Comunicata Scientiae
Horticultural Journal

ORIGINAL ARTICLE
published: 12 June 2022
https://doi.org/10.14295/CS.v13.3637

Joint application of fungicide and rhizobacteria on tomato seeds

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Abstract

The tomato (*Solanum lycopersicum)* is among the most consumed and industrialized vegetables. The treatment of seeds with fungicides and rhizobacteria stands out among the technologies to increase crop productivity and promote plant growth. This study aimed to evaluate the effect of the joint application of fungicide and rhizobacteria on the germination and vigor of tomato seeds. The trials were assembled in Completely randomized experimental design in a factorial scheme in a 10 x 2 + 1 (10 rhizobacteria, with or without fungicide and additional treatment: only fungicide), with four repetitions. The experiment assessed the following characteristics: water content, germination, first germination count, germination rate, seedling emergence, and accelerated aging. In general, the treatment of seeds with rhizobacteria stimulates the physiological quality of tomato seeds. The rhizobacteria Paenibacillus *lentimorbus-69 and Bacillus subtilis-34* were compatible with the fungicide favoring germination and vigor of the seeds compared to the fungicide treatment.

Keywords: *Solanum lycopersicum,* microbiolization, germination, vigor

Introduction

The tomato (*Solanum lycopersicum*) belonging to the Solanaceae family is widely consumed and cultivated in almost all regions of Brazil and other parts of the world (Filgueiras et al., 2017).

Brazil stands out as the ninth world's highest tomato production. The country produces 2.5% of world production and has approximately 64,400 hectares of planted tomatoes (IBGE, 2019).

The seeds' physiological quality is highly relevant in establishing a crop in the field (Nóbrega et al., 2018). In addition, the vigor of seeds can affect the establishment and influence the growth, development, and productivity of plants (Souza et al., 2009). Germination and vigor, in turn, depend both on genetic factors inherent to the seed and on cultural practices that can alter them. Among the cultural practices, inoculation with beneficial microorganisms has received particular attention in recent years because it is a low-cost and environmentally acceptable alternative, a far-reaching tool for establishing sustainable agricultural systems.

The benefits of inoculation of microorganisms in seeds can be indirect, through the suppression of pathogens (Sottero et al., 2006) or direct, by increasing the absorption of nutrients or inducing changes in the plant's hormonal balance (Ahmad et al., 2005). Such changes may promote germination induction (Schlindwein et al., 2008), rooting improvement, growth promotion, and the establishment of seedlings in the field after transplantation (Mafia et al., 2007). These benefits can be achieved through techniques such as seed microbiolization, cuttings, or seedlings.

Several studies highlighted the positive effects of rhizobacteria on seed germination and the increase in the productivity of several agronomic crops, such as corn or cucumber (Breedt et al., 2017; Islam et al., 2016).

Received: 06 October 2020
Accepted: 24 January 2022

Seed treatment with fungicides and insecticides is widespread in crop management: besides controlling important pathogens and insects transmitted by seed, it is an efficient practice to ensure adequate plants populations during sowing, even under adverse conditions. However, the widespread use of pesticides in agriculture can harm the soil microbiota. Ignoring the effect of some of these treatments on the seeds' physiological behavior, their influence on the establishment of the crop, its growth, and yield may lead to failure to take advantage of these new technologies with significant losses to the agricultural activity.

After a previous treatment with chemicals such as Captan®, Tomato seeds are marketed to protect seedlings during the emergence from soil fungi such as Pythium, Rhizoctonia, and others that cause toppling. The association of fungicides with rhizobacteria can add growth promotion attributes in plants and ensure seed quality. Some works in the literature have studied the compatibility between fungicides and rhizobacteria, displaying variable results (Soares et al., 2012; Coelho et al., 2013; Martins et al., 2018).

Considering this uncertainty, the present study aimed to evaluate the effect of the joint application of fungicide and rhizobacteria on the germination and vigor of tomato seeds.

Material and Methods

The trials were carried out in the Phytopathology and Microbiology Laboratory and the Laboratory of Seed Analysis of the Universidade Estadual de Montes Claros (Unimontes), Campus de Janaúba/MG. The region is located in the Brazilian semiarid biome, having the municipality the coordinates of 15°47'18" of south latitude and 43°18'18" of west longitude, with an altitude of 515 meters.

The experimental design was completely randomized in a 10 x 2 + 1 factorial scheme and four repetitions. The treatments consisted of 10 rhizobacteria, with or without fungicide, plus an additional treatment (only seeds with fungicide without rhizobacteria).

Multiplication of rhizobacteria

The rhizobacteria used were isolated from the rhizosphere of banana trees from different municipalities in the North of Minas Gerais and showed promising results in the development of banana trees and nematodes control (Ribeiro et al., 2012). The rhizobacteria were *Bacillus pumilus (isolates* 1, 3, 10, 60 and 76), *Paenibacillus lentimorbus (isolates* 17, 24, and 69), *Bacillus subtilis-34*, and *Bacillus* sp. -36.

The bacteria were kept in TSA medium (Triptic *Soy Agar) in* test *tubes for 24 hours at 28 °C*. The bacteria were transferred to Erlennmeyers flasks containing TSB medium (Triptic Soy Broth) and maintained at 100 rpm at 28 °C for 48 hours to obtain bacterial suspensions. The suspension was then centrifuged for 10 minutes at 10,000 rpm to precipitate the bacterial cells. Saline solution (0.85% Nacl, 99.5% minimum purity) was added to the supernatant under aseptic laminar flow chamber conditions. The concentrations of bacterial cells in the suspension were adjusted by the absorbance reading at a 540 nm wavelength (λ) in a spectrophotometer, at an 0.8 ABS optical density (OD).

Treatment of tomato seeds with rhizobacteria and fungicide

Tomato seeds were used to cultivate Santa Cruz Kada' without chemical treatment and a minimum 70%germination percentage. The seeds were disinfected in alcohol 70% for 1 minute and sodium hypochlorite at 5% for another 1 minute. After their disinfection, the seeds were rinsed three times in distilled water and placed on a sterile filter paper layer overnight in a laminar flow chamber for drying.

The chemical treatment of the seeds was performed by applying the fungicide Orthocide 750® in wettable powder (1.5 g of the active ingredient Captan 750 TS per kg seeds). First, the seeds were treated manually in plastic bags by adding 1% water. Then the wettable powder was added. After the bags' closure, these were shaken to distribute the product on the seeds uniformly, except for the control, which received no chemical product. Subsequently, the seeds were placed to dry on pre-sterilized filter paper overnight in a laminar flow chamber.

For seed microbiolization, the seeds were weighed and separated in plastic bags containing 5 grams of seeds each and stirred for 30 seconds for homogenization. The bags containing the seeds treated with the different bacteria remained at rest for 2 hours.

The rhizobacteria presence in the seeds was confirmed by placing five seeds of each treatment in tubes containing 0.85% NaCl saline and sonicated in an ultrasound bath to induce the bacterial cell wall lysis. After 30 seconds, the suspension was collected, and a 1:10 dilution was performed. Next, 1 ml of the obtained suspension was spread on TSA Agar plates Tryptic Soy agar). The plates were incubated at 28 °C for 48 hours. After this, the number of colony-forming units (CFU) was counted, presenting the data as CFU/seed.

Evaluation of the quality of tomato seeds treated with rhizobacteria or fungicide.

Water content - determined by the oven method at 105 3 °C, for 24 hours (Brazil, 2009), with four repetitions of 2.0 g of seeds each, the results being expressed as a percentage.

Germination test - after the treatment with fungicide and rhizobacteria, four repetitions of 50 seeds were arranged in gerbox plastic boxes, on two sheets of germitest® paper, moistened at 2.5 times the paper dry weight rate. The gerbox boxes were kept in a germinator at a 25 °C constant temperature. The results were obtained as the number of normal seedlings (with complete, developed, proportional, and healthy essential structures), determined at the seventh (first germination count) and fourteenth day after test assembly. Results were expressed as a percentage (Brazil, 2009).

The germination speed index (GSI) - was assessed together with the germination test, summing the number of seeds germinated each day, divided by the days between sowing and germination, according to the Maguire formula (1962).

Seedling emergence – This part of the experiment has been performed under laboratory environmental conditions (26 °C; 65 RH). The seeds have been sown at 1.0 cm deep in gerbox boxes containing washed and sterilized sand as substrate humified with water equivalent to 50% of the retention capacity. Humidity was maintained by daily sprayers irrigation (Brazil, 2009). The experiment used four repetitions of 50 seeds per treatment. The results were represented as percentage of emerged seedlings obtained on the fourteenth day after the test set. Seedlings with protrusion of the aerial part of 5 mm length or more were considered as emerged.

Accelerated aging - the seeds were placed in a single layer on an aluminum mesh coupled to a plastic gerbox box containing 40 mL distilled water. The boxes were capped and incubated in a *Biochemical Oxygen Demand* (B.O.D) incubator at 41 °C for 48 hours (Panobianco & Marcos Filho, 2001). After this incubation process, the seeds were submitted to the germination test described above. The results were described as the percentage of normal seedlings obtained on the fifth day after sowing.

The results were submitted to analysis of variance by the Sisvar program (Ferreira, 2011) and the means compared by the Scott-Knott test at 5%. The means of the witnesses were compared with the other treatments by the Dunett test at 5% significance.

Results and Discussion

In seeds treated only with rhizobacteria, the number of CFU (Table 1) per seed varied from 6.0 (*P. lentimorbus-17) to* 12.6 (*P. lentimorbus*-69). The seeds treated with the rhizobacteria and the fungicide Captan displayed between 1.0 (*Bacillus* sp.-36) to 10.6 (*B. pumilus*-60) CFUs. Seeds treated only with fungicide and those which received no treatment (absolute control) displayed no bacteria.

Table 1. Colony-forming units (CFU) per tomato seed as a function of fungicide and rhizobacteria treatments.

Rhizobacteria	Fungicide-free	With fungicide
Bacillus sp. -36	62	10
Bacillus pumilus-76	90	76
Bacillus pumilus-03	92	48
Paenibacillus lentimorbus-69	126	100
Bacillus pumilus-60	82	106
Bacillus subtilis-34	84	22
Bacillus pumilus-01	116	76
Paenibacillus lentimorbus-24	114	78
Bacillus pumilus-10	76	70
Paenibacillus lentimorbus-17	60	20
Fungicide	0	0

The initial seeds' moisture in the present study was 6.7%. The relatively low water content, as occurred in tomato seeds, gives greater reliability to the physiological quality tests results. On the other hand, high water levels may favor seed performance during the analysis (Amaro et al., 2015). The water content was not a determining factor in this study, its determination is important. This fact is important for the execution of the analyses as the seeds' longevity is strictly linked to the water content: it interferes directly in physiological processes, reducing seed quality, and affecting directly the vigor and even the germinative power (Marcos Filho, 2015).

The analysis of variance for rhizobacteria factors x fungicide treatment highlighted a significant interaction for physiological quality tests (germination, first germination count, germination rate, and accelerated aging) except for the seedlings' emergence, which did not present an effect for the interaction between the factors nor for the isolated factors.

The paired treatments analysis by the 5% Dunnett test for seed germination (Table 2) pointed out that, with the exception of *B. pumilus*-60 and *B. pumilus*-76, the treatment of seeds with other rhizobacteria in the absence of fungicide provided significant increases in the percentage of seeds' germination compared to those treated only with the fungicide, meeting the minimum acceptable standards for the production and marketing of tomato seeds: 70% germination (Brazil, 2013). The observed increments ranged from 34.09% (*B. pumilus*-76) to 77.27% (*Bacillus* sp.-36).

Table 2. Germination percentage of tomato seeds according to fungicide and rhizobacteria treatments.

Rhizobacteria	Fungicide-free	With fungicide
Paenibacillus lentimorbus-69	71.5 aA*	72.0 aA*
Bacillus subtilis-34	76.0 aA*	68.0 aA
Bacillus sp. -36	78.0 aA*	57.5 bB
Bacillus pumilus-1	74.0 aA*	52.0 bB
Paenibacillus lentimorbus-17	75.0 aA*	46.0 cB
Bacillus pumilus-60	68.0 aA	43.5 cB
Bacillus pumilus-76	59.0 aA	39.5 cB
Bacillus pumilus-03	75.0 aA*	38.0 cB
Bacillus pumilus-10	74.0 aA*	37.0 cB
Paenibacillus lentimorbus-24	72.0 aA*	27.5 cB
CV (%)	18.6	
Fungicide	44.0	

Means followed by the same lowercase letter in the column do not differ by the Scott-Knott test at the 5% significance level. Likewise, means followed by the same uppercase letter in the line do not differ by the Tukey test at 5%.* Significant difference to the control (seed with fungicide) by the Dunnett test at 5% significance.

This stimulus to germination, observed with the use of bacterial isolates, may associate with the production of hormones such as gibberellins and auxins (Araújo et al., 2005). Soares et al. (2012) obtained up to 88% germination in rice seeds treated with *Bacillus* sp rhizobacteria, representing a 14% increase to the control treatment.

The double seeds' treatment with the rhizobacterium P. *lentimorbus-69* and the fungicide Captan provided a significant 63.64% germination increase compared to the seeds treated with only the fungicide (Table 2). Furthermore, analyzing the factorial and fixing the rhizobacteria, proof that P. *lentimorbus-69 and B. subtilis-34* were not influenced by the fungicide application, demonstrating that such bacteria are compatible with the fungicide in the tomato seeds germination.

Fixing the fungicide factor pointed out no significant difference between the rhizobacteria in seed germination without chemical treatment (Table 2). However, the rhizobacteria P. lentimorbus-*17, B. pumilus-60,* B. pumilus-76, *B. pumilus*-03, *B. pumilus*-10, and *P. lentimorbus*-24 promoted the lowest germination percentages in the seeds with the fungicide presence.

The *5%* Dunnett test highlighted that, in a similar way to germination, the vigor of the seeds evaluated by the first germination count test (Table 3) was influenced by the treatments studied. The normal seedlings percentage obtained in the first germination count was significantly higher in seeds microbiolised with any rhizobacteria without the chemical treatment than those treated only with the fungicide. These data suggest that rhizobacteria favored the tomato seeds' vigor. The seeds treated with rhizobacteria and fungicide displayed no significant difference in the results of the first germination count compared to seeds treated only with fungicide.

Table 3. First germination count (%) of tomato seeds microbiolized with rhizobacteria and treated or not with fungicide.

Rhizobacteria	Fungicide-free	With Fungicide
Paenibacillus lentimorbus-69	34.0aA*	19.5 aB
Bacillus subtilis-34	37.5 aA*	17.5 aB
Bacillus sp. -36	49.0 aA*	10.0 aB
Bacillus pumilus-60	32.5 aA*	9.5 aB
Paenibacillus lentimorbus-17	45.0 aA*	3.5 bB
Bacillus pumilus-1	42.0 aA*	1.5 bB
Bacillus pumilus-76	30.0 aA*	1.5 bB
Paenibacillus lentimorbus-24	45.0 aA*	1.0 bB
Bacillus pumilus-03	44.0 aA*	0.0 bB
Bacillus pumilus-10	40.5 aA*	0.0 bB
CV (%)	42.1	
Fungicide	0.5	

By analyzing the factorial and fixing the rhizobacterial factor, the fungicide presence reduced normal seedlings' percentage in the first count, negatively affecting the seeds' vigor (Table 3). Fixing the fungicide factor highlighted no significant difference between the rhizobacteria studied without chemical treatment. In the presence of fungicide, P. *lentimorbus-69, B. subtilis-34, Bacillus* sp. *-36,* and *B. pumilus-60* rhizobacteria promoted higher normal seedlings percentages.

The *5%* Dunnett test verified that those seeds treated with all rhizobacteria without fungicide, and P. *lentimorbus-69 and B. subtilis-34* with fungicide provided

higher seeds' germination rates (Table 4). The higher indexes indicate that seeds germinated more quickly and uniformly, being, therefore, more vigorous.

Table 4. Germination speed index (GSI) of tomato seeds microbiolized with rhizobacteria and treated or not with fungicide.

Rhizobacteria	Fungicide-free	With fungicide
Paenibacillus lentimorbus-69	8.1 aA*	6.4 aB*
Bacillus subtilis-34	8.1 aA*	6.1 aB*
Bacillus pumilus-60	7.4 aA*	5.3 aB
Bacillus sp. -36	8.8 aA*	4.9 bB
Bacillus pumilus-76	7.6 aA*	4.8 bB
Paenibacillus lentimorbus-17	8.6 aA*	4.7 bB
Bacillus pumilus-03	8.7 aA*	4.2 cB
Bacillus pumilus-10	8.4 aA*	4.0 cB
Bacillus pumilus-1	8.2 aA*	3.5 cB
Paenibacillus lentimorbus-24	8.3 aA*	3.4 cB
CV (%)	12.3	
Fungicide	4.1	

Means followed by the same lowercase letter in the column do not differ from the Scott-Knott test at the 5% significance level. Means followed by the same uppercase letter in the line do not differ by the Tukey test at 5%. * Significant difference to the control (seed with fungicide) by the Dunnett test at 5% significance.

Fixing the rhizobacterial factor highlights that the fungicide reduced the germination rate of the seeds in all seeds (Table 4). The absence of fungicide caused no GSI values differences among the rhizobacteria studied. However, when the seeds received chemical treatment, the rhizobacteria *P. lentimorbus-69, B. subtilis-34*, and *B. pumilus-60* stood out, increasing the seed germination speed, indicating that these rhizobacteria provided a faster initial development of tomato seedlings. Seedlings that emerge faster can become less vulnerable to adverse conditions of the medium by spending less time in the initial stages of development (Moreira et al., 2015).

In the evaluation of accelerated aging (Table 5), the 5% Dunnett test shows that in comparison to seeds treated only with fungicide, seed microbiolization with *P. lentimorbus*-17, *B. pumilus*-76, *P. lentimorbus*-24, *B. pumilus*-10, *Bacillus* sp. -36, *B. pumilus*-03 without fungicide, and *B. subtilis*-34 with fungicide promoted seed germination after accelerated aging compared to fungicide treatment only.

Table 5. Accelerated aging (%) of tomato seeds microbiolized with rhizobacteria and treated or not with fungicide.

Rhizobacteria	Fungicide-free	With Fungicide
Paenibacillus lentimorbus-17	58.0 aA*	1.0 cB
Bacillus pumilus-76	47.5 aA*	4.0 cB
Paenibacillus lentimorbus-24	40.0 aA*	4.5 cB
Bacillus pumilus-10	39.0 aA*	4.0 cB
Bacillus sp. -36	31.5 bA*	6.0 cB
Bacillus pumilus-03	27.0 bA*	3.5 cB
Bacillus subtilis-34	24.0 bA	32.5 aA*
Paenibacillus lentimorbus-69	23.0 bA	18.0 bA
Bacillus pumilus-1	22.0 bA	4.5 cB
Bacillus pumilus-60	21.5 bA	16.5 bA
CV (%)	44.5	
Fungicide	5.0	

Means followed by the same lowercase letter in the column do not differ from the Scott-Knott test (1974) at the level of 5% significance. Means followed by the same uppercase letter in the line do not differ by the Tukey test at 5%. *Significant difference to the control (seed with fungicide) by Dunnett's test at 5% significance.

Factorial analysis shows that the germination of aged seeds was not affected by the presence of fungicide on the bacteria *B. subtilis*-34, *P. lentimorbus-60,* and *B. pumilus-60,* demonstrating that such bacteria are compatible with the fungicide, as reported above. However, the fungicide treatment affected germination after aging in the other rhizobacteria.

The results stress that the treatment of seeds with rhizobacteria without the fungicide displayed the highest efficiency, pointing out the beneficial effect of bacterial treatment on the seeds' physiological quality. Furthermore, several studies claimed improvements in germination, emergence, and production of crops from seeds treated with rhizobacteria (Islam et al., 2016; Rudolph et al., 2015; Breedt et al., 2017).

P. *lentimorbus-24, Bacillus* sp-36, *B. subtilis-34, and B. pumilus*-60 (Silva et al., 2017) isolates produce lipases: enzymes involved in the germination process. During germination, lipases hydrolyze triglycerides to form glycerol and fatty acids; some of these compounds

are later transformed into sugars, releasing energy for germination (Coelho et al., 2013).

In general, the fungicide application negatively influenced the rhizobacteria performance in all variables evaluated. Some studies reported the negative effect of treating seeds with fungicides on rhizobacteria such as *Bradyrhizobium japonicum* in soybeans, reducing nodules' number and dry matter (Costa et al., 2013).

However, it is worth mentioning that fungicide application is crucial in controlling fungi in seeds. In this sense, the rhizobacteria, *P. lentimorbus-69,* and *B. subtilis-34,* stood out in the seeds' treatment as being compatible with the fungicide and increasing germination and vigor of tomato seeds. Furthermore, the compatibility between the application of fungicides and rhizobacteria in seeds has been documented on the production of crops in disease control (Martins et al., 2018) and nodulation by *Rhizobium tropici* in beans (Araujo et al, *2007)*.

Conclusions

In general, the treatment of seeds only with rhizobacteria stimulates the physiological quality of tomato seeds.

The association of the rhizobacteria P. *lentimorbus* and *B. subtilis-34* promotes an increase in the physiological quality of tomato seeds to fungicide treatment.

Acknowledgments

The authors thank the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES; Financial Code 001) for granting of scholarships.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.