

# Biotic and Abiotic Products for Bean Angular Spot Control

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**Abstract**—Beans (*Phaseolus vulgaris*) are one of the most important crops in human food, but the occurrence of disease can greatly reduce their productivity. The objective of this work was to evaluate the use of biotic and abiotic products to control angular spot (*Pseudocercospora griseola*) in bean crop. Hight Roots<sup>®</sup> treatments; V6<sup>®</sup>; Wert Plus<sup>®</sup>, potassium phosphite; manganese phosphite; Copper phosphite, manganese, fungicide (fentin hydroxide), *Ascochylla nodosum*, *Bacillus subtilis* and *Bacillus thuringiensis*, acibenzolar-S-methyl (ASM) and *Trichoderma asperellum* were used in greenhouse and field experiments with IPR Uirapuru cultivar. Biotic to abiotic products controlled angular leaf spot in bean plants. In greenhouse, the lowest values of Area Under the Disease Progress Curve (AUDPC) were obtained with fungicide application, *A. nodosum*, *T. asperellum* and copper phosphite, *B. subtilis*, acibenzolar-S-methyl, potassium phosphite, Hight Roots<sup>®</sup>, V6<sup>®</sup> and Wert Plus<sup>®</sup>. In field, in the first sowing season the treatments with fungicide, *A. nodosum*, *B. thuringiensis* and manganese resulted in lower AUDPC in relation to the other products. In the second sowing season, the tested products did not reduce the severity of the angular spot, since the productivity was higher for fungicide treatment. In the health quality of seeds, potentially pathogenic fungi such as *Fusarium* sp., *Colletotrichum truncatum* and *Phomopsis* sp., as well as the saprophytic fungus *Aspergillus* sp.

**Keywords**— *Ascochylla nodosum*, *Bacillus* spp., phosphite, *Phaseolus vulgaris*, *Pseudocercospora griseola*, *Trichoderma* spp.

## I. INTRODUCTION

The bean (*Phaseolus vulgaris* L.) is a plant originating from Latin America, grown mainly in tropical and subtropical regions of the globe. This culture is of great importance to Brazil, involving aspects ranging from cultural habit to food security issues. Beans are considered a healthy food, their consumption is a widespread habit in Brazilian society, configuring itself as an important item of the food basket [1].

Factors limiting bean crop productivity include soil correction, fertilization, plant architecture, weed competition, pest occurrence, especially disease occurrence. [4]. The climatic characteristics of Paraná state favor the occurrence of diseases that cause damage to farmers, such as the angular spot [*Pseudocercospora griseola* (Sacc.)], plant shoot pathogen which is a major bean crop disease [3,5].

Typical symptoms of angular leaf spot can be observed in the leaves, stems and pods of plants. In the leaves, lesions

appear as angle-delimited patches of grayish brown coloration, surrounded by a yellowish halo delimited by the ribs. The fungus produces dark sycamons and conidiophores on lesions and when many lesions are present, they may coalesce causing necrosis and premature defoliation. [2].

The control of angular leaf spot can be accomplished with the use of resistant cultivars, integration of cultural practices, such as the use of good quality seeds. However, the most used method is chemical control, with the application of fungicides [6,2].

The development of new technologies to reduce production costs and increase the spectrum of disease control components is a strategy for Brazilian agricultural production. Thus, studies of alternatives for disease control, which go beyond classical chemical control, are of utmost importance [7,8].

Seeking a lower environmental impact and selection pressure of microorganisms, biological control has been

pointed as a method to minimize the use of pesticides and promote crop protection, such as *Trichoderma* fungi, bacteria of the genus *Bacillus*, the algae *Ascophyllum nodosum*, among others [9,11,12]. In addition to biotic products, there are abiotic formulations used for plant disease control, such as acibenzolar-S-methyl (ASM), balanced plant nutrition with micronutrients and phosphites [10,13].

The objective of this work was to evaluate different biotic and abiotic treatments in the control of angular leaf spot in bean plants in greenhouse and field, as well as to evaluate the health quality of seeds from the field.

## II. MATERIAL AND METHODS

The experiments were conducted in a greenhouse and field belonging to the Ponta Grossa State University (UEPG)/Brazil. In greenhouse, three seeds of the IPR Uirapurucultivar, black bean variety, with an average cycle of 86 days, susceptible to angular leaf spot, were sown in plastic pots (3.0 L capacity) with vegetable substrate (pine, eucalyptus bark and ash). Two repetitions of this experiment were performed.

The treatments used were: High Roots® (N 18%; K<sub>2</sub>O 6%), V6® (Mn 2.5%; Zn 1.9%; Mo 0.16%), Wert Plus® (Cu 4%; Mn 6%; Zn 3.9%), potassium phosphite (P<sub>2</sub>O<sub>5</sub> 26%; K<sub>2</sub>O 19%), manganese phosphite (P<sub>2</sub>O<sub>5</sub> 36%; Mn 7.0%), copper phosphite (N 11%; P<sub>2</sub>O<sub>5</sub> 22%; S 1.76%; Cu 4%), Manganese (Mn 10%; S 5.48%), fungicide (Mertin® - phentim hydroxide, 40 m/m i.a), the algae *Ascophyllum nodosum* (Acadian® - K<sub>2</sub>O 5.3%; total organic carbon 6.0%), *Bacillus subtilis* (Serenade® - 13.68 g L<sup>-1</sup>, 1.34% - 1 x 10<sup>9</sup> CFU g<sup>-1</sup>), *Bacillus thuringiensis* (Dipel® - 32 g Kg<sup>-1</sup>, 3.2% - 25 billion viable spores g<sup>-1</sup>), *Trichoderma asperellum* (Quality® - isolated SF 1.0 x 10<sup>9</sup> UFC g<sup>-1</sup>), acibenzolar-S-methyl - 50 m/m i.a (Bion®) and control (distilled water). Products were sprayed when the bean plants were in the vegetative stage V3 (first developed trifoliate).

The inoculation of *P. griseola* pathogen was performed by spraying conidia suspension, produced in tomato culture medium [14], three days after the application of treatments throughout the plant. After inoculation, the plants were kept in a humid chamber with moistened transparent plastic bags at room temperature for 48h [15].

Disease severity assessments began with the onset of symptoms, and cotyledonary leaves were evaluated up to the third trifoliate of each plant, with a two-day interval at each evaluation, using a diagrammatic scale [16], totaling 10 evaluations. From the severity data the area under the disease progress curve (AUDPC) was calculated [17].

The experimental design was completely randomized, with fourteen treatments and ten replications, where each pot with three plants was considered a repetition.

The field experiment was conducted at the Capão da Onça Experimental Farm, which belongs to the Ponta Grossa State University (25°5'49" south latitude, 50°3'11" east longitude and 1,025 m altitude) in the municipality of Ponta Grossa-PR/Brazil. The predominant climate according to Köppen is Cfb type, with cool summers and frequent frosts during winter, with no defined dry season. Maximum and minimum temperatures are 22 and 13°C, annual average precipitation from 1,600 to 1,800 mm [18]. Field evaluation for angular spot control was carried out in two sowing seasons, first season occurred on November 4, 2015 and second season on December 21, 2015, with the IPR Uirapurucultivar. The experimental design was randomized blocks with 12 treatments and 4 replications. Crop sowing was performed in direct sowing system in the straw, with row spacing of 0.45 m and population of 15 plants m<sup>-1</sup>. The treatments used were the same as those used in the greenhouse, except for the *Trichoderma asperellum* treatment which was not used under field conditions.

The first crop applications were made at 15 days after emergence (DAE) (stage V3), 32 DAE (R6 - first flower) and at 47 DAE (R7 - pod formation). In the second season, applications were made at 15 DAE (V4), 29 DAE (R6) and 47 DAE (R7). Five angular leaf spot severity assessments were performed, estimating the percentage of leaf tissue attacked, with the aid of diagrammatic [16] and after was calculated AUDPC [17].

At the end of the crop cycle, data were collected on yield components plants per meter, pods per plant, grains per pod. Plants were harvested from a useful area of 8.0 m<sup>2</sup>. The first crop was harvested at 93 DAE and the second at 96 DAE.

The seeds harvested in the field were submitted to the Blotter pathology test method [19]. 200 seeds were used from each treatment, divided into eight repetitions of 25 seeds. The seeds were individually placed on two sterilized blotting paper sheets moistened with sterile distilled water inside the gerbox. The gerbox seeds were incubated in a BOD chamber at 24 ± 2°C for seven days under 12 hours of light and 12 hours of darkness. The evaluation was performed after seven days of incubation, individually examining all seeds. Fungi were identified at the genus level, based on their morphological characteristics [20], the incidence being expressed as a percentage.

For analysis the pathogen incidence data in the seeds were transformed into arc sen  $\sqrt{(x + 0.5) / 100}$ . All data were subjected to analysis of variance by the F test and means

compared by the Scott-Knott test at 5% significance, using the statistical software R version 2.13.2.

### III. RESULTS AND DISCUSSION

The severity of angular leaf spot in a greenhouse was low, only 14 days after pathogen inoculation, it was possible to observe symptoms in the first experiment and 18 days after inoculation in the second experiment.

In the first experiment, symptoms were initially observed in cotyledonary leaves and later in the first trifolium (Table 1). The values observed in the 1<sup>st</sup> trifolium were lower than the values observed in the cotyledon leaf, however higher than the 2<sup>nd</sup> and 3<sup>rd</sup> trifolium, indicating that treatments reduced the disease development in some way (Table 1).

In the first trifolium the treatments with lower values of AUDPC were the biological treatments with *A. nodosum*, *B. subtilis* and *T. asperellum*, the ASM inducer and copper and potassium phosphites. The other treatments did not differ statistically from the control, except Wert Plus<sup>®</sup> and manganese phosphite, which presented higher values in severity. In the second trifolium no statistical difference was observed between the treatments, while in the third trifolium the *B. subtilis* treatments, and the nutrients Hight Roots<sup>®</sup>, V6<sup>®</sup> and Wert Plus<sup>®</sup>, were the only treatments that did not differ statistically from the control.

An alternative to control plant diseases is the use of algae and among them stands out *A. nodosum* [21]. Seaweed polysaccharides have been shown to be potential resistance inducers in plants [22]. Borsato, Di Piero and Stadnik [23] evaluated the ability of ulvane algae to induce resistance in three bean cultivars against *Uromyces appendiculatus* (Pers) Unger., Causal agent of rust. Polysaccharide spraying did not affect the number of rust pustules/cm<sup>2</sup>, but promoted an average reduction of 23.8% in rust diameter in bean plants.

Among the biological agents in disease control, we highlight the fungi of the genus *Trichoderma*. De Meyer et al. [24] applied *T. harzianum* T30 conidia seven days before the inoculation of *Botrytis cinerea* (De Bary) Whetzel on beans and observed significant reductions in disease. The authors reported a 35% reduction in disease severity and, as they did not find leaf fungus, the authors attributed the decrease in disease symptoms to antagonist-activated resistance induction.

Phosphites may act in a fungistatic manner in addition to fungitoxic. In bean culture, Gadaga et al. [13] evaluated the effect of different phosphite formulations on plant protection against anthracnose. The authors observed lower AUDPC in plants that received applications of K and Mn phosphites compared to control, being the K, Zn

and K phosphites + salicylic acid also effective in controlling the disease.

In an experiment conducted in a greenhouse, Gontijo Neto et al. [6] observed that bean plants treated with ASM had 23% lower severity than treatment where there was no control of angular leaf spot. Plants that were sprayed with fungicide (methyl thiophanate + epoxiconazole + piraclostrobin) achieved a 40.84% reduction in disease progress.

In the second experiment (Table 2), disease development was slower (18 days after inoculation), which justifies zero values in some tests for cotyledon leaf evaluation, since it is introduced in senescence before disease. Symptoms in the 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trifolium were more expressive when compared to experiment 1 (Table 1).

The control showed greater severity of the disease in all trifoliums (Table 2), presented higher values, but did not differ statistically from treatment *B. subtilis* and copper phosphite in the 2<sup>nd</sup> trifolium. It is no longer the third trifolium, in addition to the controls mentioned, manganese treated plants with higher disease severity.

In the first trifolium (Table 2) the lowest AUDPC values were observed in the procedures with fungicide and potassium phosphate. Already in the 2<sup>nd</sup> trifolium, there was a reduction of the disease in all cases with the lowest value of AUDPC: fungicide, *A. nodosum*, *B. thuringiensis*, *T. asperellum*, ASM, manganese and potassium phosphate, in addition to the nutrients Hight Roots<sup>®</sup> and Wert Plus<sup>®</sup>. The other controls did not differ statistically from the witness. In the 3<sup>rd</sup> trifolium all treatments showed a reduction in AUDPC, except *B. subtilis*, copper phosphite and manganese nutrient, which did not differ statistically from the control.

Most pathogenic fungi invade the apoplast releasing pectolytic enzymes that dissolve the leaf's middle lamella [25]. The activity of these enzymes is extremely inhibited by calcium, also present in Hight Roots<sup>®</sup> fertilizer, which strengthens the plant cell wall, explaining a negative correlation between calcium content and disease severity [26].

Potassium phosphite treatment also showed significant disease control in all trifolium. Potassium is the only macronutrient that presents consistent positive results in reducing the incidence of disease, as its deficiency causes accumulation of soluble amino acids, which are pathogen nutrients. [26].

Regarding the field experiments, at the time of the first application of the products, the presence of angular stain symptoms had not yet been found. Anthracnose (*Colletotrichum lindemuthianum* (Sacc. & Magn. Lams. Scrib) was the disease that occurred first and most severely

in both experiments sowing seasons. Negative correlation between anthracnose and angular leaf spot was observed in plants where high anthracnose rates were observed, low angular spot rates were found and vice versa, possibly due to competition for feeding site [13].

The first symptoms of angular leaf spot were observed only in the last two evaluations in the first season, at 51 DAE and 59 DAE; and in the second sowing season in the last three assessments, at 45, 55 and 64 DAE (Table 3).

In the first sowing season all treatments presented lower AUDPC compared to control (Table 3). Treatments with better disease control were chemical control with fungicide, biological controls with algae *A. nodosum*, bacterium *B. thuringiensis* and fertilizer V6®. The other treatments did not differ statistically among themselves.

The use of seaweed has been an ecologically sustainable alternative in the control of plant diseases. Several studies have pointed out the potential use of algal extracts, such as *A. nodosum*, to increase plant development, sometimes with consequent increases in yield, also increased tolerance of plants to biotic and abiotic stresses. [21, 28].

Due to the growing search for control alternatives to phytopathogens, the biological method has been gaining large space and bringing positive results. Species of the genus *Bacillus* are considered potential biocontrol agents of leaf and soil pathogens [28]. These bacteria produce a wide range of bioactive lipopeptides, compounds that suppress plant pathogens through the mechanism of antibiosis.[29].

Another treatment with lower angular leaf spot severity was V6® leaf fertilizer (Table 3). This treatment consists of manganese (2.5%), zinc (1.9%) and molybdenum (0.16%). The effects of mineral nutrition on crop growth and productivity have been reported and can act by altering plant resistance against disease. [30].

The nutrient molybdenum can also indirectly contribute to disease prevention, as it participates in the activation of the enzymes nitrate reductase and nitrogenase, which are key to higher plant nitrogen metabolism [25]. Zinc is one of the main micronutrients of plant nutrition, as it participates in various metabolic processes, as well as contributing to decrease or intensify the incidence of some diseases. [31].

Also as an alternative to chemical control of diseases, there is the use of phosphites. These can act directly or indirectly on the pathogen, inducing systemic resistance in plant by the synthesis of phytoalexins, phenolic compounds and PR-proteins (pathogenesis-related proteins)[32].

At the end of the culture cycle yield components were evaluated. In the first sowing season there was a statistical difference for the number of pods per plant and number of

beans per pod variables. There was no significant difference between treatments for plants per meter and productivity variables (Table 4).

In the second sowing season the fungicide, *B. subtilis*, copper phosphite and Wert Plus® treatments presented lower number of plants per meter. The other treatments did not differ statistically among themselves. For number of pods per plant, the only treatments that differed statistically from the control were *A. nodosum*, *B. subtilis* and manganese phosphite. For the variable grain per pod, treatments that did not differ from the control were *A. nodosum*, *B. subtilis* and manganese (Table 4).

According to Kuhn & Pascholati [33] a variety of situations can occur while developing a plant along with the resistance induction process. One of the changes can be mainly due to the variation in plant growth conditions. Due to this, productivity shows dependence on the inducer dose, nutritional condition and biological interaction to which they are subjected.

In the harvested seeds evaluation, in both sowing seasons, there was incidence of pathogenic and saprophytic fungi (Table 5). Seeds are the most efficient spreading agent of pathogens and aid their survival [34]. The distribution of infected seeds is random and provides primary outbreaks of field infection in the early phase of the crop [35].

In the first sowing season, the potentially pathogenic organisms found in the pathology test [19] were *Fusarium* sp. and *Colletotrichum truncatum* (Table 5). *Fusarium* sp. had higher incidence in the control, in the treatment *A. nodosum* and *B. subtilis*, potassium phosphite and High Roots® fertilizer. The treatments with the lowest incidence of the pathogen were ASM inducer and V6® and Wert Plus® leaf fertilizers. The other pathogen detected in the present work was *Colletotrichum truncatum*. The highest incidence was found in fungicide treatments, biological control with *A. nodosum*, *B. thuringiensis*, ASM inducer, copper phosphite and Wert Plus® nutrient (Table 6).

In the second sowing season there was an incidence of the pathogens *Fusarium* sp., *Phomopsis* sp. and *Aspergillus* sp. (Table 6). The treatments *B. thuringiensis*, ASM, manganese phosphite, Hight Roots®, V6® and Wert Plus® presented lower incidence of *Fusarium* sp. in relation to the other treatments. For the incidence of *Aspergillus* and *Phomopsis* there was no statistical difference between treatments.

The genus *Fusarium* sp. may cause bean crop to wilt or yellowing of fusarium caused by the species *Fusarium oxysporum*(Schlecht.) f. sp. *phaseoli* (Kendrick & Snyder). This pathogen occurs in virtually all producing regions of

Brazil, its importance has increased mainly in intensive cultivation of the same crop in the same area [36].

*Colletotrichum truncatum* causes bean disease called bean scab, with damage that can reach 100% of the crop [37].

Trade and use of saved seeds is a reality in developing countries. In this system, over 80% of farmers are involved in the selection, production, dissemination, sales, exchanges or donations that occur in the local community [38]. In Brazil, bean farmers usually use part of the grain produced as seeds in later crops [39].

#### IV. CONCLUSION

Biotic to abiotic products controlled angular leaf spot in bean plants.

In greenhouse, the lowest AUDPC values were obtained with fungicide (fentin hydroxide), *A. nodosum*, *T. asperellum* and copper phosphite, B. subtilis, acibenzolar-S-methyl, potassium phosphite, Hight Roots®, V6® and Wert Plus® treatments.

In field, in the first sowing season the application of fungicide, *A. nodosum*, *B. thuringiensis* and manganese resulted in smaller AUDPC. In the second sowing season, the tested products did not reduce the severity of the angular spot, since the yield was higher when applied fungicide.

There was an incidence of potentially pathogenic fungi such as *Fusarium* sp., *C. truncatum* and *Phomopsis* sp. and the saprophytic fungus *Aspergillus* sp.

#### ACKNOWLEDGEMENTS

To State University of Ponta Grossa (UEPG) and CAPES (Coordination of Improvement of Higher Education Personnel).

#### REFERENCES

- [1] Conab - Companhia Brasileira de Abastecimento (2018). Acompanhamento da safra brasileira de grãos – terceiro levantamento, safra 2018/19. Conab, Brasília, Brasil. p. 114. (ISSN 2318-6852).
- [2] Wendland, A., Moreira, A. S., Bianchini, A., Giampan, J. S., Lobo Jr, M. Doenças do feijoeiro. (2016). IN: Amorim, L., Rezende, J. A. M., Bergamin Filho, A., Camargo, L. E. A. Manual de Fitopatologia: doenças de plantas cultivadas. 5. ed. Ouro Fino: Agronômica Ceres. pp. 383-396.
- [3] Viecegli, C. A., Stangarlin, J.R., Kuhn, O. J., Schwan-Estrada, K. R. F. (2010). Indução de resistência em feijoeiro a mancha angular por extratos de micélio de *Pycnoporus sanguineus*. Summa Phytopathologica, 36(1), 73-80. <http://dx.doi.org/10.1590/S010054052010000100013>
- [4] Silva, O. F. da.; Wander, A. E. (2013). O feijão-comum no Brasil: passado, presente e futuro. Retrieved from: <https://ainfo.cnptia.embrapa.br/digital/bitstream/item/89747/1/seriedocumentos-287.pdf> (ISSN : 1678-9644).
- [5] Dalla Pria, M., Silva, O. C. (2010) Cultura do Feijão: doenças e controle. Ponta Grossa: UEPG, pp. 57-67.
- [6] Gontijo Neto, G. F.; Andrade, M. J. B. de.; Pozza, E. A.; Martins, F. A. D., Soares, B. L., Belan, L. L., Cardilho, B. E. da. S. (2016). Controle da antracnose e da mancha angular do feijoeiro comum com indutores de resistência. Nucleus, 13(2), pp. 199-218. (DOI: 10.3738/1982.2278.1635).
- [7] Ballaré, C. L. (2014). Light regulation of plant defense. Annual Review of Plant Biology, Palo Alto, 65(1), pp. 335-363. (DOI: 10.1146/annurev-arplant-050213-040145).
- [8] Borsato, L. C., Di Piero, R. M., Stadnik, M. J. (2010). Mecanismos de defesa eliciados por ulvana contra *Uromyces appendiculatus* em três cultivares de feijoeiro. Tropical Plant Pathology, Brasília, 35(5), pp. 318-322. (<http://dx.doi.org/10.1590/S1982-56762010000500008>).
- [9] Sharma, V. Salwan, R., Sharma, P. N., Kanwar, S. S. (2017). Elucidation of biocontrol mechanisms of *Trichoderma harzianum* against different plant fungal pathogens: universal yet host specific response. International Journal of Biological Macromolecules, 95(1), pp. 72-79. (DOI: 10.1016/j.ijbiomac.2016.11.042).
- [10] Myresiotis, C. K.; Vryzas, Z.; Papadopolou-Mourkidou, E. (2014). Enhanced root uptake of acibenzolar-S-methyl (ASM) by tomato plants inoculated with selected *Bacillus* plant growth-promoting rhizobacteria (PGPR). Applied Soil Ecology, Amsterdam, 77(1) pp. 26-33. (<https://doi.org/10.1016/j.apsoil.2014.01.005>).
- [11] Subramanian, S.; Sangha, J. S.; Gray, B. A.; Singh, R. P.; Hiltz, D.; Critchley, A. T.; Prithiviraj, B. (2011). Extracts of the marine brown macroalga, *Ascophyllum nodosum*, induce jasmonic acid dependent systemic resistance in *Arabidopsis thaliana* against *Pseudomonas syringae* pv. *tomato* DC3000 and *Sclerotinia sclerotiorum*. European Journal of Plant Pathology, Netherlands, 131(2), pp. 237-248. (DOI: 10.1007/s10658-011-9802-6).
- [12] Gao, X.; Han, Q.; Chen, Y.; Qin, H.; Huang, L.; Kang, Z. (2013). Biological control of oilseed rape *Sclerotinia* stem rot by *Bacillus subtilis* strain Em7. Biocontrol Science and Technology, London, 24(1), pp. 39-52. (<https://doi.org/10.1080/09583157.2013.844223>).
- [13] Gadaga, S. J. C., Abreu, M. S. de., Resende, M. L. V. de. & Ribeiro Júnior, P. M. (2017). Phosphites for the control of anthracnose in common bean. Pesquisa Agropecuária Brasileira, 52(1), 36-44. (<http://dx.doi.org/10.1590/s0100204x2017000100005>).
- [14] Dalla Pria, M.; Canteri, M. G.; Bergamin Filho, A.; Amorim, L. (1997). Avaliação de diferentes meios de cultura na esporulação de *Colletotrichum lindemuthianum*, *Phaeoisariopsis griseola* e *Alternaria* sp. Summa Phytopathologica, Botucatu, 23(2), pp. 181-183. (ISSN:0100-5405).
- [15] Stangarlin, J. R.; Pascholati, S. F.; Labate, C. A. (2000). Efeito de *Phaeoisariopsis griseola* na atividade de ribulose-1,5-bisfosfato carboxilase-oxigenase (rubisco), clorofilase,  $\beta$ -1,3-glucanase e quitinase em cultivares de *Phaseolus vulgaris*. Fitopatologia Brasileira, Brasília, 25(1), pp. 59-66.

- [16] Godoy, C.V.; Carneiro, S. M. T. P. G.; Imauti, M. T.; Dalla Pria, M.; Amorim, L. R. D.; Bergamin, A.; Godoy, C.V. (1996). Diagrammatic scales for bean diseases: Diagrammatic scales for bean diseases: development and validation. *Journal of Plant Diseases and Protection*, Heidelberg, 104(4), pp. 336-345. (<https://www.jstor.org/stable/43215167>).
- [17] Shaner, G.; Finney, R. E. (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. *Phytopathology*, Saint Paul, 67(8), pp. 1051-1056. (DOI: 10.1094/Phyto-67-1051).
- [18] Iapar (Instituto Agronômico do Paraná). Cartas Climáticas do Paraná. Retrieved from: <http://www.iapar.br/modules/conteudo/conteudo.php?conteudo=863>.
- [19] Mapa (Ministério da Agricultura, Pecuária e Abastecimento). Manual de Análise Sanitária de Sementes. Retrieved from: <https://www.abrates.org.br/files/manual-de-analise-sanitaria-de-sementes.pdf> (ISBN 978-85-99851-64-7).
- [20] Barnett, H. L., Hunter, B. B. (1998). *Illustrated Genera of Imperfect Fungi*. 4. ed. St. Paul: American Phytopathology Society, 240p.
- [21] Carvalho, M. E. A.; Castro, P. R. C.; Gallo, L. A.; Ferraz Junior, M. V. C. (2014). Seaweed extract provides development and production of wheat. *Agrarian*, Dourados, 7(23), pp. 166-170. (<http://ojs.ufgd.edu.br/index.php/agrarian/article/view/2459>).
- [22] Paulert, R.; Talamini, V.; Cassolato, J. E. F.; Duarte, M. E. R.; Nosedá, M.; Smania Júnior, A.; Stadnik, M. J. (2009). Effects of sulfated polysaccharide and alcoholic extracts from green seaweed *Ulva fasciata* on anthracnose severity and growth of common bean (*Phaseolus vulgaris* L.). *Journal of Plant Diseases and Protection*, Germany, 116(6), pp. 263-270. (<https://www.jstor.org/stable/43229075>).
- [23] Borsato, L. C.; Di Piero, R. M.; Stadnik, M. J. (2010). Mecanismos de defesa eliciados por ulvana contra *Uromyces appendiculatus* em três cultivares de feijoeiro. *Tropical Plant Pathology*, Brasília, 35(5), pp. 318-322. (<http://dx.doi.org/10.1590/S198256762010000500008>).
- [24] De Meyer, G.; Bigirimana, J.; Elad, Y.; Höfte, M. Induced systemic resistance in *Trichoderma harzianum* T39 biocontrol of *Botrytis cinerea*. *European Journal of Plant Pathology*, Dordrecht, v. 104, n. 5, p. 279-286. 1998. (DOI: 10.1023/A:1008628806616).
- [25] Taiz, L., Zeiger, E., Moller, I. M., Murphy, A. *Fisiologia e Desenvolvimento Vegetal*. 6. ed. Porto Alegre: Artmed, 2017. 888 p.
- [26] Dornelles, M. S. Avaliação do estado nutricional e do controle da mancha angular em feijoeiro pulverizado com biofertilizantes líquidos. 2005, 150 f. Tese (Doutorado) – Universidade Estadual do Norte Fluminense, Rio de Janeiro, 2005.
- [27] Khan, W.; Rayirath, U. P.; Subramanian, S.; Jithesh, M. N.; Rayorath, P.; Hodges, D. M.; Critchley, A. T.; Craigie, J. S.; Norrie, J.; Prithiviraj, B. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulation*, New York, 28(4), pp. 386-399. (<https://link.springer.com/article/10.1007/s00344-0099103x>).
- [28] Mcspadden Gardener, B. B.; Driks A. (2004) Overview of the nature and application of biocontrol microbes: *Bacillus* spp. *Phytopathology*, St. Paul, 94(11), pp. 1244. (<https://apsjournals.apsnet.org/doi/pdfplus/10.1094/PHYTO.2004.94.11.1244>).
- [29] Chen, Y.; Gao, X.; Chen, Y.; Qin, H.; Huang, L.; Han, Q. Inhibitory efficacy of endophytic *Bacillus subtilis* EDR4 against *Sclerotinia sclerotiorum* on rapeseed. (2014). *Biological Control*, San Diego, 78(1), pp. 67-76. (<https://doi.org/10.1016/j.biocontrol.2014.07.012>).
- [30] Marschner, H. Mineral nutrition of higher plants. 2. ed. London: Academic, 1995. 889p.
- [31] Carvalho, V. L.; Canha, R. L. da.; Guimarães, P. T. G.; Carvalho, J. P. F. (2008). Influência do zinco na incidência de doenças do cafeeiro. *Ciência e Agrotecnologia*, Lavras, 32(3), pp. 804-808. ([http://www.sbicafe.ufv.br/bitstream/handle/123456789/2098/166733\\_Art163f.pdf?sequence=1](http://www.sbicafe.ufv.br/bitstream/handle/123456789/2098/166733_Art163f.pdf?sequence=1)).
- [32] Carmona, M.; Sautua, F. (2011). Os fosfitos no manejo de doenças nas culturas extensivas. *Revista Plantio Direto*, 126(1), pp. 19-22. (<https://wp.ufpel.edu.br/consagro/files/2012/02/Os-fosfitos-no-manejo-de-doencas-nasculturas-extensivas.pdf>).
- [33] Kuhn, O. J.; Pascholati, S. F. (2010). Custo adaptativo da indução de resistência em feijoeiro mediada pela rizobactéria *Bacillus cereus* ou acibenzilar – S – metil atividade de enzimas, síntese de fenóis e lignina e biomassa. *Summa Phytopathologica*, Botucatu, 36(2), pp. 107-114. (<http://dx.doi.org/10.1590/S0100-54052010000200001>).
- [34] Silva, M. S.; Carvalho, F. C. Q.; Silva, J. R. da; Lins, S. R. de O.; Oliveira, S. M. A. de. (2014). Uso de antagonistas e produtos alternativos no manejo pós-colheita de podridão mole em pimentão. *Revista Ciência Agronômica*, 45(1), pp. 718-725. (ISSN 1806-6690).
- [35] Barros, F. C.; Sagata, E.; Ferreira, L. C. C.; Juliatti, F. C. (2010). Indução de resistência em plantas contra fitopatógenos. *Bioscience Journal*, Uberlândia, 26(2), pp. 231-239. (ISSN 1981-3163).
- [36] Paula Júnior, T. J. de.; Vieira, R. F.; Teixeira, H.; Lobo Júnior, M.; Wendland, A. Doenças do feijoeiro: estratégias integradas de manejo. In: Carneiro, J.E.; Paula Júnior, T.J.de; Borém, A. Feijão - do plantio à colheita. Editora UFV: Viçosa, p.270-299. 2015.
- [37] Mota, S. F. Variabilidade de *Colletotrichum* spp. no feijoeiro comum. Dissertação 96 f (Mestrado em Genética e Melhoramento de Plantas). Universidade Federal de Lavras. Lavras-MG, 2013.
- [38] Louwaars, N. P.; De Boef, W.S. (2012). Integrated seed sector development in Africa: a conceptual framework for creating coherence between practices, programs, and policies. *Journal of Crop Improvement*, Manhattan, v. 26, n. 1, p. 39-59. (<https://doi.org/10.1080/15427528.2011.611277>).

[39] Zambolim, L. Sementes: qualidade fitossanitária. 1. ed.

Viçosa: Editora UFV. 2005. 502.

Table 1 - Area under the disease progress curve (AUDPC) of *Pseudocercosporagriseola* in bean plants (*Phaseolus vulgaris*), on cotyledon leaf, first trifolium, second trifolium and third trifolium, as a function of the products performed in beans under greenhouse conditions – second repetition.

Treatments	Cotyledonleaf	1 <sup>st</sup> Trifoliumleaf	2 <sup>nd</sup> Trifoliumleaf	3 <sup>rd</sup> Trifoliumleaf
Control	5.98 b <sup>1</sup>	3.42 b	0.88 <sup>2</sup>	0.08 b
Fungicide	1.38 d	2.43 b	0.76	0.00 b
<i>Ascochyllum nodosum</i>	0.95 d	1.35 c	0.92	0.04 b
<i>Bacillus thuringiensis</i>	3.82 c	2.51 b	0.10	0.00 b
<i>Bacillus subtilis</i>	5.89 b	1.60 c	0.52	0.22 a
<i>Trichoderma asperellum</i>	1.61 d	1.91 c	0.10	0.00 b
Acibenzolar-S-methyl (ASM)	2.30 c	1.16 c	0.00	0.00 b
Copper phosphite	0.95 d	1.18 c	0.93	0.00 b
Manganese phosphite	7.20 b	6.29 a	0.63	0.06 b
Potassium phosphite	3.12 c	1.04 c	0.02	0.00 b
Hight Roots <sup>®</sup>	2.68 c	2.96 b	0.10	0.30 a
Manganese	2.54 c	2.49 b	0.42	0.00 b
V6 <sup>®</sup>	6.03 c	2.94 b	1.55	0.30 a
Wert Plus <sup>®</sup>	10.48 a	5.12 a	1.67	0.48 a
C.V (%) <sup>2</sup>	33.89	35.46	16.42	4.96

(1) Means with the same letter in the column do not differ significantly by Scott -Knott ( $p>0.05$ ).

(2) Not significant;

(3) Coefficient of variation.

Table 2 - Area under the disease progress curve (AUDPC) of *Pseudocercosporagriseola* in bean plants (*Phaseolus vulgaris*) on cotyledon leaf, first trifolium, second trifolium and third trifolium, as a function of the products performed in beans under greenhouse conditions – second repetition.

Treatments	Cotyledonleaf	1 <sup>st</sup> Trifoliumleaf	2 <sup>nd</sup> Trifoliumleaf	3 <sup>rd</sup> Trifoliumleaf
Control	0.00 b <sup>1</sup>	7.93 a	3.14 a	1.44 a
Fungicide	1.58 b	0.66 d	0.46 b	0.60 b
<i>Ascochyllum nodosum</i>	0.00 b	2.74 c	0.82 b	0.50 b
<i>Bacillus thuringiensis</i>	0.00 b	2.74 c	1.55 b	0.03 b
<i>Bacillus subtilis</i>	0.72 b	3.73 c	2.17 a	1.15 a
<i>Trichoderma asperellum</i>	3.76 a	4.00 c	0.93 b	0.20 b
Acibenzolar-S-methyl (ASM)	0.92 b	2.26 c	0.32 b	0.12 b
Copper phosphite	0.12 b	2.95 c	2.70 a	1.30 a
Manganese phosphite	0.00 b	5.48 b	1.42 b	0.24 b
Potassium phosphite	0.00 b	1.24 d	0.62 b	0.20 b
Hight Roots <sup>®</sup>	0.00 b	5.03 b	1.21 b	0.00 b
Manganese	0.00 b	2.36 c	1.92 a	1.70 a
V6 <sup>®</sup>	0.00 b	2.38 c	1.46 b	0.26 b
C.V (%) <sup>2</sup>	32.59	36.33	15.26	12.51

(1) Means with the same letter in the column do not differ significantly by Scott -Knott ( $p>0.05$ ).

(2) Coefficient of variation.

Table 3 - Area under the disease progress curve (AUDPC) of *Pseudocercosporagriseola* in common bean (*Phaseolus vulgaris*) on the whole plant as a function of the products performed in beans under field conditions, IPR Uirapuru cultivar - first and second season.

Treatments	1 <sup>st</sup> Season	2 <sup>nd</sup> Season
Control	15.10 a <sup>1</sup>	7.55 <sup>2</sup>
Fungicide	0.35 c	7.94
<i>Ascochyllum nodosum</i>	0.09 c	7.86
<i>Bacillus thuringiensis</i>	2.94 c	5.26
<i>Bacillus subtilis</i>	7.10 b	7.22
<i>Trichoderma asperellum</i>	5.28 b	5.09
Acibenzolar-S-methyl (ASM)	7.19 b	5.68
Copper phosphite	4.27 b	6.95
Manganese phosphite	5.98 b	6.93
Potassium phosphite	4.33 b	7.02
Hight Roots <sup>®</sup>	5.76 b	7.21
Manganese	3.51 c	6.89
V6 <sup>®</sup>	7.13 b	6.62
C.V (%) <sup>3</sup>	50.34	27.76

(1) Means with the same letter in the column do not differ significantly by Scott -Knott ( $p>0.05$ ).

(2) Not significant;

(3) Coefficient of variation.

Table 4 - Yield components: plants per meter, pods per plant, grain number and productivity (kg ha<sup>-1</sup>) as a function of products in beans (*Phaseolus vulgaris*), IPR Uirapuru, first and second season.

1 <sup>st</sup> Season				
Treatments	Plants per meter	Pods per plant	Grainnumber	Productivity (kg ha <sup>-1</sup> )
Control	10.75 <sup>2</sup>	121.25 a <sup>1</sup>	673.25 a	3946.13 <sup>2</sup>
Fungicide	9.00	89.00 b	504.25 b	2843.48
<i>Ascochyllum nodosum</i>	13.25	93.75 b	542.25 b	3552.67
<i>Bacillus thuringiensis</i>	7.75	79.50 b	441.25 b	3868.59
<i>Bacillus subtilis</i>	9.00	91.00 b	502.00 b	3847.95
<i>Trichoderma asperellum</i>	10.00	91.75 b	501.50 b	2529.13
Acibenzolar-S-methyl (ASM)	10.25	113.00 a	619.75 a	3407.70
Copper phosphite	10.25	121.00 a	692.00 a	3305.38
Manganese phosphite	10.00	126.25 a	687.75 a	3172.07
Potassium phosphite	11.50	122.25 a	491.50 b	2840.41
Hight Roots <sup>®</sup>	9.00	84.25 b	468.00 b	3484.94
Manganese	9.50	86.75 b	495.50 b	2885.94
V6 <sup>®</sup>	8.75	89.75 b	469.25 b	2704.35
C.V (%) <sup>3</sup>	17.22	17.44	13.51	12.74
2 <sup>nd</sup> Season				
Treatments	Plants per meter	Pods per plant	Grainnumber	Productivity (kg ha <sup>-1</sup> )
Control	14.00 a	109.75 a	529.75 b	5001.09 c
Fungicide	11.25 b	112.00 a	595.25 a	8524.17 a
<i>Ascochyllum nodosum</i>	14.25 a	98.50 b	491.50 b	4984.27 c
<i>Bacillus thuringiensis</i>	13.50 a	121.00 a	552.25 a	5361.03 c
<i>Bacillus subtilis</i>	12.50 b	103.25 b	498.25 b	4802.52 c
<i>Trichoderma asperellum</i>	14.25 a	127.00 a	603.00 a	6215.05 b
Acibenzolar-S-methyl (ASM)	11.50 b	114.00 a	594.25 a	5624.34 b

Copper phosphite	13.75 a	93.00 b	546.50 a	6189.08 b
Manganese phosphite	13.75 a	114.75 a	575.50 a	6032.77 b
Potassium phosphite	13.75 a	111.50 a	599.25 a	4672.08 c
Hight Roots®	14.25 a	98.00 b	486.00 b	4817.85 c
Manganese	13.50 a	112.25 a	491.00 b	5709.11 b
V6®	11.00 b	96.75 b	458.50 b	4716.57 c
C.V (%)	11.88	9.62	11.62	14.63

(1) Means with the same letter in the column do not differ significantly by Scott -Knott ( $p>0.05$ ).

(2) Not significant;

(3) Coefficient of variation.

Table 5 – Incidence (%) of seed pathogens as a function of the products performed in the field in the bean (*Phaseolus vulgaris*) in the experimental area Fazenda Capão da Onça, first and second season growth.

1 <sup>st</sup> Season			
Treatments	Fungi		
	<i>Fusarium</i> sp.	<i>Aspergillus</i> sp.	<i>Colletotrichum truncatum</i>
Control	80.50 a <sup>1</sup>	1.00 <sup>2</sup>	2.00 b
Fungicide	61.50 b	0.50	5.00 a
<i>Ascochyllum nodosum</i>	82.50 a	0.50	3.50 a
<i>Bacillus thuringiensis</i>	61.00 b	0.00	6.50 a
<i>Bacillus subtilis</i>	77.00 a	0.00	1.00 b
<i>Trichoderma asperellum</i>	29.00 d	0.50	5.50 a
Acibenzolar-S-methyl (ASM)	37.00 c	0.50	6.00 a
Copper phosphite	57.00 b	0.00	0.00 b
Manganese phosphite	77.50 a	1.00	0.00 b
Potassium phosphite	73.50 a	1.50	2.00 b
Hight Roots®	47.00 c	0.00	0.50 b
Manganese	18.50 d	0.00	2.50 b
V6®	28.50 d	0.00	3.50 a
C.V (%) <sup>3</sup>	26.16	13.70	23.78
2 <sup>nd</sup> Season			
Treatments	Fungi		
	<i>Fusarium</i> sp.	<i>Aspergillus</i> sp.	<i>Phomopsis</i> sp.
Control	33.50 a	1.50 <sup>2</sup>	0.00 <sup>2</sup>
Fungicide	21.00 a	0.00	2.00
<i>Ascochyllum nodosum</i>	14.50 a	0.50	0.50
<i>Bacillus thuringiensis</i>	0.00 b	0.00	0.00
<i>Bacillus subtilis</i>	22.50 a	0.00	1.50
<i>Trichoderma asperellum</i>	7.00 b	1.00	2.50
Acibenzolar-S-methyl (ASM)	23.50 a	1.00	0.00
Copper phosphite	8.00 b	0.00	0.50
Manganese phosphite	20.50 a	3.00	0.50
Potassium phosphite	0.00 b	0.00	0.00
Hight Roots®	20.50 a	0.50	0.50
Manganese	9.00 b	0.00	1.00
V6®	0.00 b	0.00	0.00
C.V (%) <sup>3</sup>	26.20	14.56	17.28

(1) Means with the same letter in the column do not differ significantly by Scott -Knott ( $p>0.05$ ); original data, for analysis, were transformed into  $\arcsin \sqrt{(x + 0.5)/100}$ ;

(2) Not significant;

(3) Coefficient of variation.