

In vitro Sensitivity of Three *Alternaria* Spp. Vegetable Isolates to Agents of Biological Control and Fungicides



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Abstract

The *in vitro* responses of three *Alternaria* spp. isolates to 16 biological control agents and eight fungicides were evaluated. A split plot experimental design was used, with a factorial arrangement for each pathogen. Factor A was to fungi isolates and Factor B to control agents. The mean comparisons were by Tukey test. The *Alternaria* spp. isolates were sensitive to Dicloran, Boscalid, Cyprodinil-Fludioxonil, and Tebuconazole fungicides, because they not allowed any develop of mycelial growth at 264 hours after confrontation. The biological control agents with the best fungistatic effects against to the isolates of *Alternaria* spp. were four Trichoderma agents, they significantly affected the mycelial growth at 264 hours after confrontation. There were other biological agents such as *Bacillus subtilis*, *B. cereus*, *B. megaterium* (Rhizobac combi), *Trichoderma harzianum* (Triko root) and *Gliocadium virens* (Soil gard), but with the lowest or null fungistatic effects.

Keywords: Onion; Pepper; Tomato; Bio fungicides; Chemical control

Introduction

Guanajuato State has an exceptional potential for horticulture; it is in among the first places in the production of 70 species of agricultural importance, some of the most outstanding are onions, garlics (*Allium sativum* L.), lettuces and potato [1]. However, diseases are one of the most risk factors in the vegetables production. Since few years ago the fungal diseases have caused major economic losses in the production of different vegetable species in Mexico as well as the rest of the world [2]. They have increased their presence in the productive areas of the country, with the Bajío as one of the zones with the highest incidence in that kind of diseases [3]. The most farmers use fungicides as a disease control method, but its use in excess might lead to pathogens resistance, while generating toxic residues in foods and the environment, thus becoming a risk to human health [2]; this has led to a search for new fungicides from other organisms (bio fungicides) [4]. The biological control agents (BCA) of phytopathogens is an efficient and affordable alternative, risk-free in the face of numerous and increasing problems by the excessive agrochemicals use [5]. Based on the problem described above, the aim of this work was to determine the *in vitro* sensibility to biological control agents and isolated fungicides of two *Alternaria* species in onion, pepper and tomato plantations. The investigation was carried out in commercial vegetable fields from Abasolo and Penjamo, Guanajuato and in the Plant Pathology Lab of the Life Sciences Division of the Irapuato-Salamanca Campus of the University of Guanajuato.

Obtaining Isolates

A. porri was isolated from symptomatic onion plants. *A. solani* was isolated from symptomatic pepper and tomato plants. From each isolate, a part of the tissue with disease symptoms was taken; all samples were disinfected and planted in Potato Dextrose Agar (PDA) for eight days at 20±2 °C at room temperature. BCA and fungicides evaluated (Table 1) were applied based on commercial recommendations.

In vitro Sensibility Test

The BCA and fungicides were poured into Petri dishes. When the agar solidified, disks, 1 cm in diameter were placed in the center of the dish from the periphery of the colonies obtained from each isolation and incubated at 20±2°C.

Evaluation of Mycelium Growth

The colony diameter was measured in two directions – the longest and shortest length – and mycelial growth was obtained as the average of both values at 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, and 264 hours after placing the disk with the fungi on the edge of each Petri dish.

Statistical Analysis

A split plot experimental design was used, with a factorial arrangement with three repetitions. There was a factorial

arrangement: factor A was assigned to fungal isolations, which three levels; factor B was assigned to the BCA and fungicides, which 25 levels. This gave a total of 75 treatments. The multiple comparisons of averages were carried out by Tukey's test ($P \leq 0.05$).

Results and Discussion

Alternaria porri and *Alternaria solani*

The three isolates of *Alternaria* were sensitive to four of the eight fungicides (Dicloran, Boscalid, Cyprodinil-Fludioxonil, and Tebuconazole), considering this as those which did not develop a mycelial radial average growth (Mrag), 264 hours after the confrontation (Table 1). Contrasting results were found in the behavior of *Alternaria* spp. towards the fungicides reported by [6], who showed the presence of insensitivity of the pathogen towards the iprodione. The biological control agents with the greatest fungistatic effects towards *Alternaria* spp. isolates, that is, those which caused the least Mrag 264 hours after confrontation, were: *Trichoderma harzianum* (Biotricho H), *Trichoderma* sp. (Trichoderma), *Trichoderma harzianum* (Natu control), *Trichoderma viride* (Esporalis) with 1.15a, 1.16a, 1.26a,

and 2.06 c, respectively; in comparison to *Bacillus subtilis*, *B. cereus*, *B. megaterium* (Rhizobac combi), *Trichoderma harzianum* (Triko root) and *Gliocadium virens* (Soil gard), with 7.21k, 7.38k and 8.42l, respectively, which had the lowest or null fungistatic effects towards the isolates (Table 1). In relation to the response of *Alternaria* spp. towards the biological control agents evaluated, a wide variability was found, observing that the biological products to which there was a higher sensitivity to *in vitro* were those formed with *Trichoderma* spp.; the results obtained in this study coincide with those by [7]; the cause of this greater beneficial effect may be the bio controlling action of *Trichoderma* spp., in which there have been several forms of action described that regulate the development of phytopathogenic fungi. Among these, the main ones are the competition for space and nutrients, micro parasitism and antibiosis, which have a direct action on the phytopathogenic fungi [8]; it is also known that *Trichoderma* spp. displays other mechanisms, the bioregulatory action of which is indirect, such as the biochemical defense mechanisms, that consist in the deactivation of enzymes of the phytopathogenic fungi during the infection process [8]; this could explain the high inhibitory percentage of mycelial growth of *Alternaria* spp.

Table 1: *In vitro* Mycelial radial average growth (Mrag) of three *Alternaria* spp. isolates obtained from onion, pepper and tomato, 16 BCA treatments and eight fungicides.

No.	Common name	Tradename	Mrag (cm)				
			168 Hours	192 Hours	216 Hours	240 Hours	264 Hours
1	Dicloran	Botran	1.00 a				
2	Benomilo	Blindaje 50	2.05 d	2.32 d	2.58 ef	2.87 g	3.18 e
3	Boscalid	Cabrio	1.00 a				
4	Clorotalonil-Cymoxanil	Strike	1.34 b	1.40 b	1.50 c	1.57 cd	1.65 b
5	Ciprodinilo-Fludioxonilo	Swish	1.00 a				
6	Tebuconazole	Tebucur	1.00 a				
7	Iprodione	Rovral	1.22 ab	1.27 ab	1.37 bc	1.48 bc	1.62 b
8	Clorotalonil	Trevanil	1.68 c	1.76 c	1.82 d	1.86 de	1.95 bc
9	<i>Actinomadura viridis</i> <i>B. subtilis</i> , <i>Streptomyces</i> sp.	Baktillis	3.70 gh	4.11 hi	4.50 hi	5.01 ij	5.51 g
10	<i>T. harzianum</i>	Natucontrol	1.26 ab	1.26 ab	1.26 abc	1.26 abc	1.26 a
11	<i>Streptomyces lydicus</i>	Actinovate	4.43 j	4.94 k	5.46 k	5.97 m	6.44 j
12	<i>Bacillus</i> sp., <i>Glomus</i> sp.	Soil cure	3.83 hi	4.28 ij	4.76 ij	5.21 jk	5.68 gh
13	control	control	3.45 fg	3.90 gh	4.31 gh	4.72 hi	5.15 f
14	<i>B. subtilis</i>	Bacillus subtilis	4.02 i	4.47 j	4.88 j	5.45 kl	6.00 hi
15	<i>T. harzianum</i>	Biotricho-H	1.15 ab	1.15 ab	1.15 ab	1.15 a	1.15 a
16	<i>Trichoderma</i> sp.	Trichoderma	1.16 ab	1.16 ab	1.16 ab	1.16 ab	1.16 a
17	<i>T. viride</i>	Esporalis	2.06 d	2.06 d	2.06 d	2.06 e	2.06 c
18	<i>B. subtilis</i>	Probacil	3.62 fgh	3.95 gh	4.38 gh	4.77 hi	5.16 f
19	<i>T. harzianum</i>	Triko root	5.24 l	5.80 l	6.30 l	6.92 n	7.38 k
20	<i>B. subtilis</i>	Natu bac-s	3.37 f	3.78 g	4.16 g	4.57 h	5.01 f
21	<i>B. subtilis</i>	Serenade max	2.48 e	2.65 f	2.78 f	2.91 g	2.98 e
22	<i>B. subtilis</i>	Probac bs	4.41 j	4.84 k	5.31 k	5.76 lm	6.21 ij
23	Micro-organisms	BPG-Plus	2.38 e	2.40 ef	2.41 e	2.41 f	2.41 d
24	<i>B. subtilis</i> , <i>B. cereus</i> , <i>B. megaterium</i>	Rhizobac combi	4.93 k	5.55 l	6.14 l	6.70 n	7.21 k
25	<i>Gliocadium virens</i>	Soil gard	5.66 m	6.40 m	7.25 m	7.98 o	8.42 l

Each value represents the average value of three isolates. Data with different letter within columns are significantly different by Tukey test at $P \leq 0.05$.

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