

Application effects of biofertilizers on blackleg disease and the development of potato plants

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Abstract

This study aimed to evaluate the effects of various forms of biofertilizer applications on blackleg disease, *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), as well as the growth and development of potato plants. The experimental design was randomized blocks with the following forms of biofertilizer applications: in-furrow at planting, via foliar, in-furrow at planting + via foliar. The control treatment consisted of foliar applications of a chemical fungicide. In total the experiment consisted of four treatments and five replications. The evaluation of fresh and dry mass of leaves, stems and tubers, the length of the biggest stem, the number of stems and tubers was made via extraction of plants 49, 61, 73 and 85 days after planting. The incidence of blackleg disease was obtained by reviews every 15 days with the aid of diagrammatic scale. The harvest was carried out 106 days after planting followed by an evaluation of tuber yield and classification of tubers. The assessed forms of application did not significantly affect the productivity of tubers, which varied from 32-36 t ha⁻¹. However, the forms of application were significantly correlated with the incidence of the blackleg disease. The results indicate that foliar applications of biofertilizers can considerably control the blackleg disease and positively affect the growth of potato plants.

Keywords: *Solanum tuberosum*, *Pectobacterium* sp., biofertilizers, tubers, foliar nutrition.

Abbreviations: AUDPC_Area under the disease progress curve; DAP_days after plantating; DML_ dry mass of leaves; DMS_ dry mass of stems; DMT_ dry mass of tubers; FML_fresh mass of leaves; FMS_fresh mass of stems; FMT_fresh mass of tubers; NPK_nitrogen, phosphorus, potassium; NS_ number of stems; NT_ number of tubers.

Introduction

The potato is a vegetable of a great economic importance. In Brazil it is planted on about 150,000 hectares, producing about 3.6 million tons of tubers (Ibge, 2014). In the management of potato plants special attention should be given to disease control due to the high susceptibility of this culture to a range of pathogens with potentially devastating consequences. Among these pathogens are pectobacteria, *Pectobacterium* spp, which cause blackleg disease (Tassa and Duarte, 2006). *Pectobacterium* survive in water, soil, and debris of infected crops in the region of rhizosphere of the host plant. These bacteria spread in potato crops by multiplication of contaminated seed tubers or plant injuries. *Pectobacteria* survive a wide range of temperatures and are viable for long periods of time. Therefore, chemical control has low efficacy and the development of new products is slow (Gomes et al., 2005). Thus, an integrated management of blackleg should be adopted by combining various measures from planting to the marketing of the tubers (Beriam, 2007; Mello et al, 2011). Among all control measures, chemical control is the most popular in Brazil through a combination of protective and systemic fungicides. However, due to the complexity of the epidemiology of the disease the results have often fallen short of the desired.

Because it is difficult for potato farmers to control the pathogen, there is a need for new integrated management practices aimed at minimizing economic damage caused by pectobacteria, and which are less harmful to the environment

as well as to the plant. Among the management methods, the use of biofertilizers is a promising alternative to control blackleg and to promote resistance in plants. The induced resistance has shown potential in the control of plant diseases triggered by microbial, natural and chemical agents. This resistance is expressed locally at the site of pathogen attack and systematically in uninfected parts of the plant (Mauch-Mami and Metraux, 1998). The defense mechanisms engage a combination of physical changes such as cell wall lignification, formation of papillae or induction of various proteins involved in metabolic processes. Advances in research of induced resistance in plants have helped develop new commercial products (Resende et al., 2006). Biofertilizers are liquid organic compounds used as additional source of micronutrients for plants. They can also boost pest and disease resistance in plants due to the presence of toxic substances in their compounds (Pinheiro and Barreto, 1996 ; Nunes and Leal, 2001). Biofertilizer applications in passion fruit produce plants which are higher, with increased diameter, number of branches, flowers and fruits and lower incidence of bacterial and fungal diseases (Collard et al., 2000). Nascimento et al. (2013) found better effectiveness of biofertilizers in relation to chemical fungicides to control anthracnose in tomato. Several studies have found satisfactory effects in disease control with the use of biofertilizers in plants such as watermelon, cucumber, pepper, peas and others (Murphy et al 2003; Barra et al.,

2009; Ling et al. 2011; Ramachandran et al. 2011; Aravindi et al. 2009).

In this context, this study aimed to evaluate the effects of various application forms of biofertilizers on blackleg as well as the growth and productivity of potato.

Results and Discussion

Incidence of blackleg disease

The effect of different biofertilizer application methods on the incidence of blackleg varied considerably relative to the chemical fungicide (Table 1). The application of chemical fungicide produced larger area below the progress curve of the disease in relation to the biofertilizer application methods, which was 31% higher than the in-furrow + foliar application and 40% higher than the foliar application alone (Table 1). Promising results with the use of biofertilizers were observed in the control of: (i) *Xanthomonas axonopodis* pv. *passiflorae* using Agro Mos™ biofertilizers in yellow passion fruit; (ii) powdery mildew in melon, (iii) alternaria in Mucorte tangerine and (iv) late blight and gray mold in tomatoes (Mesquita et. al 2005; Johnstson et al., 2005; Tosun, 2005; Junqueira et al. 2011).

Biofertilizers generally inhibit the action of pectobacteria in potatoes probably because they contain a copper biocomplex which is fully available for plants (Table 1 and 2). Copper regulates several enzymes involved in plant defense against infections, and also plays an important role in the production of antimicrobial compounds and in general resistance to disease (Ventura and Zambolim, 2012).

Management practices adopted during the development of potato plants in our experiment influenced the incidence of blackleg. Comparing the forms of application we observed that the foliar application was 29% more effective to control the progression of blackleg relative to the furrow application (Table 1). Copper, which induces the resistance of plants, as already mentioned, has little redistribution in phloem, which may explain the higher efficiency of foliar application because copper in the composition of biofertilizers it is in the form of chelate (Camilo, 2009). Another fact that can increase the efficiency of foliar application is the provision of nutrients which are present in the biofertilizers. These nutrients combined with the nutrients from the soil solution efficiently meet nutritional needs of potato plants during their growth and development (Rocha et al., 2009) (Table 1). The fact that the treatments with foliar applications produced healthier plants is relevant because such practices can be performed with an application of chemical pesticides, commonly used in potato crops.

Biofertilizers are products with considerably low environmental impact. They also induce the resistance of susceptible plants to a broad spectrum of pathogens (Roese et al., 2012). For this reason, their use may become a promising alternative to control blackleg in potatoes and mitigate environmental impacts caused by over-application of pesticides.

Fresh and dry mass of plants

The foliar applications produced better results regarding fresh mass of leaves stems and tubers, with verified statistical difference for fresh mass of leaves. Maintaining shoots of potatoes (stems + leaves) is essential to obtain high yields of tubers because photosynthates and mineral nutrients are

translocated to tubers (Filgueira, 2008). For this reason, the treatment with foliar application increased the fresh mass of tubers by 12%, and by 14% when the foliar application was combined with the in-furrow application. Similar results were found by Silva et al. (2011) evaluating the development of bean plants on the basis of biofertilizer application. They found higher efficiency of foliar application regarding plant height, the number of leaves per plant and the number of grains per plant comparing with an application to soil.

The assessment made during the sampling periods showed quadratic interaction of fresh mass of leaves and stems with maximum accumulation of 400.66 and 201.39 g plant⁻¹ 65 and 71 DAP, respectively. Fernandes et al. (2010) observed growth of stems and leaves of Asterix cultivar up to 69 DAP, a result similar to the present study. The fresh mass of tubers showed growth by 36.69 g plant⁻¹ each day after planting (Figure 1).

Dry mass of leaves, stems and tubers ranged from 30 - 40, 12 - 15 and 185 - 222 g plant⁻¹, respectively (Table 3). It can be seen that potato plants treated with biofertilizers showed higher dry mass of leaves, stems and tubers relative to plants treated with the chemical fungicide, which enhances the efficiency of biofertilizers to control blackleg (Table 3).

Dry mass of leaves and stems produced a quadratic function. It shows an accumulation of 40.9 and 17.48 g plant⁻¹ of dry mass of leaves and stems 67 and 65 DAP, respectively, and further decrease. With the growth of tubers, there is a change in the distribution of dry matter among the organs of the plant. The accumulation of dry matter in shoots is reduced, photoassimilates are drained to the tubers and the plant as a whole starts entering senescence phase (Coraspe-Leon et al, 2009; Silva et al, 2010). The tubers were the main drains 61 DAP. Dry matter accumulation in tubers increased reaching the maximum 254.15 g plant⁻¹ 85 DAP. Fernandes et. al (2010), Barcelos et. al (2007) and Yorinori (2003) also observed decreased accumulation in leaves and stems as potato plants reached maturity (Fig 2a).

Height, number of stems and tubers

Height, the number of stems and tubers were not influenced by the treatments (Table 3). The plants had 4 to 5 stems 71 - 75 cm long producing 11 to 13 tubers. The number of stems produced by each plant is directly associated with the size of seed tubers as well as sprouting after planting (Feltran and Lemos, 2005).

The number of stems and tubers increased during the potato growth cycle reaching the maximum of 5.45 and 13.15, respectively. The maximum length of stems (74.04 cm) was observed 76.58 DAP (Fig 3a). It is noteworthy that potato productivity is constrained by the number of stems per area from well-sprouted tubers or smaller spacing between tubers. More stems produce more tubers, but of smaller diameter (Bussan et al., 2007; Filgueira, 2008).

Yield and classification of tubers

There was no significant interaction among the application methods of biofertilizers, the fungicide, and the harvest period with respect to the growth, yield and classification of tubers (Table 4). Although no statistical difference was observed, it was noted that the number of tubers classified as Special was 21% higher with the foliar application of biofertilizer relative to the in-furrow application, reaching a difference of 3.74 t ha⁻¹ (Table 4). Importantly, in the

Table 1. The area under the disease progress curve (AUDPC) for different forms of management of blackleg disease.

Application	AUDPC
Fungicide via foliar	913.8 c
Biofertilizer in-furrow	770.7 b
Biofertilizer via foliar	543.6 a
Biofertilizer in-furrow + via foliar	624.7 a
Mean	713.2
CV (%)	9.63%

*Means followed by the same lowercase letters in the column do not differ by Tukey test at 5%

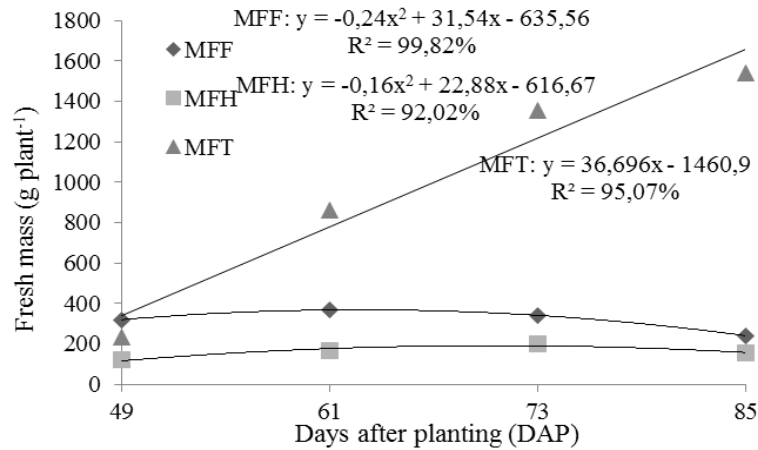


Fig 1. Fresh mass of leaves (FML), fresh mass of stems (FMS) and fresh mass of tubers (FMT) of potato plants with respect to days after planting.

Table 2. Fresh mass of leaves (FML), fresh mass of stems (FMS) and fresh mass of tubers (FMT) of potatoes for different forms of management of blackleg disease.

Application	FML	FMS	FMT
		g plant ⁻¹	
Fungicide via foliar	263,80 b	147,30 a	930.87 a
Biofertilizer in-furrow	308,15ab	161,36 a	901.67 a
Biofertilizer via foliar	336,65ab	164,37 a	1067.49 a
Biofertilizer in-furrow + via foliar	358,05a	164,37 a	1090.99 a
MAD	76,62	43,05	258.26
CV (%)	28,95	32,32	30.97

* Means followed by the same lowercase letters in the column do not differ by Tukey test at 5%. MAD: mean absolute deviation

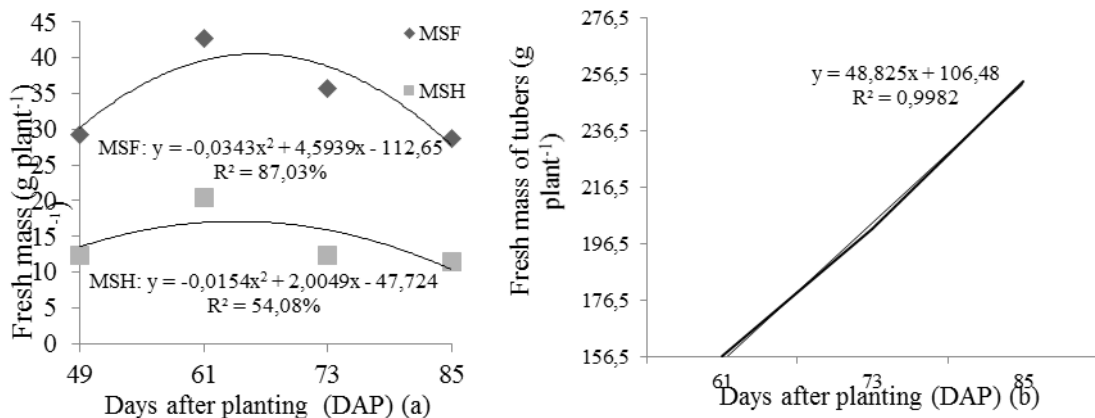


Fig 2. Dry mass of leaves, dry mass of stems (a) and dry mass of tubers (b) with respect to days after planting.

Table 3. Plant height, number of stems (NS) and tubers (NT), and dry mass of leaves (DML), stems (DMS) and tubers (DMT) of potatoes for different forms of management of blackleg disease.

Application	Height cm	NS	NT	DML Amount (g plant ⁻¹)	DMS	DMT
Fungicide via foliar	71.82 a	4.90 a	11.20 a	30.14 a	12.96 a	187.8 a
Biofertilizer in-furrow	74.20 a	5.05 a	12.20 a	30.46 a	15.62 a	205.3 a
Biofertilizer via foliar	75.40 a	4.85 a	11.50 a	34.98 a	14.47 a	200.7 a
Biofertilizer in-furrow + via foliar	71.95 a	4.85 a	12.55 a	40.73 a	13.60 a	222.8 a
MAD	5.88	1.42	3.16	12.38	4.26	66.72
cv (%)	9.60	34.64	31.89	43.46	36.03	33.51

* Means followed by the same lowercase letters in the column do not differ by Tukey test at 5%. MAD: mean absolute deviation

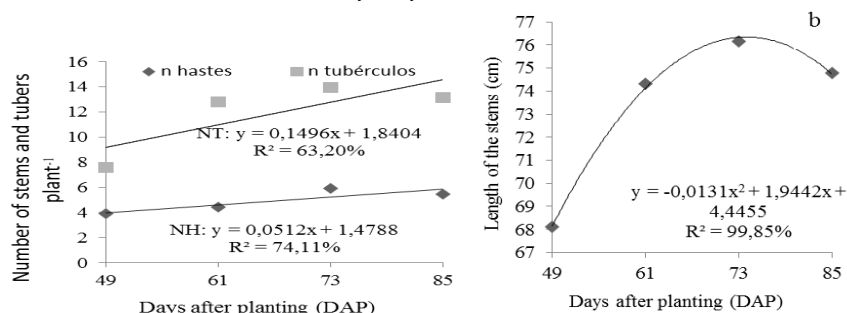


Fig 3. Number of potato stems and tubers (A) and height of the stems (B) of potato plants with respect to days after planting.

Table 4. Clasification, commercial and total productivity of potato tubers for different forms of management of blackleg disease.

Application	Special	Second	Diverse	Lollipop t ha ⁻¹	Discard	Jumbo	Total
Fungicide	16.20 a	17.68 a	1.80 a	0.31 a	4.25 ab	0.67 ab	40.93 a
In-furrow	13.30 a	14.60 a	3.00 a	0.20 a	5.45 a	1.01 ab	37.57 a
Via foliar	17.04 a	9.41 b	2.04 a	0.37 a	4.21 ab	1.54 a	34.62 a
In-furrow + foliar	12.78 a	18.37 a	1.48 a	0.22 a	2.61 b	0.09 b	35.55 a
MAD	4.95	3.97	1.79	0.31	2.77	1.31	7.38
CV (%)	20.61	16.33	15.03	19.23	14.51	26.12	12.27

*Means followed by the same lowercase letters in the column do not differ by Tukey test at 5%. MAD: mean absolute deviation

Table 5. Doses, number of applications and the period of biofertilizer application.

Application	Biofertilizer	Doses	Number of applications	Period DAP*
In-furrow	Compost-Aid®	3.0 kg ha ⁻¹	1	At planting
	Soil-Set™	2.0 l ha ⁻¹	1	At planting
Foliar	Compost-Aid®	1.5 kg ha ⁻¹	1	44
	CopperCrop™	0.3 l ha ⁻¹	2	66 and 76
	Soil-Set™	1.0 l ha ⁻¹	3	44, 66 and 76
	Agro-Mos™	1.0 l ha ⁻¹	3	44, 59 and 73

Table 6. Composition of biofertilizers.

-----Compost Aid®-----		
Bacteria	UFC g ⁻¹	Enzymes
<i>Lactobacillus plantarum</i>	1.5 x 10 ⁶	Cellulose
<i>Bacillus subtilis</i>	1.5 x 10 ⁶	Amylase
<i>Streptococcus faecium</i>	1.5 x 10 ⁶	
-----Soil Set™-----		
Minerals	%	g L ⁻¹
Sulfur	3.75	46.12
Copper	2.00	24.60
Iron	1.60	19.60
Manganese	0.8	9.84
Zinc	3.2	39.36
-----Agro Mos™-----		
Sulfur	2.28	28.04
Copper	3.00	36.90
Zinc	2.00	24.60
Organic Carbon	3.20	
-----Copper Crop™-----		
Nitrogen	4.09	5491
Copper	10.0	134.00

production of tubers a diameter greater than 42 mm is a desired class (Pereira and Campos, 1999).

Further, we observed that the foliar application produced less tubers classified as Discard, which corroborates the results found in this study regarding the development traits of potato plants (Tables 2 and 3). The use of biofertilizers to control blackleg in potatoes can be beneficial even for the storage period of tubers. During post-harvest, under favorable temperature and moisture conditions, there is a rapid decay of tubers with pectobacteria in a latent stage considerably reducing storage time (Paiva, 1997). The disease can spread quickly through contact between infected and healthy tubers, leading to losses before and after classification (Beneli et al. 2004). The overuse of chemical pesticides in agriculture promotes the contamination of natural resources, damaging the quality of life of rural and urban populations (Caporal and Costabeber, 2004). In this context, the consumer market has created a demand for healthier foods which are produced with minimal impact on the environment. Therefore, the incorporation of sustainable production practices is essential to meet these requirements.

Materials and Methods

Experimental site and the plant material

The experiment was conducted between February 16 and May 31, 2012 on Fazenda Lagoinha, Minas Gerais, located at 19°05' S and 47°20' W, at an altitude of 1050 m.

Before the deployment of the experiment soil samples were collected from the layer of 0-20 cm for chemical analyzes, conducted according to the methodology of Embrapa (2011). The soil presented the following characteristics: pH (H₂O) = 5.8; P=1.7 mg dm⁻³; K⁺= 27 mg dm⁻³; Ca²⁺= 9 mmol dm⁻³; Mg²⁺= 5 mmol dm⁻³; organic matter = 1.7%; base saturation = 50%; and cation exchange capacity = 3.12%.

The soil in the experimental area was prepared with two plowings followed by a harrowing. Potato seed tubers 30 to 40 mm in diameter of Cupid III cultivar were used.

Preparation and application of treatments

The experimental design was randomized blocks, with four treatments and five repetitions. The experimental plots were composed of 8 rows each 10 m long and 0.8 m apart, with a total area of 64 m². The treatments consisted of the following forms of applications: into the furrows at planting, via foliar, into the furrows at planting + via foliar, and the control with a foliar application of a chemical fungicide, Agrimicina® (oxitetraciclina + streptomycin sulfate), at a dose 2.4 kg ha⁻¹ (Table 5). The treatments with the application of biofertilizers into the furrows at planting were done using one application of Compost-Aid® (3.0 kg ha⁻¹) and one application of Soil-Set™ (2.0 l ha⁻¹).

Hilling of the plants to stimulate tuberization and fertilization with 300 g m⁻¹ of 10-00-10 NPK fertilizer applied into the furrows were done 30 DAP. The treatments with foliar application of biofertilizers consisted of one application of Compost-Aid® (1.5 kg ha⁻¹ 44 DAP), two applications of CopperCrop™ (0.3 l ha⁻¹ 66 and 76 DAP), three applications of Soil-Set™ (1.0 l ha⁻¹ 44, 66 and 76 DAP) and three applications of Agro-Mos™ (1.0 l ha⁻¹ 44, 59 and 73 DAP). The treatments with the in-furrow + via foliar applications were done using all rates and products described above. The composition of each biofertilizer is described in table 6. Plant samples were collected 49, 61, 73 and 85 DAP to evaluate the number of tubers and stems, the length of the

longest stem and fresh mass of leaves, stems and tubers. Dry mass was obtained by drying the samples in an oven with forced air circulation at 65°C for 96 hours.

The incidence of blackleg was obtained by ratings every two weeks from the first symptoms of the disease until the senescence of the plants. Quantification of the severity of the disease obeyed visual criteria and was defined as a percentage of shoots covered with symptoms of the disease. The evaluations were carried out using a diagrammatic scale (Godoy et al., 2006). The evaluations were performed on the 2nd, 3rd, 6th and 7th row of each parcel excluding 0.5 m from each end (total area 28.8 m²). The average severity values from each plot were used to calculate the area under the disease progress curve (AUDPC) (Campbell and Madden, 1990). The harvest took place 106 DAP. The tubers were collected from the fourth and the fifth row to evaluate the yield and to classify the tubers, disregarding 0.5 m from each end (total area 14.40 m²).

Statistical analysis

The data were subjected to the analysis of variance and regression (F test), at 0.05 significance. Productivity, classification and incidence of blackleg were evaluated comparing the means by Tukey test. Statistical analyses were done using SISVAR software (Ferreira, 2010).

Conclusions

The application of biofertilizers is effective in reducing the incidence of blackleg disease.

The application of biofertilizers via foliar alone or when combined with in-furrow application provide better development of potato plants.

The plants reached vegetative peak 69 days after planting in the production of dry matter in shoots and also increased dry matter accumulation in tubers.

Acknowledgements

To Fapemig for financial support of this research, to Rochetto group for providing the site for the experiment, and Improcrop for supplying biofertilizers.

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