Effects of application of *Bradyrhizobium japonicum*, phosphorus and potassium fertilization on soybean bacterial leaf pustule caused by *Xanthomonas axonopodis* pv. *glycines*

Gildas Codjo TCHEMADON[1]*, Valerien Amégnikin ZINSOU[1], Pierre G. TOVIHOUDJI[2], André Antoine FANOU[1], Paterne Experat Dossou TCHAKPA[1]

[1]*Laboratoire de Phytotechnie, d'Amélioration et de Protection des Plantes (LaPAPP), Faculté d'Agronomie, Université de Parakou. BP 123 Parakou, Benin.*

[2]*Laboratory of Hydraulics and Environmental Modelling (HydroModE Lab), Faculté d'Agronomie, Université de Parakou. BP 123 Parakou, Benin.*

***Corresponding author:** *gildast092@yahoo.fr ; Tél : (+229) 66622781*

Key words: Bacterial leaf pustule, Soybean, *Bradyrhizobium japonicum*, Phosphorus, Potassium.

Date of Acceptance 6/07/2021, Publication date 30/09/2021, http://m.elewa.org/Journals/about-japs/

1 ABSTRACT

Bacterial leaf pustule (*Xanthomonas axonopodis* pv. *glycines*), is one of the most devastating diseases in soybean producing areas around the world. In order to effectively control this disease, the effects of *Bradyrhizobium japonicum* inoculation and phosphorus and potassium fertilizer application were tested on the disease. Therefore, two separate experiments were conducted from March to September 2020 under greenhouse conditions at the experimental farm of the Faculty of Agronomy of the University of Parakou. Through the first experiment, the isolated or combined effect of *B. japonicum* with phosphorus and potassium was evaluated using a split plot design in three replications with Bradyrhizobium inoculation as the main factor; and mineral fertilizers, the sub-factor. The second experiment was conducted using a Completely Randomized Design (CRD) with three inoculation treatments and nine replications. Disease severity as well as plant height were evaluated according to the experiments. *B. japonicum* and potassium (375mg per plant) taken alone reduced disease severity by 28.77 and 36.39%, respectively. But their combination (coated * potassium) induces a much more significant effect (reduction of more than 62%). The second experiment reveals a very highly significant difference (P = 1.07.10^{-4}) between the two strains of *Bradyrhizobium* tested and the control. These strains reduce the disease severity from 40.58 to 46.05%; with better growth of plants in height. Effective management of soybean bacterial leaf pustule could therefore be achieved by coating the seeds with *B. japonicum* or applying, 375 mg of potassium per plant, or better yet, by combining *B. japonicum* with potassium (375 mg per plant).

2 INTRODUCTION

Legumes, largely defined by theirs unusual floral structure, fruit, and ability to form nodules with Rhizobia, rank second among the most widely cultivated plants in the world, after grasses (Graham and Vance, 2003). Within this family (Fabaceae, anc. Leguminosae), soybean constitutes one of the most important species. Indeed, soybean [*Glycine max* (L.) Merril] as an important source of vegetables and oils for human and animal consumption (Couto et *al*., 2011), is highly valued on the world market (Abbasi et *al*., 2010). It contains around 40%

protein nutrients with all essential amino acids (Raghuvanshi and Bisht, 2010) but also trace elements such as magnesium, phospholipids, vitamins and minerals (Ali, 2010). In addition to its nutritional values, soybean as a nitrogen fixing crop is also involved in restoring and maintaining sustainable soil fertility and promotes improved crop yields (Smaling et *al*., 2008). In Benin, soybean represents a choice crop with an annual production estimated at 230,000 tons from 210,000 ha, for an average yield of around 1.1 $t.ha^{-1}$ in 2019 (FAOSTAT, 2021). But this low yield obtained fora potential yield of 3 $t.ha^{-1}$ (INRAB, 1995), is the consequence of the several abiotic and biotic constraints affecting negatively its growth, productivity and quality (Gutierrez-Gonzalez et *al*., 2010; Manavalan et *al*., 2009; Tran and Nguyen, 2009). Among them, the soybean bacterial leaf pustule caused by *Xanthomonas axonopodis* pv *glycines* (*Xag*) is widespread in most soybean producing countries (Wrather et *al*., 2001), and induces yield losses in the order of 15.9 to 50% (Dirmawati, 2004) in Indonesia and 2.7 to 28.1% in Benin (Zinsou et *al*., 2016). Among the various control strategies proposed, host nutrition often influences disease tolerance or resistance in plants (Singh, 2015). Thus, the application of mineral and bio fertilizers in the soil provides nutrients to the plant, which creates a positive reaction, a way to induce plant resistance to disease and as a result, the plants can recover from disease or can overcome the disease epidemic (Hossain et *al*., 1995; Kundu et *al*., 1996). As proof, potassium (K) constitutes the cationic nutrient, the most abundant and crucial univalent essential for crop yield and tolerance to biotic stresses (Nieves-Cordones et *al*., 2019). Thus, sufficient application of potassium reduces the incidence of bacterial and fungal diseases by about 70% (Amtmann et *al*., 2008; Veresoglou et *al*., 2013) and is very effective in the control of *Xanthomonas oryzae* pv. *oryzae* (Begum et *al*., 2011) and several species of *Helminthosporium* (Imrani et *al*., 2014; Zinsou et *al*., 2020). The effect of P in plant resistance to diseases, however, appears to be inconsistent (Singh, 2015). But, according to Wasnikar et *al*. (1993), the incidence of bacterial leaf spot caused by *Xanthomonas campestris* pv. *betlicola* decreased significantly as phosphorus (P) level increased. Phosphorus (P) has property of increasing the resistance of plants and thus reducing their infection by various fungal diseases (Brennan, 1995; Csöndes et *al*., 2008; Spagnoletti et *al*., 2018). But besides mineral fertilization, bio fertilization also has an effect in the plant diseases management. Indeed, the biological control of plant diseases through the application of various *Rhizobium* species has also been reported through several studies. Thus, *Rhizobium leguminosarum* bv. *phaseoli* and *Rhizobium* sp. were shown to be very effective, respectively against *Xanthomonas axonopodis* pv. *phaseoli* (Osdaghi et *al*., 2011) and *Pseudomonas savastanoi* (Mourad et *al*., 2009). Moreover, *Bradyrhizobium* sp. has been shown to be effective in controlling *Macrophomina phaseolina, Rhizoctonia solani, Fusarium solani* and *F. oxysporum* (Parveen et *al*., 2019). However, the effect of nutrients on soybean bacterial leaf pustule is not properly studied. Therefore, the application of phosphorus, potassium but also coating the seeds of soybean with *Bradyrhizobium japonicum*, could they have a significant effect on the soybean bacterial leaf pustule?

3 MATERIALS AND METHODS

3.1 Study area: Two pot experiments were carried out under greenhouse conditions at the experimental farm of the Faculty of Agronomy of the University of Parakou (N 9°20'18.1"; E 2°38'50.2") from March to September 2020. The relative humidity varied from 37 to 90% and the temperature was between 21.9 and 39.3°C during P, K, *B. japonicum* fertilizer experiment. In contrast, the relative humidity ranged from 37 to 95% and the temperature from 20.5 and 39.3°C during *B. japonicum* fertilizer experiment.

3.2 Mineral and bio fertilization experiment: In greenhouse, the first experiment was carried out using a split plot design (Howard et *al*., 1998; Jones and Nachtsheim, 2009) with 3 replications. The main factor was Bradyhizobium with 2 levels namely: coated and uncoated. The sub-factor was mineral fertilizers with 4 levels: control without fertilizer (T_0); Phosphorus at the rate of 375 mg per plant (T_1); Potassium at the rate of 375 mg per plant (T_2); Phosphorus + Potassium at the rate of 375 mg per fertilizer and per plant (T_3). Three (03) blocks each subdivided into 2 sub-blocks and 08 treatments were designed. Fertilizer rates (375mg per plant of phosphorus and potassium) used in the trial correspond the recommended rates of 50 $kg.ha^{-1}$ of P and K by GIZ and COMPACI (2013) for soybean production in Benin. Phosphorus and potassium were applied in the form of Triple Superphosphate (TSP: 22% P_2O_5) and Potassium Chloride (KCl: 60% K_2O), respectively. Each elementary plot was represented by a $1.5dm^3$ pot containing two plants. Two successive blocks were separated by 2m while the sub-blocks as well as the elementary plots were separated by alleys of 1m. Coated or uncoated seeds of a soybean variety (TGX 1984-77F) susceptible to bacterial leaf pustule (Zinsou et *al*., 2016), were sown in pots containing substrate (soil) sterilized at 65°C for 72 hours. Three weeks after sowing, plants were inoculated using a hand-held sprayer with a bacterial suspension of *X. axonopodis* pv. *glycines* (Zinsou et *al*., 2015) at a concentration of $1.0.10^8$ $CFU.ml^{-1}$ (bacteria/ml). Fertilizers were applied one week before the plants inoculation. Manual weeding was carried out if needed.

3.3 Rhizobium strain experiment: This experiment was carried out using a Completely Randomized Design (CRD) (Kirk, 2013; Sadik and Alwan, 2020) with the strain of *B. japonicum* as factor. The design consisted of three (03) treatments: control without Bradyhizobium (T_0); IITA Bradyhizobium (T_1); NITRO-FIX® Bradyhizobium (T_2), with nine (09) replications. Two successive elementary plots were separated by alleys of 1m. On each elementary plot, represented by a 1.5 dm^3 pot containing a substrate (soil) sterilized at 65°C for 72 hours, two seeds of a soybean variety susceptible to bacterial leaf pustule (TGX 1984-77F) (Zinsou et *al*., 2016), were manually sown. These seeds were uncoated (T_0); coated with Bradyhizobium marketed from IITA-Ibadan (T_1); or coated with Bradyhizobium marketed under the name NITRO-FIX® by Chemtura Corporation at a concentration of 2.10^8 cells/gr. (T_2), taking into account the manufacturer recommendations. A bacterial suspension of *X. axonopodis* pv. *glycines* (*Xag*) (Zinsou et *al*., 2015) at a concentration of $1.0.10^8$ CFU ml^{-1} (bacteria/ml) were inoculated to all the plants, three (03) weeks after sowing. According to the needs, manual weeding was carried out.

3.4 Soil sampling and analysis: Before sowing the seeds, a sample of the substrate (soil) used for the two tests was taken in order to get an idea of the soil status before the different treatments application. All visible organic residues were removed by hand before sample transporting to the laboratory. Sample was analyzed by the "Laboratoire des Sciences du Sol, Eaux et Environnement (LSSEE/ CRA-Agonkanmey / INRAB)" according to the methods of Tran and Boko (1978).

3.5 Data collection: Disease severity was assessed five (05) times during the experiments: the first, one week after inoculation with *Xag*, and the others, fifteen (15) days apart. The assessment method developed by Prathuangwong et *al.* (1993) was applied to two plants per elementary plot. Indeed, a stencil card of 4 cm x 7 cm with 9 circles of 1 cm in diameter was placed on the infected leaflet surface and the number of lesions obtained was then reported to the leaf surface. The leaves from the lower, middle and upper parts were assessed per plant, and the average severity value was assigned to the plant. The Area Under Severity Progress Curve (AUSPC) was then calculated using the disease severity values obtained at each date, according to the formula:

$$\mathbf{AUSPC = \sum_i [(S_i + S_{i-1}) * (t_i - t_{i-1})] / 2}$$

with: S_i = mean of the severity at date t_i; t_i = the different assessment dates (Jeger and Viljanen-Rollinson, 2001, Shaner and Finney, 1977).

Soybean plant height was assessed every 15 days from the age of three weeks, by measuring height of each plant from the base until its apex; using a ruler graduated in centimeters. Five assessments were carried out and the heights were expressed in cm.

3.6 Data statistical analyses: Analysis of variance (ANOVA) was performed using R software version 3.6.1 (R Core Team, 2019) on severity values (AUSPC) to compare the means at the 5% level. Tukey's test was then completed in order to separate the means in the event of a significant difference ($P \leq 0.05$). The values in the tables are the actual means with their standard errors.

4 RESULTS

4.1 Soil characteristics: The soil used for the experiments is a loamy-sand with approx. 6% clay, acidic with low organic carbon and total nitrogen, medium phosphorus and potassium content (Table 1).

Table 1: Results of soil sample analysis

Characteristics	Soil contents
Clay (%)	6.10
Silt (%)	9.36
Sand (%)	84.54
pH-H_2O	6.87
Organic carbon (%)	1.12
Total nitrogen (%)	0.085
C/N	13
Organic material	1.93
Exchangeable Ca^{2+} (meq/100g)	6.937
Exchangeable Mg^{2+} (meq/100g)	0.678
Exchangeable K^{+} (meq/100g)	0.187
Assimilable P (ppm)	59
Sum of cations (meq/100g)	7.911
CEC (meq/100g)	8.500

4.2 Effect of *B. japonicum,* P and K fertilization on disease severity: Results show that there is a highly significant ($P = 0.004$); ($P = 4.5.10^{-3}$) and significant ($P = 0.021$) difference between the AUSPC values when considering the Bradyrhizobium; Bradyrhizobium * Mineral fertilizers and mineral fertilizers factors, respectively (Table 2). Thus, plants from coated seeds by *B. japonicum*, perform differently in the face of the disease compared to those from uncoated seeds. Plants from coated seeds have lower AUSPC (on average 196.35 ± 23.20) than those from uncoated seeds (on average 275.67 ± 16.79). *B. japonicum* therefore significantly reduced the severity of soybean bacterial leaf pustule by 28.77% compared to the control (uncoated). Likewise, the effect of fertilizers on disease varies between treatments. The lowest AUSPC value was observed in the T_2 (potassium) treatment (169.64 ± 31.85) while the T_3 (phosphorus + potassium) treatment gave the highest value (280.06 ± 20.67). It therefore appears that the application of potassium at 375 mg per plant reduces more effectively the severity of soybean bacterial leaf pustule by 36.39% compared to the control (without fertilizer). Like the application of potassium, phosphorus application (375 mg per plant), reduces the severity of soybean bacterial leaf pustule by 14.62% compared to the control (without fertilizer). Similar to the factors taken in isolation, the Bradyrhizobium * Mineral fertilizers combination greatly influences the disease severity. Indeed, the control (uncoated * without fertilizers) presented the highest value of AUSPC (343.75 ± 24.37) while the lowest value was noted at the level of the combination (coated * potassium) with an AUSPC of (129.46 ± 60.46). In addition, a reduction in the disease severity is observed ranging from 17.58 (coated * potassium + potassium) to 62.34% (coated * potassium) compared to the control. It is therefore found that all the combinations reduce the disease severity with the combination (coated * potassium), as the one which has the most significant effect on the soybean bacterial leaf pustule. From all this, it appears that although coating the seeds with *B. japonicum* or applying potassium (375 mg per plant) each reduces the disease severity, the result is even better when these two different treatments are combined (reduction of more than 62% compared to the control).

Table 2: Severity (AUSPC) of soybean bacterial leaf pustule according to mineral and bio fertilization

Treatments		AUSPC	AUSPC Reduction rate (%)
Bradyrhizobium (B)	**Uncoated (U)**	275.67 ± 16.79b	-
	Coated (C)	196.35 ± 23.20a	28.77
	F	9.28	
	P	0.004	
Mineral fertilizers (F)	**T_0 (without F)**	266.67 ± 34.99ab	-
	T_1 (Phosphorus)	227.68 ± 25.86ab	14.62
	T_2 (Potassium)	169.64 ± 31.85a	36.39
	T_3 (Phosphorus + Potassium)	280.06 ± 20.67b	-
	F	3.62	
	P	0,021	
Interaction B*F	**U-T_0 (Control)**	343.75 ± 24.37b	-
	U-T_1	272.32 ± 37.51ab	20.78
	U-T_2	209.82 ± 12.78ab	38.96
	U-T_3	276.79 ± 35.04ab	19.48
	C-T_0	189.58 ± 49.14ab	44.85
	C-T_1	183.04 ± 27.16ab	46.75
	C-T_2	129.46 ± 60.46a	62.34
	C-T_3	283.33 ± 25.45ab	17.58
	F	1.61	
	P	$4.5.10^{-3}$	

Values followed by the same lowercase letter in a column are not significantly different ($P \leq 0.05$); AUSPC = Area Under Severity Progress Curve.

4.3 Effect of *B. japonicum* on disease severity according to the tested strains: Table 3 shows that there is a very highly significant difference ($P = 1.07.10^{-4}$) between the two strains of Bradyrhizobium tested and the control. Indeed, the two strains from IITA Bradyrhizobium and NITRO-FIX® Bradyrhizobium exhibit the lowest severity values (497.66 ± 30.99 and 548.10 ± 66.60, respectively). However, it should be noted that although there is not a significant difference between these two strains, IITA Bradyrhizobium strain reduced the disease severity more (46.05%) than NITRO-FIX® Bradyrhizobium strain (40.58%).

Table 3: Severity (AUSPC) of bacterial leaf pustule according to the *B. japonicum* strains tested

Treatments	AUSPC	AUSPC reduction rate (%)
Control	922.43 ± 94.45b	-
IITA Bradyrhizobium	497.66 ± 30.99a	46.05
NITRO-FIX® Bradyrhizobium	548.10 ± 66.60a	40.58
F	11.28	-
P	$1.07.10^{-4}$	-

Values followed by the same lowercase letter in a column are not significantly different (P ≤ 0.05); AUSPC = Area Under Severity Progress Curve.

4.4 Effect of *B. japonicum* strains on soybean plant height: Figure 1 shows that the soybean plants height progress according to the various treatments. From the analysis of this figure, it appears that irrespective of the date of measurement, the plants resulting from coated seeds with IITA Bradyrhizobium and NITRO-FIX® Bradyrhizobium strains, show greater height values than the control. In fact, the plant height varied from 44.45 to 84.64cm for IITA Bradyrhizobium, 35.22 to 80.39 cm for NITRO-FIX® Bradyrhizobium and 32.53 to 63.93cm for the control. Coating the seeds with *B. japonicum* therefore favoured the plant development compared to the control. In addition, it is noted that the plants resulting from coated seeds with IITA Bradyrhizobium strain develop a little more in height than those resulting from coated seeds with NITRO-FIX® Bradyrhizobium.

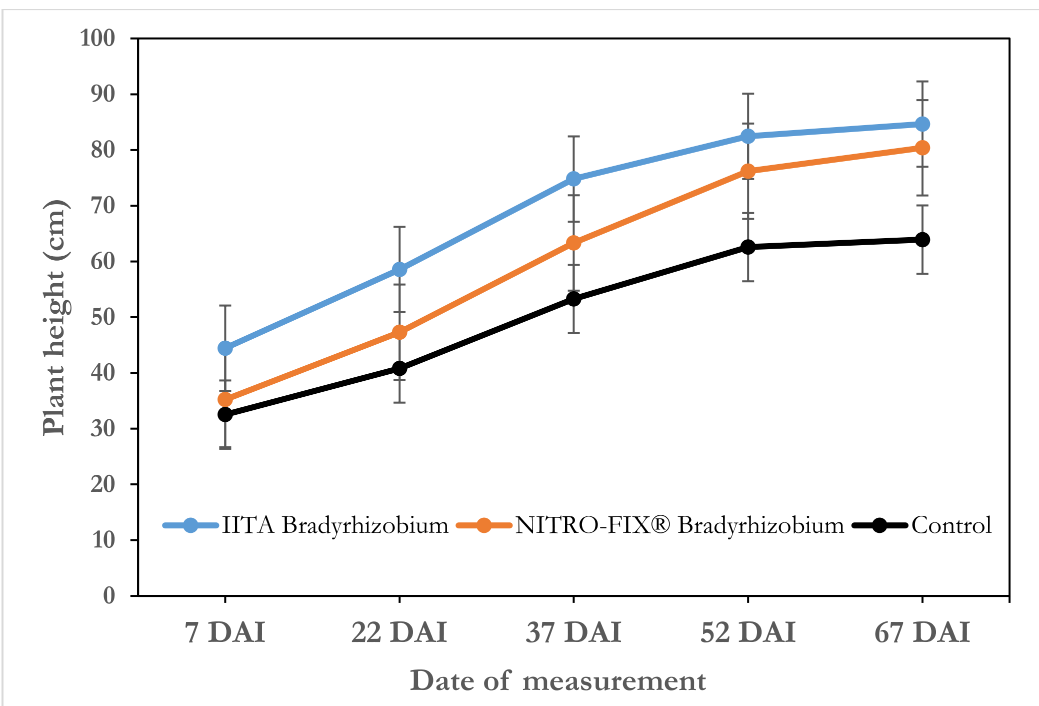

Fig. 1: Progress of the soybean plants height according to the different treatments following the assessment dates **(**DAI: Days after inoculation)

5 DISCUSSION

The severity (AUSPC) of soybean bacterial leaf pustule varies depending on the different mineral and bio fertilizers tested. This could be explained by the fact that there is a relationship between plant nutrition and plant pathogens. Indeed, according to Navarrete et *al.* (2010), the susceptibility of crops to diseases would be influenced by the management of plant nutrition. In addition, Anderson (2002) proved that there are interactions between plants, nutrients and pathogenic species. Thus, according to Hossain et *al.* (1995) and Kundu et *al.* (1996), mineral and organic fertilizers application in the soil provides nutrients to the plant, which creates a positive reaction, a way to induce resistance in plants to diseases and as a result, the plants can recover disease or overcome the disease epidemic. In addition, the results show that the application of potassium (375 mg per plant) effectively reduced (36.39%) the soybean bacterial leaf pustule severity compared to the control (without fertilizers). These results corroborate those of various authors who have also noted the effectiveness of potassium (K) in the management of several diseases. Indeed, potassium has been shown to be very effective in controlling *Xanthomonas oryzae* pv. *oryzae* (Begum et *al*., 2011) and several species of *Helminthosporium* (Imrani et *al*., 2014; Zinsou et *al*., 2020). This efficiency could be explained by the fact that according to Nieves-Cordones et *al.* (2019), potassium (K) is the cationic nutrient, the most abundant and crucial univalent essential for crop tolerance to biotic stresses. In addition, FAO et *al.* (2003) showed that plants well supplied with potassium are less susceptible to disease. Also, according to Amtmann et *al.* (2008) and Veresoglou et *al.* (2013), bacterial and fungal diseases incidence is reduced by approximately 70% by sufficient application of potassium. Although this is low (14.62%), the application of phosphorus (375 mg per plant) nevertheless reduces the soybean bacterial leaf pustule severity, compared to control. These results confirm those of various authors who have demonstrated that the application of phosphorus (in the form of phosphate salts or other) induces resistance in various plants and against several pathogenic agents (Csöndes et *al*., 2008; Spagnoletti et *al*., 2018). Indeed, phosphorus application increased resistance in certain crops as tobacco to *Candidatus liberibacter asiaticus* (Zhao et *al*., 2013). Also, the incidence of bacterial leaf spot caused by *Xanthomonas campestris* pv. *betlicola* decreased significantly as phosphorus (P) level increased (Wasnikar et *al*., 1993). Moreover, according to Gottstein and Ku (1989), the application of phosphorus induces a systemic resistance in cucumber plants and beans to *Colletotrichum lagenarium* (pathogen responsible for anthracnose) and to *Uromyces viciaefabae* (pathogen of rust) (Walters and Murray, 1992), respectively. This situation could be explained by the fact that according to Brennan (1995); Csöndes et *al.* (2008) then Spagnoletti et *al.* (2018), phosphorus (P) would have the property of increasing the resistance of plants and thus reducing their infection by various fungal diseases. Indeed, this property would be based on the fact that phosphorus can affect the expression of resistance genes and the defensive traits of plants through the jasmonate signalling pathway, and thus regulate fungal diseases (Turner et *al*., 2002). Coating the seeds with *B. japonicum* significantly reduced (28.77%) the soybean bacterial leaf pustule severity. These same results were found by Osdaghi et *al.* (2011) and Mourad et *al.* (2009), who revealed the efficacy of *Rhizobium leguminosarum* bv. *phaseoli* and *Rhizobium* sp., respectively against the common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* and the olive knot disease due to *Pseudomonas savastanoi*. Indeed, according to Bhattacharyya and Jha (2012), PGPR (Plant Growth Promoting Rhizobacteria) have a beneficial effect on plants through the control of plant diseases by indirectly suppressing pathogens or by inducing systemic resistance in plants. Additionally, the antibiotics produced by rhizobia have been found to play an important role in disease control. Indeed, *B. japonicum* protects the soybean crop against infection by *Macrophomina phaseolina* (Chakraborty and Purkayastha, 1984; Parveen et *al*., 2019) through the direct action of antibiotics such as rhizobitoxin (Chakraborty and Purkayastha, 1984; Deshwal et *al*., 2003). Moreover, along with nitrogen fixation efficiency of rhizobia, it also have a good potential of use as biological control agents against soil borne plant pathogens (Noreen et *al*., 2016). Some of the Rhizobium strains are known to reduce disease severity caused by *Pythium ultimum* (Ozkoc and Deliveli, 2001), *Phytophthora clandestine* (Simpfendorfer et *al*., 1999), *Fusarium solani* (Al-Ani et *al*., 2012), *Fusariuum oxysporum*, *Rhizoctonia bataticola* and *Pythium* sp. (Nautiyal, 1997). Ehteshamul-Haque and Ghaffar (1993) found that *Bradyrhizobium japonicum*, *Sinorhizobium meliloti* and *Rhizobium leguminosarum* used either as seed dressing or as soil drench reduced infection of *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp., in both leguminous and non-leguminous plants. Combination (coated * potassium), as the one which has the most significant effect on the soybean bacterial leaf pustule (reduction of more than 62% compared to the control). This high efficiency could be explained by the synergy of action between *Bradyrhizobium japonicum* and potassium, which individually already reduces the disease severity. Indeed, according to various authors, inoculation of PGPR (such as *B. japonicum*) promotes plant uptake of several mineral elements including K, P, Ca, Fe, Cu, Mn and Z (Mantelin and Touraine, 2004; Ahemad and Kibret, 2014; Zaidi et *al*., 2009). Coating the seeds with *B. japonicum* favoured plant development compared to the control. In addition, it is noted that the plants resulting from seeds coated with IITA Bradyrhizobium strain developed a little more in height than those resulting from seeds coated with NITRO-FIX® Bradyrhizobium. These results corroborate those of various authors who have reported that coating seeds with Rhizobium promotes plant growth by significantly increasing the height of soybean (Alam et *al*., 2015; Parveen et *al*., 2019), lentil (Hoque and Haq, 1994) and chickpea (Shinde and Bhilare, 2003; Togay et *al*., 2008) plants. In addition, according to Rehman et *al*. (2010), *B. japonicum* would promote nitrogen fixation and would be therefore considered as an element for improving the growth and yield of soybean plants. In the same vein, various other works have reported that strains of *Rhizobium* by colonizing the roots of tomato and chilli plants, not only promote their growth, but also improve the yield and quality of the fruits (Garcia-Fraile et *al*., 2012; Parveen et *al*., 2008). This situation could be due to the adequate amount of nitrogen fixed by the rhizobium. In addition, a greater number of leaves per plant could also positively contribute to a higher plant height in plants grown from seeds coated with *Rhyzobium* (Sajid et *al*., 2011).

6 CONCLUSION

The application of *Bradyrhizobium japonicum* or potassium (375 mg per plant) reduced the soybean bacterial leaf pustule severity compared to the control, by 28.77% and 36.39%, respectively. However, the combined application of organic (*B. japonicum*) and mineral (potassium) fertilizers contributes to better management of the soybean bacterial leaf pustule (reduction of 62.34%). In addition, coating of soybean seeds with the strains of *B. japonicum* while reducing the disease severity from 40.58 to 46.05%, also promotes better plant growth in height. Soybean bacterial leaf pustule could be managed more effectively by combining seed coating with *Bradyrhizobium japonicum* and fertilizing soybean plants with potassium, at the rate of 375 mg per plant.

7 REFERENCES

Abbasi M.K., Manzoor M., Tahir M.M. 2010. Efficiency of Rhizobium inoculation and P fertilization in enhancing nodulation, seed yield, and phosphorus use efficiency by field grown soybean under hilly region of Rawalakot Azad Jammu and Kashmir, Pakistan. Journal of Plant Nutrition. 33(7):1080-1102. Doi: 10.1080/01904161003729782.

Ahemad M., Kibret M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King Saud University Science. 26(1):1–20

Alam F., Bhuiyan M.A.H., Alam S.S, Waghmode T.R., Kim P.J., Lee Y.B. 2015. Effect of *Rhizobium* sp. BARIRGm901 inoculation on nodulation, nitrogen fixation and yield of soybean (*Glycine max*) genotypes in gray terrace soil. Bioscience, Biotechnology and Biochemistry. 79(10):1660-1668. DOI: 10.1080/09168451.2015.1044931.

Al-Ani R., Adhab M.A., Mahdi M.H., Abood H.M. 2012. *Rhizobium japonicum* as a biocontrol agent of soybean root rot disease caused by *Fusarium solani* and *Macrophomina phaseolina*. Plant Protection Science. 48(4):149-55.

Ali N. 2010. Soybean Processing and Utilization. In Singh G (ed,) The Soybean: Botany, Production and Uses, CAB International, USA. ISBN: 9781845936457, pp: 345-374.

Amtmann A., Troufflard S., Armengaud P. 2008. The effect of potassium nutrition on pest and disease resistance in plants. Physiologia Plantarum. Review. pp 1-10. ISSN 0031-9317. Doi: 10.1111/j.1399-3054.2008.01075.x.

Anderson S. 2002. The Relationship between nutrients and other elements to plant diseases. Tree Care Industry. pp. 26-32.

Begum N., Rahmanb M.M., Basharc M.A., Hossaind M.A., Uddin M.N. 2011. Effect of Potassium Fertilizer on Development of Bacterial Blight of Rice. Bangladesh Journal of Scientific and Indus experiment Research. 46(1):69-76.

Bhattacharyya P., Jha D. 2012. Plant growth promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology & Biotechnology. 28(4): 1327–1350. https://doi.org/10.1007/s1127 4-011-0979-9.

Brennan R.F. 1995. Effect of levels of take-all and phosphorus fertilizer on the dry matter and grain yield of wheat. Journal of Plant Nutrition. 18(6):1159-1176.

Chakraborty U., Purkayastha R.P. 1984. Role of rhizobitoxine in protecting soybean roots from *Macrophomina phaseolina*, Canadian Journal of Microbiology. 30(3):285-289.

Couto C., Silva L.R., Valentão P., Velázquez E., Peix A., Andrade P.B. 2011. Effects induced by the nodulation with *Bradyrhizobium japonicum* on *Glycine max* (soybean) metabolism and antioxidant potential. Food Chemistry 127(4):1487–1495. doi:10.1016/j.foodchem.2011.01.135.

Csöndes I., Balikó K., Degenhardt A. 2008. Effect of different nutrient levels on the resistance of soybean to *Macrophomina phaseolina* infection in field experiments. Acta Agronomica Hungarica. 56(3):357-362.

Deshwal V.K., Dubey R.C., Maheshwari D.K. 2003. Isolation of plant growth-promoting strains of *Bradyrhizobium* (Arachis) sp. with biocontrol potential against *Macrophomina phaseolina* causing charcoal rot of peanut. Current Science. 84:443-448.

Dirmawati S.R. 2004. Study on the effectiveness of several components of environmentally friendly control methods against soybean bacterial pustule disease [PhD Desertation] Postgraduate School, Bogor Institute of Agriculture, Indonesia.

Ehteshamul-Haque S., Ghaffar A. 1993. Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mungbean. Journal of Phytopathology. 138(2):157-63.

FAO, IFA, IMPHOS. 2003. Les engrais et leurs applications: Précis à l'usage des agents de vulgarisation agricole Quatrième édition, version révisée. IFA Paris. 7632p. ISBN: 92-5104414-7.

FAOSTAT. 2021. http://fao.org/faostat/fr/data/QC. Accessed at 2021.02.19.

Garcia-Fraile P.L., Robledo C.M., Ramírez-Bahena M.H., Flores-Félix J.D., Fernández M.T., Mateos P.F., Rivas R., Igual J.M., Martínez-Molina E., Peix A., Velázquez E. 2012. Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One, 7: e38122. Doi: 10.1371/ journal. Pone. 0038122.

GIZ, COMPACI. 2013. Les bonnes pratiques de production du soja. Pp 1-4. http://www.fao.org/3/CA2805FR/ca2805fr.pdf. Accessed at 2021.04.30.

Gottstein H.D., Ku J. 1989. Induction of systemic resistance to anthracnose in cucumber by phosphates. Phytopathology 79(2):176-179.

Graham P., Vance C. 2003. Legumes: importance and constraints to greater use. Plant Physiology. 131(3):872–877.

Gutierrez-Gonzalez J.J., Guttikonda S.K., Tran L.S., Aldrich D.L., Zhong R., Yu O., Nguyen H.T., Sleper D.A., 2010. Differential expression of isoflavone biosynthetic genes in soybean during water deficits. Plant and Cell Physiology. 51(6):936-948.

Hoque M.M., Haq M.F. 1994. Rhizobial inoculation and fertilization of lentil in Bangladesh. Lens Newsletter. 21:29-30.

INRAB. 1995. Cultures vivrières: céréales, légumineuses à graine et tubercules. Fiche Technique.

Jeger M.J., Viljanen-Rollinson S.L.H. 2001. The use of the area under the disease progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theoretical and Applied Genetics. 102(1):32-40.

Jones B., Nachtsheim C.J. 2009. Split-plot designs: What, why, and how. Journal of quality technology. 41(4):340-361.

Hossain A., Yahia I.G., Jahiruddin M. 1995. Effect of copper, boron and molybdenum on leaf spot disease and grain set of wheat. Bangladesh Journal of Scientific and Indus experiment Research. 46(1):69-76.

Howard D.D., Chambers A.Y., Lessman G.M. 1998. Rotation and fertilization effects on corn and soybean yields and soybean cyst nematode populations in a no‐tillage system. Agronomy journal. 90(4):518-522.

Imrani N, Ouazzani Chahdi A.L, Chliyeh M., Touati J., Ouazzini Touhami A., Benkirane R., Douira A. 2014. Effet de la fertilisation par différents niveaux de NPK sur le développement des maladies foliaires du riz. Journal of Animal and Plant Sciences. 23(1):3601-3625. ISSN: 2071-7024.

Kate S., Azontonde A.H., Dagbenonbakin G.D., Sinsin B. 2016. Effets des changements climatiques et des modes de gestion sur la fertilité des sols dans la commune de Banikoara au nord-ouest du Bénin. International Journal of Biological and Chemical Sciences. 10(1):120-133.

Kirk R.E. 2013. Completely randomized design. In Experimental design: Procedures for the behavioral sciences pp. 125-153. SAGE Publications, Inc., https://www.doi.org/10.4135/9781483384733.

Kundu S., Hossain I., Jahiruddin M. 1996. Effect of boron on leaf blotch (*Bipolaris sorokiniana*) and grain yield of wheat cv. Kanchan. Progress. Agric. 7(2):171-175.

Manavalan L.P., Guttikonda S.K., Tran L-S.P., Nguyen H.T. 2009. Physiological and Molecular Approaches to Improve Drought Resistance in Soybean. Plant and Cell Physiology. 50(7):1260-1276. doi:10.1093/pcp/pcp082.

Mantelin S. and Touraine B. 2004. Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. Journal of experimental Botany. 55(384):27–34.

Mourad K., Fadhila K., Chahinez M., Meriem R., Philippe D.L., Abdelkader B. 2009. Antimicrobial activities of *Rhizobium* sp. strains against *Pseudomonas savastanoi*, the agent responsible for the olive knot disease in Algeria. Grasas y Aceites. 60:139–146

Nautiyal C.S. 1997. Rhizosphere competence of *Pseudomonas* sp., NBR 19926 and *Rhizobium* sp. NBRI9513 involved in the suppression of chickpea (*Cicer arietinum* L.) pathogenic fungi. FEMS Microbiology Ecology. 23(2):145-58.

Navarrete M., Lecompte F., Collange B., Tchamitchian M. 2010. Systèmes de culture et qualité de la laitue: comment repenser les systèmes de culture pour réduire l'usage de pesticides et les risques de résidus? Innovations Agronomiques 9:67-84.

Nieves-Cordones M., Ródenas R., Lara A., Martínez V., Rubio F. 2019. The combination of K+ deficiency with other environmental stresses: what is the outcome? Physiologia plantarum 165(2):264-276.

Noreen R., Shafique A., Ali S.A., Habiba, Sultana V., Ara J., Ehteshamul-Haque S. 2016. Role of mungbean root nodule associated fluorescent *Pseudomonas* and rhizobia in suppressing the root rotting fungi and root knot nematode affecting chickpea (*Cicer arietinum* L.). Pakistan Journal of Botany. 48(5):2139-45.

Osdaghi E., Shams-bakhsh M., Alizadeh A., Lak M.R., Maleki H.H. 2011. Induction of resistance in common bean by *Rhizobium leguminosarum* bv. *phaseoli* and decrease of common bacterial blight. Phytopathologia Mediterranea. 50(1):45-54.

Ozkoc I., Deliveli M. H. 2001. In vitro inhibition of mycelial growth of some root rot fungi by *Rhizobium leguminosarum* biovar *phaseoli*. Turkish Journal of Biology. 25(4):435-445.

Parveen G., Ehteshamul-Haque S., Sultana V., Ara J., Athar M. 2008. Suppression of root pathogens of tomato by rhizobia, *Pseudomonas aeruginosa* and mineral fertilizers. International Journal of Vegetable Science. 14(3):205-215.

Parveen G., Noreen R., Shafique H.A., Sultana V., Ehteshamul-Haque S., Athar M. 2019. Role of rhizobia in suppressing the root diseases of soybean under soil amendment. Planta daninha. 37:e019172336. Doi: 10.1590/S0100-83582019370100038. ISSN 0100-8358 (print). 1806-9681 (online).

Prathuangwong S., Preecha C., Thaveechai N. 1993. Development standard method and format for measuring severity of soybean bacterial pustule, Abstract 6.4.1. In Proceedings. 6th International Conference of Plant Pathology. 1993. Montreal, Canada.

Preecha C., Sadowsky M.J., Prathuangwong S. 2010. Lipopeptide surfactin sroduced by *Bacillus amyloliquefaciens* KPS46 is required for biocontrol efficacy against *Xanthomonas axonopodis* pv. *glycines*. Agriculture and Natual Resources. 44(1):84-99.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Raghuvanshi R.S., Bisht K. 2010. Uses of Soybean: Products and Preparation. In: Singh G (Ed). The Soybean: Botany, Production and Uses, CAB International, USA, pp: 345-374.

Rehman A., Hamid N., Jawaid F. 2010. Growth and nitrogen dynamics of *Glycine max* inoculated with *Bradyrhizobium japonicum* and exposed to elevated atmospheric carbon dioxide. Pakistan Journal of Botany. 42(3):1977-1982.

Sadik N.J., Alwan I.M. 2020. Applied multivariate analysis of variance in experiment of randomized design. Periodicals of Engineering and Natural Sciences (PEN). 8(1):365-373.

Sajid M., Rab A., Wahid F.-I., Shah S.N.M., Jan I., Khan M.A., Hussain S.A., Khan M.A., Iqbal Z. 2011. Influence of rhizobium inoculation on growth and yield of groundnut cultivars. Sarhad Journal of Agriculture. 27(4):573-576.

Shaner G., Finney R.E., 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knows wheat. Phytopathology. 67(8):1051-1056.

Shinde S.H., Bhilare R.L. 2003. Response of chickpea to soil and foliar application of DAP. Madras Agriculture Journal. 90(4-6):352-354.

Simpfendorfer S., Harden T.J., Murray G.M. 1999. The in vitro inhibition of *Phytophthora clandestine* by some rhizobia and the possible role of *Rhizoium trifolii* in biological control of *Phytophthora* root rot of subterranean clover. Australian Journal of Agricultural Research, 50(8):1469-1474.

Singh D.P. 2015. Plant nutrition in the management of plant diseases with particular reference to wheat. In Awasthi L. P. (eds) Recent Advances in the Diagnosis and Management of Plant Diseases. Springer, New Delhi. Pp 273-284. https://doi.org/10.1007/978-81-322-2571-3_20.

Smaling E.M.A., Roscoe R., Lesschen J.P., Bouwman A.F., Comunello E. 2008. From forest to waste: Assessment of the Brazilian soybean chain, using nitrogen as a marker. Agriculture, Ecosystems & Environment, 128(3):185-197. doi:10.1016/j.agee.2008.06.005.

Spagnoletti F.N., Leia M., Chiocchio V., Lavado R.S. 2018. Phosphorus fertilization reduces the severity of charcoal rot (*Macrophomina phaseolina*) and the arbuscular mycorrhizal protection in soybean. Journal of Plant Nutrition and Soil Science. 181(6):855–860.

Togay N., Togay Y., Mesut-Cimrin K., Turan M. 2008. Effects of Rhizobium inoculation, sulfur and phosphorus applications on yield, yield components and nutrient uptakes in chickpea (*Cicer arietinum* L.). African Journal of Biotechnology. 7(6):776-782.

Tran V.A., Boko A. 1978. Procédures d'analyses physiques et chimiques des sols, eaux et végétaux, CENAP/INRAB: Agonkamey, 251p.

Tran L.S., Nguyen H.T. 2009. Future Biotechnology of Legumes. In: Emerich WD, Krishnan H (eds) *Nitrogen fixation in crop production*. ASA-CSA-SSSA, Madison, pp 265-308.

Turner J.G., Ellis C., Devoto A. 2002. The jasmonate signal pathway. The Plant Cell. 14(1):153-164.

Veresoglou S.D., Barto E.K., Menexes G., Rillig M.C. 2013. Fertilization affects severity of disease caused by fungal plant pathogens. Plant Pathology. 62(5):961-969.

Walters D.R., Murray D.C. 1992. Induction of systemic resistance to rust in *Vicia faba* by phosphate and EDTA: effect of calcium. Plant Pathology. 41(4):444-448.

Wasnikar A.R., Khatik S.K., Nayak M.L., Vishwakarma S.K., Puneka L.K. 1993. Effect of Phosphorus on bacterial leaf spot disease incidence, and chemical composition and storage quality of *Piper betle* leaves. Phytoparasitica. 21(1):75-78

Wrather J.A., Anderson T.R., Arsyad D.M., Tan Y., Ploper L.D., Porta-Puglia A., Ram H.H., Yorinori J.T., 2001. Soybean disease loss estimates for the top ten soyabean-producing countries in 1998. Canadian Journal of Plant Pathology. 23(2):115-121.

Zaidi A., Khan M., Ahemad M., Oves M. 2009. Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica Immunologica. Hungarica. 56(3):263–284.

Zhao H., Sun R., Albrecht U., Padmanabhan C., Wang A., Coffey M.D., Girke T., Wang Z., Close T.J., Roose M., Yokomi R.K., Folimonova S., Vidalakis G., Rouse R., Bowman K.D., Jin H. 2013. Small RNA profling reveals phosphorus defciency as a contributing factor in symptom expression for citrus huanglongbing disease. Molecular Plant. 6(2):301–310.

Zinsou V.A., Afouda L.C.A., Zoumarou-Wallis N., Pate-Bata T., Dossou L., Götz M., Winter, S. 2015. Occurrence and characterisation of *Xanthomonas axonopodis* pv. *glycines*, causing bacterial pustules on soybean in the guinea savanna of Benin. African Crop Science Journal. 23(3)203-210.

Zinsou V.A., Afloukou F., Sekloka E., Dannon F., Zoumarou-Wallis N., Afouda L.A.C., Dossou L. 2016. Sélection des variétés de soja pour la résistance à la pustule bactérienne au Bénin. Tropicultura. 34(1)69-79.

Zinsou V.A., Djago G.K., Tchemadon G.C., Tovihoudji P.G., Afouda L.A.C, Dossa H.A., Soumaïla F. 2020. Effect of different potassium rates on the severity of Northern Corn Leaf Blight caused by *Helminthosporium turcicum* in North Benin. Journal of Applied Biosciences 152: 15621-15629. ISSN 1997-5902.