amendments. 21-Pre- and post-emergence damping-off was significantly increased and % survival seedlings and healthy standing were significantly decreased by increasing time, between soil amendment and sesame sowing time from 10 to 20 or 30 days after soil amendment. The incidence of charcoal rot was not affected significantly by sowing date or the interaction between sowing date and soil amendment. 22-The fungicidal seed treatments were better than soil treatments. Treating sesame seeds with benlate and rizolex-T resulted in 0.0% and 3.3% pre-emergence; 3.3% and 6.7% post-emergence; 96.7% and 90.0% survived seedlings; 3.3% and 6.7% rotted plants and 93.3% and 83.3% healthy standing, respectively. While treatment soil with rizokex-T and vitavax-T had no significant effect on disease at seedling stage and produced the lowest effect on controlling the disease at maturity stage i.e., 13.3% and 13.3% rotted plants against control (23.3%) and 46.7% and 43.3% healthy standing plants against control (26.7%). 23- Most tested antagonistic fungi and bacteria were significantly effective in controlling disease incidence at seedling and/or maturity stages. The maximum survival seedlings was produced by *Trichoderma harzianum* (100.0%) followed by *Chaetomium bostrycoides* (96.7%) and *Trichoderma* sp. No. -5 (93.3%) followed by *T. hamatum* (86.7%). However, the lowest significant increases were produced by *Trichoderma* sp 3 (60.0%) followed by *Trichoderma* sp 8 (63.3%) and *Gliocladium penicilloides* (63.3%). *T. harzianum*, *C. bostrycoides*, *T. hamatum*, *T. viride*, *Trichoderma* sp 5 and *Trichoderma* sp 6 were the best for reducing % charcoal rotted plants (3.3-10.0%). The charcoal rot was not significantly affected by *Bacillus subtilis*, *Trichoderma* sp 8, *Trichoderma* sp 10, *Bacillus* sp3, *B. megdella* and *Gliocladium penicilloides*. 24-*Trichoderma harzianum* and *C. bostrycoides* produced the highest % healthy standing plants i.e., 96.7 and 90.0%, followed by *Trichoderma* sp 5 (83.3%) and *T. hamatum* (80.0%). However, the lowest increase in healthy standing plants was induced by *Trichoderma* sp 3, *Trichoderma* sp 8, *B. megdella* (43.3%), *G. penicilloides* (36.7%) and *Trichoderma* sp 10 (30.0%) compared with control (16.7%). 25- Inoculation with G1+G2+G3+G4 followed by 01 (*G. macrocarpum*) alone and G3+G4 were the best treatments for controlling charcoal-rot disease incidence at the seedling stage. These 3. VAM treatments decreased % pre-emergence damping-off to 0.0, 3.3 and 6.7% and increased survived seedlings to 93.3; 86.7 and 83.3%, respectively. Percentage of post-emergence damping-off (at seedling stage) and charcoal-rotted plants (at mature stage) was not significantly affected by all tested soil VAM preparations. The VAM treatment G1+G2+G3+G4 produces the maximum % healthy standing plants (86.7%) followed by G1 alone (76.7%), G3+G4 (70.0%), G2+G3 (63.3%), and G4 "mati VAM" (63.3%). The soil preparation containing G2 alone has no significant effect on healthy standing plants (43.3%) meanwhile, G2 alone, G3 alone and G1+G2 produced the lowest significant increases i.e., 43.3%, 3.3% and 46.7%, respectively compared with 36.7% in control treatment. 26-The different levels of VAM mixture Of axenic cultures of 4 isolates of VAM fungi caused significant improvement in disease control at seedling and maturity. Applying this preparation at rates of 1, 2, 4 and 8 g/Kg potted soil reduced % pre-emergence damping-off to 12.5, 12.5, 10, and 17.5% and increased % survived seedlings to 70.0, 72.5, 72.5 and 62.5% and healthy plants to 62.5, 70.0, 57.5 and 42.5%, respectively. The

charcoal rotted plants were significantly decreased at rates 1 and 2 g/Kg soil meanwhile not significantly affected at the highest 2 levels i.e., 4 and 8 g/Kg when compared with control treatment. 27-The roots of sesame plants grown in sterilised potted soil infested with the charcoal rot pathogen showed extensive numbers of M phaseolina-sclerotia and few numbers of vesicles, a characterizing structure of the VAM fungi. These VAM-vesicles were detected also in roots of sesame plants grown in the pathogen free-sterilized potted soil. The sclerotia was nearly absent while extensive VAM-vesicles were noticed in roots of the mycorrhizal sesame plants that inoculated with any of the tested VAM soil or axenic culture preparations and grown in potted soils infested with charcoal rot pathogen. 28-Soaking sesame seeds in the tested autoclaved (A) and filtered (F) plant extracts decreased disease incidence significantly compared with control. In case of thyme, garlic and marjoram, the filtered extracts were more effective than autoclaved extracts for reducing charcoal rot infection and increasing healthy standing plants. On contrast, the autoclaved extracts of cumin, azedarach, roselle and clove were more effective than the filtered extracts in this respect. Both filtered and autoclaved extracts of roselle and anise were approximately equal in controlling disease incidence and increasing healthy plants. The obtained results concluded that the best control of charcoal rot disease on sesame plants could be obtained by soaking sesame seeds in filtered extracts of thyme, rhubarb or garlic (healthy standing plants 83.3-86.7%) or autoclaved extracts of cumin or azedarach (healthy standing plants 86.7%) 29-Soaking seeds in IAA at 100 ppm; IBA at 100, 200, and 400 ppm; SA at 2, 4, and 8 mM were significantly equal (90.0-100.0%) but the superior treatments were IBA at 100 ppm (100.0%) and 200 ppm (96.7%) and SA at 4 mM (96.7%). SA and IBA were the best for minimizing the charcoal rot disease incidence 1.7% and 6.7% and maximizing healthy plants to 89.2% and 84.2%, respectively. While, H₂O₂ and KCl were the least effective as they decreased charcoal rot to 13.3% and 17.5% compared with 23.3% in control treatment. Tanic acid, IAA, and Bion, decreased charcoal rot to 8.3%, 9.2% and 10.8% and increased healthy plants to 69.2%, 71.7%, and 56.7%, respectively 30-Activity of the oxidative enzymes peroxidase, polyphenol oxidase and catalase was obviously higher in tissues of sesame plants that were grown from seeds treated with any of the tested chemical inducers especially IBA, IAA and SA than those grown from untreated seeds (control). 31-Phenolic contents (free, conjugated and total) were higher in SA treatment followed by IBA; Tanic acid; Bion; IAA; KCl; H₂O₂, respectively. As for tested concentrations, the highest amounts of phenol contents were produced by SA (2 mM), Tanic acid (2 mM), ABA (100 ppm) and IAA (100 ppm), KCl (4.0%) and Bion (1 mM) and H₂O₂ (0.5%). 32-Most tested chemical inducers caused obvious increase in sugars content compared with control treatment. The highest amounts of reducing sugars were induced by the lower concentration of H₂O₂, KCl and IAA and or by the second concentration of IBA, SA and Bion. While, Tanic acid does this at its higher concentration. This trend was quietly varied in case of non-reducing and total sugars contents. 33- The tested cultivars and strains of sesame reacted differently throughout the different stages of disease development. Based on percentage of healthy standing plants, the screened sesame entries could be classified as follow: 1 —Highly susceptible entries including 8 entries i.e., strain 806, strain 792, strain 779, strain 799, B 11, strain 772, Giza 32, and Tushka 2. 2 - Susceptible entries including 7 entries i.e., strain 773, strain 786, Tushka 1, strain 796, strain 774, strain 775, and strain 797. 3 — Moderately susceptible entries including 6 entries, i.e., strain 783, strain 791, strain 794, Taka 1, Aceteru-M, and Taka 3. 4 :Moderately resistant entries including 5 entries i.e., B35, Mutation 48, Shandaweel 3, strain 785, and strain 787. 5 —Resistant entries including 4 entries i.e., strain 771, Tushka 3, Adnan 1(5/91), and Taka2. 34-The above classification of descriptive resistance could be confirmed and completely supported by the newly suggested accumulative resistance which calculated in similar way as previously described in accumulative virulence of the tested isolates of M phaseolina. 35-The amounts of free phenols, total phenols, reducing and total sugars, in general were obviously higher in the sesame entries that were classified as highly resistant "HR" and resistant "R" than those classified as susceptible "S" and high susceptible "HS" sesame entries. 36-Protein bands derived from the gel electrophoretic pattern of soluble proteins of the selected 15 sesame entries classify them into 2 main separate clusters with similarity 57.01%. The first main cluster consists of 3-sub clusters. The first and second sub clusters included the high susceptible and susceptible sesame

entries strain 806, Giza 32, strain 779, B 11, Tushka. 2, and Tushka 1 . While the third sub cluster consists 2 sub sub clusters which included the high resistant and resistant sesame entries Shandaweel 3, Taka 2, Adnan 1(5/91), strain 771 and Tushka 3. The second main cluster included the resistant and moderately resistant entries strain 787, Mutation 48, Aceteru' M., and strain B35.37-Under field conditions, the sesame entries Aceteru-M "R", Adnan 1 (5/91) "HR", Taka 2 "R" during the growing both seasons and Mutation 48 "R" in season 2000 only were the most resistant at Tahrir location "Behira Province". While, Aceteru-M "R", B35 "R", Taka 2 "R" in both growing seasons and Mutation 48 "R" and Tushka 2 "S" in season 1999 showed the resistance response at Sedes location "Beni-Suef Province" without significant variation between entries during the same season and location.38-The sesame entry strain 806 (HS in greenh use test) exhibited the highest % infection under field conditions during both seasons at both locations followed by the entry Giza 32 "S" during season 2000 at Tahrir location. However, the sesame entry Tushka 2 "S" could resist the natural infection significantly better than Taka 1 "MR" during both seasons and-strain 787 "R" during season 1999 at Tahrir location. Meanwhile, Tushka 2 "S" was significantly comparable to Adnan 1 (5/91) "HR", Aceteru-M "R", B35 "R", Mutation 48 "R", and Taka 2 "R" during season 1999 at Sedes location.39- At Tahrir location, the highest total seed yield was produced by the sesame entries Taka 2 "R" (237.9 Kg) and Mutation 48 "R" (234.3 Kg) in season 1999 without significant variation in between followed by Adnan 1 (5/91) "HR" (218.1 Kg). While Mutation 48 "R" (248.4 Kg) was best of all in season. 2000 at the same location followed by Adnan 1 (5/91) "HR" (207.0 Kg) and Taka 2 "R" (205.8 Kg). At Sedes location, the highest seed yield was produced by Mutation 48 followed by Taka 2 with significant differences only in season 1999. On the other hand, the highly susceptible entry strain 806 "HS" produces the lowest seed yield followed by B 11 "S", strain 779 "S", Giza 32 "S", Tuska 1 "MS" and Aceteru-M "R", respectively during both seasons at Tahrir location. At Sedes location, the lowest seed yield was produced also by strain 806 "HS" followed by strain 773 "MS", Giza 2 "S", B 11 "S" and-strain 779 "S" during 1999 season and Giza 32 "S", strain 779 "S", Taka 1 "MR", and strain 773 "MS" during 2000season. The seed yield produced by the susceptible sesame entry Tushka 2 was significantly higher during both growing seasons at both locations than that produced by some resistant entries such as Shandaweel 3, Strain 787 and Aceteru-M. 40-Percentages of oil content were significantly higher in seeds of healthy (52.9-62.9%) than diseased (48.2-59.4%) plants of all sesame tested entries. The average reduction in seed oil content due to charcoal rot infection was ranged between 3.95% in Tushka 2 "S" to 11.28% in strain 806 "HS". Reduction percentages were considerably varied according to season, location and sesame entry. The lowest reduction in oil content was associated with the sesame entries Shandaweel 3 (2.95-4.30%), Mutation 48 (2.93-4.66%) and Giza2 4.32-4.54%) at Tahrir location and Taka 3 (2.86-3.17%) and Tushka 2 (2.53-. 4.55%) at Sedes location. On contrast, the highest reduction in oil content at both locations was associated with strain 806 "I-IS" (10.93-11.79%) during both seasons, Adnan 1 (5/91) "HR" (9.44-10.17%) during season 1999 and Taka 1 "MR" during both seasons (7.16-10.24%) at Sedes location, 41-Under field conditions, the fungicidal seed treatments were significantly better in improving and seed yield than soil fungicidal treatments. Rizolex-T, vitavax-T and amconil were more effective in controlling sesame charcoal rot disease and increasing sesame seed yield when used as seed dressing than as soil drench. However, the best results were obtained when theyused in dual combinations. The combined seed/soil treatments rizolex-T/amconil, rizolex-T/rizolex-T, benlate/vitavax-T and benlate/amconil could be recommended for controlling sesame charcoal rot disease and increasing seed yield at both Tahrir and Sedes locations.