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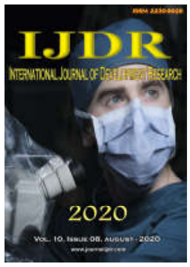

RESEARCH ARTICLE OPEN ACCESS

DISEASE CONTROL IN POD BEAN (*PHASEOLUS VULGARIS* L.) WITH *TRICHODERMAASPERELLUM*, MILK *IN NATURA* AND FUNGICIDES

Gislaine Gabardo[1*], Maristella Dalla Pria[2], André Manosso Correa Prestes[3] and Henrique Luis da Silva[4]

[1]Doutoraem Agronomia, Universidade Estadual de Ponta Grossa (UEPG), Av. General Carlos Cavalcanti, 4748 - Uvaranas, - CEP 84030-900, PontaGrossa - PR, Brasil,[2]Doutoraem Fitopatologia, UEPG, [3]Engenheiro Agronomo (Grupo Pitangueiras), [4]Mestreem Fitotecnia, IDR-Instituto de Desenvolvimento Rural

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****Corresponding author: Gislaine Gabardo,***

ABSTRACT

The aimed of this work was to evaluate the effect of foliar application of milk *in natura*, *Trichodermaasperellum* and different fungicides in the control of angular leaf spot (*Pseudocercosporagriseola*), white mold (*Sclerotiniasclerotiorum*) and anthracnose (*Colletotrichumlindemuthianum*), in the cultivar of common beans low butter pod, in the field. The design used was a randomized block, with 7 treatments and 4 repetitions. The treatments used were: 1- witness, 2- weekly sprays of *Trichodermaasperellum* (2.0×10^6spores mL^{-1}); 3- weekly sprays of 20% cow's milk; 4- weekly sprays of 20% milk, plus vegetable oil (1.6 mL L^{-1}); 5- sprays of azoxystrobin (80 g a.i. ha^{-1}); 6- sprays of mancozeb (2.0 kg a.i. ha^{-1}) and 7- sprayings of diphenoconazole (0.3 L a.i. ha^{-1}). Severity of anthracnose and white mold in pods and angular leaf spot on leaves and productivity were evaluated. With the severity data of *C. lindemuthianum* and *P. griseola*, the area under the disease progress curve (AUDPC) was calculated. Alternative products (*T. asperellum* and milk, with and without vegetable oil), reduced AUDPC from angular leaf spot and anthracnose, but less AUDPC was observed in treatments with mancozeb and diphenoconazole. There was no effect of treatments for the incidence of white mold, number and weight of pods.

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INTRODUCTION

Pod beans (*Phaseolus vulgaris* L.) belong to the same botanical species as beans for dry grains. It is characterized for being harvested when the seeds are still immature, since the pods are the edible part, with all its content (Moreira *et al*., 2009). Despite not being rich in proteins and calories like common bean grains, it is rich in vitamins and minerals, which are lacking in most staple foods (Peixoto*et al*., 2001). Pod beans are the thirteenth vegetable in terms of economic importance and the sixth in volume produced in Brazil (Oliveira *et al*., 2007). One of the main factors responsible for the reduction in crop productivity is the occurrence of diseases that limit production and reduce the product's physiological, health, nutritional and commercial quality (Sartorato, Nechet&Halfeld-vieira 2006). Pod beans are subject to a large number of diseases, including anthracnose (*Colletotrichumlindemuthianum* Sacc & Magnus), angular leaf spot (*Pseudocercosporagriseola* (Sacc) Crous& U. Braun) and white mold (*Sclerotiniasclerotiorum*(Lib.) De Bary) (Sartorato, Nechet & Halfeld-vieira 2006; Zhang *et al*., 2018). Anthracnose is a disease of great importance for culture, which can cause losses of up to 100%, in addition to being transmitted by seeds (Bianchini *et al*., 2005). The angular leaf spot can cause losses of 7 to 70%. (Bianchini *et al*., 2005). And white mold causes losses in grain yield that reach an average of 50% (Canteri*et al*., 1999). Among disease control strategies, chemical control is the most used in Brazil (Machado *et al*., 2017). Among the fungicides used to control diseases in the crop, there are azoxystrobin, mancozeb and diphenoconazole. However, this control method can cause damage to the environment, problems related to the selection of resistant strains of the pathogen, environmental contamination, food and applicator (Carniel *et al*., 2019). Brazil is a major holder of biodiversity (Mascarin *et al*., 2019). Fungi of the genus *Trichoderma* spp. present characteristics of free life and asexual reproduction, being found more easily in soils of temperate and tropical regions, they can be used in the control of phytopathogens, due to their effects on parasitism, antibiosis, competition and induction of resistance of plants against diseases (Sriram, Roopa & Savitha, 2011; Peterson &Nevalainen 2012; Steyaert*et al*., 2013; Dos Santos, 2019). Another alternative product is cow's milk. Milk can have a direct effect due to its germicidal properties because it contains various salts and amino acids, can induce plant resistance and / or directly control the pathogen, can also stimulate natural biological control, forming a microbial film on the surface of the leaf or change the physical, chemical and biological characteristics of the leaf surface (Zatarim*et al*., 2005). Research that evaluates the effectiveness of these alternative products can contribute to the rational control of phyto-pathogens and the reduction of pollution (Newitt *et al*., 2019; Gabardo *et al*., 2020), because in Brazil, the bean is grown mainly by small producers, with high demand for labor and based on traditional planting methods and intensive use of chemical inputs (De Sant'anna *et al*., 2019). Within this context, the aimed of the present work was to evaluate the effect of foliar application of *Trichodermaasperellum*, cow's milk *in natura* and different fungicides in the control of angular leaf spot, white mold and anthracnose, in the field, in the cultivar of bean pod butter in Ponta Grossa, Paraná, Brazil.

MATERIAL AND METHODS

The experiment was conducted at Fazenda Escola Capão da Onça belonging to the State University of Ponta Grossa, in the municipality of Ponta Grossa, PR, Brazil. The sowing of pod beans, cultivating low butter, occurred on February 5, 2010, carried out mechanically in rows spaced 0.80 m apart, at a depth of 0.04 to 0.05 m, with a population of 10 plants m^{-2} and 1000,000 ha^{-1} plants. The plots had 4.0 x 6.0 m (24.0 m^{-2}). The design used was a randomized block, with 7 treatments and 4 repetitions. The treatments used were: 1- witness (water), 2- weekly sprays of *T. asperellum* ($2.0x10^6$spores mL^{-1}); 3- weekly sprays of 20% cow's milk *in natura*; 4- weekly sprays of cow's milk *in natura* 20%, plus vegetable oil (1.6 mL, L^{-1}); 5- fungicide sprays azoxystrobin (80 g a.i. ha^{-1}); 6- sprays of mancozeb fungicide (2.0 kg a.i. ha^{-1}) and 7- spraying of diphenoconazole fungicide (0.3 L a.i. ha^{-1}). Fungicides were applied in accordance with MAPA recommendations. The spraying started when the first trefoil appeared. In total, nine applications were made with *T. asperellum*; nine sprays with 20% milk; nine sprays with 20% milk plus vegetable oil; six sprays of azoxystrobin fungicide; nine sprays of mancozeb fungicide and four sprays of diphenoconazole fungicide. 40 kg ha^{-1} of urea (30% N) was applied in the experiment 45 days after emergency (DAE). The experiments were irrigated by spraying when required. The crop was kept clean throughout its cycle, with weeding weekly, with a culture cycle of 76 DAE.

Five evaluations were carried out to produce pods and incidence or disease severity, at seven-day intervals, on 8 plants chosen at chance on the central lines of each plot. For the angular spot on the leaves, the percentage of leaf tissue attacked was estimated using a scale diagrammatic by DallaPria and Amorim (2010). With the severity of the angular spot, the area under the disease progress curve (AUDPC) was calculated, with the aid of the equation proposed by Shaner& Finney (1977). The intensity of anthracnose in the pods was estimated by a scale of grades proposed by Canteri, DallaPria& Silva (1999) and the values obtained transformed in Disease Index by McKinney's equation (1923). With the data of anthracnose index in the pods, it was also calculated the AUDPC. The incidence of white mold in the useful area of the plot was also evaluated at 37 and 44 DAE. In the production evaluation, the number and weight of pods of the eight plants in the central lines of the plot's useful area were determined. For analysis, the percentage data were transformed into arc sen $\sqrt{x / 100}$. The data were submitted to analysis of variance and the differences between the means, when significant, compared by the Tukey test at 5% probability in the SASM-Agri program.

RESULTS AND DISCUSSIONS

The following diseases occurred in the experiment: anthracnose, angular leaf spot and white mold (Table 1). Anthracnose and angular leaf spot were the diseases that attacked the culture of beans with greater intensity. The lowest AUDPC values for the angular leaf spot were observed in the treatments with the fungicides diphenoconazole, mancozeb and azoxystrobin, which reduced the disease's AUDPC by 81.70%, 65.49% and 40.32%, respectively. Kendra (2009), in India, showed the effectiveness of controlling the angular stain in the bean culture with the spraying of hexaconazole, propiconazole, mancozebe, diphenoconazole and carbendazim. Ito *et al*. (2000) also found good effectiveness in the control of angular stain with the mixtures of fungicide tank fluquinconazole and fentine hydroxide, azoxystrobim and chlorotalonil, tetraconazole and methyl thiophanate. Chemical control is the most effective method for controlling angular leaf spot (Table 1). However, treatments with *T.asperellum* and milk *in natura* and milk *in natura* plus vegetable oil, showed intermediate AUDPC values (between the witness and the fungicides). There was a reduction of 25.35 %, 28.87% and 35.04 % in the treatments with *T. asperellum* and milk and milk plus vegetable oil, respectively. According to Jasper; Pria& Silva (2009), the use of adjuvant (mineral oil) increases the efficiency of fresh cow's milk, however, it can form residue deposits on the leaves that vary with the dose of the adjuvant, which was not observed in this experiment. In the present study, there was no difference between fresh milk and fresh milk plus mineral oil for the control of the angular spot (Table 1).

T. asperellum and cow's milk *in natura*can be an alternative to control angular leaf spot, in the organic production of green beans, or can be used in association with chemical control, contributing to the rational use of fungicides. The incorrect use of fungicides both in relation to the use of high doses and in an excessive number of applications, has increased the selection pressure and consequently the emergence of resistant individuals, in addition to the contamination of the agro-ecosystem (Carniel*et al*., 2019; Newitt *et al*., 2019; Gabardo *et al*., 2020). Regarding anthracnose in the pods, all treatments reduced the disease's AUDPC, which varied was the intensity of the witness (Table 1). There was a reduction of 31.99%, 42.27 %, 31.99 %, 36.97 %, 67.15 % and 82.76 % in the treatments with *T. asperellum*, 20% milk, 20% milk more oil, azoxystrobin, mancozeb and diphenoconazole, respectively. The control obtained with *T. asperellum* was similar to that of milk (with or without the addition of oil) and azoxystrobin. The addition of oil did not improve the efficiency of cow's milk in reducing the AUDPC of anthracnose (Table 1).

Table 1. Area under the disease progress curve (AUDPC) of the angular leaf spot (*Pseudocercosporagriseola*), anthracnose (*Colletotrichumlindemuthianum*) and incidence (%) of white mold (*Sclerotiniasclerotiorum*) after different treatments. Ponta Grossa, PR, 2010

Treatment	Angular leaf spot	Anthracnose	White mold	White mold
	AUDPC	AUDPC	37 DAE[1]	44 DAE
1.Witness (water)	56,80 a*	63,62 a	0,25 a	0,25 a
2.*Trichoderma asperellum*	42,40 ab	43,27 ab	0,25 a	0,25 a
3. milk 20%	40,40 ab	36,73 bc	0,0 a	0,0 a
4.milk 20% + vegetable oil	36,90 ab	43,57 ab	1,0 a	0,0 a
5. azoxystrobin	33,90 abc	40,10 bc	0,0 a	0,0 a
6. mancozeb	19,60 bc	20,90 cd	0,0 a	0,0 a
7. difenoconazol	10,40 c	10,97 d	0,0 a	0,0 a
C. V. %	29,90	24,89	22,5	11,10

[1]DAE= days after crop emergence; * Means followed by the same letter in the column, did not differ by Tukey's test at 5% probability; C.V. = coefficient of variation.

Table 2. Weight of pods (grams) of beans (*Phaseolus vulgaris*) after application of different treatments, in five evaluations. Average of 4 repetitions. Ponta Grossa / PR. 2010

Treatment	1ªEval.	2ªEval.	3ªEval.	4ªEval.	5ªEval.
	37 DAE[1]	44 DAE	55 DAE	62 DAE	69 DAE
1. Witness (water)	18,4 a*	26,7 a	18,3 a	23,8 a	22,2 a
2. *Trichodermaasperellum*	18,1 a	25,0 a	16,7 a	26,3 a	21,4 a
3. milk 20%	18,1 a	24,3 a	16,1 a	23,9 a	20,5 a
4. milk 20% + vegetable oil	16,6 a	19,3 a	15,5 a	22,2 a	20,0 a
5. azoxystrobin	15,4 a	18,0 a	18,3 a	18,2 a	18,0 a
6. mancozeb	15,5 a	26,5 a	18,8 a	27,8 a	19,9 a
7. difenoconazol	16,8 a	34,3 a	19,9 a	23,7 a	22,8 a
C. V. %	38,3	35,6	21,8	29,9	37,7

[1]DAE= days after crop emergence; * Means followed by the same letter in the column, did not differ by Tukey's test at 5% probability; C.V. = coefficient of variation.

Table 3. Number of green bean pods (*Phaseolus vulgaris*), per plant, after different alternative treatments and with fungicides. Average of 4 repetitions. Ponta Grossa / PR. 2010

Treatment	1ªEval.	2ªEval.	3ªEval.	4ªEval.	5ªEval.
	37 DAE[1]	44 DAE	55 DAE	62 DAE	69 DAE
1. Witness (water)	2,7 a*	5,4 a	4,0 a	4,9 a	5,8 a
2. *Trichodermaasperellum*	3,3 a	6,1 a	3,9 a	5,7 a	5,3 a
3. milk 20%	3,2 a	6,0 a	3,7 a	5,4 a	5,9 a
4. milk 20% + vegetable oil	3,0 a	5,8 a	3,7 a	5,2 a	5,6 a
5. azoxystrobin	2,9 a	5,1 a	3,4 a	5,0 a	5,6 a
6. mancozeb	2,8 a	4,2 a	3,9 a	4,3 a	5,2 a
7. difenoconazol	3,4 a	5,9 a	3,9 a	4,5 a	5,5 a
C. V. %	42,3	43,0	42,3	27,4	37,4

[1]DAE=dias após a emergência da cultura; *Médias seguidas da mesma letra na coluna, não diferiram entre si pelo teste de Tukey a 5% de probabilidade; C.V.= coeficiente de variação.

Some species of Trichoderma showed great potential as antagonists in experiments conducted to control *Colletotrichum* spp. (Shovan*et al*., 2008; Kushwaha*et al*., 2014). In the present experiment, its efficiency in controlling *C.lindemuthianum* in field conditions was observed (Table 1). Therefore, this antagonist and cow's milk have the potential as an alternative to fungicide applications for anthracnose control. Bettiol, Astiarrraga and Luiz (1999) used aqueous solution with raw cow's milk, in concentrations of 5 to 50% to control powdery mildew (*Sphaerothecafuliginea*Schlecht. (Poll.)) In zucchini (*Cucurbita pepo* L.) and obtained 95 and 99%, respectively, of disease control under controlled conditions. In this experiment, with the pod bean culture, cow's milk 20% reduced anthracnose in 57.7% in relation to the control (Table 1). Zatarim*et al*. (2005), in a field study to evaluate the efficiency of several types of cow's milk on the powdery mildew of the pumpkin cultivar Piramoita, caused by *Sphaerothecafuliginea*, concluded that milk is a viable alternative in the control of powdery mildew, even after the beginning of infection, in field conditions and that its use in the form of raw milk is more efficient than in the form of long life. Among the fungicides tested for anthracnose, difeconazole showed the highest percentage of reduction in the disease's AUDPC (82.76%) (Table 1).

Diphenoconazole is a systemic fungicide belonging to the group of triazoles that interfere with ergosterol synthesis. The main lipid component of the fungal plasma membrane is ergosterol and its synthesis is accomplished through the catalytic action of acetyl-CoA. A reduction in the availability of ergosterol results in the rupture of the membrane and the leakage of ionic solutes, resulting in the death of the pathogen (FRAC, 2020). With regard to white mold, its occurrence was observed in the evaluations performed at 37 and 44 DAE (Table 1). All treatments tested did not control the disease. This fungus is normally controlled with synthetic chemical fungicides that pose risks to the environment and can be harmful to human health and can also induce resistance to pests (Sumida *et al*., 2018). McCreary *et al*., (2016), working with biofungicides and fungicides to control white mold in beans, with seed treatment obtained the highest AUDPC values in untreated control and treatment with biofungicide, the authors report that the most effective products in relation to disease suppression and response to yield there were two applications of boscalid, all rates of fluazinam and thiophanate-methyl, and two applications of the high rate of fluopyram + protioconazole. Biological control with the fungus *T. asperellum* did not reduce the severity of white mold (Table 1). According to Peterson &Nevalainen (2012), the presence of antagonistic microorganisms such as Trichoderma in the soil affects the long-term survival of sclerotia of *S. sclerotiorum*, making sclerotia unviable, thus affecting the survival of sclerotia, contributing to decrease their inoculum. Sumida *et al*., (2018), worked in vitro by dual culture tests, with two strains of *Trichodermaasperelloides* (T25 and T42) as a biocontrol agent for white mold disease in soybeans. Both strains of *T. asperelloides* exhibited a high potential for antagonism (60 to 100%). The present experiment was conducted in the field, with foliar spraying of the antagonist, which may justify the lack of response. Other researches have been carried out with the use of the bacterium *Bacillus amyloliquefaciens* in the biological control of white mold, Rahman *et al*., (2016) tested *B*. *amyloliquefaciens* subsp. *plantarum* against *S. sclerotiorumin vitro*, Bacillus isolates inhibited mycelial growth and suppressed sclerotia formation. In vitro seed bacterialization with Bacillus protected mustard seedlings (*Sinapsis alba* L.) up to 98% against *S. sclerotiorum*. In a pot experiment, the damage of mustard plants against the pathogen decreased by up to 90% after foliar spraying of Bacillus isolates. Although there is a difference between treatments for the severity of diseases (*P. griseola* and *C. lindemuthianum*) (Table 1). There was no effect of treatments on weight and number of pods (Tables 2 and 3), in any of the evaluations. Five harvests were made, the last harvest was made at 69 DAE. The weight of the pods varied from 15.4 to 34.3 grams in the evaluations performed at 37 and 44 DAE, respectively (Table 2). Regarding the number of pods per plant, it varied from 2.7 in the control at 37 DAE, to 6.1 in the treatment with *T. asperellum* at 44 DAE (Table 3).

Conclusion

Alternative products (*Trichodermaasperellum* and cow's milk*in natura*, with and without vegetable oil), reduced the área under the disease progress curve (AUDPC) of angular leaf spot (*Pseudocercosporagriseola*) and anthracnose (*Colletotrichumlindemuthianum*), but less AUDPC was observed in treatments with axoxystrobin, mancozeb and diphenoconazole. There was no effect of treatments for the incidence of white mold (*Sclerotiniasclerotiorum*) and for the number and weight of pods.

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