RESEARCH ARTICLE Agricultural Science Digest, Volume 42 Issue 5: 598-603 (October 2022)

Effect of Thiram on the Bacteria-Pea (*Pisum sativum*) Symbiotic Complex

S. Laabas, D. Boukirat, H. Chaker, F. Berber
10.18805/ag.DF-453

ABSTRACT

Background: Algerian agriculture, like many developing countries, has developed production systems based on the use of phytosanitary products. But today, the use of these products is called into question, with the awareness of the risks they can generate for agriculture, the environment and even for human health.

Methods: A trial was conducted in the greenhouse on two varieties of pea (*Pisum sativum*), with and without thiram treatment (recommended dose), to estimate the effect of the latter on the establishment of symbiosis and biological nitrogen fixation. In addition, a rhizospheric soil microflora count was carried out to determine and assess the bacterial concentration including PGPRs in each sample from two varieties tested with and without treatment.

Result: The fungicide treatment and variety choice have no effect on the number of nodules and the fresh and dry weight of the aerial and root parts and the low nodulation can be attributed to the low population of native rhizobia in the soil. A low bacterial concentration was observed in the rhizospheric region of the Gros Vert variety treated with the fungicide compared to the untreated control and Onward variety; this result may be due to the effect of fungicide thiram influencing the abundance of microflora in the soil and the differential response between cultivars of the same species.

Key words: Nodulation, PGPR, *Pisum sativum*, Rhizospheric soil, Thiram.

INTRODUCTION

Food pulses have become an essential component of food and feed for the whole world and especially for low-income populations (Asia and Africa) (Pachico, 2005). Among these legumes, the pea is an appreciated vegetable and constitutes a staple food and its consumption is linked to its dietetic virtue. On the other hand, food legumes constitute a means of improving soil fertility and the yields of crops associated with them (Baudoin, 2001), thanks to their characteristic feature of symbiotic nitrogen fixation with BNL (Bacteria Nodulating Legumes) (Graham and Vance, 2003), which has earned it a prominent place as the crop of choice for sustainable agriculture (Babar *et al.* 2009).

However, this natural process of symbiotic nitrogen fixation promote the growthand the soil microbial community like PGPRs (Plant Growth-Promoting Rhizobacteria) are affected by several constraints that reduce their efficiency via disruption of nodulation processes and thereby hindering natural nitrogen fixation.

Edaphic factors (Hatimi, 2001) and toxicity due to certain phytosanitary products (Wani *et al.* 2005), including fungicides which in treating fungal diseases (Rocher, 2004); are among these constraints. In Algéria thiram is a fungicide widely used in the cultivation of legumes, including the pea (*Pisum sativum*).

A comparative trial was carried out in a greenhouse on two varieties of peas (Onward and Gros Vert) with and without treatment with the fungicide (thiram). The objective of this study is to evaluate the effect of treating pea seeds with this fungicide on the establishment of symbiosis via the comparison of the number of nodules formed on the roots and the comparison of dry and fresh weight from the aerial and root parts of the two varieties tested.

Laboratory of Agriculture and Environment, Department of Nature and Life Science, Faculty of Science and Technology, University of Ahmed Ben Yahia El Wancharissi, Tissemsilt-380 00, Algeria.

Corresponding Author: S. Laabas, Laboratory of Agriculture and Environment, Department of Nature and Life Science, Faculty of Science and Technology, University of Ahmed Ben Yahia El Wancharissi, Tissemsilt-380 00, Algeria.
Email: saadialaabas@yahoo.fr

How to cite this article: Laabas, S., Boukirat, D., Chaker, H. and Berber, F. (2022). Effect of Thiram on the Bacteria-Pea (*Pisum sativum*) Symbiotic Complex. Agricultural Science Digest. 42(5): 598-603. DOI: 10.18805/ag.DF-453.

Submitted: 26-01-2022 **Accepted:** 05-07-2022 **Online:** 16-07-2022

Physico-chemical analyses of the soil were carried out to estimate its natural fertility and to look for possible correlations between the presence, absence and abundance of nitrogen-fixing bacteria and PGPRs.

A bacterial microflora count was performed to evaluate the concentration of microorganisms present in the rhizospheric soils of plants from two varieties tested with and without thiram treatment.

MATERIALS AND METHODS

Soil sampling

Sampling was conducted from an agricultural field set aside for fallow, having durum wheat as the previous crop, in

Tissemsilt region (Algeria) (50° 35′ 62. 23″ N 27°1′ 54. 042″ E), during March 2021. A transect is drawn at field level (10 m/ 10 m) and soil samples were taken randomly at 10 cm depth on both sides of this transect. A mixture of soils that was formed was homogenized and used for the pot experiment and physico-chemical analysis. Soil analyses for physical (granulometric) and chemical (carbon, pH, conductivity, organic matter, nitrogen, phosphorus, *etc*) parameters were carried out at the FERTIAL agronomic laboratory (Annaba).

Plant material

The pea (*Pisum sativum*) seed cultivars used in this experiment (Onward and Gros Vert) originated from New Zealand and France, respectively and were delivered by SARL CASAP (Algiers). The seeds tested were treated with thiram at a recommended dose of 0.2% (w/w), by preparing fungicide slurry a 5 ml water/kg of seed (Graind *et al*. 2007).

Greenhouse experiment

Planting seedlings

The seeds were sown in pots of 500 g capacity containing sampled soil, each pot was sown with 3 seeds of pea (Onward and Gros Vert varieties), six replicates were made for each variety tested, (the same varieties used without seed treatment with thiram is considered as control). The experiment was arranged in a randomized block and the seedlings were watered every week till flowering.

Estimation of biological nitrogen fixation

At flowering stage, the plants were dug up and the rhizospheric soils were recovered and stored at -20°C for enumeration of the native soil microflora. The nodules formed at the roots were detached and counted to estimate the symbiotic power, then kept dry in tubes containing $CaCl_2$ (Vincent, 1970). Biological nitrogen fixation was estimated by comparing the fresh and dry weights of the aerial and root parts of plants treated with thiram and controls.

Statistical analyses

Statistical analysis was performed using SAS software (version 09). A one-way analysis of variance test was conducted (varietal effect and the effect of thiram treatment on the nodules number and the fresh and dry weight of aerial and root parts) and the probability threshold used to determine significance is $p \leq 0.05$.

Quantification of the microflora of rhizospheric soils

Rhizospheric soil is the soil adhering to the roots of plants, intense microbial activity is observed. The aim is to evaluate the concentration of this microflora present in the rhizospheric soils of two varieties of plants tested with and without seed treatment with thiram. 10 g of soil was stirred in 90 ml of physiological (NaCl 0.9%) water for 30 minutes. From this stock suspension, cascade dilutions of 10^{-1} to 10^{-5} were made, 0.1 ml of each dilution was spread on a solid medium (Nutrient Agar) and incubated at 30°C for 24 hrs. The plates containing a number of colonies between 30 and 300 are taken into consideration, then the number of microorganisms is expressed in number of Colony Forming Units (Bonnefoy *et al*. 2001).

RESULTS AND DISCUSSION

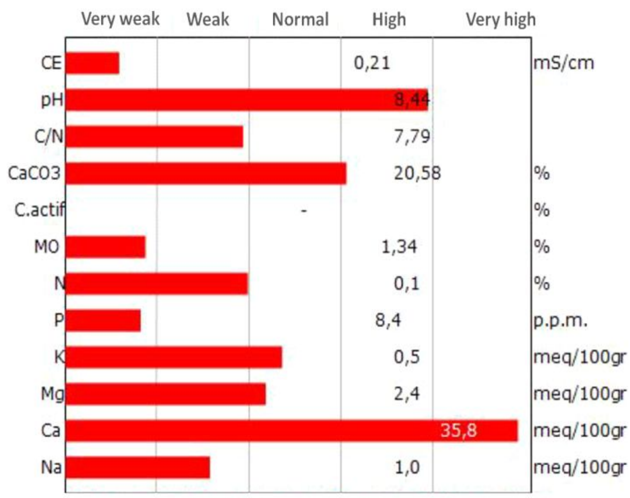
Physico-chemical analysis of soils

All physico-chemical parameters have been included in Table 1. These analyses make it possible to search for possible relationships between the abundance of rhizospheric bacteria and the natural fertility of the soil studied (Soltner, 2005).

The results show that according to the texture triangle, the soil sampled is clay soil (72%); it is a very heavy soil, with very poor internal drainage and a very high water retention capacity. The soil tested has a low total nitrogen content (0.1%) and very low phosphorus concentration (8.4 ppm). It is a non-saline soil (0.21 ms/cm), with an alkaline pH (8.44). The limestone content is high (20.58%) and its organic matter content is very low (1.34%). According to Robert, (1996), this is an indication of low fertility and does not favor microbial activities.

Table 1: Results of chemical analyzes of sampled soil.

Sand 8%	Silt 20%	Clay 72%
Conductivity (1/5 mS/cm)	0.21	Non saline
pH eau (1/2.5)	8.44	Alkaline
C/N	7.79	Weak
	%	p.p.m.
Carbonates	20.58	205800.00
Active limestone	-	-
Organic material	1.34	13400.00
Total nitrogen	0.1	1000.0
	Meq/100gr	p.p.m.
Phosphorus (Olsen)	0.03	8.4
Exchangeable potassium	0.5	203.3
Magnésium échangeable	2.4	289.4
Exchangeable calcium	35.8	7166.3
Exchangeable sodium	1.0	239.2



Analytical data interpreted by the Siddra system

Estimation of biological nitrogen fixation

Nodule number

The differences in the responses of the root systems regarding the establishment of symbiosis is considered by the number of nodules formed at the root level. Low nodulation was observed in this experiment for both the varieties tested (treated or untreated with thiram) indicating statistically, the varietal effect on nodulation was not significant (p= 0.15). Nevertheless, the highest nodulation rate was recorded in the roots of the Gros Vert variety (Fig 1) compared to the Onward variety.

According to Andrade and Hungria (2002), the rate of nodulation varies with variety sown and genetic factors of the plant. The potential of symbiotic nitrogen fixation depends on symbiotic partners Aaccording to Maj *et al*. (2010), when *Rhizobium* is present in a population of legume crop that is specific to *Rhizobium* species, it prefers certain genotypes to form root nodosities.

The low or total absence of nodulation in certain plants may be due to low population of native and efûcient rhizobial strains in the soil; survival and persistence of rhizospheric bacteria are major characteristics to infect the roots of the plant and form nodules (Date, 1976). The low of nodulation my also due to the inhibition of the establishment of symbiosis by some unfavorable environmental factors such as the physical and chemical properties of soil (salinity, clay soil, *etc*) (O'hara *et al*. 1988; Peoples and Crawell, 1992). Similarly, the nitrogen concentration is low to prevent the establishment of symbiosis because it is widely accepted that a high ammonium concentration is always depressive for nodulation (Munns, 1977).

According to Tibaoui and Zouaghi, (1989), phosphorus improves symbiotic nitrogen fixation through increased nodulation. Deficiency of this element nodular activity, therefore resulting in the reduction of symbiotic nitrogen fixation (Christiansen and Graham, 2002).

It is well known that factors such as chemical fertilizers, heavy metals and pesticides compromise the survival, growth and nitrogen-fixing capacity of rhizobia strains (de Lajudie, 2004). Thiram treatment had an effect on the inhibition of nodulation in the case of the Onward variety in this experiment (Fig 2) but this effect was not significant statistically (p= 0.65); Isoi and Yoshida (1988), have shown that thiram has a negligible effect on the growth of rhizospheric bacteria like *Rhizobium leguminosarum* bv *phaseoli.* Other studies have also shown positive effects of pesticide seed treatment especially borax on weight and nodule number of blackgram (*Vigna mungo* L.) (Yamini and Anilkumar, 2022). However, Lal and Lal (1988) afûrmed that pesticides influence the *Rhizobium*-legume symbiosis and in turn nitrogen fixation through direct action on free living soil *Rhizobium* population that subsequently affects the degree of infection and the number of nodules formed. These results presumably imply that the sensitivity to thiram is very different among *Rhizobium* species (Isoi and Yoshida, 1988).

Fresh weight of aerial and root parts

The highest aerial and root fresh weight was recorded in the Onward variety compared to the Gros Vert variety (Fig 3), but statistically, this response had no significant effect in this study (p>0.05). McMichael and Quisenberry (1993) suggest that the growth and development of the root system and the plant, in general, are under genetic control and can be altered by factors such as soil composition.

Contrasting results have been obtained by other researchers who have shown that varietal choice will have important consequences on the functioning of aerial and root system whose result may explain the differences in performance between varietal types. The difference in response between varieties of the same species has been observed in corn and soybeans crops (Bushamuka and Zobel, 1998) and peas too (Tsegaye and Mullins, 1994).

The soil structure also affects the development of the aerial and root system and the yellowing of the leaves in the two varieties tested, may be due to the low concentration of phosphorus (8.4 ppm), or to the deficiency of nitrogen (0.1%) in the soil used (Kolef, 1974).

Seed treatment with thiram did not significantly influence fresh aerial and root weights (p>0.05); this result is in agreement with Lal and Lal (1988), who showed that

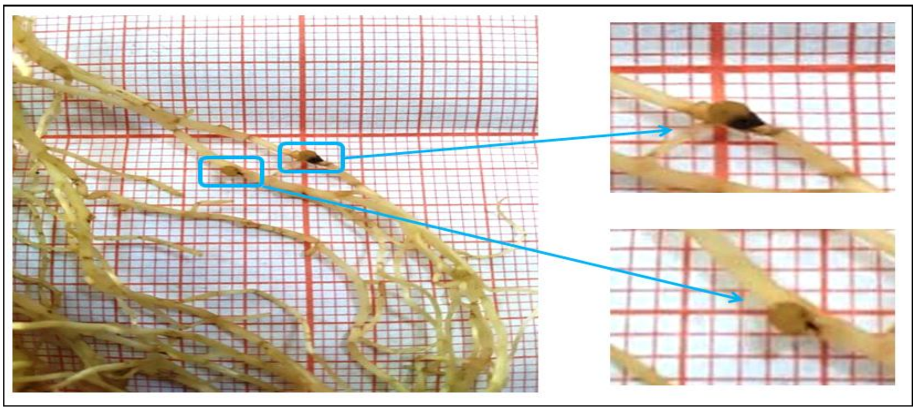


Fig 1: Appearance of nodules formed at the roots of a pea (*Pisum sativum*) variety Gros Vert.

treatment with fungicides may not affect plant growth and their influence differs depending on the class, chemical formulation and applied dose. On the other hand, remarkable negative effect of fungicides, especialy thiram, was observed on the performance of nitrogen-fixing bacteria, root biomass (Gaind *et al*. 2007), plant biomass and grain yield of chickpea (Aamil *et al*. 2004), bean (*Phaseolus vulgaris*) (Guene *et al*. 2003) and pea (*Pisum sativum*) (Ahmad *et al*. 2006).

Dry weight of aerial and root parts

The treated onward variety showed a slightly higher root dry weight compared to the Gros Vert variety (Fig 4), however, this effect was not statistically significant (p= 0.88). The same result was observed on the aerial dry weight of treated plants and without treatment (p=0.44), which explains why varietal choice has no effect on the development and behavior of the plant in this study. The result obtained confirms the suggestion of McMichael and Quisenberry (1993), that the development and growth of the plant are genetically controlled and can be modified, by certain soil factors such as clay texture (72%), low concentration of phosphorus (8.4 ppm) and nitrogen (0.1%) and the low content of organic matter (1.34%) which are indicators of low soil fertility and which are essential for microbial activity according to Robert (1996).

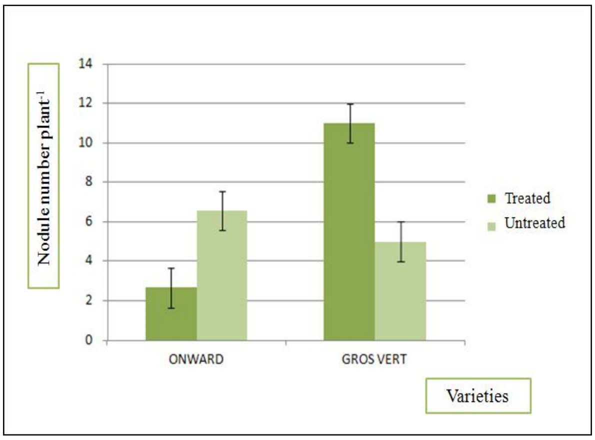


Fig 2: Number of nodules on the roots of two varieties of peas (*Pisum sativum*) with and without thiram treatment.

A slight reduction in aerial dry weight was observed in both varieties treated with fungicide compared to untreated controls; however, the effect of thiram on air-dry weight is not significant statistically (p=0.60). On the other hand, the root dry weight was more in the Onward variety treated with thiram compared to the untreated control, but without any statistically significant effect (p=0.66); the same observation was made when measuring the fresh root weight and this result was attributed to the dose of fungicide used (Lal and Lal, 1988).

Quantification of the microflora of rhizospheric soils

This step consists in determining and evaluating the bacterial concentration in each rhizospheric soil samples (from two varieties: Onward and Gros Vert with and without thiram treatment). A low bacterial concentration is observed in the tested soils, the soil type may be the cause of such results as it was suggested by Hatimi, (2001), who found that edaphic factors such as low organic matter content (1.34%), alkaline pH, soil texture (Clay soil) significantly affect the development of soil microbial flora essential for plant growth and nutrition (PGPR) and nitrogen-fixing bacteria.

The lowest bacterial concentration was found in the rhizospheric soil of the Gros Vert variety treated with thiram, with a concentration of 1.40×10^5 cells/ml (10%), compared to the untreated control where the concentration was 1.21×10^6 cells/ml (90%); this result may be due to the seed treatment with thiram which affects the abundance of microflora in the soil. The development and inhibition of nitrogen-fixing bacteria and PGPR activities by fungicides, including thiram has been proven by several researchers, Drouin *et al*. (2010) showed that fungicides such as thiram, captan and mancozeb significantly reduce the number, growth and survival of *Rhizobium, Mesorhizobium* and *Sinorhizobium* strains.

Unlike the Gros Vert variety, thiram treatment has no effect on the bacterial microflora of the rhizospheric soil of the onward variety and their concentration was almost equal

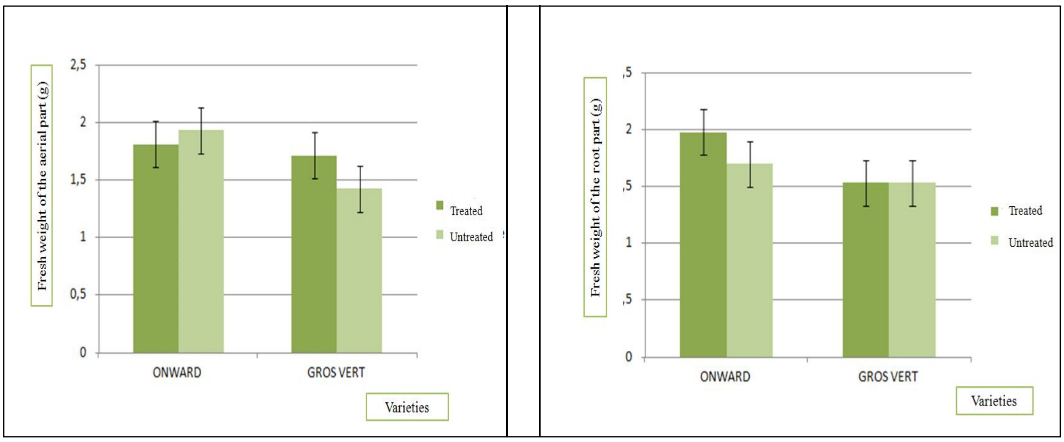


Fig 3: Fresh weight of the aerial and root parts of two varieties of peas (*Pisum sativum*) with and without thiram treatment.

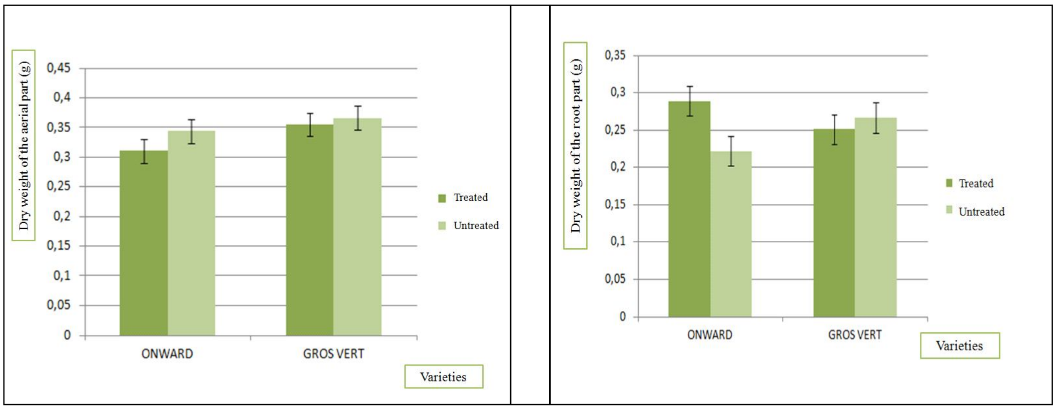


Fig 4: Dry weight of the aerial and root parts of two varieties of peas (*Pisum sativum*) with and without thiram treatment.

to that recorded in the control (50%), according to Ahemad and Khan, (2012), fungicides at recommended doses have a minor reducing effect on beneficial bacterial microflora.

A remarkable difference in bacterial concentration was observed between the rhizospheric soils of the cultivars tested (Onward, Gros Vert), which explains the difference in response between varieties of the same species and that plants select their own rhizosphere community (Maougal *et al*. 2020). According to Khan *et al*. (2009), the growth of plants and microorganisms in the rhizosphere, reciprocally depend on their secreted molecules such as carbohydrates, amino acids, vitamins and plant growth regulators. Most of them act as attractants to harbor the various microbial communities. The composition of these exudates depends on the physiological state and the species of plants and soil microorganisms (Zaidi *et al*. 2009).

CONCLUSION

During this study, low nodulation was observed for both varieties tested; suggesting the presence of a low population of native rhizobia in the soil, or inhibition of the development of both the symbiotic partners because of unfavorable factors of the environment such as the soil structure (clay texture, low phosphorus and nitrogen concentration, and low organic matter content). On the other hand, a difference was observed in the number of nodules formed between the treated and untreated plants of each variety, however the analysis of variance did not reveal any significant effect of thiram treatment on the number of nodules, which confirms some hypotheses that show that pesticides, especially thiram, have negligible effects on the establishment of symbiosis. Fungicide treatment with a recommended dose and varietal choice also has no significant effect on the fresh and dry weight of aerial and root parts of plants, suggesting, that the development and growth of the plant are genetically controlled, can be altered, by certain unfavorable factors, and the influence of fungicide differs depending on the class, chemical formulation and the dose applied. These results confirm that the recommended dose of thiram has no significant effect on the *Rhizobium*-legume symbiosis, nitrogen fixation and subsequently the degree of infection and the number of nodules formed. However, a remarkable difference was observed between the bacterial concentrations of the rhizospheric soils from the cultivars tested, which explains the difference in response between the varieties, which in turn depends on the molecular dialogue established between the variety and the microbial community present in the soil. On the other hand, the lowest bacterial concentration is observed in the rhizospheric soil of the Gros Vert variety treated with thiram, compared to the untreated control; which shows that the fungicide affects the abundance of microflora in the soil.

Conflict of interest: None.

REFERENCES

Aamil, M., Zaidi, A., Khan, M.S. (2004). Effects of herbicide dose rate on nodule bacteria and growth, nodulation and yield of chickpea (*Cicer arietinum* L.). Annals of Plant Protection Sciences. 12: 186-191.

Ahemad, M., Khan, M.S. (2012). Effect of fungicides on plant growth promoting activities of phosphate solubilizing *Pseudomonas putida* isolated from mustard (*Brassica compestris*) rhizosphere. Chemosphere. 86: 945-950.

Ahmad, M.S.A., Javed, F., Ashraf, M., Hafeez, F.Y. (2006). Effect of fungicide seed treatments on N_2 -fixation and nodulation in pea, *Pisum sativum* L. Bulletin of Environmental Contamination and Toxicology. 77: 896-904.

Andrade, D.S., Hungria, M. (2002). Maximizing the Contribution of Biological Nitrogen Fixation in Tropical Legume Crops. In: Nitrogen Fixation, Global Perspectives. [Finan, T.M., O'Brian, M.R., Layzell, D.B., Vessey, J.K., Newton, W. (Eds.)], CABI Publishing, Wallingford. UK, pp. 341-345.

Babar, B.M., Shah, T.M., Abbas, G., Ahsanul-Haq, M. (2009). Genotype × environment interaction for seed yield in *Kabuli* chickpea (*Cicer arietinum* L.) genotypes developed through mutation breeding. Pakistan Journal of Botany. 4: 1883-1890.

Baudoin, J.P. (2001). Contribution des ressources phytogénétiques à la sélection variétale de légumineuses alimentaires tropicales, Biotechnologie, Agronomie, Société et Environnement. 5(4): 221-230.

Bonnefoy, C., Guillet, F., Leyral, G., Verne-Bourdais, E. (2001). Microbiologie et qualité dans les industries agro-alimentaires. CRDP d'Aquitaine- Doin. 231 p.

Bushamuka, V.N. and Zobel, R.W. (1998). Differential genotypic and root type penetration of compacted soil layers. Crop Science. 38: 776-781.

Christiansen, I and Graham, P.H. (2002). Variation in di-nitrogen fixation among Andean bean (*Phaseolus vulgaris*) genotypes grown at low and high of phosphorus supply. Field Crops Resherch. 73: 133-142.

Date, R.A. (1976). Principles of *Rhizobium* Strain Selection. In: Nutman PS. (Eds) Symbiotic Nitrogen Fixation in Plants. IBP 7. Cambridge Univ. Press. Cambridge. pp. 137-150.

de Lajudie, P. (2004). Taxonomie des microsymbiotes des légumineuses: Historique et dernier développement, 2eme conférence Méditéranienne de rhizobiologie, 23-25 Mai. Oran Algérie, 2.

Drouin, P., Sellami, M., Prévost, D., Fortin, J., Antoun, H. (2010). Tolerance to agricultural pesticides of strains belonging to four genera of Rhizobiaceae, Journal of Environmental Science and Health. Part B. 45: 757-765.

Gaind, S., Rathi, M.S., Kaushik, B.D., Nain, L. and Verma, O.M.P. (2007). Survival of bio-inoculants on fungicides-treated seeds of wheat, pea and chickpea and subsequent effect on chickpea yield. Journal of Environmental Science and Health Part B. 42: 663-668.

Graham, P.H., Vance, C.P. (2003). Legumes: Importance and constraints to greater use. Plant Physiology. 131: 872-877.

Guene, N.F.D., Diouf, A., Gueye, M. (2003). Nodulation and nitrogen fixation of field grown common bean (*Phaseolus vulgaris*) as influenced by fungicide seed treatment. African Journal of Biotechnology. 2: 198-201.

Hatimi, A. (2001). Caractérisation de souches de *Rhizobiums* autochtones des dunes: Effet sur la croissance et la nutrition azotée d'*Acacia cyanophylla* Lindl. Acta Botanica Gallica. 148 (3): 191-199.

Isoi, T., Yoshida, S. (1988). Effect of thiram (tetramethyl-thiuram-disulphide) application on nodulation in soybean and kidney bean plants: Observationusing the root-box-culture technique. Soil Science and Plant Nutrition. 34**(**4): 633-637.

Khan, M.S., Zaidi, A., Wani, P.A., Ahemad, M., Oves, M. (2009). Functional Diversity among Plant Growth-promoting Rhizobacteria. In: Microbial Strategies for Crop Improvement. [Khan, M.S., Zaidi, A., Musarrat, J. (Eds.)], Springer, Berlin Heidelberg. pp. 105-132.

Kolef, N. (1974). Culture maraichère. Fam. Légumineuses, Haricot. FAO. Alger. pp. 15-71.

Lal, R. and Lal S. (1988). Pesticides and nitrogen Cycle. CRC Press, Inc. Boca Raton. Florida. Vol. III : 48-119.

Maj, D., Wielbo, J., Marek Kozaczuk, M., Skorupska, A. (2010). Response to flavonoids as a factor influencing competiveness and symbiotic activity of *Rhizobium leguminosarum*. Microbiological Research. 165: 50-60.

Maougal, R.T., Kechid, M., Baziz, K., Djekoun, A. (2020). Do *Phaseolus vulgaris* development stages influence the total rhizospheric bacterial and the phytate utilising community? Agricultural Science Digest. 40: 370-375.

McMichael, B.L. and Quisenberry, J.E. (1993). The impact of the soil environment on the growth of root systems. Environmental and Experimental Botany. 33(1): 53-61.

Munns, D.N. (1977). Mineral Nutrition and the Legume Symbiosis. In: A Treatise on Dinitrogen Fixation, Sect IV. Agronomy and Ecology. [Hardy, R.W.F and Gibson, A.H. (Eds.)], Wiley Interscience. New York. pp. 353-391.

O'hara, G., Boonkerd, N., Dil Worth, M.J. (1988). Mineral constraints to nitrogen fixation. Plant and Soil. 108: 93-110.

Pachico, D.H. (2005). Towards a Conceptual Model of Optimal Investment in the Conservation of Agricultural Gentenic Resources. In: Internationel Conference on Agricultular Biotechnology: Internationel Trade and Domestic Production. Rome. Italy. p19.

Peoples, M.B and Craswell, E.T. (1992). Biological nitrogen fixation: Investments, expectations and actual contributions to agriculture. Plant Soil. 141: 13-39.

Robert, M. (1996). Le sol interface dans l'environnement, ressource pour l'environnement. Dunod. Masson. Paris. 240 p.

Rocher, F. (2004). Lutte chimique contre les champignons pathogènes des plantes: Evaluation de la systémie phloémienne de nouvelles molécules à effet fongicide et d'activateurs de réaction de défense. Thèse de Doctorat de l'Université de Poitiers. 163.

Soltner, D. (2005). Les bases de la production végetale. Tome I (Le sol et son amélioration). Sciences et techniques agricoles. 472 p.

Tibaoui, G., Zouaghi, M. (1989). Rôle de l'inoculation dans l'amélioration des rendements des légumineuses fourragères dans la région de Mateur. Revue de l'I.N.A.T. 4: 2.

Tsegaye, T. and Mullins C E. (1994). Effect of mechanical impedance on root growth and morphology of two varieties of pea (*Pisum sativum* L.). New Physiology. 126: 707-713.

Vincent, J.M. (1970). A Manual for the Practical Study of Root-nodule Bacteria. In: International Biological Programme Handbook n°. 15. Blackwell Scientific Publications Ltd. Oxford. pp. 73-97.

Wani, P.A., Zaidi, A., Khan, A.A., Khan, M.S. (2005). Effect of phorate on phosphate solubilization and indole acetic acid releasing potentials of rhizospheric microorganisms. Annals of Plant Protection Sciences. 13: 139-144.

Yamini, V. and Anilkumar, A.S. (2022). Effect of seed treatment and nutrient schedule on the productivity of blackgram (*Vigna mungo* L.). Agricultural Science Digest. 42: 58-62.

Zaidi, A., Khan, M.S., Ahemad, M., Oves, M. (2009). Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica et Immunologica Hungarica . 56: 263-284.