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Role of Chemicals for Plant Resistance, Trichoderma Bioagents and Cow Whey Milk in Combination on Management of Tomato Powdery Mildew

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ABSTRACT

The tested inducer resistance chemicals (IRCs) Bion, chitosan, and salicylic acid, Trichoderma strains ,i.e. T. album, T. hamatum, T.koningii ,T. harzianum and T.viride. and cow milk ,i.e. full cream, skim and whey resulted in significant reduction to the germinated conidia of Leveillula taurica (anamorph Oidiopsis taurica,) with control treatment. This reduction was gradually increased by increasing the tested concentration.

In field experiments during 2016 and 2017 growing seasons at Dakahlia governorat, Egypt, spraying of tomato plants with the tested IRC Bion , the bioagents T.koningii and T.harzianum and cow whey milk , each alone or in different combinations, resulted in significant reduction to the severity of the disease with significant increase to the produced fruit yield . Furthermore, spraying of any of these compounds alone was of less effect in this regard compared with spraying of their combinations. However, the fungicide Sumi-8 was the superior treatment in this regard, being 3.2 % disease severity and fruit yield 185 kg. / plot (42 m2) followed by the mixture of the three treatments, being 4.0 % disease severity and fruit yield 181 kg. / plot (42 m2).

There were considerable increase in chlorophyll a, chlorophyll b and carotenoids as well as free and total ascorbic acid (vitamin-C) due to the tested treatments, i.e. both bioagents, BTH and cow whey milk in tomato plants under the natural infection by powdery mildew in comparison with those of untreated plants. BTH was the best treatment for minimizing the reduction in photosynthesis pigments as well as free and total ascorbic acid in plants infected by the disease compared with un-infected plants by the causal fungus (control).

Keywords: *Tomato, ascorbic acid, cow milk, inducer resistance chemicals, photosynthesis pigments, powdery mildew and Sumi-8, Trichoderma spp;*

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is considered one of the most important vegetable crops in Egypt for local consumption and exportation. Therefore, improving the bio production of this crop is one of the objectives in agriculture in many countries. The total cultivated area with tomato in Egypt estimated by about 440.200 feddan during 2016 growing season with a total production of about 7.300.000 ton with the rate of 16.583 ton /feddan (Agric. Statics Dept., Agric. Min.& Land Recl.,2016).

Tomato is liable to be attack by many bacterial, fungal, viral and nematode diseases as well as physiological disorder. However, powdery mildew is considered one of the major devastative and destructive disease affecting crop production, where the infection become serious during fruit formation and ripening (Hannah *et al.*, 2001and Jones *et al.*, 2014).).

Tomato powdery mildew is a common fungal disease affecting tomato plants. This type of mildew is caused by many types of fungi that do not require high humidity to get established, and prosper under mild conditions. These qualities make them more prevalent than many other plant diseases. Researches shown a direct correlation between the percentage of powdery mildew infection of the leaves and yield loss, where one percent mildew infection on the leaves would result in a one percent yield loss or more. An early, heavy infection with mildew had about 50% loss of production compared to a later, lighter infection (Cerkauskas *et al*., 2011 and Karkanis, *et al.*, 2012). Tomato growers need to follow an intensive disease prevention plan because it is very important that powdery mildew never gets out of hand. The use of chemical control against such diseases sometimes gives good results. However, improper use of fungicides leads mostly to environmental pollution, disasters throughout the world and the phenomena of resistance to the plant pathogens (Brewer and Larkin, 2005). Hence, to overcome these difficulties, it is urgent to using resistance cultivars, apply biological control , chemical elicitors , natural products as alternative safe efficient methods against such diseases (Alharbi and Alawlaqi ,2014). Biological control is considered an important approach of agricultural biotechnology in recent years for controlling many fungal plant pathogens (,Barakat *et al.*, 2014; Abada and Eid,2014 and Abada and Ahmed,2014, Abada and Attia, 2017 and Abada *et al.*, 2018).

Tomato powdery mildew grows unseen, within the leaf tissue for a latency period of up to two weeks. Disease monitoring, early detection and prevention from powdery mildew is critical. In addition, tomato plants can become defoliated and do not recover as quickly as other vegetable crops when infected with powdery mildew (Jones *et al.*,2014). The disease does not infect the fruits but can quickly destroy unprotected leaves and eventually the entire fruit crop. The fungus can cause disease in a wide range of temperature and relative humidity. Under favorable conditions, the fungus reproduces rapidly and spores can germinate and infect a plant in less than 48 hours. Wind-dispersed spores cause secondary infections, which help spread the disease. The disease is most severe at any time throughout the season if environmental conditions are favorable and severe infection early in the season can result in heavy yield loss (Jones *et al.*,2014).

Resistance to plant disease is supposed to be a dynamic and multi factorial process. It is assumed that plant defense response can be activated by specific recognition of some microorganisms by the plant. There may be whole organisms or products secreted by microorganisms under the influence of which plants initiate defense response (Akram *et al.*,2013).

This work was planned to management of tomato powdery mildew by induce systemic resistance by fungal bioagents, IRCs and cow whey milk. Also, the effect of the tested treatments on photosynthesis pigments and vitamin-C were taken into consideration.

MATERIALS AND METHODS

Isolation of the Antagonistic Trichoderma Bioagents

Microorganisms naturally grown on tomato leaf surface were isolated from the phylloplane of healthy plants, collected from Aga county, Dakahlia governorate, Egypt. Serial dilution plate technique was used to isolate fungi on PDA medium.

Trichoderma spp. were selected from the isolated fungi then purified using hyphal tip method and identified depending on their morphological features and the description of Rifai (1969) .The identification was confirmed by the Biolog-System technique (Biological Control of Faba-Bean Chocolate -Spot Disease Project, Plant Pathol. Res. Instit., A.R.C., Giza, Egypt).

Effect of the Non-Volatile Substances of *Trichoderam* spp., IRCs and Cow Milk on the Conidial Germination of the Causal Pathogen

Tomato leaves naturally infected by the disease were collected from a field located at Dakahlia governorate to test the effect of Trichoderma bioagents, IRCs and cow milk on conidial germination of *Leveillula taurica*. Powdery mildew spores from the abaxial side of tomato leaves were collected.

- The inducer resistance chemicals (RICs), *i.e.* Bion (Benzothiadiazole; BTH), chitosan (cellulose with the hydroxyl at position C2 substituted with an acetamido group) and salicylic acid (monohydroxybenzoic acid) were prepared at 0.0, 5.0, 10 , 20, 30 and 40 mM depending on their molecular weight.
- The tested *Trichoderma* spp., *i.e T. album,T. hamatum ,T.koningii ,T. harzianum* and *T.viride* were grown on gliotoxin fermentation medium (GFM) as described by Brain and Hemming (1945) for 14 days. Twenty ml. of sterile water were added to each Petri-dish and growth (spores and mycelium) was gently crushed by sterilized camel brush and collected in sterile 500 ml conical flask. The collected growth was filter through 3 layers of cheese cloth. The fungal filtrate was sterilized using 0.25 µm syringe filter. The concentrations of 0.0, 20, 40, 60 and 80% were prepared from the sterilized filtrate for each bioagent using sterilized water. Freshly

conidiospores of the pathogen were added to each concentration of the tested Trichoderma bioagents .

- Cow milk, *i.e.* full cream, skim and whey was diluted to 0.0, 20, 40, 60 and 80 % by adding sterilized distilled water.

Freshly collected conidia by sterilized camel brush from the infected leaves were put in each concentration of the tested IRCs, Trichoderma bioagents and the different kinds of cow milk. One m1.of conidial suspension of *L.taurica* was placed on two sterilized slides, borne on two glass rods in a sterilized Petri -dish containing a piece of wetted cotton by sterilized distilled water to provide high relative humidity. The same was made for a spore suspension put in distilled sterilized water only as control treatment. Preparations were incubated in an incubator at 28±1 ˚C for 48 h. One drop from lacto-phenol cotton blue stain was added at the time of slide examination to fix and killing the germinated conidia. Percentage of conidial germination was counted in a total of 100 conidia. The germinated conidia were counted and percentages mean of germination was calculated and recorded for each treatment.

Field Experiments

Field experiments were carried out at Aga county, Dakahlia governorate, Egypt during 2016 and 2017 growing seasons, where symptoms of severe infection by the disease occurs annually.

A piece of land was prepared for planting tomato as recommended by Min. of Agric. and Land Reclamation The land was divided into plots of 42 m^2 (7 ridges of 6 m long). Tomato plants transplants (777 cv.) of 30 days old grown in Foam trays were transplants (one transplant in each hill) at 50 cm. apart during beginning of September of both seasons. Three plots were used for each treatment. The plants were left for the natural infection by the causal fungus. The prepared two bioagents *T.harzianum* and *T.koningii* at $1x10^6$ conidia / ml. water , the IRC Bion at 40 mM and cow whey milk at 80 % (amended with calculated aliquots of an adhesive surfactant super-film at 50 ml/100 L water) were sprayed on tomato plants at the rate of 40 L / plot (42 m^2) . Three plots were used for each treatment using split design .Spray began at beginning of the initial of powdery mildew symptoms (protective) and just after their appearance and repeated three times each two weeks. All agricultural practices, *i.e.* irrigation, weeds and pests control as well as fertilization were applied according to the standard recommendations of Min. of Agric. and Land Reclamation.

Disease severity was assessed just before the following spray as mentioned under disease assessment and the average was recorded. Also, the produced fruit yield was weighed each harvest and the average was recorded.

Disease Assessment

Plants were examined periodically and disease measures were determined using the modified scale (0-5) adopted with Horsfall and Barratt (1945) , where 0 = No symptoms appear ; 1 = 0.1 to 5% of the leaflets area covered by the infection ; 2 = more than 5 to 10 % of the leaflets area covered by the infection ; 2 = more than 10 to 25% of the leaflets area covered by the infection; 4 = more than 25 to 50% of the leaflets area covered by the infection; 5 = more than 75% of the plant growth covered by the infection and the most leaves of the plants defoliated.

The severity of the disease was assessed using the following formula:

$$\text{Disease severity \%} = \frac{\Sigma\,(nxv)}{5N} \times 100$$

Where: n = Number of infected leaflets in each category.

v = Numerical values of each category.

N = Total number of the infected leaflets.

Estimation of Photosynthetic Pigments and Ascorbic Acid (vitamin-C)

The method of Metzner *et al.* (1965) was used to estimate photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) spectro photometrically.

Twenty gram fresh weight of tomato leaves, taken from five randomly plants and represent the categories of the diseasse, were homogenized in 85% aqueous acetone for 10 min. The homogenized tissues were centrifuged (10.000 rpm for 10 minutes), and the supernatant was adjusted to 200 mL with 85% acetone and measured against a blank of pure 85% aqueous acetone at three wavelengths of 452.5, 644 and 663 nm. Dilutions were used to determine the concentrations of the pigment fractions.

Both free (FAA) and total ascorbic acid (TAA) were estimated spectrophotometrically according to the procedure mentioned by Hodges *et al*. (2001). Fresh fruit tissues (50 g) were weighed and placed in a 250 mL centrifuge tube and 100 mL of ice-cold 5% (w/v) metaphosphoric acid was added, followed by homogenization at the speed of 15,000 rpm for 2 min in an ice−water bath by use of a homogenizer. The homogenized tissues were centrifuged at 7000 rpm for 15 min at 4 °C, and the supernatant was filtered through Whitman No. 4 filter paper.

The filtrate was used for estimating FAA and TAA by converting dehydro-ascorbic acid (DAA) to FAA with dithiothreitol. Both FAA and TAA were estimated spectrophotometrically at 525 nm. The concentrations of FAA and TAA were calculated by using the standard curve (all $R^2 \geq 0.99$) of L-ascorbic acid and their difference was equal to the concentration of DAA. The content of chlorophyll a, chlorophyll b and carotenoids was calculated using the formula of Arnon (1949):

Chlorophyll a (mg/ml) = $12.7\Delta A663 - 2.69\Delta A645$,

Chlorophyll b (mg/ml) = $22.9\Delta A645 - 4.68\Delta A663$, and

Carotenoids (mg/ml) = $4.75\Delta A452.5 - 0.226C(a + b)$,

where,

ΔA is the absorbance at the respective wavelength.

Statistical Analysis

Data were statistically analyzed using the standard procedures for split and split split designs as mentioned by Snedecor and Cochran (1967). The averages were compared at 5% level using least significant differences (L.S.D) according to Fisher (1948).

RESULTS

Effect of Non-Volatile Substances Trichoderma Bioagents on Conidial Germination of the Causal Pathogen

Table (1) reveals that non-volatile substances of all the tested Trichoderma bioagents, *i.e. T.album,T.hamatum, T.koningii, T.harzianum* and *T.viride* resulted in significant inhibition to the germinated conidia of *L.taurica* compared with control treatment. This reduction was gradually increased by increasing the tested concentration. In addition, Both *T.harzianum* and *T.koningii* were the most efficient ones in this regard, being 40.4 and 41.1 % conidial germination followed by *T,viride*, being 48.1 % then *T.hamatum* , being 52.9% conidial germination. Meanwhile, *T.album* was the lowest efficient one being, 54.1% conidial germination. Control treatment recorded 94.6% conidial germination. Therefore, both T.harzianum and T.koningii were used in managing the disease under field conditions.

Table1. *Effect of the tested Trichoderma bioagents on conidial germination of Leveillula taurica , 48 hours after incubation at 28±1ºC.*

***Trichoderma* spp.**	**Average percentage of conidial germination* at conc. (%)**					**Mean**
	0.0	**20**	**40**	**60**	**80**	
Trihoderma album	94.6	83.6	49.2	28.0	15.2	54.1
T.hamatum	94.6	82.8	47.4	27.2	12.6	52.9
T.harzianum	94.6	78.6	28.6	0.0	0.0	40.4
T.koningii	94.6	80.0	29.8	0.0	0.0	41.1
T.viride	94.6	81.0	40.2	23.8	0.0	48.1
Mean	94.6	81.2	39.0	15.5	5.6	----

** Initial germination percentage was 1.8 %*

L.S.D. at 5 % for : Trichoderma (T)= 2.3,Concentrations (C)=2.9 and T x C = 3.3.

Effect of IRCs on Conidial Germination

Results tabulated in Table (2) reveal that all the tested IRCs , *i.e.* Bion (BTH), chitosan and salicylic acid resulted in significant reduction to the germinated conidia of *L.taurica* compared with the control treatment.

This reduction was gradually increased by increasing the tested concentration.

In this regard, BTH was the most efficient one in this regard, being 41.6% conidial germination followed by chitosan, being 42.5 % conidial germination. Meanwhile, salicylic acid was the lowest effective one, being 45.5% conidial germination. Control treatment recorded 94.4% conidial germination. Therefore, both *T.harzianum* and *T.koningii* were used in managing the disease under field conditions.

Table2. *Effect of three inducer resistance chemicals on conidial germination of leveillula taurica , 48 hour after incubation at 28±1˚C.*

Inducer resistance chemicals	Average percentage of conidial germination at conc. (mM)						Mean
	0.0	**5**	**10**	**20**	**30**	**40**	
Bion	94.2	85.8	63.0	34.0	00.0	0.0	46.2
Chitosan	94.2	88.2	66.4	40.4	23.2	0.0	53.6
Salicylic acid	94.2	89.0	62.2	46.6	27.0	8.8	50.7
Mean	94.2	87.7	63.8	49.6	16.7	3.6	----

** Initial germination percentage was 1.4 %.*

L.S.D. at 5 % for : Inducer resistance chemicals (I) = 2.7,Concentrations (C)=2.5 and I x C = 3.2.

Effect of Cow's milk on Conidial Germination

Results shown in Table (3) show that the tested component of cow milk ,*i.e.* full cream, skim and whey caused significant reduction to the germinated conidia of the causal fungus. Full cream milk was more efficient in reducing the germinated conidiospores of the causal pathogen the skim milk and whey milk, being .36.1, 43.9 and 57.0, respectively. In addition, control treatment recorded 93.8 % conidial germination. Although, cow whey milk was the lowest efficient in reducing the germinated conidiospores of the causal pathogen, but considers a waste product and easyally to obtain abundant amounts with low price.

Effect of the Bioagents T.harzianum and T.koningii, the IRC Bion (BTH) and Whey Milk on the Severity of Tomato Powdery Mildew and the Produced Fruit Yield

The effect of the bioagents *T.harzianum* and *T.koningii*, the IRC BTH and whey milk on the severity of tomato powdery mildew and the produced fruit yield was carried out during 2016 and 2017 growing seasons at Aga county, Dakahia governorate, Egypt(Tables 4 and 5).

Result (Table 4) show that spraying of tomato plants with the tested two bioagents , BTH and cow whey milk, , each alone or in different combinations, resulted in significant reduction to the severity of the natural infection by powdery mildew compared with control treatment. In general, protective sprays were significantly efficient in reducing the infection by the disease than curative sprays, being 13.2 and 12.4 %, respectively.

In addition, spraying any of the two bioagents, the IRC Bion and whey milk each alone was of low efficiency in this regard than spraying their different combinations.

The highest reduction was obtained by the combination among the tested treatments (the two bioagents , the IRC Bion and whey milk), being 4.0 % disease severity nearby the effect of the systemic fungicide Sumi-8, being 3.2 % disease severity .

The severity of the disease was significantly higher during the first season than of the second season, being 13.2 and 12.4 %, respectively .Control treatment recorded 50.8% disease severity.

Table3. *Effect of different components of cow milk on conidial germination of Leveillula taurica, 48 hours after incubation at 28±1ºC*

Cow milk Component	Average percentage of conidial germination at conc. (%)*					Mean
	0.0	**20**	**40**	**60**	**80**	
Full cream	93.8	54.8	32.0	0.0	0.0	36.1
Skim	93.8	60.2	45.4	20.2	0.0	43.9
Whey	93.8	74.0	60.2	41.8	15.0	57.0
Mean	93.8	61.0	46.0	20.7	5.0	----

** Initial germination percentage was 1.8 %.*

L.S.D. at 5 % for : Cow milk component (M) = 2.7,Concentrations.

(C)=2.5 and M x C = 3.2.

Table4. *Effect of spraying tomato plants with the combination among the bioagents Trichoderm harzianum and T.koningii IRC Bion and cow whey milk on the severity of powdery mildew, field experiments at Dakahlia governorate, during 2016 and 2017growing seasons*

Treatments	% Disease severity after spraying the tested material during				Mean		General mean
	2016		2017				
	Protective	Curative	Protective	Curative	Protective	Curative	
Trichoderma harzianum (TH)	11.3	13.2	10.7	12.9	11.0	13.1	12.1
T.kningii(TK)	11.7	13.5	10.6	12.6	11.2	13.1	12.2
Bion (B)	12.5	14.0	10.0	12.2	11.3	13.1	12.2
Whey milk(WM)	13.1	13.7	12.8	13.1	13.0	13.4	13.2
TH+TK	10.6	11.0	7.9	10.1	9.3	10.6	10.0
TH+B	9.6	9.8	8.8	9.2	9.2	9.5	9.4
TH+WM	9.0	9.2	9.0	9.2	9.0	9.2	9.1
TK+B	9.5	9.7	9.2	9.7	9.4	9.7	9.6
TK+WM	9.7	9.9	9.3	9.5	9.5	9.7	9.6
B+WK	10.0	10.2	9.7	10.2	9.8	10.2	10.0
TH+TK+B+ WM	4.0	4.2	3.7	4.0	3.9	4.1	4.0
Sumi-8	3.1	3.3	3.0	3.2	3.1	3.3	3.2
Control	51.6	51.6	50.0	50.0	50.8	50.8	50.8
Mean	12.8	13.5	11.9	12.8	12.4	13.2	-----
General mean	13.2		12.4		--------------		-------

L.S.D. at 5 % for: Treatments(T)= 2.1 , Season (S)= 0.6 , Kind of spray (K) = 0.7 , TxS=2.2 , TxK= 2.0, SxK= 1.8 and TxSxK= 2.3.

Table5. *Effect of spraying tomato plant with the combination among the bioagents Trichoderma harzianum and T. koningii , the IRC Bion and cow whey milk on the produced fruit yield (under condition of the infection by powdery mildew) , field experiments at Dakahlia governorate during 2016 and 2017 growing seasons.*

Treatments	plant after spraying /Average fruit yield (g.) the tested materials during				Mean		General mean
	2016		2017				
	Protective	Curative	Protective	Curative	Protective	Curative	
Trichoderma harzianum (TH)	165	171	173	172	169	172	171
T.kningii(TK)	164	176	172	174	168	175	172
Bion (B)	172	179	174	177	173	178	176
Whey milk(WM)	163	165	165	168	164	167	166
TH+TK	167	169	170	172	169	171	170
TH+B	165	171	167	170	166	171	169
TH+WM	165	167	167	170	166	169	168
TK+B	165	168	168	172	167	170	169
TK+WM	164	167	165	168	165	168	167
B+WK	164	166	166	170	165	168	167
TH+TK+B+ WM	76	180	180	186	178	183	181
Sumi-8	179	186	85	189	82	188	185
Control	112	112	118	118	115	115	115
Mean	163.2	167.5	166.9	169.7	165.1	168.6	-------
General mean	185.8		184.8		------------		---------

** Sprayed with water only.*

L.S.D. at 5 % for: Treatments (T)= 3.6 , Season (S)= 1.5 , Kind of spray (K) =2.0, TxS= 3.1, TxK= 2.7 , SxK=2.1 and TxSxK= 4.1 .

Table (5) reveals that spraying of tomato plants with the bioagent *T.koningii* and *T.harzianum*, the IRC Bion and cow whey milk, , each alone or in combinations, resulted in significant increase to the produced fruit yield compared with control treatment . Spraying any of the two bioagents , the IRC BTH and whey milk alone was of low efficiency in this regard than spraying their different combinations. The highest produced fruit yield was obtained by the combination among the tested treatments (the two bioagents , the IRCBion and whey milk), being 181 kg. fruit yield/ pot (42 m^2) nearby the effect of the systemic fungicide Sumi-8, being 285 kg./ plot (42m2). Control treatment produced 115 kg. Fruit yield/ pot (42 m^2).

Estimation of Photosynthesis Pigments and Ascorbic Acid

Data presented in Table (6) reveal the effect of the tested treatments compared with the infected control on photosynthesis pigments of the leaves and free and total ascorbic acid of the fruits of tomato plants. The IRC Bion (BTH) resulted in the highest concentration of photosynthetic pigments and ascorbic acid for plants infected with *L.taurica* compared with the other treatment, *i.e.* Trichoderma bioagents and cow whey milk and the infected control. Meanwhile, whey milk had the lowest concentration of photosynthetic pigments and total ascorbic acid. The infected control had a reduction in photosynthetic pigments and a reduction in ascorbic acid.

Discussion

Management strategy of tomato powdery mildew, in most cases, depends mainly on fungicides. But due to the great hazard on human health and harmful effect on the environment from the fungicides residue, non-traditional and safe materials supposed to manage this disease for reducing the inoculum density and produce healthier tomato fruits. Therefore, production of healthy and safe food free from toxic substances is the desire of the consumer especially that consume freshly like tomato.

Therefore, to avoid the use of hazard chemicals against diseases, certain protective or curative procedures could be conducted using different non-chemical methods to control such diseases. In this regard, Trichoderma bioagents, inducer resistance chemicals (IRCs) and cow whey milk were evaluated for management tomato powdery mildew. However, in most cases, using such untraditional management methods did not give adequate results when used alone. In this respect, the use of these methods is preverbal to use as a mixture.

Table6. *Effect of two Trichoderma bioagents, Bion and cow whey milk on photosynthesis pigments (chlorophyll a, chlorophyll b and carotenoids) and total ascorbic acid of tomato plants naturally infected by Leveillula taurica*

Treatment	Photosynthesis pigments *			Ascorbic acid **	
	Chlorophyll -a	Chlorophyll -b	Carotenoids	Free	Total
T.harzianum	1.42	1.19	0.84	31.9	44.6
T.koningii	1.43	1.18	0.86	32.2	45.3
Bion (BTH)	1.51	1.23	0.88	33.8	46.9
Cow whey milk	1.40	1.16	0.82	32.1	42.2
Control (Infected)	1.03	0.90	0.58	24.6	31.7
Control (uninfected)	1.57	1.25	0.93	37.0	53.5

**mg/g fresh leaves weight, **mg/100g fruit fresh weight .*

The tested bioagents, IRCs and cow whey milk caused significant reduction to conidial germination of *Leveillula taurica* (imperfect stage = *Oidiopsis taurica*) the causal of tomato powdery mildew compared with control treatment. This reduction was gradually increased by increasing the tested concentration. Field experiments were carried out under the natural infection by powdery mildew during 2016 and 2017 growing seasons at Aga county, Dakahlia governorate, Egypt. The obtained data revealed that spraying of tomato plants with the bioagents *T.koningii* and *T.harzianum*, the IRC Bion (BTH) and cow whey milk each alone or in combinations, resulted in significant reduction to the severity of the disease with significant increase to the produced fruit yield. Furthermore, spraying of any of these compounds were less efficient in this regard compared with spraying their combinations. However, the fungicide Sumi-8 was the superior in this regard followed by the mixture of the bioagents, the IRC Bion and cow whey milk.

Chemical control is highly recommended because downy mildew is an aggressive and destructive disease and satisfactory control without the use of fungicides is unlikely. The role of fungicides in reducing the disease is well known (Mc Grath, 2001 and 2004).

But due to the great hazard on the human health due to the residue of agrochemicals in the consume food, fungicides become unlikely to use. Therefore, great efforts by agro-scientists are spend to search about alternative safely methods to management plant pests. In this respect, this work aimed to evaluate spraying tomato plants with the bioagents *T.harzianum* and *T.koningii*, the IRC Bion and cow whey milk, each alone or in different combinations , on management of the infection by powdery mildew of tomato under the natural infection in the field.

Biological control has emerged as an alternative and most promising means of the management of plant pathogens. The earlier studies revealed that antimicrobial metabolites produced by *Trichoderma* spp. are effective against a wide range of phytopathogenic fungi. In addition, *Trichoderma* spp. are known to control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and enhancing plant defensive mechanisms and antibiosis, or directly by inhibition of growth and sporulation of the pathogen mechanisms such as mycoparasitism and enzyme production. In addition, the inhibitory activity of the tested bioagent Trichoderma on the development of germ tube of the pathogen could be explained by the ability of Trichoderma spp. to produce volatile substances that are able to limit and even stop the development of the pathogen. Also it is found that there is large variety of volatile secondary metabolites produced by *Trichoderma* strains such as ethylene, carbon dioxide, hydrogen cyanide, aldehydes and ketones, which play an important role in controlling the plant pathogens (Junid *et al*., 2013 and Bhattacharjee and Dey, 2014).In addition, *Trichoderma* spp. are known to control pathogens either indirectly by competing for nutrients and space, modifying the environmental conditions, or promoting plant growth and enhancing plant defensive mechanisms and antibiosis, or directly by inhibition of growth and sporulation of the pathogen mechanisms such as mycoparasitism and enzyme production (Zimand *et al*., 1994; Bouhassan *et al.*, 2004).

Protection of plants from plant pathogens by induction of systemic resistance is a new approach and is of much less harmful to the environment and plant products as compared to deadly agrochemicals applied to control plant diseases. The obtained results revealed a significant reduction in disease severity of powdery mildew on tomato plants treated with the two bioagents and the IRC Bion and cow whey milk either sprayed protective or curative treatment compared with control treatment.

Larcke (1981) found that unlike elicitors of phytoalexines accumulations, which are elicited at the site of application may be responsible for localized protection and induces systemic acquired resistance that sensitizes the plant response rapidly after infection. These responses induced phytoalexines accumulation and lignifications and induce enhance activities of chitinase and β-glucanase (Dean and Kuc, 1985 and Metranx and Boller, 1986). Doubrava *et al.* (1988) mentioned that induced acquired resistance is persistent and generally is pathogen nonspecific. Furthermore, Kessmann *et al*. (1994) reported that the mechanism of systemic acquired resistance is apparently multifaceted, likely resulting in stable broad spectrum disease control and they could be used preventatively to bolster general plant health, resulting in long lasting protection. Iriti and Faoro (2003) reported that bion was used to induce resistance in bean against rust caused by *Uromyces appendiculatus*. Histochemical and cytochemical investigations showed that BTH causes hydrogen peroxide (H_2O_2) accumulation in the treated tissues. H_2O_2 deposits were localized in situ for the first time in the apoplast of the leaf epidermis. No cell death was detected at BTH concentrations below the phytotoxicity threshold, suggesting that acquired resistance against bean rust is mainly related to the enhanced activity of anionic peroxidases, promoted by H_2O_2 accumulation, thereby leading to cell wall strengthening. This hypothesis is also supported by the long induction phase required to establish complete resistance.

Feskanich *et al*. (2014) mentioned that the major whey proteins in cow milk are ß-lactoglobulin and a-lactalbumin. a-Lactalbumin is an important protein in the synthesis of lactose and its presence is central to the process of milk synthesis. ß-Lactoglobulin's function is not known. Other whey proteins are the immunoglobulins (antibodies; especially high in colostrum) and serum albumin (a serum protein). Whey proteins also include a long list of enzymes, hormones, growth factors, nutrient transporters, disease resistance factors, and others. El-Dakar and Mahmoud (2015) reported that cow's full cream, skim and milk whey resulted in significant reduction to the germinated conidia of *Golovinomyces cichoracearum* var. *cichoracearum* (D.C.) Heluta (syn. *Erysiphe cichoracearum* D.C.), the causal of cucumber powdery mildew compared with control treatment. They added that spraying of the combination of the bioagent *B.subtilis*, the IRC potassium mono phosphate and cow whey milk was of the highest efficiency in reducing disease severity and producing the highest fruit yield following the fungicide Punch.

Cow milk has been shown to be effective against powdery mildew (*Sphaerotheca fuliginea*) in greenhouse grown zucchini (Bettiol 1999), pepper (*Leveillula taurica*) powdery mildew (Al-Harbi and Alawlaque, 2013), pumpkins (*Podosphaera xanthii*) powdery mildew (DeBacco, 2013) and cucumber (*Erysihe cichoracearum*) powdery mildew (El-Dakar and Mahmoud ,2015) . They attribute the controlling mode of action in this instance as possibly being a direct effect of milk due to its germicidal properties or the salts and amino acids contained in milk providing the controlling mechanism. Alternatively, Bettiol (1999) mentioned that numerous small studies from around the world have validated the use of milk sprays on powdery mildew on a wide range of plants. Most recently, a spray made of 40% milk and 60% water was as effective as chemical fungicides in managing powdery mildew of pumpkins and cucumbers grown in mildew areas. Like other fungicides, milk sprays work best when used preventatively, before the disease can gain a foothold. He added that it does not matter if the milk you use is skim or whole because it is the protein rather than the milk fat that is working on your behalf. He reasons the application of milk may induce systemic acquired resistance in the crop. Also, Pleasant (2012) used milk in managing many powdery mildew diseases on different hosts.

The photosynthesis pigments including chlorophyll a, chlorophyll b and carotenoids as well as free and total ascorbic acid were greatly increased due to the tested treatments, *i.e.* both bioagents , BTH,and cow whey milk in tomato plants grown under natural infection by *l.taurica*. BTH was the best treatment for reduction the impact of the disease on photosynthesis pigments in the leaves as well as free and total ascorbic acid in the fruits . Un-infected plants without any another treatment recorded the highest values of photosynthesis pigments and total ascorbic acid. The impact of the treatments on photosynthetic pigments and ascorbic acid may be due to the reduction on the activity of green plastids and vitamin-C metabolism. Similar results were obtained by Pennypacker *et al.* (1990) , Hunter *et al.* (2011) and Abada *et al.*2018). Metzner *et al.* (1965) repoted that when a plant is subject to physical or physiological stress, the free ascorbic acid can be oxidized into dehydro-ascorbic acid

CONCLUSION

Protection of plants from disease by induction of systemic resistance is a new approach. This is much less harmful to the environment as compared to deadly agrochemicals applied to control plant diseases especially those consume fresh like tomato fruits. However, management strategy of tomato powdery mildew, in most cases , depends mainly on fungicides . But due to the great hazard on human health and harmful to the environment from the fungicides residue , non-traditional and safe materials supposed to mange this disease for reducing the inoculum density and produce healthier tomato fruits and are not harmful to the environment or human health. The use of Trichoderma bioagents , IRC Bion and cow whey milk could be used in combination as a management strategy or a part of an integrated management program. These treatments provide eco-friendly alternatives to reduce the incidence and severity of powdery mildew on tomato plants and produce healthier fruits.

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