



SPRAYING SOME ORGANIC COMPOUNDS ON KING RUBY GRAPEVINES FOR POWDERY MILDEW RESISTANCE

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Keywords: Grapevine, *Uncinula necator*, *T. harzianum*, *T. viride*, *Bacillus subtilis* and blight stop

INTRODUCTION

Grapevine (*Vitisvinifera* L.) is one of the most important fruits grown in Egypt. Grapevine is subjected to the infection with powdery mildew caused by *Uncinula necator* Schwe in Burrill, teleomorph of *Oidiumtuckeri* Berk. Recently, renamed *Erysiphenecator* Schw. and placed into the section *Uncinula* of the genus (Braun and Takamatsu, 2000). Powdery mildew is a worldwide economically important fungal disease in the grapevine farms and the most enduring and widespread problem. It costs million dollars annually to vine growers, due to crop losses and an intensive usage of fungicides for its control (Rumbolz et al 2000; Miazzi et al 2003; Hajjeh et al 2005; Péros et al 2005 and Crisp et al 2006). The damage caused by the pathogen, *U. necator*, has been noticed from the studies of the disease on cultivars of the European grape species (Calonnec et al 2004). In Egypt, powdery mildew was found on different varieties of grapevine causing considerable losses in grape production (David et al 2001; Gadoury et al 2001 and Saleh et al 2007). Powdery mildew can result in reducing vine growth, yield, fruit quality and winter hardiness. Uncontrolled, the disease can be devastating on susceptible varieties under the proper environmental conditions. However, infection caused by this fungus is developed in high humidity conditions but not by free water (Ellis et al 2008). In addition, the release of ascospores has always been associated with rainy periods where cumulative rainfall ranged between 2 and 58.5 mm. Therefore, rain is necessary for ascospore release that is a primary in ooclasia (Jailloux et al 1999). *E. necator* has been reported

ABSTRACT

This investigation was conducted for two successive seasons (2013 and 2014) in a private vineyard located at 64 Km of Cairo-Alexandria desert road to study the possibility of using organic products to reduce powdery mildew, which it is reflected in reducing yield and fruit quality of King Ruby grapevines. The chosen vines were ten-years-old, grown in a sandy loam soil, spaced at 1.5 X 3.0 meters apart and irrigated by the drip irrigation system, trained to bilateral cordon with spur pruning, and trellised by the "Y" shape system. The vines were pruned during the last week of January with bud load of (60buds/vine). Application of different biocontrol agents *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis* as well as blight stop a commercial biocide which contains different isolates of *Trichoderma* forms in Arabic gum and potassium soap were obtained kindly from central lab. of organic agriculture, ARC. Giza, in an attempt to reduce powdery mildew of the plants.

The results showed that all vital bioagents treatments significantly reduced the powdery mildew disease compared with micron sulphur and control in both seasons. However, spraying mixture of *Trichoderma harzianum* + *Trichoderma viride* and blight stop gave the least disease incidence and severity which it is reflected to increase yield, achieve the best physical characteristics of bunches as well as improving the physical and chemical properties of berries.

(Received 4 November, 2015)
(Revised 11 November, 2015)
(Accepted 19 November, 2015)

to overwinter as mycelium or conidia in dormant buds and/or as cleistothecia on infected tissues, on the bark of vines or in the soil (Delye et al 1997; Miazzi et al 2003 and Cortesi et al 2005).

Several antagonistic microorganisms were recorded to be promising candidate and effective for controlling wide range of plant pathogens including these bioagents species of *Trichoderma* (Chet et al 1997) and *Bacillus* spp. (Sharifi and Ramezani 2003). The use of synthetic chemicals to control plant diseases is to be coming more restricted. The added costs for controlling diseases make losses even more important economically. Development of resistance in the pathogens, residual effects and environmental pollution which led to an increase of health hazards due to their phytotoxic residual and pollution effects along of the cost of controlling diseases are problems associated with use of these chemicals. Recently, search for substances alternative have been initiated. Therefore using some other means of disease control instead of agrochemical is strongly encourage.

Biological control of plant diseases using microorganisms is a very promising alternative to the extended use of fungicides. The biological control of plant pathogenic fungi has received considerable attention as an alternative strategy (El-Rafai Ilham et al 2003). Among the biological control agents *Trichoderma* spp. is the most promising and effective biocontrol agent. *Trichoderma* spp. as antagonist controlling wide range of microbes and their mechanism of mycoparasitism is much more complex, involves nutrient competition, hyperparasitism, antibiosis, space and cell wall degrading enzymes. *Trichoderma harzianum* Rifai is a cosmopolitan species that might be found in the ground. *Trichoderma harzianum* can be considered as identical biocontrol agent for its ideal characteristic. This antagonist is very easy to be isolate and grows rapidly on any organic staff. *T. harzianum* act through different mode of actions i.e. mycoparasitism (Abd El-Moity & Shatla, 1981; Benhamoud & Chet, 1993 and Abada, 2002), production of antifungal (Hayes 1992 and Robinson et al 2009), also it owns enzyme system causes destruction for the pathogens (Abd El-Moity, 1981; Bolar et al 2000 and Ziedan et al 2005). In addition to these modes of action *Trichoderma* spp. also act as inducer for resistance in treated plants against certain pathogen organs (Harman 2006). It is also clear that it can grow within wide range of temperature and other environmental conditions (Singh et al 2010). Use of *Trichoderma* spp. as a tool in the biological control of many plant diseases

has been a subject of many workers (Osman et al 2001 and Abd El-Moity et al 2003).

Mixing antagonists with each other's (*Trichoderma harzianum* Rifai and *Trichoderma viride* Harz.) might be lead to antagonistic effect consequently decrease efficacy of treatment (Robinson et al 2009) or lead to synergistic effect and increase the efficacy (Latha et al 2009). This increase or decrease is due to harmony and compatibility factors between bioagents.

Bacillus subtilis is widely spread in nature. It might be used for suppressing plant diseases caused by bacteria and fungi. This bacterium suppresses harmful microorganisms through the competition for food and directly populating them. It is not toxic for humans and environment (EPA, 2009). *B. subtilis* Cohn is antagonistic to plant pathogenic fungi and bacteria. *Bacillus* spp. produced at least 66 different antibiotic compounds (Ferreira et al 1991). The antagonistic effect of *B. subtilis* against many pathogenic fungi was examined *in vitro* and *in vivo* (Abd El-Moity et al 2003 and Hussein et al 2007). The antagonistic mechanism of these bacteria for plant pathogens involved antibiosis competition for nutrients or space, enhancement of root and plant development, induction of plant resistance, solubilization and sequestration of inorganic nutrients and/or inactivation of the pathogen enzymes (Intana et al 2008).

Sulphur can be applied as a spray or dust. Dusting sulphur is popular in the USA but used rarely in Australia, even though it is considered an effective product (Possingham, 2002). Sulphur works by killing the spores of powdery mildew, thus protecting the vines from new infections. It does not kill the fungus itself. The best use of sulphur therefore, is to prevent vines from becoming infected, rather than to suppress infections once they have developed. Existing mature fungal colonies will begin producing more spores as soon as a week after a sulphur spray is applied. Sulphur is a popular fungicide because it is relatively cheap and provides good results under less than ideal application conditions. The latter point is due to the fact that under suitable conditions (temperatures 25°C to 30°C), sulphur becomes volatile, and to a degree it 'fumigates' the foliage and bunches, including areas that were not actually contacted by the spray or dust.

The present work was designed to find out effective, nontoxic, in harmony with organic production rules. Products used to control diseases which can replace toxic chemical substances now in

grapevine field systems. These safe products will help in protecting biological balance, keep our environment clean, in addition it will help in organic production. Organic products in markets have special high prices, so this work will also improve the income farmers and consequently lead to increase organic products which will lead to increase in the national income. In this work, different single bio agents or mixture of bio agents with others were tested under Egyptian conditions. goal of this study is the use of vital bio agents treatments to control powdery mildew, which it is reflect to increase vine growth, yield and fruit quality of King Ruby grapevines compare with chemical treatment (micron sulphur) and untreated vines (control).

MATERIAL AND METHODS

This investigation was conducted for two successive seasons (2013 and 2014) in a private vineyard located at 64 Km of Cairo-Alexandria desert road to study the possibility of using organic products to reduce using powdery mildew, which it is reflect to evaluate vine growth, yield and fruit quality of King Ruby grapevines. The chosen vines were ten-years-old, grown in a sandy loam soil, spaced at 1.5 X 3.0 meters apart and irrigated using drip irrigation system, trained to bilateral cordon with spur pruning, and trellised by the "Y" shape system. The vines were pruned during the last week of January with bud load of (60 buds/vine) in both seasons according to **Fawzi et al (1984)**.

All vines received the same agricultural practices already applied in the vineyard. Different bio control agents *Trichoderma harzianum*, *Trichoderma viride*, *Bacillus subtilis* and blight stop as commercial biocide were kindly obtained from Central Lab. of Organic Agriculture, ARC. Giza. *Trichoderma harzianum* and *Trichoderma viride* were grown in liquid fermentation medium (GFM) developed by **Brain and Hemming (1945)** for 11 days under complete darkness condition, at 25°C. *B. subtilis* was grown on nutrient glucose broth (NGB) prepared by **Dowson (1957)** for 2 days, at 25°C. Mixture of *T. harzianum* and *T. viride* were mixed at the rate of 1:1. Biocontrol agents or mixture of bioagents were prepared in form consists of bioagent plus water just to modify the number of propagules/each ml to be contain 30×10^6 per each ml of the preparation in (**Abd El-Moity and Shatla, 1981**). Adjusted preparations and blight stop as commercial biocide were diluted to be at

the rate of 1: 100L. Blight stop contains different isolates of *Trichoderma* with other formula consist of adjusted culture plus 5 % Arabic gum and 0.5 % potassium soap to increase adhesive capacity and improve distribution of bio agents on the surface of treated plants.

The application rate for wet table sulphur were diluted adjusted preparations were used to spray grape leaves. Recent local research (**Emmett et al 2003a & 2003b**) has shown that management of powdery mildew can be improved through the use of the newer, higher label rate of sulphur, *i.e.* 600g/100L, when applied at the volumes required to achieve thorough coverage of the vine foliage. This rate is registered for some sulphur products and growers can choose between the two application rates depending upon the disease pressure in their vineyard.

Each four vines acted as a replicate and each three replicates were treated by one of the following treatments where six treatments were applied as follows:

1. *Bacillus subtilis*
2. *Trichoderma harzianum*
3. *Trichoderma viride*
4. Mixture of *Trichoderma harzianum* + *Trichoderma viride*
5. Blight stop with
6. Micron sulphur.
7. Untreated vines (control)

The following plant parameters were adopted to evaluate the tested treatments

Disease assessment

For powdery mildew assessment, the evaluations on leaves were carried out 15 days after the last application.

% Disease incidence (DI) was determined according to the following formula:

$DI \% = (\text{Number of infected leaves} / \text{Total No studied leaves} \times 100)$.

The percentage of disease severity for each particular treatment was calculated using the following formula:

$DS \% = (\text{Sum} (n \times v) / 11N \times 100)$

Where: n=number of plants in every grade

v = Numerical grade

N=total number of examined leaves

11=Maximum disease grade

Leaf infection was evaluated based on the scale on ten leaves for each in fection replicate was evaluated according to **Horsfall and Barrat**

(1945). Five trees were estimated for disease symptoms from each replicate, leaves were selected randomly from each treatment and classified to score on a (12) leaves rating scale, representing a percentage of surface infected area.

Representative random samples of 6 bunches/vine were harvested at maturity when total soluble solids (TSS) reached about 16-17% according to **Tourky et al (1995)**.

The following plant parameters were determined

1. Yield and physical characteristics of bunch

Yield/vine (kg) was determined as number of bunches/vine X average bunch weight (g). Also, bunch length and width (cm) were determined.

2. Physical characteristics of berries

Average berry weight (g), berry size (cm³) and berry dimensions (length and diameter) (cm) were determined.

3. Chemical characteristics of berries

Total soluble solids in berry juice (TSS %) by hand refractometer and total titratable acidity as tartaric acid (%) (**A.O.A.C. 1985**). Hence TSS /acid ratio and total anthocyanin of the berry skin (mg/100g fresh weight) were calculated according to **Husia et al (1965)**.

Statistical analysis

The complete randomized block design was adopted for the experiment. The statistical analysis of the present data was carried out according to **Snedecor and Cochran (1980)**. Averages were compared using the new LSD values at 5% level.

RESULTS AND DISCUSSION

1. Disease incidence

The efficacies of test bio control agents as well as commercial biocide under natural conditions were determined in two seasons 2013 and 2014. Results in **Table (1)** show that all bio agents treatments vital significantly reduced the disease incidence of powdery mildew compared with micron sulphur and control in both seasons. However, significant differences were found among the bio

agents vital treatments. Data also showed that, mixture of *T. harzianum* and *T. viride* reveals the first rank when compared with the other bioagents in reducing disease incidence percentage at first season (2013) which recorded 10%. *Trichoderma* spp. long time have been known for their abilities in controlling plant pathogenic fungi mechanisms primarily have include direct effects upon target fungi via competition, mycoparasitism and antibiosis (**Abd El-Moity, 1981 and Harman, 2006**). In consequence these fungi have been shown to directly increase plant growth (**Chang et al 1986**). On the other hand, blight stop which forms in Arabic gum and 0.5 % potassium soap recorded the highest effect (10.5%) at the second season (2014) followed by the mixture of both *Trichoderma* spp. forms in water. Obtained results also showed that using the antagonist as suspension in Arabic gum plus potassium soap gave better results compare with using the same antagonist as water suspension. This is due to Arabic gum and potassium soap create a slimy thin film surround treated roots and improve establishment of antagonistic propagules on the treated surfaces. Many investigators reported efficacy of bio control agents in plant protection against different air borne pathogens. Some investigators explain this protection effect as antibiosis action occurred in court of infection (**Matei and Matei, 2008**). Some other investigators reported changes in plant physiology and chemical components in plants treated with these bio agents (**Hafez et al 2012**), where the other act through production of antifungal substances such as Endo chitinase, Beta -glucosidase, alpha -1,3-glucanase (**Monteiro et al 2011**) and trichodermin (**Balode 2010**) other isolates compete for space or nutrients. Mixing different isolates increase the scope of mode of action consequently increase efficacy of the treatment.

2. Disease severity

As shown data in **Table (1)** clear that the disease severity of powdery mildew was significantly decreased by all bio agents treatments vital in both seasons. Blight stop records the highest effect in the first season (2013) being (22.33%). *B. subtilis* was the superior bio agents resulting a decrease in the disease severity at second season (2014) being (17.15%). This might be due to that bacteria produced more antibiotic (bacteriosin and subtilisin) which act as inhibitors to pathogenic fungi (**Ferreira et al 1991; Aska & Shoda 1996 as well as Abd El-MoneimMaisea, et al 2006**). *B. subtilis*

might also grows very fast and occupied the court of infection and consumes all available nutrients while prevent pathogens to invade the plant. Application of *B. subtilis* was the most effective if compared with the other treatments. This can be explained on the light of fact that *B. subtilis* grow very fast and occupied the court of infection and compete for spaces and nutrients so prevent pathogens to invade plants (Wolk and Sorkar 1994). In addition *B. subtilis* produces some growth regulators that might increase all growth parameters compared with control treatment (Asaka & Shoda 1996 and Grosch & Grote 1998).

Table 1. Disease incidence and severity of powdery mildew on King Ruby grapevines at seasons 2013 and 2014 as affected by some bioagent treatments

Treatments	Season 2013		Season 2014	
	% Disease incidence	% Disease severity	% Disease incidence	% Disease severity
<i>Bacillus subtilis</i>	12.33	26.69	16.7	17.15
<i>T.harzianum</i> (T1)	15	32.59	23.3	26.69
<i>T. viride</i> (T2)	16.67	33.26	20	24.47
Mixture of (T1 and T2)	10	28.92	13.3	31.11
Blight stop	11.67	22.33	10.7	20
Micron sulphur	17.67	36.67	25	34.33
Control	26.7	41.24	33.33	49.66
L.S.D at 0.05	1.8	1.3	5.7	5.3

3. Yield and physical characteristics of bunch

The results presented in **Table (2)** reveal that yield and physical characteristics of bunch *i.e.* average of bunch weight, bunch length and bunch width were significantly affected by the conducted treatments in both seasons. Blight stop as commercial biocide indicate the highest significant yield value increased in both seasons (30.8 and 29.6 kg). Blight stop also increased bunch weight at first season (751.2g) while spraying with *B. subtilis* was the most effective at the second one (778.6 g). Average bunch length increased when treated with blight stop at the first season (32.8cm). While mixture of *Trichoderma harzianum* + *Trichoderma viride* (36.3cm). Average bunch width increased by spraying with mixture of *Trichoderma harzianum* +

Trichoderma viride resulted at first season (18.7cm). These synergistic effects are due to the dual effect of bio agent which produce growth regulators (Karlidag et al 2012) in addition to their effect as resistant inducer (Constantinescu et al 2009). On the other hand, application of *B. subtilis* recorded the highest values ones at the second season (19.1cm) compared with micron sulphur and control. These effects might be ascribed to the positive action of compounds application in reducing of disease incidence as well as improving fruit characteristics. Some of these applied compounds contain nutrient elements that have a positive action for improving the yield and quality of grapes (Ahmed et al 1991). On the other hand, using of these compounds for controlling powdery mildew were accompanied with protecting bunches from fruit rots which reflected in decreasing fruit dropping and succeeded in gaining heaviest bunches and high yield. This good system increase photosynthesis consequently amount of sugars and lead eventually to good yield. This increase in yield is due to vigorty of healthy plants, in addition to growth regulators are produced by *Trichoderma* spp. and *B. subtilis* which improve photosynthesis metabolism in treated plants (Hernandez et al 2011). This improvement led to increase in yield and dry matter.

4. Physical characteristics of berries

Results in **Table (3)** show that all vital activities significantly improved physical characteristics of berries *i.e.* average berry weight, berry size, berry length and berry diameter compared with micron sulphur treatment and control in both seasons. Significant differences were found among the organic material vital activities as according to Geza et al (1984). Spraying with *Trichoderma harzianum* + *Trichoderma viride* and blight stop gave the highest values for these parameters followed in a descending order by spraying with *Bacillus subtilis*, while, micron sulphur and control recorded the least values in both seasons. This high potentiality in antagonism might be due to the compatible and synergistic relation between *Trichoderma* isolates which acts through different mechanisms including mycoparasitism (Benhamoud and Chet 1993 and Harman 2006), and production of antifungal substances (Hayes, 1992). *Trichoderma* spp. might also act through production of destructive enzymes *i.e.* chitinase (Bolar et al 2000). Tronsmo et al (1993) mentioned that, *T. harzianum* isolates produce chitinase and 1,3 gluconase enzymes which

Table 2. Yield and physical characteristics of bunch on King Ruby grapes at seasons 2013 and 2014 as affected by different bio agent treatments

Treatments	Yield/vine (kg)		Average bunch weight (g)		Average bunch length (cm)		Average bunch width (cm)	
	2013	2014	2013	2014	2013	2014	2013	2014
<i>Bacillus subtilis</i>	20.4	18.3	733.9	778.6	32.3	35.7	18.2	19.1
<i>T. harzianum</i> (T1)	19.3	18.1	726.7	751.8	31.9	35.4	17.9	18.4
<i>T. viride</i> (T2)	19.2	17.2	705.1	728.7	31.7	35.1	17.7	18.1
Mixture of (T1+T2)	21.6	19.6	746.5	773.3	32.6	36.3	18.7	18.9
Blight stop	21.8	20.6	751.2	758.1	32.8	36.2	18.4	18.6
Micron sulphur	18.2	16.3	663.3	684.5	31.1	34.3	17.4	17.7
Control	17.7	15.9	647.4	669.2	30.7	33.8	17.1	17.5
L.S.D at 0.05	1.5	1.7	16.3	17.1	0.5	0.6	0.4	0.3

Table 3. Physical characteristics of berries on King Ruby grapes at seasons 2013 and 2014 as affected by different bioagent treatments

Treatments	Average berry weight (g)		Average berry size (cm ³)		Average berry length (cm)		Average berry diameter (cm)	
	2013	2014	2013	2014	2013	2014	2013	2014
<i>Bacillus subtilis</i>	3.01	3.18	2.99	3.01	1.70	1.74	1.58	1.63
<i>T. harzianum</i> (T1)	2.93	3.08	2.75	2.89	1.68	1.71	1.64	1.59
<i>T. viride</i> (T2)	2.85	3.01	2.71	3.14	1.67	1.69	1.55	1.58
Mixture of (T1+T2)	3.09	3.32	2.94	3.08	1.72	1.79	1.61	1.65
Blight stop	3.17	3.27	2.85	2.85	1.75	1.77	1.57	1.67
Micron sulphur	2.79	2.96	2.63	2.79	1.64	1.65	1.52	1.54
Control	2.77	2.93	2.59	2.76	1.61	1.61	1.50	1.51
L.S.D at 0.05	0.13	0.11	0.12	0.09	0.05	0.04	0.05	0.03

are responsible for dissolving cell wall of the pathogenic fungi, then it can grow and consume the inner contents of the pathogen. The effect of bio-control agent might due to production of antifungal as viridin, gliotoxine and bassellin (Sivan and Chet 1992 and Tourky et al 1995). Mixing bioagents with each other's might be lead to antagonistic effect consequently decrease treatment efficacy (Robinson et al 2009) or lead to synergistic effect and increase the efficacy. This might be due to compatible relation between mixture of *Trichoderma* isolates, leading to synergistic effect between them and improving all vegetative growth and yield. Action of *B. subtilis* is due to its growth very fast and occupied the court of infection in addition to production number of antibiotics (Ryder et al 1999). Treatment with *B. subtilis* proved to be the most effective one compared to other ones. This

might be due to that *B. subtilis* grows very fast and occupies the court of infection and consumes all available nutrients. These actions prevent pathogen spores to germinate and reach susceptible tissues. Also its effect might be due to competition for spaces or nutrients (Wolk and Sorkar, 1994). In addition *B. subtilis* produces some growth regulators that increased all plant growth parameters compared with control treatment (Asaka & Shoda 1996; Sankar & Jeyarajan 1996 and Grosch & Grote 1998). Disease reduction led to increase in fruit yield of treated plants. This increase in yield is due to vigorty of healthy plants, in addition to growth regulators produced by *Trichoderma* spp. and/or *B. subtilis* which improve photosynthesis metabolism in treated plants (Govindappa et al 2011). This improvement led to an increase in yield and dry matter. Bioagents do not only affect the

outer treated plants but also affect inner plant metabolism leading to changes in plant component (Hafez et al 2012).

5. Chemical characteristics of berries

As shown data in **Table (4)** it is apparent that all berry chemical properties *i.e.* total soluble solids, titratable acidity, TSS/acid ratio and anthocyanin was significantly improved by all bioagents treatments in both seasons while the lowest values of acidity were obtained by spraying with blight stop followed by spraying with mixture of *Trichoderma harzianum* + *Trichoderma viride*. Micron sulphur treatment and control recorded the least values of total soluble solids, TSS/acid ratio and anthocyanin and the highest values of acidity in both seasons. These synergistic effects might be due to the dual effect of bioagent which produce growth regulators in addition to their effect as resistant inducer (Constantinescu et al 2009). Bioagents do not only affect outer surface plant but also affect plant metabolism leading to changes in plant components (Hafez et al 2012). Data obtained in **Table (4)** also indicated that all treatments led to an increase in the all tested parameters of fruits in compared with micron sulphur and control treatments. Obtained data also reveal that blight stop was the most effective showing the highest ratios in total soluble solids, TSS/acid ratio and anthocyanin. The other treatments varied in their effects on different parameters. Blight stop show the least values in titratable acidity of successive examined seasons as well as mixture of *Trichoderma harzianum* + *Trichoderma viride* of the first one. This might be due to their effect on powdery mildew pathogen and have a profound effect on crop health (Kloepper et al 2004).

Tronsmo et al (1993) mentioned that, *T. harzianum* isolates produce chitinase and 1,3 glucanase enzymes which are responsible for dissolving cell wall of the pathogenic fungi, then grow and consume its inner contents causing destruction of the pathogen. Fruit quality must be considered when any treatments take place on harvested fruit. In this experiment samples of grape fruits obtained from different treatments were analyzed to be sure that the quality parameters did not disturbed. Obtained data showed that all quality parameters (TSS, TSS/acid ratio and anthocyanin) were increased when any of used treatment was applied while decrease titratable acidity in compared with micron sulphur and control. Total soluble solids and anthocyanin was significantly increased. This increase might be due to the healthy and vigour of treated plants. This vigour improve photosynthesis and other biological activity in plant physiology consequently increases TSS and anthocyanin (Fawzi et al 2010).

Economical justification of the contribution of different vital bioagent treatments in raising vine productivity by reduced the powdery mildew disease compared with micron sulphur and control

It can be shown from the data presented in **Table (5)**, that spraying mixture of *Trichoderma harzianum* + *Trichoderma viride* or blight stop gave the maximum net profit compared with micron sulphur and control in both seasons. In spite of the high costs of all vital bioagents treatments over control. Hence, it can be anticipated that the added cost of establishment will be offset by an increase in vine productivity.

Table 4. Chemical characteristics of berries on King Ruby grapes at 2013 and 2014 seasons affected by different bioagent treatments

Treatments	TSS (%)		Acidity (%)		TSS/acid ratio		Anthocyanin (mg/100g F.W.)	
	2013	2014	2013	2014	2013	2014	2013	2014
<i>Bacillus subtilis</i>	16.5	16.9	0.65	0.64	25.4	26.4	34.1	36.9
<i>T. harzianum</i> (T1)	16.4	16.7	0.67	0.66	24.5	25.3	33.8	34.9
<i>T. viride</i> (T2)	16.3	16.5	0.69	0.67	23.6	24.6	32.5	34.2
Mixture of (T1+T2)	16.7	17.2	0.63	0.62	26.5	27.7	35.4	38.1
Blight stop	16.9	17.3	0.63	0.61	26.8	28.4	37.2	39.5
Micron sulphur	16.1	16.2	0.71	0.69	22.7	23.5	31.7	33.1
Control	15.9	16.1	0.74	0.71	21.5	22.7	31.3	32.9
L.S.D at 0.05	0.4	0.3	0.03	0.02	1.4	1.3	2.4	2.1

Table 5. Economical justification of the contribution of different vital bioagent treatments in raising vine productivity by reduced the powdery mildew disease compared with micron sulphur and control

Per Feddan	Season 2013						
	<i>Bacillus subtilis</i>	<i>T. harzianum</i> (T1)	<i>T. viride</i> (T2)	Mixture of (T1+T2)	Blight stop	Micron sulphur	Control
*Vital bioagent treatments (L)	24	24	24	48	24	---	---
**Micron sulphur (Kg)	---	---	---	---	---	6.0	---
***Vital bioagent treatments (L.E.)	840.0	840.0	840.0	1680.0	840.0	---	---
****Price of Micron sulphur (L.E.)	---	---	---	---	---	150.0	---
Labour cost (L.E.)	100.0	100.0	100.0	100.0	100.0	100.0	---
Cost of cultural practices (L.E.)	8000	8000	8000	8000	8000	8000	8000
Total cost (L.E.)	8940	8940	8940	9780	8940	8250	8000
Yield (Kg)	19039.3	18012.7	17919.4	20159.3	20345.9	16986.1	16519.4
Kg (L.E.)	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Yield (L.E.)	57118.0	54038.1	53758.1	60477.8	61037.8	50958.2	49558.2
The net profit (L.E.)	48178.0	45098.1	44818.1	50697.8	52097.8	42708.2	41558.2
Per Feddan	Sseason 2014						
	<i>B. subtilis</i>	<i>T. harzianum</i> (T1)	<i>T. viride</i> (T2)	Mixture of (T1+T2)	Blight stop	Micron sulphur	Control
*Vital bioagent treatments (L)	24	24	24	48	24	---	---
**Micron sulphur (kg)	---	---	---	---	---	6.0	---
***Vital bioagent treatments (L.E.)	840.0	840.0	840.0	1680.0	840.0	---	---
****Price of Micron sulphur (L.E.)	---	---	---	---	---	150.0	---
Labour cost (L.E.)	120.0	120.0	120.0	120.0	120.0	120.0	---
Cost of cultural practices (L.E.)	8500	8500	8500	8500	8500	8500	8500
Total cost (L.E.)	9460	9460	9460	10300	9460	8770	8500
Yield (Kg)	17079.4	16892.7	16052.8	18292.7	19226.0	15212.8	14839.5
Kg (L.E.)	3.25	3.25	3.25	3.25	3.25	3.25	3.25
Yield (L.E.)	55508.0	54901.4	52171.5	59451.2	62484.4	49441.6	48228.3
The net profit (L.E.)	46048.0	45441.4	42711.5	49151.2	53024.4	40671.6	39728.3
*Vital bioagent treatments (L)	6 L X 4 dates = 24 L						
**Micron sulphur (Kg)	1.5 Kg X 4 dates = 6 Kg						
***Vital bioagent treatments (L.E.)	24 L X 35 L.E. = 840 L.E.						
****Price of Micron sulphur (L.E.)	6 Kg X 25 L.E. = 150.0 L.E.						

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