

5. SUMMARY

This work comprises two laboratory experiments and greenhouse one as the following :

Laboratory experiments

Part I

Part 1 deals with study of the effect of seed-coat diffusates of some leguminous seeds on growth and survival of their specific rhizobial strains.

The tested rhizobial strains were *Rhizobium leguminosarum* bv. *trifolii* (ARC 103, USDA 2101 and USDA 2128) for clover (*Trifolium alexandrinum*) var. Sakha 4, *Rhizobium leguminosarum* bv. *viceae* for faba bean (*Viceae faba*) variety Giza 4 and lentil var. Giza 370 (*Lens esculanta*) with corresponding strains ICARDA 441 ARC and 205, *Rhizobium leguminosarum* bv. *phaseoli* for bean var. Giza 6 (*Phaseolus vulgaris*) strain 302 and *Bradyrhizobium japonicum* 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 their specific rhizobia strains, however others inhibit them.
- 2) The filtrate of pre-soaked clover seeds enhanced the growth of clover rhizobial strains USDA 2101, USDA 2128 and ARC 103 (Fig. 1).
- 3) The filtrates of pre-soaked faba bean and lentil seeds encourage the growth of the rhizobial strains ICARDA 441 and ARC 205, respectively (Fig. 2).
- 4) The filtrate of pre-soaked bean seeds did not affect the growth of the rhizobia strain ARC 302 and its growth was as usual (Fig. 3).
- 5) The filtrate of pre-soaked soybean seeds exhibited different effects on the specific rhizobial strains that is the seed diffusate stimulate vigorously the growth of the strain USDA 110 around the treated spots followed by lower growth of the strain RCR 3407 and finally the strain ARC 501 with the least growth (Fig. 4).

Part II

Survival of rhizobia on stored pre-inoculated and pelleted faba bean and soybean seeds with different carrier-based inocula and adhesive agents.

Seeds of both faba bean and soybean were pre-inoculated and pelleted with different 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) FABA BEAN

1- Fine peat

The carrier-based inoculum fine peat achieved the highest viable rhizobial cells count at zero shelf-time storage with all adhesive agents (gum arabic, molasses, mineral oil and water). The corresponding rhizobial cells/seed were 9.1×10^5 , 4.1×10^5 and 3.2×10^5 . The lowest number of 6 cells/seed was carried at 120 days shelf-time period using mineral oil as an adhesive agent.

2- Vermiculite

Application of vermiculite as a carrier-based inoculum in combination with any of adhesive agents tested gave viable number of rhizobial cells ranged between 8.3×10^5 to 70 cells/seed. The superior viable rhizobial cells/seed had been achieved at zero and 15 days shelf-time storage periods. These results was recorded with all adhesive agents tested.

3- Talc powder

The combination of talc powder with all adhesive agents tested gave the highest viable rhizobial count/seed at zero shelf-time storage period. The corresponding numbers were 5.1×10^5 (gum arabic), 4.3×10^5 (molasses) and 2.5×10^5 for mineral oil and water.

4- Calcium carbonate

When calcium carbonate powder combined with any of the adhesive agents tested, viable numbers of rhizobial cells/seed were splendid at zero shelf-time

storage period. These numbers were 7.2×10^5 , 6.2×10^5 , 3.1×10^5 and 3.1×10^5 for gum arabic, molasses and mineral oil or water, respectively.

In general, seed pelleting using the tested carrier-based inocula and the different adhesive agents, when stored from zero up to 120 days, the best viable rhizobial cells/seed was recorded at zero shelf-time storage period. However, the viable counts recorded at 15 days shelf-time storage period were able to induce nodulation.

II) SOYBEAN

1- Fine peat

Results revealed that the highest viable rhizobial count /soybean seed was at zero days shelf-time storage period with all adhesive agents tested. The corresponding numbers were 6.7×10^4 , 4.4×10^4 , 6.5×10^4 and 4.1×10^4 cells/seed for gum arabic, molasses, mineral oil and water, respectively.

Increasing shelf-time storage periods decreased the numbers of viable rhizobial cells. However, the viable number of rhizobial cells/ seed recorded after 30 days storage period was able to induce nodulation.

2- Vermiculite

The same trend noticed for fine peat with gum arabic and molasses had been achieved with vermiculite. The highest viable rhizobial cells/seed were 6.9×10^4 and 7.8×10^4 for gum arabic and molasses at zero days shelf-time storage period, respectively. While both of them recorded the lowest viable number cells/seed of 2.7×10^3 and 1.1×10^3 at 120 days shelf-time storage period, respectively. Mineral oil as an adhesive agent gave reasonable viable rhizobial cells/seed up to 75 days shelf-time storage period. On the other hand, storing pelleted seeds for more than 75 and up to 120 days shelf-time storage periods dramatically declined the viable rhizobial cells/seed for the adhesive agents tested.

3) Talc powder

Talc powder data revealed also that the highest viable rhizobial cells/seed had been recorded at zero days shelf-time storage period using all adhesive agents tested. The corresponding values were 4.2×10^4 , 4.6×10^4 , 4.1×10^4 and 4.2×10^4 cells/seed for gum arabic, molasses and mineral oil or water, respectively. However, the lowest viable rhizobial cells/seed were noticed with talc powder at 120 days shelf-time storage period for all tested adhesive agents.

4) Calcium carbonate

The results of calcium carbonate as a carrier-based inoculum indicated that at zero shelf-time period, all adhesive agents recorded the highest viable rhizobial cells/seed of 7.6×10^4 , 4.4×10^4 , 3.7×10^4 and 2.2×10^4 for gum arabic, molasses and mineral oil or water, respectively. It was also noticed that all adhesive agents when mixed with calcium carbonate in seed pelleting gave the lowest viable rhizobial cells/seed at 120 days shelf-time storage period.

Greenhouse experiments

Effect of seed pelleting with different carrier-based inocula and different adhesive agents on the growth and nodulation of faba bean (Giza 4) and soybean (Crawford) under different shelf-time storage periods:

Greenhouse experiments were carried out to investigate growth, nodulation and nitrogen content of faba bean and soybean plants as affected by rhizobial inoculation and pelleted with different carrier-based inocula and different adhesive agents under different shelf-time storage of initial, 30, 45 and 60 days before 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 enhanced the growth at 45 and 90 days sampling time (DAP).

b) Storing the pelleted seed before sowing had significantly increased the root and shoot dry weight, number and dry weight of nodules and shoot nitrogen

content for both faba bean and soybean plants at 45 and 90 DAP. These increases were more pronounced at 30 days shelf-time storage period for both crops.

1- Faba bean experiment

1.1. Fourty five days sampling time:

1.1.1 With faba bean, mineral oil as an adhesive agent gave the highest root dry weight (2.02 g/plant) compared to the other adhesive agents tested.

1.1.2. All the tested carrier-based inocula had significantly increased the roots dry weight for faba bean plants. However, fine peat-based inoculum gave the highest roots dry weight (2.4 g/plant).

1.1.3. The number of nodules/plant for faba bean achieved by using gum arabic or molasses as adhesive agents significantly exceeded both of mineral oil and water. Also using fine peat and vermiculite, as carrier-based inocula were statistically 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 of shelf-time storage periods, generally increased significantly the dry weight of nodules.

1.1.5. The values of fine peat and vermiculite recorded for faba bean shoots dry weight were significantly different from each other and were significantly higher and different from those of talc powder or calcium carbonate. This trend was more obvious at 30 days shelf-time storage.

1.1.6. The combination of gum arabic as adhesive agent with fine peat gave the highest nitrogen content for faba bean plants. The values were 73.67 and 72.0 mg/plant.

1.1.7. All adhesive agents tested for faba bean negatively affected the dry weight of roots at initial, 30 and 45 days shelf-time storage. However, storage of pelleted seeds for 60 days, and application of molasses as an adhesive agent gave the highest dry weight of roots (1.89 g/plant) and was significantly different from all the other adhesive agents tested. On contrast, all the tested carrier-based

inocula showed positive effects on the dry weight of faba bean roots at all shelf-time storage up to 60 days before sowing.

1.2. Ninety days sampling time

1.2.1. The highest number of nodules/plant for faba bean (14 nodules plant) had achieved with the combination of gum arabic x fine peat x zero shelf-time, molasses x fine peat x zero shelf-time, gum arabic x fine peat x 30 shelf-time, gum arabic x vermiculite and molasses x vermiculite.

1.2.2. All adhesive agents and carrier-based inocula used in seed pelleting for faba bean significantly increased the dry weight of nodules and the dry weight of shoots with priority of the combination of molasses or gum arabic with fine peat in case of shoot dry weight although their values were in equal at 30 days shelf-time storage period.

1.2.3. Concerning nitrogen content at all shelf-time storage periods for faba bean, it was concluded that the combination of adhesive agents and carrier-based inocula in exchange had significantly increased the amount of nitrogen/plant. This trend was clear with the use of molasses x fine peat (141.25 mg/plant), gum arabic x fine peat (138.75 mg/plant) and molasses x vermiculite (130.25 mg/plant).

2- Soybean experiment

2.1. Fourty five sampling time

2.1.1. The combination of adhesive agents and carrier-based inocula significantly increased the roots dry weight of soybean. The greatest values ranged 1.13 to 1.27 g/plant were obtained when mineral oil was combined with any of vermiculite, talc powder or calcium carbonate.

2.1.2. The application of adhesive agents significantly increased the root dry weight of stored pelleted soybean seeds. The highest root dry weight was recorded for mineral oil (1.01 g/plant) and water (0.81 g/plant) compared to either molasses or gum arabic.

2.1.3. The application of adhesive agents and carrier-based inocula significantly increased the number of nodules/plant at all shelf-time intervals investigated.

2.1.4. Molasses as an adhesive agent gave the greatest number of nodules/plant at all shelf-time storage periods.

2.1.5. The combination of adhesive agents with carrier-based inocula significantly increased the number of nodules/plant with splendid values of 25 and 20 nodules/plant for molasses and gum arabic at zero shelf-time, respectively.

2.1.6. All adhesive agents and carrier-based inocula significantly increased the dry weight of nodules at all shelf-time storage periods.

2.1.7. The glorious dry weight of nodules had achieved by the combination of fine peat x gum arabic (93.83 mg/plant) and fine peat x molasses (89.44 mg/plant).

2.1.8. The application of adhesive agents and carrier-based inocula in soybean seed pelleting significantly increased the shoots dry weights of soybean plants at all shelf-time storage periods.

2.1.9. Any of adhesive agents and carrier-based inocula when alternated together in pelleting soybean seeds had significantly increased the nitrogen content 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 two treatments had no significant differences between each other.

2.2. Ninety days sampling time

2.2.1. The adhesive agents used for pelleting soybean seeds did not affect significantly the roots dry weight at zero shelf-time. However, increasing shelf-time storage of pelleted seeds stored for 30 days before sowing gave the highest roots dry weight of 1.70 and 1.59 g/plant with gum arabic and molasses, respectively.

Increasing shelf-time storage period up to 60 days before sowing elevated the roots 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 ranked discendingly as gum arabic, molasses and then mineral oil with priority to gum arabic at the shelf-time storage of 30 days before sowing.

2.2.3. Fine peat as a carrier-based inoculum recorded the highest roots dry weight of 2.02 g/plant at 30 days shelf-time storage before sowing.

2.2.4. The combination of all carrier-based inocula with the adhesive agents tested 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 days shelf-time storage.

2.2.6. The highest number of nodules/plant was achieved with gum arabic and molasses at zero shelf-time storage. The corresponding values were 18 and 16 nodules/plant. Increasing the shelf-time storage up to 60 days had decreased the number of nodules/plant with an order of gum arabic, molasses and mineral oil.

2.2.7. All carrier-based inocula tested significantly increased the number of nodules/plant. However, fine peat, as a carrier-based inoculum was significantly higher than the other carrier-based inocula included. The highest values of nodules/plant were 20 (fine peat), 19 (vermiculite), 14 (talc powder) and 10 (calcium carbonate) at 30 days shelf-time storage before sowing. Increasing shelf-time storage up to 60 days significantly increased also the number of nodules/plant with respective values of 18 and 17 nodules/plant for fine peat and vermiculite at 60 days shelf-time storage, respectively.

2.2.8. When adhesive agents and carrier-based inocula used together in alternation, the highest three values for number of nodules/plant were 32 (gum arabic 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 at all shelf-time storage periods before sowing. The priority was for gum arabic followed by molasses, mineral oil and finally water (the control treatment). The highest 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. The lowest dry weight of nodules were 47.71, 44.33, 38.89 and 37.73 mg/plant at 60 days shelf-time storage, which corresponded to gum arabic, molasses, mineral oil and water as adhesive agents.

2.2.10. Generally the use of the carrier-based inocula in seed pelleting significantly increased the dry weight of nodules at all shelf-time storage before sowing. The highest dry weights of nodules were 116.88, 108.56, 55.75 38.37 and 0.00 mg/plant for fine peat, vermiculite, talc powder calcium carbonate and uninoculated seeds at zero shelf-time storage, respectively. Increasing the shelf-time storage before sowing up to 60 days led to decrease the dry weight of nodules with priority to the thirty days storage period, which was nearly equal to those of zero-time storage with no significant difference between them. The corresponding values were 11.59 (fine peat), 100.42 (vermiculite), 41.80 (talc powder), 26.92 (calcium carbonate) and 0.00 mg/plant (uninoculated seeds).

2.2.11. The combination of adhesive agents with carrier-based inocula at different shelf-time storage intervals had achieved the highest significant dry weight of nodules at the cases of zero-time x molasses x fine peat, 30 days x molasses x fine peat and zero-time x gum arabic x fine peat respective to the

values of 137.0, 135.33 and 135.10 mg/plant. These values were not significantly different from each other.

2.2.12. The use of adhesive agents in soybean seeds pelleting significantly improved 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 were higher and significantly different from the other adhesive agents tested.

2.2.13. The highest dry weight of shoots values achieved by gum arabic were 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 shelf-time storage periods were for zero-time (2.63 g/plant), 45 days (2.62 g/plant) and 30 days (2.25 g/plant).

2.2.14. The use of carrier-based inocula also significantly increased the shoots dry weight of soybean plants at all storage periods before sowing. Fine peat and vermiculite gave the highest dry weight of shoots at zero and 30 days shelf-time storage intervals followed by talc powder and calcium carbonate. The values of shoots dry weight were 3.46 and 3.44 g/plant (fine peat), 2.82 and 3.08 g/plant (vermiculite) at zero-time and 30 days, respectively. It is clear that fine peat and vermiculite as carrier-based inocula were the splendid carrier materials in respect to the shoots dry weight either at zero-time or 30 days hence their values at these two intervals were not significantly different.

2.2.15. The combination of adhesive agents and carrier-based inocula in soybean seeds pelleting had significantly improved the shoots dry weight in the cases 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 g/plant).

2.2.16. All the adhesive agents used in soybean pelleting had significantly increased the nitrogen content/plant at all shelf-time storage periods in the order of gum arabic, molasses, mineral oil and then water. The highest nitrogen content/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 45 days shelf-time storage. It is obvious that gum arabic and molasses are the most recommended adhesive agents, which can be used in seed pelleting effectively other than mineral oil and water.

2.2.17. The tested carrier-based inocula showed that fine peat had a splendid effect on the nitrogen content/plant at all shelf-time storage periods and recorded 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 those of the other carrier-based inocula used in soybean seed pelleting.

2.2.18. The use of adhesive agents with carrier-based inocula in soybean seed pelleting had significantly increased the nitrogen content of soybean plants and this was clear with the cases of gum arabic x fine peat (128.42 mg/plant) and molasses x fine peat (117.50 mg/plant). However, these two values were not significantly different from each other.

6. GENERAL CONCLUSION

Laboratory experiments:

PART I

1. Some leguminous seed-diffusates encourage the growth of their specific rhizobial strains and others inhibit them.
2. It may be beneficial to pay attention towards the legume seeds that their diffusates encourage the growth of their specific rhizobial strains before sowing such as faba bean, clover and some varieties of soybean seeds.
3. There is no difference to previously immerse or do not the legume seeds, which their diffusates had not affected their specific rhizobial strains before sowing such as bean and some varieties of soybean seeds.

PART II

1. For adhesive agents, any of molasses or mineral oil could replace gum arabic, the common adhesive agents in legumes seed pelleting in case of non-availability of gum arabic.
2. Water as an adhesive agent in seed pelleting is not beneficial to keep enough viable rhizobial cells/seed.
3. Vermiculite as a carrier-based inoculum could substitute fine peat the common carrier-based inoculum in legumes seed pelleting.
4. Storage the legume pelleted seeds before sowing at room temperature seems to ensure more viable cells/seed specially at shelf-time of zero or 30 days before sowing, hence their corresponding results were not significantly different from each other.
5. The combination of gum arabic as adhesive agent with fine peat or vermiculite rhizobial inocula in legumes seed pelleting ensures the highest viable rhizobial cells/seed.

6. Whenever gum arabic, as an adhesive agent, is not available, it may be replaced by molasses or mineral oil to combine fine peat or vermiculite as carrier-based inocula in seed pelleting.

7. It is recommended that legume seed pelleting protects rhizobial strains against 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 is preferable.

2) Storing of pelleted seeds of any of faba bean or soybean before sowing is more beneficial at 30 days shelf-time storage.

3) Gum arabic as an adhesive agent is the most recommended one in faba bean and soybean seeds pelleting. It is also noticed that molasses followed by mineral 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 and soybean seeds pelleting followed by vermiculite that may compensate fine peat shortage.

5) Gum arabic followed by molasses and then mineral oil could combine fine peat or vermiculite in alternation for faba bean or soybean seeds pelleting. However, the combination of gum arabic with fine peat seems to be more acceptable in faba bean and soybean seed pelleting.

6) Water as an adhesive agent and any of talc powder or calcium carbonate as carrier-based inocula are weakly recommended in faba bean and soybean seed pelleting.

7) Seed pelleting process significantly increased the roots dry weight, number and dry weight of nodules, dry weight of shoots and the nitrogen

content/plant of faba bean and soybean plants. This was clear up to storage of 30 days for the pelleted inoculated seeds.

Generally, the legume seed diffusates materials need to be defined as this will enable scientists to avoid or encourage the release of these materials according to their effect on the viable rhizobial cells/seed. So, more work should be done to catch the extreme benefits of the seed coat-diffusates materials. However, the task of legume inoculation with pelleting methods is still a matter of arguments that requires a scope of more research and studies.
