

COMPARATIVE EVALUATION OF *Trichoderma* spp. AND *Bacillus subtilis* IN CONTROLLING POWDERY MILDEW DISEASE OF GRAPE

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ABSTRACT

Five different biocontrol preparations and the commercial bioagent Plant guard varied in their effects in controlling powdery mildew of grapes, incited by *Uncinula necator* (Schw.) Burr.

A mixture of *Trichoderma harzianum* isolate number 5 and isolate number 28 with *T. hamatum* isolate number 17 proved to be more effective than the single isolates and even was the most potent of the tested bioagents. However, the least potentiality was recorded with *Bacillus subtilis* application.

Number of sprays increased the disease control up to three sprays, whereas the fourth spray did not increase the efficiency of the bioagent.

Concerning with the bioagent concentrations, five various doses had been tested and the most effective was 250 ml of the bioagent preparation in 100 liter of water. No great differences were obtained if the concentration was increased to 350 ml/100 L water.

Two different scales had been applied for disease detection; disease incidence and disease severity. The latter scale was more reliable. In general, all the bioagent treatments gave similar or near similar potentiality while great differences occurred with the disease incidence.

INTRODUCTION

Grape is one of the most important fruit trees grown in many parts of the world. The importance of the grape is not only due to the consumption of the fresh fruits but also due to the processing industries depending on, like wines, juices, dehydrated and caked fruits.

Powdery mildew is the most destructive disease attacking grape cultivations in Egypt resulting heavy losses if not well controlled. This disease have been known since centuries and sulfur was one of the different materials have been applied against it, and still successfully used Gaikwand and Karkil (1994). Yet many fungicides have been developed for powdery mildew control. Patil *et al.* (1993) used different synthetic fungicides with various chemical groups. They reported that the most effective control was obtained by hexaconazol (as Anvil). Redl *et al.* (1994) used some fungicides that inhibited the sterol biosynthesis and mentioned that the efficacy was increased when the spray mixture was enriched with CO₂. Reuveni and Reuveni (1995) compared the effect of mono and dipotassium phosphate and potassium hydroxide with the effect of some systemic fungicides. They found that phosphate solutions were as effective as the fungicide Dorado 480EC (pyrifenoxy). Minuto *et al.* (1998) used a mixture of the fungicide Topas (penconazol) and sulfur to reduce disease severity.

Since last decades there have been a considerable increasing demand to search for other compounds or materials that would be more safe to the environment and the public health too. Hence, certain biocontrol agents have been investigated and some of them had been developed. *Trichoderma* spp. and *Bacillus subtilis* were the most promising agents that produced commercially and used mostly against soil and seed borne diseases. Nevertheless, they show good results against some foliage diseases. Tronsmo and Dennis (1977) reduced the effect of *Botrytis cinerea* in the field using *T. harzianum*. Baker *et al.* (1985) used *B. subtilis* to control rust disease of beans. Abd-El-Moity *et al.* (1997) controlled downy mildew and purple blotch of onion and garlic under field conditions with *T. harzianum*.

The present research was designed to evaluate the effect of different biocontrol agents in controlling powdery mildew of grape particularly at an organic farm, where the use of agricultural chemicals is not allowed.

MATERIAL AND METHODS

The present study was carried out in registered organic farm belongs to SEKEM company, located at El-Adlia, Sharkeia Governorate, during 1998 and 1999 seasons.

Five grape trees, Thompson var., 2 years old, were considered as one replicate. Three different isolates were identified as *Trichoderma harzianum* (isolate T5), *Trichoderma harzianum* (isolate T28) and *Trichoderma hamatum* (isolate T17) were kindly obtained from Abd-El-Moity, Tawfik, Plant Pathology Res. Institute, Agric. Res. Center, Giza, Egypt. An isolate of the bacteria *Bacillus subtilis* was also obtained from the same source. The commercial product "Plant guard" contains wild Egyptian isolate of *Trichoderma harzianum* was obtained from El-Nasr Company for Biocides and Fertilizers, El-Sadat City, Egypt. This commercial product contains 30×10^6 spores/ml *Trichoderma* spores/ml of the product.

Propagation and preparation of the bioagents:

All *Trichoderma* isolates were grown on liquid gliotoxin fermentation medium "Brian and Hemming (1945)" under complete darkness conditions to stimulate toxin production (Abd-El-Moity and Shatla, 1981). After 12 days, all cultures were blended using electric blender for 3 min. Blended suspensions were adjusted, using sterile water to contain 30×10^6 spore/ml. *Trichoderma* mixture was prepared by mixing equal parts of each isolate. Also mixture was adjusted to contain 30×10^6 spores/ml. *Bacillus subtilis* was grown on nutrient broth for 48 hrs. The number of cells was also adjusted to 30×10^6 c.f.u/ml. The following experiments were carried out during 1998 and repeated in 1999.

The experiments:

Two linked experiments were carried out, the first was designed to evaluate the efficiency of the tested microbial fungicides when applied at the rate of 200 ml/100 liter of water and sprayed upon the trees as follows:

- i. Trees subjected to one spray and not more.

- ii. Trees subjected to two sprays at 15 days intervals.
- iii. Trees subjected to three sprays at 15 days intervals.
- iv. Trees subjected to four sprays at 15 days intervals.
- v. Check trees were sprayed with water only.

The second experiment was planned to evaluate the efficiency of different concentrations of the tested bioagents. Five concentrations were applied; 50, 150, 200, 250 and 350 ml of the bioagents to 100 liters of water. The check trees received only water. Two successive sprays were carried out with an interval of two weeks.

The spray started in the two experiments on March 15, 1998 and 1999. The numbers of trees in each treatment was 5 and the replicates were also five.

The treatment with the concentration of 200 ml/100 liter water in the second experiment was considered a double purpose treatment that served in the first experiment as well.

At ripening stage, end of June, 100 leaves were randomly taken from each replicate and the percentage of diseased leaves represented the disease incidence. The disease severity was estimated according to the infected area on the given leaves. The formula developed by Micheal (1962) and Townsend and Heuberger (1943) were applied using disease index of five categories. The evaluation of the bioagents were detected as relative disease reduction compared to the check treatment as follows:

$$\% \text{ RDR} = \frac{\text{DI or DS in the check} - \text{DI or DS in the treatment}}{\text{DI or DS in the check}} \times 100$$

Where:

RDR = Relative disease reduction

DI = Disease incidence

DS = Disease severity

RESULTS

Effect of number of sprays on the disease incidence:

Results shown in the Table (1) and Fig (1) indicate that, at the season of 1998, the mixture of the Trichoderma (T28 + T17 + T5) gave the most relative reduction of the disease incidence after three successive sprays recording 87.2% reduction. Two sprays gave reduction of 79.5%. There was no difference between the reduction after three and four sprays as the figure was 87.2% in both treatments.

All bioagents when used singly were almost less effective than the mixture spray. The bioagents started to reduce the disease incidence after one spray and the mixture gave the most reduction (53.9%), and *T. hamatum* T17 followed it as it gave 43.6% reduction. *B. subtilis* was the least potent with one spray as well as two and three sprays, but its effectiveness after four sprays was almost equal to the other single bioagents with unworthy differences among them. Plantguard was more effective than *B. subtilis* except in case of four sprays.

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Fig 1

Table (1): Effect of different number of sprays with the bioagents on the disease incidence.

The bioagents	% Relative reduction of the disease incidence							
	1998				1999			
	No. sprays				No. sprays			
	1	2	3	4	1	2	3	4
<i>T. harzianum</i> (T28)	35.9	59.0	71.8	70.5	32.3	50.0	48.4	53.2
<i>T. hamatum</i> (T17)	43.6	69.2	69.2	66.7	21.0	67.8	48.2	51.6
<i>T. harzianum</i> (T5)	29.5	50.0	66.7	64.1	3.2	51.6	50.0	46.8
T28 +T17 +T5	53.9	79.5	87.2	87.2	53.2	82.3	87.1	83.9
<i>B. subtilis</i>	23.0	42.3	57.7	68.0	19.4	17.8	27.4	53.2
Plantguard	35.9	56.4	60.3	62.8	27.4	37.1	40.3	54.8

% Disease incidence without any spray (check = 78% (1998)
= 62% (1999)

On the year 1999, the results indicated also the superiority of the mixture treatment even with one spray as it gave 53.2% reduction and after two sprays the reduction was the most (82.3%), that no increase in the percent of the relative reduction thereafter with three or four sprays. Then, *T. hamatum* (T17) showed the highest reduction with two sprays (67.8%) but with three or four sprays the reduction was markedly low (48.2% and 51.6%) respectively, meanwhile on the season 1998, the reduction was almost the same with the different numbers of spray. *T. harzianum*, T28, and T5 expressed moderate effectiveness even with four sprays, a case that did not happen in the experiment of 1998 where the reduction on the four sprays reached successively 70.5 and 64.1%. *B. subtilis* showed its high potentiality on the treatment of four sprays, but the reduction was, high as compared with single bioagents, not high at all. The commercial product "Plantguard" acted similarly to the bioagent *B. subtilis* with four sprays despite the weak superiority happened to occur with other number of spray; one, two and three sprays.

Effect of number sprays on the disease severity:

Table (2) and Fig. (2) point out that the best reduction of the disease severity took place with the mixture (T28 + T17 + T5). A high superiority started with the two sprays recording 78.2% on the season of 1998, and 85.4% on the season 1999. The maximum reduction was reached by four sprays on the season 1998, but without worthy differences than three sprays as the reduction was respectively 94.6% and 93.0%. On the season 1999 the most reduction occurred with three sprays (97.9%) that was almost the same as four sprays treatment (97.5%). All single bioagents, reached their highest potentiality, with almost similar reduction, on the sprays number three and four with unworthy differences among those two sprays. All treatments started their meaningful effectiveness on the disease severity with two sprays, and the relative reduction on the season 1998 ranged between 40.7% and 78.2%, while range on the season 1999 was between 44.0% and 85.4%.

Plantguard, the commercial product of *T. harzianum* and *B. subtilis* expressed high potency on the disease severity as the rest bioagents despite the low activity occurred on the disease incidence (Table 1).

Table (2): Effect of number of sprays of the bioagent on the disease severity.

The bioagents	% Relative reduction of the disease severity							
	1998				1999			
	No. sprays				No. sprays			
	1	2	3	4	1	2	3	4
<i>T. harzianum</i> (T28)	6.6	51.2	88.7	87.2	34.5	44.0	92.2	91.5
<i>T. hamatum</i> (T17)	3.8	63.5	88.8	89.1	14.1	71.9	91.5	90.3
<i>T. harzianum</i> (T5)	0.0	40.7	87.5	87.5	0.0	60.2	92.2	90.7
T28 +T17 +T5	35.0	78.2	93.0	94.6	50.5	85.4	97.9	97.5
<i>B. subtilis</i>	16.7	45.8	81.5	80.8	8.9	51.6	85.6	84.6
Plantguard	37.5	70.5	91.8	92.8	61.6	66.4	92.6	92.4

Disease severity in the check treatment without any spray = 62.4% (1998)
= 52.7% (1999)

Effect of different concentrations of the bioagents on the disease incidence:

The results presented in the Table (3) and Fig. (3) show that there was an obvious increase in the relative reduction on increasing the bioagent concentrations but it was nearly negligible with *B. subtilis* particularly on the experiment of 1999, as the given reduction was 0.0, 21.0, 17.8, 19.4 and 19.4% respectively with the concentrations of 50, 150, 200, 250 and 350 ml per 100 liter of water. The best effective concentration on 1998 was 250 ml/100 liter of water for the bioagents *T. hamatum* T17 (82.1%), *T. harzianum* T5 (84.6%) and the mixture T28 + T17 + T5 (87.2%). With *T. harzianum* T28 and Plantguard, this concentration came in the second range giving 71.8% reduction at both treatments. *B. subtilis* was the least effective at all concentrations, where the relative reduction ranged between 7.6% and 42.3%.

Table (3): Effect of different concentrations of the bioagents on the disease incidence.

The bioagents	% Relative reduction of disease incidence									
	Concentrations ml/100 liters of water									
	1998					1999				
	50	150	200	250	350	50	150	200	250	350
<i>T. harzianum</i> (T28)	12.8	29.5	59.0	71.8	76.9	0.0	11.3	66.1	66.1	71.0
<i>T. hamatum</i> (T17)	7.6	37.2	69.2	82.1	73.1	0.0	11.3	67.8	77.4	69.4
<i>T. harzianum</i> (T5)	0.0	35.9	50.0	84.6	83.3	0.0	16.1	51.6	69.4	69.4
T28 +T17 +T5	28.2	61.5	80.8	87.2	76.9	9.6	69.4	82.3	82.3	80.7
<i>B. subtilis</i>	7.6	38.5	42.3	35.9	34.6	0.0	21.0	17.8	19.4	19.4
Plantguard	35.9	55.2	56.4	71.8	75.6	21.0	43.6	37.1	72.6	82.3

Disease incidence without any spray (the check) = 78% (1998)
= 62% (1999)

The results of the second season 1999, revealed that 50 ml/100 liter of water did not express any obvious reduction except with Plantguard and the mixture as the reduction was 21.0 and 9.6%. The best reduction took place with the mixture T28 + T17 + T5 at the concentrations 200 and 250 ml (82.3%), even at 350 ml the reduction was almost the same (80.7%) followed

fig2

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fig3

by *T. hamatum* which gave higher reduction at the same concentration. *T. hamatum* gave the best reduction at the concentration at 250 ml (77.4%). *T. harzianum* T28 at the concentration of 350 ml expressed the highest reduction on the two seasons (respectively 76.9% and 71.0%). The best effect with *T. harzianum* T5 occurred with the concentrations of 250 ml and 350 ml where the relative reduction was the same (69.4%). The best concentration for Plantguard was 350 ml on the two seasons and the reduction was successively 75.6% and 82.3%.

Effect of different concentrations of the bioagents on the disease severity:

It is clear, from the results given in the Table (4) and illustrated in Fig. (4), that the concentration 250 ml per 100 liters of water was generally the best effective though the better results recorded by some bioagent on 350 ml, but without worthy differences. Plantguard, the commercial product of *T. harzianum* characterized by high effectiveness starting with the concentration of 150 ml as the relative reductions were successively 72, 70, 82.4 and 84.8% (1998) and 73.4, 85.2, 94.3 and 90.5% (1999) on the concentrations of 150, 200, 250 and 300 ml. The mixture T28 + T17 + T5 gave generally the highest effectiveness particularly on 1998 experiment and the relative reduction was 89.9% on the concentration of 250 ml and 88.5% with 350 ml. *B. subtilis* was the least effective that the highest reduction did not exceed 60%, and 62.1% at the concentration of 250 ml successively on 1998 and 1999. The rest of the single bioagents expressed a similar high potentiality particularly with the high concentrations during 1999 season.

Table (4): Effect of different concentrations of the bioagents on the disease severity.

The bioagents	% Relative reduction of disease severity									
	Concentrations ml/100 liters of water									
	1998					1999				
	50	150	200	250	350	50	150	200	250	350
<i>T. harzianum</i> (T28)	28.2	47.1	51.2	78.9	79.8	4.1	37.4	37.2	78.0	77.8
<i>T. hamatum</i> (T17)	10.3	52.9	63.5	89.5	83.2	7.0	42.6	72.0	78.8	80.1
<i>T. harzianum</i> (T5)	0.0	35.3	40.7	84.6	84.3	12.3	35.9	57.9	71.5	80.1
T28 +T17 +T5	23.2	76.0	78.2	89.9	88.5	25.6	76.5	85.0	86.5	88.6
<i>B. subtilis</i>	4.6	38.5	45.8	60.0	59.1	7.0	53.5	53.5	62.1	52.7
Plantguard	35.0	72.0	70.0	82.4	84.8	44.2	73.4	85.2	94.3	90.5

Disease severity without any spray (the check) = 62.4% (1998)
= 52.7% (1999)

fig4

DISCUSSION

The present study proved that controlling grape powdery mildew, as a foliar disease is likely to give a considerable high success by certain bioagents mainly different species and isolates of *Trichoderma* particularly when applied in a mixture form as they showed less potentiality if singly used. This superiority of the mixture may be attributed to a synergistic property or/and to the complementary mode of action of the different isolates of *Trichoderma* spp.

Abd-El-Moity (1981 and 1985) mentioned that different isolates work by different mode of action i.e. toxin production, parasitism, competition for available nutrients or others. This phenomenon may gave the combined mixture a potent consistency overhand either on disease incidence or disease severity.

Different species and isolates of *Trichoderma* bioagent were not as effective as each other. *T. hamatum* was more potent than *T. harzianum* in general, particularly when the disease incidence was concerned. The superior effect of *T. hamatum* may be contributed to the difference of the optimum temperature for both species. *T. harzianum* has an optimum temperature of 30°C while *T. hamatum* has 24°C. (Domsch *et al.*, 1980). Moreover, *T. hamatum* grows faster and its occupation of the infection court is more and faster.

The obtained results revealed the important role of the number of the successive sprays as well as the concentrations of the bioagent in the spray mixture. There was an obvious relation between the potentiality of the given bioagent and those two factors either on disease incidence or disease severity. A contributive explain, reported by Bull *et al.* (1991) and Schippers (1992) could be taken into account in this respect. They referred to the high population of the bioagent taking place on the site of infection due to the successive sprays. Yet, another factor may be thought about, it is the sustained fungal toxicity that persists during the spray intervals could be increased and prolonged by the second and third spray to express more and more potentiality till the maximum effectiveness of each bioagent brought about. In concern with the factor of the bioagent concentration, it was clear that the most effective concentration was 250 ml per hectaliter with no worthy difference than the higher concentration; 350 ml. Schippers (1992) explained such phenomenon by the increasing occupation of the infection site by the increased number of the bioagent propagules. This means that each bioagent reached its maximum effect by certain number of propagules and any more propagules do not express further activity on the infection court. Yet the maximum efficacy of the given concentrations may not be as simple as such; due to the propagules number on the infection host site. It is more reasonably, that the potentiality reaches its maximum rate by a given concentration, as the existence of extra propagules does not render them inactive.

Bacillus subtilis proved to express a very limited activity against disease incidence and disease severity. This may be explained by the fact

that this bacterium acts mainly by the antibiotics it produces (Gueldner *et al.*, 1988), and these are more specific to particular bacterial diseases and not of broad spectrum biotoxicity.

There was marked difference between the bioagents potentiality on the disease incidence than that on the disease severity. In concern with the disease incidence, the bioagents differed greatly in their activity when they were singly applied or in a mixture, a case which was lacking with the disease severity as most of the bioagents acted so potent as each other with unworthy differences. Thus the low effectiveness on the disease incidence did not accomplished by low potentiality on the disease severity. Hence, at any evaluation program the two parameters would be considered to avoid the misleading results that could be obtained if the disease incidence is the only estimated parameter giving a false under evaluation of the overall potentiality. This clear difference between the two parameters may point out to the eradivative properties of the bioagents that stopped the disease from severe spread after infection took place. However, the disease severity is more important from the economical point of view as the expected loss in the yield is greatly related to this factor.

In conclusion *Trichoderma* isolates and species, tested in the current study succeeded to control the powdery mildew of grapes. Powdery mildew is a foliar pathogens that grow superficially on the host leaves have a pathogenesis pathway that is not similar to that of the soil and seed borne pathogens, known to be controlled successfully by *Trichoderma* bioagent and corresponding different mode of action is expected.

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تقييم فاعلية أنواع من الفطر ترايكودرما والبكتريا باسلس ساتلس فى مقاومة
مرض البياض الدقيقى على العنب
نادية على فؤاد
معهد بحوث أمراض النباتات - مركز البحوث الزراعية - جيزة

اختلفت خمسة تحضيرات حيوية وكذلك المركب التجارى "بلانتجارد" فى تأثيرها على مقاومة البياض الدقيقى على العنب المتسبب عن الفطر *Uncinula nicator* . وأظهرت النتائج أن الخليط المكون من الفطر ترايكودرما هرزيانم العزلة رقم ٥ ورقم ٢٨ والفطر ترايكودرما هاماتم العزلة رقم ١٧ كان تأثيره فى مقاومة المرض متفوقا عن هذه العزلات منفردة بل كان متفوقا كذلك عن باقى المعاملات المختبرة.

وكانت البكتريا باسلس ساتلس هى الأقل فاعلية فى مقاومة المرض وباستخدام عدد مختلف من الرشاشات، واحد واثنين وثلاثة وأربع رشاشات، كان الرش ثلاث مرات هو الأكفأ فى خفض نسبة الإصابة وشدتها فى حين أن الرش الرابعة لم يصاحبها زيادة ملحوظة فى درجة الفاعلية. وعند مقارنة أربعة تركيبات مختلفة من الميكروبات المضادة تبين أن أفضل هذه التركيزات كان ٢٥٠ ملليلتر من المستحضر الحيوى لكل مائة لتر من محلول الرش ولم يصاحب زيادة التركيز إلى ٣٥٠ ملليلتر/١٠٠ لتر ماء أى زيادة ذات قيمة فى فاعلية الميكروبات المضادة. وقد اعتمد فى هذا البحث على مقياسين عند تقييم الفاعلية هما نسبة الإصابة بالمرض وشددة الإصابة. وثبت من النتائج أن شدة الإصابة هى الأكثر تعبيراً عن الفاعلية حيث أن جميع التحضيرات الحيوية كانت ذات فاعلية مرتفعة بدون فروق كبيرة بينها بعكس الحال فى نسبة الإصابة.