Efficacy of three biological control agents on black mold caused by *Aspergillus niger* fungus and growth and yield components of grapevines (*Vitis vinifera* L.) under field conditions

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Abstract

The objectives of this study were to evaluate the efficacy of three biological agents (*Pseudomonas fluorescens*, *Bacillus subtilis*, and *Trichoderma harzianum*) to control black mold disease caused by *Aspergillus niger* on grapevines, and measuring their effects on growth and production parameters of this crop under field conditions. A foliar spray application was used to inoculate the plants with the *A. niger* spore suspension. The three bio-control agents were incorporated into the infected grapevine plants soil. Results showed that infection severity has reached in all treatments of biological agents to 0.00% compared to 11.14% in the negative control and 46.13% in positive control (infected untreated plants). Treatment of T. *harzianum* gave the highest values of growth and yield parameters which were significantly different from all other treatments. The interaction between *P. fluorescens*, *T. harzianum* and *B. subtilis* was the most effective treatment among other interactions, in reducing infection severity and increasing all the growth and yield parameters.

Key words: *Pseudomonas, Bacillus Trichoderma, Aspergillus,* grapevins, biological control.

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Introduction

Grapes are among the most important industry agriculture worldwide occupying about 7086022 km^2 (8). *Vitis vinifera* L. is the most common species of the commercial grapes produced worldwide (2). They are climbing plants with running branches. with fruits produced in the form of clusters of small green flowers (22). Grapes can be infected by many pests and pathogens, causing serious economic yield losses (15). One of the common grapevines pathogens is the fungus *A. niger*, which causes black mold diseases on grapes causes an average yield loss of 5-40%. *Aspergillus* spp. exhibits a large number of highly diverse species (10). Because of disadvantages of chemicals used to control pests as the cause environmental pollution, health hazards on human, plants and animals. In addition most pests have had a resistant against these chemicals, as a result of the excessive use which is considered environmentally unacceptable action (3).

Therefore It is, necessary to seek alternatives that are safer, low cost and environmentally friendly. Among these methods, is the induced resistance, in which the defense mechanisms in the plant against pathogens and diseases will be stimulated physically and/or chemically after exposing to or inoculated with a certain pathogen at certain density level (20). Plants contain an immune system that can be stimulated by biological agents (1). Studies have shown that initial resistance of plants can be induced using biological agents including some bacteria and fungi (20).The fungus *Trichoderma harzianum*, bacteria such as *B. subtilis* and *P. fluorescens* are among the most common biological resistance inducers against many plant pathogens (17). These inducers stimulate production of the plant hormones, mainly Salicylic acid and Jasmonic acid, which directly affect pathogens and contribute to the induction of systemic acquired resistance (SAR) (21).

and reduce the virulence of the pathogen (25). Some species of *Trichoderma* spp. provide protection to plants against pathogens through competition, fungal parasitism and/or , production of antibiotics (7). Promoting plant growth and stimulating a defense against many pathogens (12). It is also known that *Pseudomonas fluorescens* bacteria produce a group of secondary metabolites such as 2, 4-diacetylphloroglucinol, hexyl, 5-propyl resorcinol, phenazines 2, and other Siderophores-based antibiotics such as pyrrolnitrin and pyoluteorin which have antifungal properties (5). These compounds on the other hand, can enhance plant root system and thus increase the absorption of nutrients (19). Root occupying bacteria *Bacillus* spp. also play an important role in promoting plant growth (13). such plant growth is very likely happening due to bacterial production of plant growth regulators such as Indol acetic acid (IAA), Gibberellins and Cytokinins (4). The aim of this study, therefore, was to evaluate the effectiveness of *P. fluorescens*, *Bacills subtilis* and *T. harzianum* compared to the fungicide Tapsen against *A. niger* in controlling the black mold disease on grapevines.

Materials and Methods

The experiment was carried out from 1st feb. to 15th Jul, 2018 on 7 year old, 0.5 hectare grapevines orchard in Al-Kufa district, Najaf province. Plants were grown in 61 rows with 3-5m distance between each two rows and each row consists of 10-15 grapevine plant var. 'Shadah'. Four controlling agents were evaluated for their effectiveness against the black mold disease on grapevines caused by the opportunistic fungus *A. niger*. The main treatments included: soil amendment with 20% spore suspension of the three bio-agents *P. fluorescens, B. subtilis* and *T. harzianum,* a chemical treatment with fungicide Tapsen70, nine interaction treatments and a negative control treatment with sterile millet seeds *Panicum miliaceum*. *A.niger* spore suspension was sprayed on the plants, while the biological and chemical treatments were loaded on sterile millet seeds and incorporated to the plant soil. The experiment design was a Complete Randomized Block Design (CRBD) with ten replicates for each treatment.

Measurements

Data collections were based on the infection severity, growth parameters during the growing season and yield quality parameters. Plant growth parameters were recorded first at 25th Feb.2018 where buds started to grow. Data were collected periodically until yield parameters at harvest in 15th Jul, 2018. The Infection severity was recorded based on a disease index where 0=healthy clusters and shoot system, 1=1-25% clusters with black mold and few infections on other plant parts, 2=25-50% heavy infection on clusters and other plant parts, 3=51-75% black molded clusters and 4=76-100% infected clusters with no healthy fruit.

However, percentage of the infection severity was calculated according to equation by Mckinney (16). Growth parameters on the other hand were: number of days until branching, numbers of branches, leaf area (cm^2) and leaf content of chlorophyll SPAD. Yield components were : number of days until fruit formation and fruit maturity, cluster weight (g), number of clusters and percent of soluble solid compound (TSD/ppm).

Statistical Analysis

All experimental collected data were analyzed and subjected to analysis of variance ANOVA using SAS (18) computing program. Means were compared according to least significant difference (L.S.D.) at 95% confidence ($P \leq 0.05$).

Results

Results (Table1 and Figure1) showed that all the treatments significantly reduced infection severity compared to both positive (plants sprayed with *A. niger* spore suspension) and negative (plant soil incorporated with sterile millet seeds) controls. At 50 days post treatment, infection was reduced by all controlling factors applied reaching 0.00% compared to 11.14% in the negative control and 46.13% in the positive control treatment.

In terms of growth and production parameters, the treatments resulted in significant increase in the growth and production parameters (number of days until branching, numbers of branches, leaf area cm^2 and leaf content of chlorophyll SPAD, number of days until fruit formation and fruit maturity, cluster weight (g), number of clusters and percent of soluble solid compound (TSD/ ppm) compared to both negative and positive control treatments. Although the three bio-agents did not differ between each other, the highest values of most growth and yield parameters were recorded in the treatment of *T. harzianum* followed by *P. fluorescens* and *B. subtilis*, respectively.

Table1.Effects of different treatments on infection severity by *A. niger*, growth parameters and yield component of grapevines

Treatments	% infection severity	** No. DPT to branching	No. of branches/plant	Leaf area cm^2	Leaf chlorophyll content	No. DPT to fruit formation	No. DPT to maturity	Cluster weight (g)	No. clusters/plant	%Soluble solid compounds TDS/ppm
Control	11.14	26	30	59	43.4	30	132	420	17	617
A.n (1)	46.13	27	15	55	26.6	31	144	380	5	422
T.h (2)	0	20	99	137	61.9	22	116	1000	37	1135
P.f (3)	0	21	92	118	60.1	23	118	950	34	1094
B.s (4)	0	20	69	93	51.8	23	117	875	27	977
Tapsen 70 w.p	0	25	40	88	47.2	28	121	565	22	811
A.n + *T.h*	0	21	87	112	57.3	23	119	920	30	991
A.n + *P.f*	0	22	73	95	55.4	24	120	870	28	969
A.n + *B.s*	0	21	68	91	49.9	24	123	800	24	937
A.n + Tapsen 70 w.p	0	26	27	79	46.5	29	124	430	19	732
P.f + *T.h* + *B.s*	0	20	104	141	63.2	22	116	1100	39	1203
P.f + *T.h* + *B.s* + *A.n*	0	22	76	115	56.7	24	117	1025	29	1168
Tapsen70 + *B.s* + *P.f* + *T.h*	0	21	94	134	60.4	25	119	925	36	1013
Tapsen 70 + *B.s* + *P.f* + *T.h* + *A.n*	0	23	89	121	59.6	25	121	850	35	947
L.S.D. 0.05	1.281	3.819	6.368	6.545	3.008	4.169	5.096	96.59	5.474	55.336

* Values are means of ten replicates.** DPT referred to day post treatment (foliar spraying). Treatments are (1) *A.. niger*, (2)*T. harzianum*, (3)*P. fluorescens*, (4)*B. subtilis*, and fungicide Tapsen70.

Results of interaction treatments showed that interaction of *P.f* +*T.h* +*B.s* had the best results in most growth and production parameters compared to all the other interaction treatments followed by interaction treatment of *P.f* +*T.h* + *B.s*+Tapsen70. However, period to fruit formation, period to maturity, cluster weight and total soluble compounds ratio were at highest values in the interaction treatment of *A.n* + *P.f* + *T.h* + *B.s*, while the interaction treatment of Tapsen70 + *A.n* was the least effective on growth and yield parameters.

Discussion

The increase in growth and production parameters in the *T. harzianum* treatment after being incorporation to plants soil, may be attributed to the suppression of *A. niger* by its secondary metabolites, which play a major role in the biological control against many plant pathogens (8 and 24) pointed out that some compounds of this fungus such as 6-Pentyl-α-Pyrone can affect plant growth by stimulating growth hormone production in the plant, thus promoting growth of plant root and shoot systems, which in turn is positively reflected on yield increase.

P. fluorescens bacteria were substantially effective in reducing the infection by *A. niger* and increasing growth and yield parameters. This might occur through several different mechanisms including: production of antibiotics and fungal antagonistic compounds, competition for colonization sites, nutrients and minerals, and increasing oxidative metabolism activities, especially hydrogen peroxide H_2O_2, which involves in a wide range of reactions and series signals required for various aspects of plant growth (6). Our findings came to be confirmative to results found by Karami (14) where an application with this bacterium resulted in an increase in total chlorophyll content and the fresh shoot weight. This bacterium was also reported to be very effective in increasing yield of treated plants (23).

In terms of the *B. subtilis* bacteria, they showed suppressive effects on *A. niger* and positive effects by increasing growth and production parameters. Our study with Hatayama (11) as they found that *B. subtilis* showed a direct effect by increasing plant growth standards and enhancing production of plant growth hormones (auxins and cytokinins). In addition, *B. subtilis* has an antagonistic ability to inhibit invasion and development of many fungal pathogens.

The interaction treatment of the three biological agents (*P.f* + *T.h* + *B.s*) showed superiority over all the transactions tested in increasing growth and production indicators. This confirm that a combination of the three bio-agents may have synergistic effects to be more efficient in reducing infection and promoting plant growth and productivity than individual agent treatment (26).

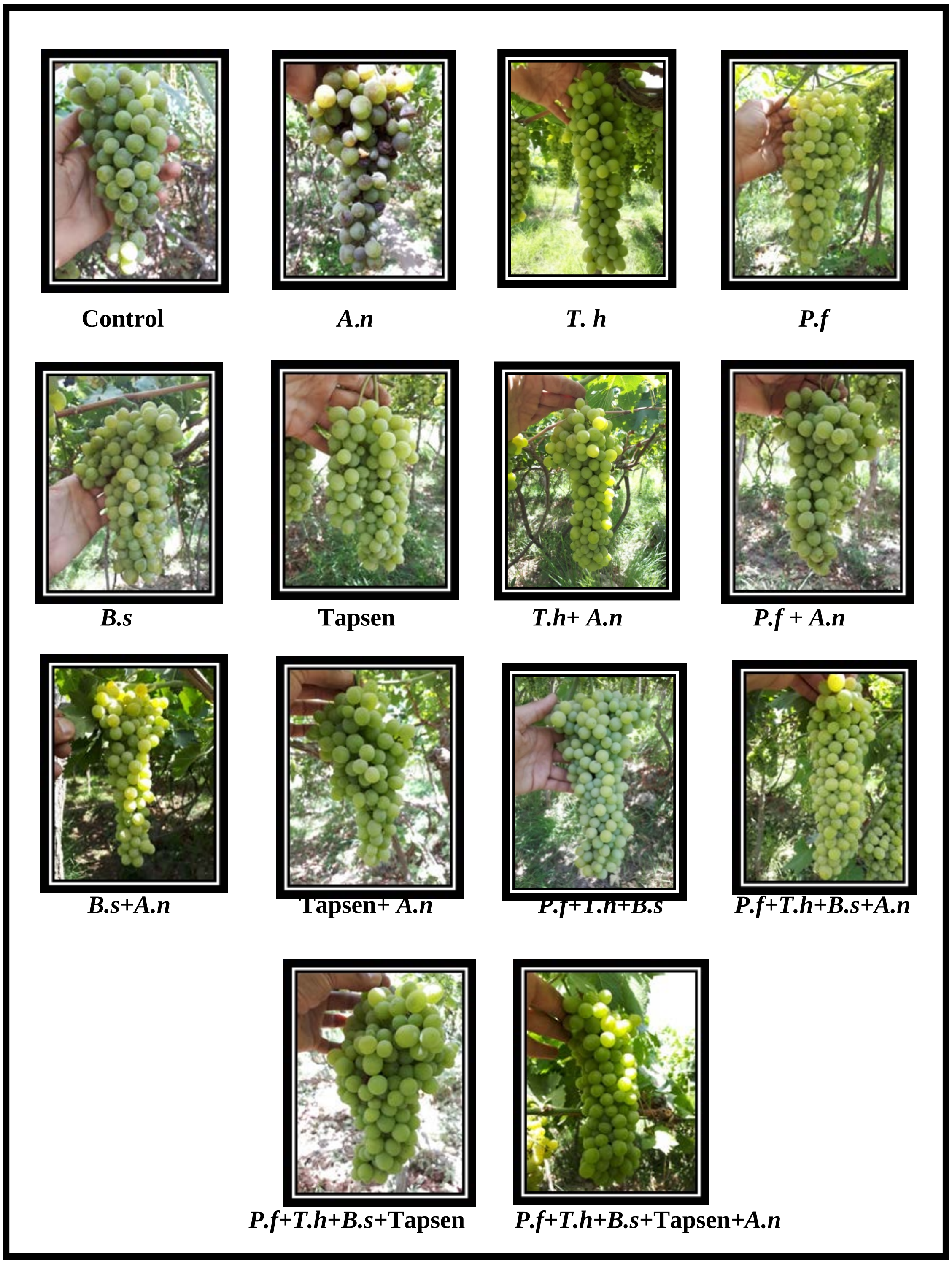

Figure1. Yield (clusters) of grapevines affected by treatments of pathogenic fungus *A. niger* (*A.n*), *P. fluorescens* (*P.f*), *B. subtilis* (*B.s*), and *T. harzianum* (*T.h*), fungicide Tapsen70 and their interaction combinations.

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