Studies on rhizobial inculation of some legumes using seed pelleting method

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This work comprises two laboratory experiments and greenhouse one as thefollowing:Laboratory experimentsPart 1Part 1 deals with study of the effect of seed-coat diffusates of someleguminous seeds on growth and survival of their specific rhizobial strains.The tested rhizobial strains were Rhizobium Ieguminosarum by. trifolii(ARC 103, USDA 2101 and USDA 2128) for clover (Trifolium alexandrinum)var, Sakha 4, Rhizobium Ieguminosarum bv, viceae for faba bean (Viceae faba)variety Giza 4 and lentil var. Giza 370 (Lens esculanta) with correspondingstrains ICARDA 441 ARC and 205, Rhizobium leguminosarum by. phaseolifor bean var. Giza 6 (Phaseolus vulgaris) strain 302 and Bradyrhizobiumjaponicum strains (RCR 3407, USDA 110 and ARC 501) for soybean var.Crawford (Glycine max (L.) Merr). The results revealed that:1) Some leguminous seeds coat diffusates encourage the growth of theirspecific rhizobia strains, however others inhibit them.2) The filtrate of pre-soaked clover seeds enhanced the growth of cloverrhizobial strains USDA 2101, USDA 2128 and ARC 103 (Fig. 1).3) The filtrates of pre-soaked faba bean and lentil seeds encourage thegrowth of the rhizobia! strains ICARDA 441 and ARC 205, respectively (Fig. 2).4) The filtrate of pre-soaked bean seeds did not affect the growth of therhizobia strain ARC 302 and its growth was as usual (Fig. 3)., 5) The filtrate of pre-soaked soybean seeds exhibited different effects on thespecific rhizobia! strains that is the seed diffusate stimulate vigorously the growthof the strain USDA 110 around the treated spots followed by lower growth of thestrain RCR 3407 and finally the strain ARC 501 with the least growth (Fig. 4).Part IISurvival of rhizobia on stored pre-inoculated and pelleted faba beanand soybean seeds with different carrier-based inocula and adhesive aeents.Seeds of both faba bean and soybean were pre-inoculated and pelleted withdifferent carrier-based inocula and adhesive agents. The survival of rhizobial cells/seed was carried out at different shelf-time storage periods of 0, 7, 30,45,60,75, 90, 105 and 120 days. Results indicated that:I) FABABEAN1- Fine peatThe carrier-based inoculum fine peat achieved the highest viable rhizobia}cells count at zero shelf-time storage ’with all adhesive agents (gum arabic,molasses, mineral oil and water). The corresponding rhizobial cells/seed were9.1 x 105, 4.1 x 105 and 3.2 x 105. The lowest number of 6 cells/seed was carriedat 120 days shelf-time period using mineral oil as an adhesive agent.2- VermiculiteApplication of vermiculite as a carrier-based inocuhnn in combination withany of adhesive agents tested gave viable number of rhizobial cells rangedbetween 8.3 x 105 to 70 cells/seed. The superior viable rhizobial cells/seed hadbeen achieved at zero and 15 days shelf-time storage periods. These results wasrecorded with all adhesive agents tested.3- Talc powderThe combination of talc powder with all adhesive agents tested gave thehighest viable rhizobial count/seed at zero shelf-time storage period. Thecorresponding numbers were 5.1 xl0s (gum arabic), 4.3 x 105 (molasses) and 2.5x 105 for .mineral oil and water.4- Calcium carbonateWhen calcium carbonate powder combined with any of the adhesive agentstested, viable numbers of rhizobial cells/seed were splendid at zero shelf-timestorage period. These numbers were 7.i x 105, 6.2 x 105, 3.1 x 105and 3.1 x lOsfor gum arabic, molasses and mineral oil or water, respectively.In general, seed pelleting using the tested carrier-based inocula and thedifferent adhesive agents, when stored from zero up to 120 days, the best viablerhizobial cells/seed was recorded at zero shelf-time storage period. However, theviable counts recorded at 15 days shelf-time storage period were able to inducenodulation.II) SOYBEAN1- Fine peatResults revealed

that the highest viable rhizobial count /soybean seed was atzero days shelf-time storage period with all adhesive agents tested. Thecorresponding numbers were 6.7 x 104, 4.4 X 104, 6.5 X 104 and 4.1 x 104cells/seed for gum arabic, molasses, mineral oil and water, respectively.Increasing shelf-time storage periods decreased the numbers of viablerhizobial cells. However, the viable number of rhizobia Icells/ seed recorded after30 days storage period was able to induce nodulation.2- VermiculiteThe same trend noticed for fine peat with gum arabic and molasses had beenachieved with vermiculite. The highest viable rhizobial cells/seed were 6.9 x 104and 7.8 x 10-t for gum arabic and molasses at zero days shelf-time storage period,respectively. While both of them recorded the lowest viable number cells/seed of2.7 x 103 and 1.1 x 103 at 120 days. shelf-time storage period, respectively.Mineral oil as an adhesive agent gave reasonable viable rhizobial cells/seed up to75 days shelf-time storage period. On the other hand, storing pelleted seeds formore than 75 and up to 120 days shelf-time storage periods dramatically declinedthe viable rhizobial cells/seed for the adhesive agents tested.1623) Talc powderTalc powder data revealed also that the highest viable rhizobial cells/seedhad been recorded at zero days shelf-time storage period using all adhesiveagents tested. The corresponding values were 4.2 x 104,4.6 x 104,4.1 x 104 and4.2 x 104 cells/seed for gum arabic, molasses and mineral oil or water,respectively. However, the lowest viable rhizobial cells/seed were noticed withtalc powder at 120 days shelf-time storage period for all tested adhesive agents.4) Calcium carbonateThe results of calcium carbonate as a carrier-based inoculum indicated thatat zero shelf-time period; all adhesive agents recorded the highest viable rhizobialcells/seed of 7.6 x 104, 4.4 X 104, 3.7 X 104 and 2.2 x 104 for gum arabic,molasses and mineral oil or water, respectively. It was also noticed that alladhesive agents when mixed with calcium carbonate in seed pelleting gave thelowest viable rhizobial cells/seed at 120 days shelf-time storage period.Greenhouse experimentsEffect of seed pelleting with . different carrier-based inocula anddifferent adhesh’e agents on the growth and nodulation of {aha hean (Giza 4)and soybean (Crawford) under different shelf-time storae;e periods:Greenhouse experiments were carried out to investigate growth, nodulationand nitrogen content of faba bean and soybean plants as affected by rhizobialinoculation and pelleted with different carrier-based inocula and differentadhesive agents under different shelf-time storage of initial, 30,45 and 60 daysbefore sowing. The plants were uprooted at 45 and 90 days after planting (DAP),results revealed that :a) Seed pelleting for both faba bean and soybean before planting enhancedthe growth at 45 and 90 days sampling time (DAP).b) Storing the pelleted seed before sowing had significantly increased theroot and shoot dry weight, number and dry weight of nodules and shoot nitrogen163content for both faba bean and soybean plants at 45 and 90 DAP. These increaseswere more pronounced at 30 days shelf-time storage period for both crops.1- Faba bean experiment1.1. Foum’ five days samplin2 time:1.1.1 With faba bean. mineral oil as an adhesive agent gave the highest rootdry weight (2.02 g/plant) compared to the other adhesive agents tested.1.1.2. All the tested carrier-based. inocula had significantly increased theroots dry weight for faba bean plants. However, fine peat-based inoculum gavethe highest roots dry weight (2.4 g/plant).1.1.3. The number of nodules/plant for faba bean achieved by using gumarabic or molasses as adhesive agents significantly exceeded both of mineral oiland water. Also using fine peat and vermiculite, as carrier-based inocula werestatistically different and higher than those of talc powder and calcium carbonate.1.1.4. The application of adhesive agents or carrier-based inocula at any ofshelf-time storage periods, generally increased significantly the dry weight ofnodules.1.1.5. The values of fine peat and vermiculite recorded for faba bean shootsdry weight were significantly different .from each other and were significantlyhigher and different from those of talc powder or calcium carbonate. This trendwas more obvious at 30 days shelf-time storage.1.1.6. The combination of gum arabic as adhesive agent with fine peat gavethe highest nitrogen content for faba bean plants. The values were 73.67 and 72.0mg/plant.1.1.7. All adhesive agents tested for faba bean negatively affected the dryweight of roots at initial, 30 and 45 days shelf-time storage. However, storage ofpelleted seeds for 60 days, and application of molasses as an adhesive agent gavethe highest dry weight of roots t 1.89 glplant) and was significantly different fromall the other adhesive agents tested. On contrast, all the tested carrier-basedinocula showed positive effects on the dry weight of faba bean roots at all shelftimestorage up to 60 days before sowing.1.2. !inety days sampling time1.2.1. The highest number of nodules/plant for faba bean (14 nodulesplant) had achieved with the combination of gum arabic x fine peat x zero shelftime,molasses x fine peat x zero shelf-time, gum arabic x fine peat x 30 shelftime,gum arabic x vermiculite and molasses x vermiculite.1.2.2. All adhesive agents and carrier-based inocula used in seed pelletingfor faba bean significantly increased the dry weight of nodules and the dry weightof shoots with priority of the combination of molasses or gum arabic with finepeat in case of shoot dry weight although their values were in equal at 30 daysshelf-time storage period.1.2.3.Concerning nitrogen content at all shelf-time storage periods for fababean, it was concluded that the combination of adhesive agents and carrier-basedinocula in exchange had significantly increased the amount of nitrogen/plant. Thistrend was clear with the use of molasses x fine peat (141.25 mg/plant), gumarabic x fine peat (138.75 mg/plant) and molasses x vermiculite (130.25mg/plant).2- Soybean experiment2.1. Fourtv five sampling time2.1.1. The combination of adhesive agents and carrier-based inoculasignificantly increased the roots dry weight of soybean. The greatest valuesranged 1.13 to 1.27 gJplant were obtained when mineral oil was combined withany of vermiculite, talc powder or calcium carbonate.2.1.2. The application of adhesive agents significantly increased the root dryweight of stored pelleted soybean seeds. The highest root dry weight wasrecorded for mineral oil (1_01 glplant) and water (0.81 glplant) compared to eithermolasses or gum arabic.1652.1.3. The application of adhesive agents and carrier-based inoculasignificantly increased the number of nodules/plant at all shelf-time intervalsinvestigated.2.1.4. Molasses as an adhesive agent gaye the greatest number of nodules/plant at all shelf-time storage periods,2.1.5. The combination of adhesive agents with carrier-based inoculasignificantly increased the number of nodules/plant with splendid values of 25and 20 nodules/plant for molasses and gum arabic at zero shelf-time, respecnvely.2.1.6. All adhesive agents and carrier-based inocula significantly increasedthe dry weight of nodules at all shelf-time storage periods.2.1.7. The glorious dry weight of nodules had achieved by the combinationof fme peat x gum arabic (93.83 mg/plant) and fine peat x molasses (8944mg/plant).2.1.8. The application of adhesive agents and carrier-based inocula insoybean seed pelleting significantly increased the shoots dry weights of soybeanplants at all shelf-time storage periods.2.1.9. Any of adhesive agents and carrier-based inocula when altemaredtogether in pelleting soybean seeds had significantly increased the nitrogencontent of soybean plants. This trend was pronounced with gum arabic x fine peat(41.17 mg/plant) and molasses x fine peat (40.00 mg/plant). These 1\'0 rreatmeotshad no significant differences between each other.2.2. Ninety days sampling time2.2.1. The adhesive agents used for pelleting soybean seeds did not affectsignificantly the roots dry weight at zero shelf-time. However, increasing shelftimestorage of pelleted seeds stored for 30 days before sowing gave the hightestroots dry weight of 1.70 and 1.59 g1plant with gum arabic and molassses,respectively.166Increasing shelf-time storage period up to 60 days before sowing elevatedthe foots dry weight for all adhesive agents compared to that of the control(water) as an adhesive agent.2.2.2. It was clear that the adhesive agents tested could be rankeddiscendingly as gum arabic, molasses and then mineral oil with priority to gumarabic at the shelf-time storage of 30 days before sowing.2.2.3. Fine peat as a carrier-based inoculum recorded the highest roots dryweight of2.02 g1plantat 30 days shelf-time storage before sowing.2.2.4. The combination of all carrier-based inocula with the adhesive agentstested in alternation did not significantly affect the roots dry weight.2.2.5. All adhesive agents significantly increased the number of nodules/plant at zero shelf-time but they lowered the number of nodules/plant at 30 daysshelf-time storage”2.2.6. The highest number of nodules/plant was achieved with gum arabicand molasses at zero shelf-time storage. The corresponding values were 18 and16 nodules/plant. Increasing the shelf-time storage up to 60 days had decreasedthe number of nodules/plant with an order of gum arabic, molasses and mineraloil.2.2.7. All carrier-based inocula tested significantly increased the number ofnodules/plant. However, fine peat, as a carrier-based inoculum was significantlyhigher than the other carrier-based inocula included. The highest values ofnodules/plant were 20 (fine peat), 19 (vermiculite), 14 (talc powder) and 10(calcium carbonate) at 30 days shelf-time storage before sowing. Increasing shelftimestorage up to 60 days significantly increased also the number ofnodules/plant with respective values of 18 and 17nodules/plant for fine peat andvermiculite at 60 days shelf-time storage, respectively.2.2.8. When adhesive agents and. carrier-based inocula used together inalternation, the highest three values for number of nodules/plant were 32 (gLUTIarabic x fine peat), 27 (molasses x fine peat) and 23 (molasses x vermiculite).2.2.9. Adhesive agents significantly increased the dry weight of nodules atall shelf-time storage periods before sowing. The priority was for gum arabicfollowed by molasses, mineral oil and finally water (the control treatment). Thehighest values were 75.65, 75.59, 61.27 and 43.13 mg/plant for gum arabic,molasses, mineral oil and water at zero shelf-time storage, respectively. Thelowest dry weight of nodules were 47.71,44.33,38.89 and 37.73 mg/plant at 60days shelf-time storage, which corresponded to gum arabic, molasses, mineral oiland water as adhesive agents.2.2.10. Generally the use of the carrier-based inocula in seed pelletingsignificantly increased the dry weight of nodules at all shelf-time storage beforesowing. The highest dry weights of nodules were 116.88, 108.56,55.75 38.37and 0.00 mg/plant for fine peat, vermiculite, talc powder calcium carbonate anduninoculated seeds at zero shelf-time storage, respectively. Increasing the shelftimestorage before sowing up to 60 days led to decrease the dry weight ofnodules with priority to the thirty days storage period, which was nearly equal tothose of zero-time storage with no significant difference between them. Thecorresponding values were 11.59 (fine peat), 100.42 (vermiculite), 41.80 (talcpowder), 26.92 (calcium carbonate) and 0.00 mg/plant (uninoculated seeds).2.2.11. The combination of adhesive agents with carrier-based inocula atdifferent shelf-time storage intervals had achieved the highest significant dryweight of nodules at the cases of zero-time x molasses x fine peat, 30 days xmolasses x fine peat and zero-time x gum arabic x fine peat respective to the16Svalues of 137.0, 135.33 and 135.10 mg/plant. These values were not significantlydifferent from each other.2.2.12. The use of adhesive agents in soybean seeds pelleting significantlyimproved the dry weight of shoots at all shelf-time storage periods before sowing.The use of gum arabic was superior and its values of shoots dry weight werehigher and significantly different from the other adhesive agents tested.2.2.13. The highest dry weight of shoots values achieved by gum arabicwere 2.70 g/plant (30 days), 2.69 g/plant (zero-time) and 2.62 g/plant (45 days) .. The corresponding shoots dry weight values recorded by molasses at these shelftimestorage periods were for zero-time (2.63 glplant), 45 days (2.62 g/plant) and30 days (2.25 g/plant).2.2.14. The use of carrier-based inocula also significantly increased theshoots dry weight of soybean plants at all storage periods before sowing. Finepeat and vermiculite gave the highest dry weight of shoots at zero and 30 daysshelf-time storage intervals followed by talc powder and calcium carbonate. Thevalues of shoots dry weight were 3.46 and 3.44 g/plant (fine peat), 2.82 and3.08g/plant (vermiculite) at zero-time and 30 days, respectively. It is clear that finepeat and vermiculite as carrier-based inocula were the splendid carrier materialsin respect to the shoots dry weight either at zero-time or 30 days hence theirvalues at these two intervals were not significantly different.2.2.15. The combination of adhesive agents and carrier-based inocula insoybean seeds pelleting had significantly improved the shoots dry weight in thecases of gum arabic x fine peat (4.17 g/plant), molasses x fine peat (3.39 g/plant)and mineral oil x fine peat (3.37 glplant).2.2.16. All the adhesive agents used in soybean pelleting had significantlyincreased the nitrogen contentplant at all shelf-time storage periods in the orderof gum arabic, molasses, mineral oil and then water. The highest nitrogencontent/plant values had achieved with gum arabic at 30 days (80.73 mg;plant)followed by 77.93 mg/plant for molasses and 73.93 mg/plant (gum arabic) at 45days shelf-time storage. It is obvious that gum arabic and molasses are the mostrecommended adhesive agents, which can be used in seed pelleting effectivelyother than mineral oil and water.2.2.17. The tested camer-based inocula showed that fine peat had asplendid effect on the nitrogen content/plant at all shelf-time storage periods andrecorded 104.33, 106.58, 105.25 and 105.58 mg/plant for zero, 30,45 and 60,respectively. These values were higher than and significantly different from thoseof the other carrier-based inocula used in soybean seed pelleting.2.2.18. The use of adhesive agents with carrier-based inocula in soybeanseed pelleting had significantly increased. the nitrogen content of soybean plantsand this was clear with the cases of gum arabic x fine peat (128.42 mg/plant) andmolasses x fine peat (117.50 mg/plant). However, these two values were notsignificantly different from each other.6. GENERAL CONCLUSIONLaboratory experiments:PARTI1. Some leguminous seed-diffusates. encourage the growth of their specificrhizobial strains and others inhibit them. .2. It may be beneficial to pay attention towards the legume seeds that theirdiffusates encourage the growth of their specific rhizobial strains before sowingsuch as faba bean, clover and some varieties of soybean seeds.3. There is no difference to previously immerge or do not the legume seeds,which their diffusates had not affected their specific rhizobial strains beforesowing such as bean and some varieties of soybean seeds.PART II1. For adhesive agents, any of molasses or mineral oil could replace gumarabic, the common adhesive agents iIi legumes seed pelleting in case of nonavailabilityof gum arabic.2. Water as an adhesive agent in seed pelleting is not beneficial to keepenough viable rhizobial cells/seed.3. Vermiculite as a carrier-based inoculum could substitute fine peat thecommon carrier-based inoculum in legumes seed pelleting.4. Storage the legume pelleted seeds before sowing at room temperatureseems to ensure more viable cells/seed specially at shelf-time of zero or 30 daysbefore sowing, hence their corresponding results were not significantly differentfrom each other.5. The combination of gum arabic as adhesive agent with fine peat orvermiculite rhizobial inocula in legumes seed pelleting ensures the highest viablerhizobial cells/seed.6. Whenever gum arabic, as an adhesive agent, is not available, it may bereplaced by molasses or mineral oil to combine fine peat or vermiculite as carrierbasedinocula in seed pelleting.7. It is recommended that legume seed pelleting protects rhizobial strainsagainst the adverse conditions such as soil acidity, pesticides, rhizobiophage,indigenous rhizobial strains competition and chemical fertilizers.Greenhouse experiments:1) Seed pelleting process for both faba bean and soybean before sowing ispreferable.2) Storing of pelleted seeds of any of faba bean or soybean before sowing ismore beneficial at 30 days shelf-time storage.3) Gum arabic as an adhesive agent is the most recommended one in fababean and soybean seeds pelleting. It is also noticed that molasses followed bymineral oil but not water could be substitute the non-available gum arabic.4) Fine peat as a carrier-based inoculum is the splendid one in faba bean andsoybean seeds pelleting followed by vermiculite that may compensate fine peatshortage.5) Gum arabic followed by molasses and then mineral oil could combine finepeat or vermiculite in alternation for faba bean or soybean seeds pelleting.However, the combination of gum arabic with fine peat seems to be moreacceptable in faba bean and soybean seed pelleting.6) Water as an adhesive agent and any of talc powder or calcium carbonateas carrier-based inocula are weakly recommended in faba bean and soybean seedpelleting.7) Seed pelleting process significantly increased the rootsdry weight,number and dry weight of nodules, dry weight of shoots and the nitrogencontent/plant of faba bean and soybean plants. This was clear up to storage of 30days for the pelleted inoculated seeds.Generally 0 the legume seed diffusates materials need to be defined as thiswill enable scientists to avoid or encourage the release of these materialsaccording to their effect on the viable rhizobial cells/seed. So, more work shouldbe done to catch the extreme benefits of the seed coat-diffusates materials.However, the task oflegume inoculation with pelleting methods is still a matter ofarguments that requires a scope of more research and studies.