Induced resistance for controling root rot disease of strawberry and their side effects on biological activities in soil

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SUMMARYTo study the effect of some abiotic and biotic inducers on systemic resistance of strawberry plants to some root-rot fungal pathogenics, trials of in vitro and ex vitro were carried out during 2003 to 2005 seasons at Laboratories and Greenhouse of Integrated Control Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.The present investigation was planned to study the following topics: 1): Isolation and identification of the root-rot pathogenic fungi from different localities at four Egyptian governorates. 2): Testing pathogenicity of the isolated fungi at different levels of soil infestation. 3): Studying the reaction of some common strawberry cultivars against root-rot infection. 4): Studying the effect of some chemicals [Salicylic acid (SA), boric acid (BA), CuSO4 and MgSO4, KH2PO4, Bion, Ethephon and Rizolex-T] and biotic inducers [T. harzianum, P. fluorescens, B. subtilis, S. aureofaciens and Kombucha fermented tea] against the tested root rot fungi in vitro and in vivo. 5): Studying the effect tested inducers on morphological characters of strawberry. 6): Studying the defense mechanism in strawberry plants due to treating with some inducers. 7): Studying the side effects of the investigated treatments on soil microflora and activity of some enzymes. The obtained results could summarized as following:1. Two hundreds and ten fungal isolates belonging to 9 fungal species namely Rhizoctonia fragariae, R. solani, Sclerotium Fusarium solani, Macrophomina phaseolina, Pythium sp., F. oxysporum, Alternaria sp. and Trichoderma harzianum were isolated from naturally infected roots of two strawberry cultivars collected from four governorates. The occurrence and frequency of isolated fungi seems to be depended on the strawberry cultivar and governorate from which it was collected. Isolation trials from frigo transplants of strawberry cv. Camarosa after 15 days post planting at Qalubia governorate yielded R. fragariae, R. solani, S. rolfsii, F.Summary153solani, F. oxysporum, M phaseolina, Pythium ultimum, Alternaria sp., Curvularia sp., F. roseum and F. semitectum. However, some fungi i.e. Alternaria sp., Curvularia sp., F. roseum, F. semitectum were never be recorded at Sharclia governorate.2.All tested fungi could infect and led to death of strawberry transplants that was increased with increasing inoculum level (IL) of different fungi. After 21 days, the mixture inocula recorded the highest % disease (54.1%) followed by S. rolfsii, R. fragariae and R. solani (47.9%), F. oxysporium and M phaseolina (39.6%), F. solani (31.2%) and P. ultimum (16.6%), respectively while S. rolfsii produces the highest % disease after 45 days (68.8%) followed by the inoculum mixture (66.7%), R. fragariae (62.5%), R. solani (60.4%), M phaseolina (56.2%), F. oxysporium (50.0%), F. solani (41.7%) and P. ultimum (37.5%), respectively.3.All tested strawberry cultivars were susceptible to different extents to infection with all tested root rot pathogens. The K14 cv. was the least susceptible followed by Sweet Charli, Shundler, Gafuta and Camarosa cvs., respectively.4.Growth of all tested fungi was not affected by MgSO4 meanwhile KH2PO4 at 10, 15 and 20mM reduced growth of F. solani only by 29.6, 44.4 and 59.2%, respectively. Also, CuSO4 used against M phaseolina and P. ultimum, Bion 50% used against S. rolfsii, M phaseolina and P. ultimum, Boric acid against M phaseolina showed no any inhibition on growth of these fungi. However, the highest growth inhibition was obtained by using Rizolex-T at > 200ppm and salicylic acid at > 15 mM (against all tested fungi) and Ethephon at > 200ppm (against M phaseolina, F.

solani and P. ultimum). Salicylic acid at 4mM shows no inhibition on growth of M phaseolina meanwhile reduced growth of P. ultimum and S. rolfsii by 43.3 and 87.0%, respectively.Summary1545.T harzianum recorded maximum antagonistic activity against F. solani followed by R. solani and R. fragriae, M phaseolina, S. rolfsii and P. ultimum, respectively. While, B. subtilis shows it highest activity against R. solani followed by S. rolfsii, R. fragriae, M. phaseolina and F. solani but it had no effect on growth of P. ultimum. However, P. fluorescens reduced growth of R. solani (33.7%), S. rolfsii (31.0%) and F. solani (17.3%) meanwhile had no any inhibition on growth of the other tested pathogenic fungi. Also, S. aureofaciens reduced growth of F. solani only by 18.1% meanwhile had no any inhibition on growth of the other tested pathogenic fungi. Using kombucha preparation shows no inhibition on fungal growth.6.MgSO4 had no effect on germination of propagules of both F. solani and S. rolfsii whereas KH2PO4 and Bion 50% had no effect on germination of the S. rolfsii-sclerotia only. Germination of sclerotia (S. rolfsii) and conidia (F. solani) was completely inhibited by using Rizolex-Tat >200 and 600ppm, respectively. Using SA (20mM), CuSO4 (20mM) and Ethephon (800ppm) completely inhibited germination of sclerotia while decrease germination of conidia by 4.4, 26.9 and 9.6%, respectively.7.T. harzianum and P. fluorescens were the most effective for decreasing germination of sclerotia and conidia whereas, kombucha preparation shows no effect on germination of both propagules. Also, S. aureofaciens had no effect on germination of sclerotia but slightly decreased germination of conidia. The Generally, germination of S. rolfsii-sclerotia seems to be more sensitive to the tested abiotic and biotic inducers more than germination of F. solani-conidia.8.Activities of the oxidative enzymes peroxidase, poly phenol oxidase, catalase and chitinase were higher in mycelia than culture filtrates of the 6 tested root rot pathogenic fungi. Activity of an155 Summaryenzyme was increased by increasing the incubation period from 7 to 21 days. S. rolfsii shows the highest activity of any of these enzymes either in mycelia or in culture filtrates after 7 or 21 days incubation followed by R. fragariae, M phaseolina, R. solani, F. solani and P. ultimum, respectively.9.All tested abiotic inducers significantly decreased percentage of dead plants comparing with the untreated control. Using SA and AA (S. rolfsii), CuSO4 and BA (R. fragariae) and CuSO4 and KH2PO4 (R. solani) recorded the lowest percentage of dead plants after 45 days from cultivation. Applying any of the tested abiotic inducers using the twice application (dipping roots + spraying shoots) method seemed to be better in most cases than the dipping roots (dipping) and spraying shoots (spraying) methods for reducing % dead plants. Also, all tested biotic inducers significantly decreased percentages of dead plants after 21 but not after 45 days from cultivation compared to the untreated control. Regardless the pathogenic fungi, the lowest decrease was induced by T harzianum followed by P. fluorescence, B. subtilis, kombucha tea and S. aureofaciens, respectively.10.All tested abiotic inducers significantly increased shoot and root lengths of strawberry plants in comparison with the untreated controls. In this respect, the highest increase in the shoot and root lengths was induced by using ethephon and CuSO4 (S. rolfsii or R. fragariae) and ethephon and AA (R. solani). In most cases, the twice application method produced the highest increase followed by methods of dipping roots and spraying shoots, respectively. Regarding with biotic inducers and regardless pathogens, kombucha produced the highest significant increase in shoot length followed by B. subtilis meanwhile, S. aureofaciens caused the lowest significant increase comparing with the untreated control. While, T harzianum was the best for increasing root lengthSummary156followed by B. subtilis and P. fluorescence meanwhile, kombucha was the least effective in this respect comparing with the untreatedcontrol.11. All tested abiotic inducers significantly increased fresh weights (FW) of shoots and roots of strawberry plants in comparison with the untreated controls. In this regard, the best results were induced by using the twice application method associated with ethephon followed by KH2PO4 (S. rolfsii), MgSO4 (R. fragariae) and bion (R. solani). The FW of shoots and roots of strawberry plants was significantly improved also by all tested biotic inducers compared to the check treatment. In this respect, S. aureofaciens and P. fluorescence recorded the highest FW of both shoots and roots followed by B. subtilis and T harzianum, respectively. Using kombucha preparation caused the lowest increase in the FW of shoots meanwhile decreased the root FW particularly when used against both R. fragariae and R. solani.12. All tested abiotic inducers significantly increased dry weights (DW) of shoots and roots of strawberry plants in comparison with the untreated controls.

Regardless pathogenic fungi, the highest increase in the shoot DW, in general, was induced by using the twice application method and ethephon. The DW of shoots and roots of strawberry plants treated with most of the tested biotic inducers was significantly higher than the untreated control. Using S. aureofaciens and P. fluorescence recorded the highest increases in the DW of both shoots and roots followed by B. subtilis and 7'. harzianum, respectively. However, kombucha had no significant effect on the DW of shoots and caused the lowest significant increase in the DW of roots comparing with the untreated control.13. Most tested chemical inducers significantly increased percentage dray matter (DM) in both shoots and root's in comparison with the untreated controls. Regardless pathogens, the highest increase in %Summary157DM in shoots was induced by using the twice application method and ethephon meanwhile the highest DM in roots was induced by using the same application method together with CuSO4 (S. rolfsii and R. fragariae), and ethephon (R. solani). Concerning the biotic inducers, S. aureofaciens and P. fluorescence recorded the highest increases in % DM in both shoots and roots. Kombucha tea recorded the lowest increase in % DM in roots meanwhile significantly decreased it in shoots comparing with the untreatedcontrol.14.The sugars content was higher in shoots and roots of plants treated with any of the tested abiotic inducers (with few exceptions) in comparison with the untreated controls. Using Rizolex-T against any of the tested pathogens induced the highest increase in the reducing, non-reducing and total sugars contents in plant shoots. However, using the twice application method together with SA induced the highest sugars contents in plant roots. The reducing, non-reducing and total sugars in shoots and roots of strawberry plants were increased also .by using all tested biotic inducers. Applying T harzianum and P. fluorescence produced the highest increase in sugars content followed by B. subtilis, kombucha and S. aureofaciens, respectively comparing with their respectiveuntreated controls.15.The phenols contents particularly the free phenols in shoots and roots under stress of infection with a known pathogen were affected differently by using the different tested abiotic inducers and application methods. In presence of S. rolfsii the free phenols increased only by using Rizolex-T, ethephon, SA and AA meanwhile decreased by the other abiotic inducers. Similar effects were noticed also in presence of R. fragariae and R. solani (with few exceptions). As for biotic inducers, the free phenols content in shoots was increased by most tested biotic inducers used against S.Summary158rolfsii, except S. aureofaciens and kombucha comparing with the untreated control. Using B. subtilis against R. fragaria. recorded the highest amounts of all phenols in roots and free and totalphenols in shoots.16. Applying bion, BA and ethephon against any of the tested root rot pathogens in addition to KH2PO4 against S. rolfsii decreased the total amino acids content in both shoots and roots while the rest of abiotic inducers increased it. Using CuSO4 recorded the highest increase in the total amino acids in shoots and roots followed by AA (S. rolfsii and R. fragaria) and MgSO4 (R. solani). In most cases, the twice application method produced the highest total amino acids followed by the dipping and spraying methods, respectively. Also, all tested biotic inducers increased the total amino acids content in shoots and roots of plants grown in soil infested with a particular pathogen. Using B. subtilis and kombucha, respectively resulted in the highest and lowest increase in the total amino acids either in shoots or roots.17.Applying the tested abiotic • inducers induced some new amino acids such as proline (salicylic acid, boric acid, ascorbic acid and CuSO4), phenylalanine (ascorbic acid, KH2PO4, bion and ethephone), tryptophane (boric acid and MgSO4) and valine, leucine and lysine (boric acid). As for biotic inducers, the amino acid serine was induced only by B. subtilis meanwhile histidine, arginine, proline and phenylalanine were induced only by kombucha. Also, the amino acids methionine, valine and tryptophan were induced only by S. aureofaciens and kombuchatreatments.18.The lignin content in roots was increased by all tested abiotic inducers pathogens in comparison with the untreated controls. The twice application method and SA used against R. fragariae and R. solani produced the highest lignin content followed by AA and theSummary159fungicide Rizolex-T, respectively. The lignin content in plant roots, regardless root rot pathogens, was increased also by any tested biotic inducers in comparison with the untreated control. The highest lignin content was induced by B. subtilis followed by P. fluorescence, S. aureofaciens, T harzianum and Kombucha, respectively comparing with the untreated control. This trend wastrue against any pathogen tested.19. The salicylic acid and phytoalexens (isoflavone) contents in roots of strawberry plants under stress of infection with S.

rolfsii were increased by all tested abiotic and biotic, inducers in comparison with the untreated control treatment. Using SA and Rizolex-T recorded the highest amounts of both salicylic acid and phytoalexens followed by BA, AA, ethephon and CuSO4, respectively. While, The 7'. harzianum was the superior one among tested biotic inducers followed by P. fluorescence, B. subtilis, S. aureofaciens and kombucha, respectively.20. All tested abiotic inducers increased peroxidase activity in shoots and roots of strawberry plants in comparison with their untreated controls. Using ascorbic acid against any of the tested root rot pathogens (S. rolfsii, R. fragariae and R. solani) induced the highest increase in peroxidase activity in shoots whereas, CuSO4 (S. rolfsii), ascorbic acid (R. fragariae) and CuSO4 and Rizolex-T (R. solani) recorded the highest activity in roots. Using the twice application method caused the highest peroxidase activity followed by the methods of dipping roots and spraying shoots each alone, respectively. The peroxidase activity in shoots and roots of strawberry plants were increased also by using all biotic inducers in comparison with their untreated controls. B. subtilis recorded the highest increase in peroxidase activity in plant shoots whereas, S. aureofaciens was the most effective for increasing peroxidaseactivity in plant roots.21. Activity of polyphenol oxidase (PPO) in shoots and roots were affected differently by tested abiotic inducers. The PPO activity in shoots was increased only by using MgSO4, SA and AA against any tested pathogen and BA and ethephon (S. rolfsii),KH2PO4 (R. ftagaria) while the rest of tested abiotic inducers decreased it in comparison with the untreated control. Degree of increase or decrease was depending on the used application method. On the other hand, all abiotic inducers except MgSO4 (S. rolfsii) and salicylic acid (R. fragariae) decreased in activity of PPO enzyme in roots. As for biotic inducers, Bacillus subtilis used against the three pathogens and P. fluorescence and S. aureofaciens used against R. fragariae and R. solani increased PPO activity in shoots meanwhile the other tested biotic inducers i.e. T. harzianum and Combucha decreased it comparing with their untreated control treatments. The tested biotic inducers showed similar effects on PPO activity inroots. Most tested abiotic inducers increased chitinase (CHT) activity in shoots and roots in comparison with their untreated controls. The CHT activity in shoots and roots was higher when the twice application method was used compared to the dipping roots and spraying shoots each alone. The chitinase activity in plant shoots was increased also by the tested biotic inducers. Applying T. harzianum against any tested root rot pathogen recorded the highest activity in shoots followed by P. fluorescence, B. subtilis, S. aureofaciens and kombucha, respectively. The chitinase activity in roots was increased only by P. fluorescence, T. harzianum and S. aureofaciens meanwhile B. subtilis and kombucha decreased it. 23. Activity of 13, 1-3-Glucanase enzyme in shoots and roots of strawberry plants was increased by all tested abiotic inducers. The highest increase was induced by using KH2PO4, Rizolex-T and ascorbic acid. In general, the twice application method increased glucanase activity in shoots and roots more than applying the dipping or spraying methods each alone. As for biotic inducers, using T. harzianum against any tested pathogen, recorded the highest activity in shoots followed by P. fluorescence, B. subtilis, S. aureofaciens and kombucha, respectively. However, the enzyme activity in roots was increased by most tested biotic inducers particularly T harzianum meanwhile decreased or not changed by using B. subtilis against any pathogen, P. fluorescence against S. rolfsii and kombucha against R. solani, 24. Activities of the dehydrogenase, cellulose and phosphatase enzymes were higher in soil cultivated with strawberry plants treated with any of the tested abiotic inducers in comparison with soil cultivated with the untreated plants. Activities of these enzymes were obviously higher after 8 days than after 30 days from cultivation. Regardless the tested abiotic inducers, the twice application method showed higher activities of these soil enzymes than the dipping method or spraying method. In general, Rizolex-T, SA and bion induced the highest activities particularly after 8 days from cultivation. This trend was approximately true under stress of infection with any tested root rot pathogen. Activities of these enzymes were increased also by using any of the tested biotic inducers. Using B. subtilis against any of the tested pathogenic fungi, in general, induced the highest activities of the dehydrogenase and phosphatase enzyme meanwhile S. aureofaciens was the most effective for increasing activity of thecellulose enzyme. 25. Treating strawberry transplants with all tested abiotic and biotic inducers increased the counted numbers of the different soil microorganism in the pathogen-infested soil planted with these treated transplants in comparison with the soil cultivated with the untreated

transplants. The total microbial count, soil fungi, bacteria, actenomycetes, azotobacter, nitrifying bacteria and spore-forming bacteria increased to different extents by prolonging time after cultivation according to kind of the tested inducer, method of application and kind of pathogen in the infested soil. The total counts of these soil microorganisms, in general, were higher after 30 and 65 days from treatment with the abiotic and biotic inducers, respectively compared to their counts after 8 days.26. Percentage dead strawberry plants determined under field conditions after 21 or 45 days from planting were significantly decreased by all tested abiotic inducers during seasons 2004 and 2005 comparing with the untreated control treatments. In 2004 season, the fungicide Rizolex-T was the best of all (after 21 and 45 days), followed by SA and AA (after 21 days) and BA, AA and SA (after 45 days). In 2005 season, Rizolex-T was the most effective followed by CuSO4 and ascorbic acid after 21 days. The lowest % dead plants was associated with the twice application method followed by the dipping and spraying methods, respectively. Also, the percentage of dead plants determined after 21 or 45 days from sowing was significantly decreased in both seasons by all tested biotic inducers. In this respect, T. harzianum was the most effective followed by S. Aureofaciens, B. subtilis, P. fluorescence and Kombucha, respectively comparing with the untreated control treatments.27. All tested abiotic inducers significantly increased the strawberry fruit yield during both 2004 and 2005 seasons comparing with the untreated control. The fungicide Rizolex-T produces the highest fruit yield in both seasons followed by AA, BA and CuSO4 in the first season and BA, AA and MgSO4, respectively in the second season. Meanwhile, ethephon and bion (2004), and ethephone, bion and SA (2005) produced the lowest significant increase in the fruit yield comparing with the untreated controls. Regardless abiotic inducers, the highest yield in both seasons was associated with applying the spraying and dipping methods in the first season without significant differences in between whereas, the spraying method was the best in the second season' followed by the dipping and twice application method, respectively with clear significant differences in between. Similarly, the fruit yield was significantly increased by tested biotic inducers comparing with the untreated controls. The highest yield in both seasons was produced by S. aureofaciens and 7'. harzianum followed by B. subtilis, P. fluorescence and kombucha, respectively.