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Effect of Some Bio-Control Agents, Natural Salts and Planting Densities on Controlling Sweet Pepper Powdery Mildew and Some Horticultural Characteristics Under Greenhouse Conditions

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The objective of this work is to study the effect of three different bio-agents, *i.e. Trichoderma harzianum* (*T. harzianum*), *Bacillus subtilis* (*B. subtilis*) and *Streptomyces griseus* (*S. griseus*), in addition to two natural salts (potassium silicate and mono-potassium phosphate, MKP) and two different plant densities of pepper on powdery mildew (PM) incidence, severity, some horticultural characteristics and yield. Original high tunnel greenhouse located at Kaha Research Station, Qalyubia governorate was used. Data revealed that, low planting density led to decrease of PM disease incidence and severity when compared with high planting density. Increase in pepper growth in terms of plant height, number of leaves, number of branches, leaf area, fresh and dry weights per plant was noticed when low planting density was used compared with high planting density. Fruit quality, *i.e.* fruit weight, length, diameter, flesh thickness and chemical characteristics were also increased with low density. The highest yield characteristics, *i.e.* early yield, number of fruits per plant and total yield were increased with low planting density. Regarding biological control and natural salts, data revealed that the highest reduction in disease incidence was obtained when *B. subtilis* was used. Disease severity was also recorded and *T. harzianum* stand as the most effective treatment in reducing disease severity. Maximum values of the vegetative growth characteristics, fruit quality and yield were recorded when foliar application with MKP followed by the application with *B. subtilis.* Laboratory analysis for treated plants showed that disease control was positively correlated with amount of phenols and sugars in leaves. Interaction between low planting density with *B. subtilis* or MKP treatment reduced PM disease incidence and severity. Significant effect on vegetative growth characteristics, early yield, number of fruits per plant and yield was recorded with this interaction.

Keywords: Agriculture method, biological control, powdery mildew and sweet pepper.

Sweet pepper (*Capsicum anuum* L.) is an important crop in Egypt either in greenhouses or net houses. Powdery mildew (PM) caused by *Leveillula taurica*, is one of the most destructive diseases that associated with higher loss in productivity of pepper under greenhouse condition (Kumer *et al*., 2008). In general powdery mildew causes losses in yield of pepper between 30 to 70 %. Direct correlation between powdery mildew incidence on leaves and yield losses was detected (Kiss, 2003).

Pepper grower's used to use heavy plant protection scheme in greenhouse to protect their investment and always keep powdery mildew out of their greenhouses or at least under their control. To achieve such results they used highly toxic substances. Dik *et al*. (2002) stated that powdery mildew does not attack fruits or stems but can quickly destroy unprotected leaves. According to the available data there are no commercial pepper varieties resistant to PM.

Using highly toxic fungicide is not acceptable any more, due to increase awareness and avoids contaminated food. Biological control can stand as suitable solution, since it can protect investment without residual toxic effect in the final product "pepper fruits". Different bio-agents such as fungi, bacteria and actinomyces can be used to control PM.

Trichoderma harzianum is considered as an ideal bio-agent for controlling many fungal diseases according to its good characteristics. *T. harzianum* acts through different modes of action, *e.g.* mycoparasitism (Abd El Moity, 1976; and Abada, 2002), production of antifungal substances (Lumsden *et al.*, 1995 and Robinson *et al*., 2005), also it owns enzymatic system causes destruction for the pathogens (Elad and Kapat, 1999 and Ziedan *et al.,* 2005). In addition to the previously mentioned modes of action, Trichoderma also acts as inducer for resistance in treated plants against certain pathogens (Abd El Moity, 1981 and Harman, 2006). *Bacillus subtilis* is well known as effective antagonistic bacterium against many plant diseases (Ali, 2013). This antagonist acts through antibiosis, secretion of volatile toxic metabolites, destructive enzymes and competition for space and nutrition (Nakkeeran *et al*., 2002). Golinska and Dahm (2011) found that most isolated strains of *Streptomyces* spp. have enzymatic activity (chitinolytic, proteolytic, pectolytic and cellulolytic). Most strains produce chitinases, catalyzing the degradation of chitin, the main component of fungal cell walls including pathogenic fungi.

Salt treatments can inhibit growth of plant pathogens or suppress mycotoxin production. Potassium silicate (K_2SiO_3) was used by Wang and Galletta (1998), to reduce the severity of strawberry powdery mildew. They found that use of Potassium silicate against PM increased chlorophyll content and plant growth vigorous. Mono potassium phosphate (MKP) as source of potassium and phosphorus is an essential plant nutrient that plays a very important role in plant growth and development. MKP has been tested against various powdery mildews with promising results. Foliar application of MKP in cucumber induces local and systemic resistance to PM (Reuveni *et al.*, 1995). Plant density and plant arrangement can influence plant development, growth and the marketable yield of peppers (Khasmakhi *et al*., 2009).

This work aims to find out:
The most effective bio-agents can replace chemical control of sweet pepper PM.
- Effect of some nontoxic chemicals (potassium silicate or MKP) on pepper PM incidence and severity.

- Correlation between planting densities on disease incidence and on some horticulture characteristics of pepper plants under greenhouse conditions.
- Effect of planting densities and bio-agents or chemicals treatments on some chemical components quality and quantity of sweet pepper fruits.

Materials and Methods

The present investigation was carried out in a clay soil at Kaha Research Station, Qalyubia governorate during two successive growing seasons (2015 and 2016) to study the effect of three bio-control agents, two natural salts and two agriculture planting densities on sweet pepper powdery mildew. Effect of these treatments on some horticultural characteristics, *i.e.* yield and chemical characteristic was also studied under greenhouse conditions.

Original high tunnel greenhouse (9x60m) was used. This high tunnel was covered with plastic sheet 200 Micron with pale yellow colour. Greenhouses in this area have history of high infestation with powdery mildew on pepper. The characteristics of the soil of Kaha were measured, the pH tends to be alkaline (pH 8.08 - 8.09) and EC 0.55-0.57 mmhos.

Sweet pepper seedlings (Gedion F1 hybrid, produced by Syngenta Company), 40 days old with three true leaves were transplanted, on September 22 and 29 for the first and the second season, respectively.

Split plot design was used with three replicates each replicate included 12 treatments to study interaction between plant density and using bio-control agents or natural chemical salts to control PM. Main plots were used for two different plant densities, *i.e.* 2 transplants / m^2 and 3 transplants / m^2. Each plot was 5 m^2 for the two agriculture methods. Each main plot was divided into 6 different sub-plots to distribute different plant protection treatments either biologically (*T .harzianum*, *B. subtilis* and *S. griseus*) or by natural chemical salts, *i.e.* Potassium silicate and Mono-Potassium phosphate, MKP) and water as control.

Lab experiments:

1- source of tested bio-agents:

Three different biocontrol agents namely, *Trichoderma harzianum*, *Bacillus subtilis* and *Streptomyces griseus* were used. *T. harzianum* and *B. subtilis* were previously isolated, purified, identified and used by Ali (2013). *S. griseus* was isolated from phyloplan of pepper plants and identified according to the morphological and physiological characteristics using methods described in Bergey's Manual of Determinative Bacteriology 9 (Cross, 2009). Purified isolates were used as bio-agents in this work.

2- Propagation of bioagents:

T. harzianum isolate was grown on liquid gliotoxin fermentation medium (GFM) developed by Brain and Hemming (1945) for 11 days under complete darkness condition at 25ºC to stimulate toxin production (Abd El Moity, 1981). *B. subtilis* isolate was grown in liquid nutrient glucose medium (NGM) developed by Dowson, (1957) for 2 days at 25ºC whereas, *S. griseus* isolate was grown in Starch nitrate medium, for 7 days at 28-30ºC (Waksman, 1959). All bioagents were prepared as suspension at concentration of 30 x 10^6 CFU / ml. Suspensions were mixed with 0.5 % potassium soap to increase distribution of bio-agent on the surface of treated plants. All adjusted preparations were diluted using water at the rate of 1: 100. Adjusted diluted preparations were used to spray pepper plants. Plants received different treatments for six times through the growing season, starting from 60 days after transplanting and repeated every 15 days. Plants sprayed with water only were acted as control.

3- Natural salts application:

Two different natural salts, *i.e.* Potassium silicate K_2SiO_3 (3%) and MKP 5% produced by Central Lab of Organic Agriculture (CLOA), were used to spray pepper plants at dilution of 1:100. All treatments were distributed on experimented plots either at low density or high plant density. The previous applications were carried out early morning. Drip irrigation and other cultural practices such as basic fertilization and pesticides were applied as recommended by Egyptian Ministry of Agriculture.

Data were recorded for the different characteristics as follows:

I - Powdery mildew assessment:

Powdery mildew was recorded after 120 days from transplanting time. Disease incidence (DI) was recorded as percentage of infected leaves. Disease severity (DS) was determined according to disease index designed by Maachia *et al*. (2010). A scale was performed with 6 classes according to infected leaf area by powdery mildew, i.e. 0= 0%, 1= 0-5%, 2= 5-25%, 3= 25-50%, 4= 50-75%, 5= 75-100%, using the following equation:

$$D.S. = \frac{\Sigma (n \times c) \times 100}{N}$$

n = number of infected leaves per category

c = category number

N = total examined leaves

II- Horticultural characteristics:

The following horticultural characteristics were taken into consideration:-

1- Vegetative growth characteristics:

Samples of pepper plants from each treatment were taken at the beginning of fruiting stage from each sub-plot to estimate average plant height (cm), number of branches and leaves per plant, leaf area (cm^2) for the above six leaves from the top of plant, that measured using LI 3000 Portable Area Meter (PAM) No. 5, produced by Li-cor Pennsylvania, fresh and dry weights (g/plant) which determined for three plants pulled off randomly from each replicate and dried at 70ºC.

2- Early and total yield :

Data were recorded on early and total yield as a number and weight of picked fruits. Early yield was recorded for picked fruits of the first three harvests, while total yield was recorded for all harvested fruits during the growing season.

3-Fruit quality:

Data concerning fruit characteristics were collected when the market stage reached the marketing fruit size. Data were recorded as average fruit length (cm), average fruit diameter (cm), flesh thickness and average fruit weight (g).

4-Chemical contents of plant and fruits:

Fruit contents of ascorbic acid and TSS were measured using fruits from the third picking. Ascorbic acid content (mg\100g fresh weight) was determined according to the method described by A.O.A.C. (2000). TSS was determined in different treatments using a hand refractometer and was expressed as percentage. Leaves content of chlorophyll was determined at the flowering stage using Minolta Chlorophyll Meter SPAD- 501 as SPAD. Total phenols and total sugars were also determined in leaves at flowering stage according to Snell and Snell (1953) and Dubois (1956), respectively.

Statistical analysis:

The obtained data were subjected to the analysis of variance procedure and means were compared using the L.S.D. at 5% level of significance according to Gomez and Gomez (1984).

Results and Discussion

Through two successive seasons, *i.e.* 2015 and 2016 trials were carried out to study the effect of planting densities, different bio-agents and some natural salts on controlling powdery mildew in sweet pepper.

I- Control of powdery mildew:

A- Effect of agriculture densities:

In general, significant reduction in disease incidence was noticed in plants grown at one row application (low plant density) compared with two rows application (high plant density). Positive correlation was noticed between reduction in disease incidence and increase in chlorophyll percentage or early yield. Data in Table 1 show that at low planting density only 38.2 and 30.3% powdery mildew disease incidence were recorded in seasons 2015 and 2016, respectively. Disease severity was also affected and only 2.5 and 2.1% were recorded during the same seasons. In case of sweet pepper planted at high density, disease incidence and severity were increased and gave 42.2 and 34% disease incidence and 3 and 2.5% disease severity. These results can be explained in the light of fact that increases in planting density lead to decrease light and increase shade on plants. This shade reduces light to reach green leaves of shaded plants, consequently reduces photosynthesis resulting in reduction in sugar content, which eventually lead to reduction in yield and quality of resulted fruit (Maynard and Scott, 1998). Powdery mildew is a low sugar disease, under shaded conditions, plant leaves contain low sugar, which is favorable to PM establishment. High plant density also leads to increase humidity surround plants which is considered as a second factor to improve disease spread out among plants. High planting density led to direct contact between diseased and healthy plants which facilitated mechanical spread out of this disease, consequently decreased early yield.

B- Effect of bio-control agents and natural salts applications:

Data in Table 1 indicate that all treatments led to significant decrease in percentages of disease incidence and severity with considerable increases in early yield and chemical components compared with control treatment. In the growing season 2015, the highest reduction in D.I. was recorded in *B. subtilis* or MKP treatment, whereas, in season 2016 the highest reduction in DI was obtained when *B. subtilis* was used and gave 19%. *T. harzianum* or MKP came in second rank. *S. graiseus* came in the last rank when compared with other bio-agents. Disease severity (D.S) was also recorded in 2015. Both of *B. subtilis* and *T. harzianum* recorded the lowest D.S (1.7 and 1.5%, respectively) compared with control (4.7%). In 2016, *T. harzianum* stand as the most effective treatment in reducing D.S followed by *B. subtilis*.

Data in the same Table were tabulated to correlate disease reduction, in different treatments, with plant physiology. Data indicating chemical analysis of total phenols and total sugars content were carried out from plants received different treatments compared with control plants. Obtained data indicate that in all tested treatments, phenolic content in treated plants was higher than in control treatment (Table1). The same situation was observed when total sugars and early yield in treated plants were compared with that recorded from the control. On the light of these finding we can explain the mechanism of disease control. This mechanism might be due to the direct effect of the tested antagonist towards the pathogen in addition to stimulate plants to resist pathogen through producing active phenols (Cherif *et al*., 2007).

C- Effect of interaction:

In general, *B. subtilis* was recorded as the highest bio-agent in one row or two rows trial compared with any other bio-agents used to control pepper PM. Regarding to the two tested natural salts, it is noticeable that MKP gave more controlling effect than potassium silicate (Table 1).

MKP is an excellent and fast source for P and K elements when applied as a foliar fertilizer. Additionally, it's able to control powdery mildew in cucumber fruits (Reuveni *et al*., 1995). Phosphorus plays a vital role in virtually every plant process that involves energy transfer. High-energy phosphate, held as a part of the chemical structures of adenosine diphosphate (ADP) as well as adenosine triphosphate (ATP). The element is a source of energy that drives the multitude of chemical reactions within the plant. When ADP and ATP transfer the high-energy phosphate to other molecules (termed phosphorylation), the stage is set for many essential processes to occur. ATP is then available as an energy source for many other reactions that occur within the plant.

In season 2015, the highest reduction in DI was recorded with *B. subtilis* or MKP treatments with one row trial. In season 2016 the highest reduction in D.I. was obtained when *B. subtilis* was used. *T. harzianum* or MKP came in the second rank.

To study the mechanism of these treatments and their effect on yield increase, two chemical analyses, *i.e*. chlorophyll and sugar contents in treated and untreated plants were determined. Obtained data in Table 1 indicate that as a result of reduction in disease incidence, chlorophyll, total phenols and sugar contents were increased in all treatments compared with the control. The increase in chlorophyll means more photosynthesis which consequently associated with more sugars content (Viiiaa *et al*., 1999). This will lead to increase in the early yield of fruits. Data also indicate that the relation between reduction in disease incidence and increase of chlorophyll, total phenol and total sugar contents was very clear when the most effective treatment (*B. subtilis*) was compared with control.

II- Horticultural characteristics:

1- Vegetative growth characteristics:

A- Effect of Agriculture method:

Data in Table 2 show that there were significant differences between the two tested agriculture methods on vegetative growth characteristics. It could be noticed that planting pepper plants on one row of the ridge significantly encouraged the physical parameters of their vegetative growth expressed as plant height, number of leaves and branches/ plant, leaf area (cm^2) as well as fresh and dry weights of plant compared with those planted in two rows of the ridge during the two studied seasons. Plants grew in one row were sufficient to allow more light penetration and increase photosynthesis efficiency. Moreover, the increments in the growth of pepper plants as a result of planting in one row method also might be attributed to the reduction of competition between plants for availability of nutrients, water and light as reported by (Elattir, 2002; De-Viloria *et al*., 2002; Preece and Read, 2005; Ara *et al*., 2007; Abubaker, 2008 and Aminifard *et al*., 2010).

B- Effect of bio-control agents and natural salts application:

Concerning the foliar application of bio-control agents and natural salts on vegetative growth characteristics data in Table 2 revealed that foliar spray with bio-control agents and natural salts led to vigorous pepper plants vegetative growth and gave best plant height, leaf area, number of leaves and branches per plant as well as fresh and dry weights per plant compared with untreated plants. In this respect, data in Table 2 also show that plants sprayed with MKP gave the highest results followed by the application with *B. subtilis*. These results might be due to that MKP contains potassium and phosphorus which are considered an essential plant nutrient source that plays a very important role in plant growth. However, potassium plays important role in photosynthesis, increase enzyme activity, improve synthesis of protein, carbohydrates and fats, enable plants to resist pests and diseases. Also, potassium is considered as a major osmotically active cation of plant cell (Mehdi *et al*., 2007). Moreover, phosphorus is a major building block of DNA molecules, thus, encouraging plant growth (Shaheen *et al*., 2007). Similar results were also obtained by Mubasher *et al.* (2013) who studied the nutrient supplement efficacy against powdery mildew of pumpcin. The obtained results also might be due to the effect of MKP as potential control for powdery mildew in field, inhibits the disease development and increase the phosphorus, nitrogen and potassium content in leaves (Nofal and Haggag, 2006 and Peyvast *et al*., 2009).

Table 1. Effect of spraying pepper plants using some bio-agents and natural salts with planting density on powdery mildew incidence and severity in relation to some chemical component and early yield in two successive growing seasons (2015 and 2016)

Treatment	* D.I. %		** D.S. %		Chlorophyll SPAD		Total Phenol (mg/100g f.w)		Total sugar (g/100g f.w)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Pd o.r. (A)	38.2	30.3	2.5	2.1	56.1	59.7	0.287	0.358	2.65	3.15
T. r. (B)	42.2	34.0	3.0	2.5	51.9	57.4	0.221	0.353	2.45	3.12
L.S.D. 5%	1.5	1.1			0.5	1.8	0.035	0.005	0.07	0.05
S.g	39.0	30.5	3.2	2.45	52.8	54.5	0.204	0.259	2.50	3.04
T.h	33.0	25.0	1.5	1.00	56.6	63.9	0.334	0.468	2.75	3.35
B.s	26.5	19.0	1.7	1.25	60.0	64.5	0.365	0.468	2.75	3.34
MKP	28.5	23.0	1.9	1.65	57.6	63.1	0.308	0.457	2.71	3.31
K_2SiO_3	41.5	37.0	3.7	3.25	51.9	56.3	0.179	0.277	2.39	3.09
Control	72.5	61.0	4.7	4.15	45.0	49.1	0.138	0.203	2.23	2.67
L.S.D. 5%	1.4	1.6			0.57	1.0	0.027	0.004	0.03	0.04
A x S.g	38.0	31.0	3.1	2.4	55.2	55.1	0.197	0.217	2.51	3.07
Ax T.h	30.0	22.0	1.2	0.8	58.6	64.2	0.386	0.495	2.84	3.39
Ax B.s	25.0	16.0	1.4	1.1	62.0	64.3	0.423	0.493	2.82	3.38
A x MPK	26.0	20.0	1.6	1.2	60.1	64.1	0.388	0.491	2.80	3.32
Ax K2Si O_3	40.0	35.0	3.5	3.1	53.6	60.1	0.181	0.249	2.48	3.06
Ax control	70.0	60.0	4.6	4.0	46.8	50.5	0.149	0.202	2.44	2.68
B x S.g	40.0	30.0	3.3	2.5	50.5	53.9	0.21	0.301	2.48	3.01
B x T.h	36.0	28.0	1.8	1.2	54.7	63.6	0.281	0.441	2.65	3.31
B x B.s	28.0	22.0	2.0	1.4	58.1	64.7	0.306	0.443	2.68	3.30
B x MKP	31.0	25.0	2.2	2.1	55.0	62.1	0.228	0.422	2.61	3.30
B x K2Si O_3	43.0	39.0	3.8	3.4	50.2	52.5	0.177	0.305	2.29	3.12
B x control	75.0	64.0	4.8	4.3	43.1	47.6	0.127	0.204	2.02	2.65
L.S.D. 5%	2.0	2.26			0.81	1.38	0.038	0.03	0.04	0.05

*D.I.= Disease incidence, **D.S.= Disease severity, Pd o.r. = Planting density One row (A) and S. g = *S. griseus*, T.h = *T. harzianum* and B.s = *B. subtilis*

C- Effect of interaction:

Regarding the interactions between the two agriculture densities, bio-control agents and natural salts application, data in Table 2 show that there were significant effects on some growth characteristics in terms of plant height, number of leaves and branches per plant, leaf area as well as fresh and dry weights per plant compared with the control. The most effective interaction treatments regarding the previous growth characteristics were recorded when plants were grown on one raw with application of MKP followed by that treated with *B. subtilis.* Moreover, all the interaction treatments of pepper plants on one raw with application of natural salts and bio-control agents gave good effect on the previous growth characteristics when compared with the control.

Table 2. Effect of spraying pepper plants using some bio-agents and natural salts with two planting densities on vegetative growth characteristics in two successive growing seasons (2015 and 2016)

Treatment	plant height (cm)		No. of leaves /plant		No. of branches /plant		Leaf area (cm^2)		Plant fresh weight (g)		Plant dry weight (g)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
P.d o.r(A)	72.78	74.78	151.39	155.00	34.11	36.61	82.41	85.94	382.28	396.34	46.56	48.89
T. r.(B)	68.56	69.67	138.22	141.50	29.28	31.06	73.76	78.18	332.67	341.50	42.56	44.39
L.S.D.5%	1.33	1.45	2.58	2.19	1.90	1.04	0.47	7.1	12.01	7.13	2.07	1.49
S. g	70.33	72.17	139.00	142.33	30.17	33.30	79.77	84.11	360.33	368.50	46.50	48.00
T. h	73.67	75.17	149.83	152.33	33.67	35.17	81.71	87.71	393.50	404.17	47.33	48.67
B.S	74.33	76.17	161.67	161.50	37.50	38.33	84.53	91.37	410.50	424.67	49.00	51.33
MKP	79.17	80.00	170.67	179.83	38.67	43.50	92.36	95.00	425.67	441.50	54.83	58.67
Si	68.33	70.17	136.83	141.00	28.67	29.67	71.17	72.99	329.00	337.50	42.00	44.00
Cont	58.17	59.67	110.83	112.50	31.50	23.00	58.98	61.18	225.73	237.33	27.67	29.17
L.S.D.5%	0.96	2.04	4.35	3.42	1.99	2.09	1.75	2.83	9.92	6.67	1.10	1.73
A x S.g	71.70	74.00	143.00	147.00	34.00	35.30	85.42	88.6	389.00	401.00	48.00	49.67
Ax T.h	77.00	79.30	159.00	161.30	37.70	38.00	85.84	89.7	421.33	443.33	48.67	50.00
Ax B.s	78.00	80.70	170.00	172.00	39.00	40.00	88.21	95.4	442.00	451.67	50.67	53.00
A x MPK	80.30	81.00	178.00	185.30	40.00	49.00	93.96	97.3	455.67	475.67	57.67	62.00
Ax Si	70.70	73.30	141.67	146.00	32.00	33.30	79.36	80.7	343.33	357.33	45.00	47.67
Ax cont	59.00	60.30	116.67	118.30	22.00	24.00	61.67	64.1	232.33	249.33	29.33	31.00
B x S.g	69.00	70.30	135.00	137.67	26.30	31.30	74.11	79.7	331.67	336.00	45.00	46.33
B x T.h	70.30	71.00	140.67	143.30	29.70	32.30	77.57	85.8	355.67	365.00	46.00	47.33
B x B.s	70.70	71.70	153.33	151.00	36.00	36.70	80.85	87.3	379.00	397.67	47.33	49.67
B x MKP	78.00	79.00	163.33	174.33	37.30	38.00	90.76	92.7	395.67	407.33	52.00	55.33
B x Si	66.00	67.00	132.00	136.00	25.30	26.00	62.99	65.3	314.67	317.67	39.00	40.33
B x cont.	57.30	59.00	105.00	106.70	21.00	22.00	56.30	58.3	219.33	225.33	26.00	27.33
L.S.D.5%	1.36	2.38	6.15	4.83	2.83	2.95	2.47	4.01	14.02	9.44	1.56	2.45

Pd o.r.= Planting density One row (A), Two row (B) = T. r. (B), S. g = *Streptomyces griseus* T.h =*Trichoderma harzianum* and B.s= *Bacillus subtilis*

2-Early and total yield:

A- Effect of Agriculture practices:

Results in Table 3 reveal that one row of plants on the ridge was more effective than two rows resulted in high yield characteristics, *i.e.* early yield, number of fruits per plant and total yield during the two studied seasons. Increasing of total yield regarding to the agriculture practices was due to the increasing of number of branches and fruits per plant. These results are in conformity with the findings of Cavero *et al*. (2001), Ara *et al*. (2007), Nasto *et al.* (2009) and Aminifard *et al*. (2010).

B- Effect of bio-control agents and natural salts application:

Foliar spray with both bio-control agents and natural salts application showed that the previous treatments increased yield characteristics, *i.e.* early yield, number of fruits per plant and total yield (kg per plant) when compared with control during the two studied seasons (Table 3). These results might be due to the effect of bio-agents on reducing in disease incidence and severity of powdery mildew of pepper as shown in Table 1. Similar results were also found by Abdel-Kader *et al.* (2012) and Alharbi and Alawlaqi (2014).

C- Effect of interaction:

The interaction between two planting densities and foliar application with bio-control agents and natural salts is shown in Table 3. The data showed significant effects on early yield, number of fruits per plant and total yield when compared with control. The most remarkable interaction treatments were noticed when MKP or *B. subtilis* treatments were sprayed on plants grown on one low planting density. Generally, one row of plants on the ridge with the application of bio-control agents or natural salts gave significant differences on the previous characteristics when compared with two rows of plants or control treatment.

3- Fruit quality:

A- Effect of Agricultural method:

Data in Table 4 indicate positive effect of one row planting density on fruit quality, *i.e.* average fruit weight, length and diameter and flesh thickness during the two studied seasons. The increasing of fruit length, diameter and weight may be due to the increase of plant stand establishment and increase of vegetative growth characteristics with one row of plants on the ridge (Vavrina *et al.*, 1994). Similar findings of increased average fruit weight with wider spacing plants were reported by Miyao (2002) and Jovicich *et al*. (2003). This is likely due to the competition associated with the higher plant populations resulting in the lower weight and volume mean fruits.

B- Effect of bio-control agent's and natural salts application

Concerning the foliar application of bio-control agents or natural salts on fruit quality, data in Table 4 indicate a positive correlation between foliar applications and average fruit length, fruit diameter and fruit weight during the two studied seasons. Data revealed that maximum values of the previous fruit quality were found with MKP application followed by spraying with *B. subtilis*, while the lowest vegetative growth was recorded with control. Vegetative growth can provide more photosynthesis activity and more synthesis of soluble matter, such as sugars, which affect fruit quality characteristics (Halil *et al*., 2011). Also, Lovatt (1999) found that foliar application of MKP improved fruit quality and yield of citrus and avocado.

Table 3. Effect of spraying pepper plants using some bio-agents and natural salts with two planting densities on early and total yield/plant in two successive growing seasons (2015 and 2016)

Treatment	Early yield/plant				Total yield/plant			
	2015		2016		2015		2016	
	No.	Weight (kg)	No.	Weight (kg)	No.	Weight (kg)	No.	Weight (kg)
P.d o.r (A)*	4.8	0.620	5.2	0.835	35.2	4.440	38.1	4.680
T. r.(B)**	4.5	0.537	4.7	0.714	30.3	3.830	32.2	4.190
L.S.D. 5%	0.3	0.04	0.1	0.020	1.6	0.210	1.1	0.140
S. g	4.8	0.583	5.0	0.843	31.7	4.080	35.3	4.440
T. h	5.0	0.712	5.6	0.935	34.7	4.430	36.7	4.745
B.S	5.4	0.755	5.8	0.966	38.5	5.140	39.3	5.324
MKP	6.1	0.883	6.2	1.000	39.7	6.000	44.3	6.322
Si	3.5	0.363	3.6	0.534	29.3	3.340	30.8	3.670
Cont	3.1	0.267	3.4	0.370	22.5	1.810	23.3	2.101
L.S.D. 5%	0.1	0.040	0.2	0.040	1.5	0.190	1.1	0.130
A x S.g	4.9	0.610	5.1	0.910	35.7	4.359	38.0	4.786
Ax T.h	5.0	0.794	5.8	1.020	38.3	4.839	39.7	4.914
Ax B.s	5.4	0.850	6.1	1.027	40.3	5.382	41.0	5.531
A x MPK	6.6	0.917	6.7	1.083	41.3	6.295	49.7	6.645
Ax Si	3.7	0.403	3.8	0.607	32.7	3.805	34.7	3.898
Ax cont	3.2	0.277	3.7	0.363	22.7	1.958	25.3	2.300
B x S.g	4.7	0.503	5.0	0.777	27.7	3.800	32.7	4.095
B x T.h	5.0	0.630	5.5	0.850	31.0	4.000	33.7	4.577
B x B.s	5.3	0.660	5.5	0.905	36.7	4.900	37.7	5.117
B x MKP	5.6	0.850	5.7	0.917	38.0	5.700	39.0	6.000
B x Si	3.4	0.323	3.5	0.462	26.0	2.900	27.0	3.435
B x cont	3.0	0.257	3.1	0.377	22.3	1.700	23.3	1.900
L.S.D. 5%	0.1	0.06	0.3	0.05	1.1	0.27	1.5	0.180

Pd o.r. (A)* = Planting density One row (A), Two row (B)** = T. r. (B), S. g = *Streptomyces griseus*, T. h = *Trichoderma harzianum* and B.s = *Bacillus subtilis*

C- Effect of interaction:

Concerning the interactions between agriculture densities and bio-agents' or natural salts application, data in Table 4 show significant effect on some fruit characteristics in terms of average fruit weight, length and diameter. The most effective interaction treatments regarding the previous fruit characteristics were recorded when pepper plants were grown on one raw with application of MKP or *B. subtilis*. All the interaction treatments gave significantly effects on the previous fruit characteristics when compared with control treatment.

Table 4. Effect of spraying pepper plants using some bio-agents and natural salts with two planting densities on some fruit quality properties in two successive growing seasons (2015 and 2016)

Treatment	Fruit weight (g)		Fruit length (cm)		Fruit diameter (cm)		Flesh thickness (cm)	
	2015	2016	2015	2016	2015	2016	2015	2016
P.d o.r(A)*	177.17	175.39	13.36	13.15	6.4	6.5	0.48	0.49
T. r.(B)**	143.33	153.44	12.72	12.42	5.7	6.0	0.43	0.44
L.S.D. 5%	10.25	4.08	0.63	0.23	0.1	0.1	0.02	0.04
S. g	158.00	167.17	13.83	12.58	6.1	6.3	0.46	0.47
T. h	169.17	171.50	12.41	12.67	6.2	6.4	0.48	0.50
B.S	177.83	173.50	13.58	14.00	6.3	6.5	0.51	0.52
MKP	181.17	198.17	12.42	14.17	6.7	6.8	0.53	0.55
Si	151.00	149.33	12.75	12.37	5.8	6.1	0.42	0.43
Cont	124.33	126.83	11.25	10.92	5.3	5.5	0.33	0.35
L.S.D. 5%	3.53	2.84	0.53	0.27	0.2	0.2	0.05	0.06
A x S.g	178.30	178.30	14.8	13.0	6.4	6.5	0.48	0.50
Ax T.h	182.30	177.30	13.2	13.2	6.6	6.8	0.50	0.53
Ax B.s	195.00	179.00	13.2	14.2	6.7	6.9	0.52	0.53
A x MPK	200.70	216.00	14.7	14.3	7.0	7.1	0.55	0.57
Ax Si	177.70	171.30	12.8	12.9	6.1	6.3	0.45	0.47
Ax cont	129.00	130.30	11.5	11.3	5.6	5.7	0.35	0.37
B x S.g	137.70	156.00	12.8	12.2	5.7	6.1	0.43	0.43
B x T.h	156.00	165.70	11.7	12.2	5.8	6.0	0.47	0.47
B x B.s	160.70	168.00	14.0	13.8	6.0	6.2	0.50	0.50
B x MKP	161.70	180.30	14.2	14.0	6.3	6.5	0.42	0.53
B x Si	124.30	127.30	12.7	11.8	5.6	5.8	0.38	0.40
B x cont	119.70	123.30	11.0	10.5	5.1	5.3	0.30	0.33
L.S.D. 5%	4.98	4.01	0.76	0.38	0.2	0.3	n.s.	n.s.

Pd o.r. (A)* = Planting density One row (A), Two row (B)** = T. r. (B), S.g= *Streptomyces griseus* T.h = *Trichoderma harzianum* and B.s= *Bacillus subtilis*

4-Chemical fruit composition:

*A- Effect of Agriculture practices***:**

Regarding the parameters of chemical composition of sweet pepper fruits, data in Table 5 clear that fruits of plant which planted in one row had a significant increase in their contents of total soluble solids (TSS) and vitamin C in the first and second seasons. These results might be due to increasing the vegetative growth characteristics as shown in Table 2.

B- Effect of bio-control agents and natural salts application:

Effect of foliar application of bio-control agents and natural salts on TSS and vitamin C represented as ascorbic acid of sweet pepper fruits as shown in Table 5 during the two studied growing seasons. Foliar application with natural salts or bio-control agents led to increase in the fruit TSS and ascorbic acid (vitamin C) contents. Data revealed that maximum values of the previous chemical contents were found with application of MKP followed by *B. subtilis.*

C- Effect of interaction:

Regarding to the interactions between agriculture methods and natural salts and bio-control agents application data in Table 5 indicate that the most significant favorable beneficial interaction treatments regarding the fruit ascorbic acid and TSS content were obtained from plants grown on one raw with application of MKP or *B. subtilis* at the first and second seasons.

Table 5. Effect of spraying pepper plants using some bio-agents and some natural salts with two planting densities on fruit chemical component in two successive growing seasons (2015 and 2016)

Treatment	TSS %		V.C Mg/100g f.w.	
	2015	2016	2015	2016
Pd o.r. (A)*	5.33	5.57	131.89	133.78
T. r. (B)	4.78	4.93	106.39	115.17
L.S.D. 5%	0.06	0.19	1.36	3.56
S. g	4.97	5.17	113.13	117.91
T. h	5.20	5.33	120.67	125.91
B.S	5.40	5.5	135.03	140.52
MKP	5.83	6.0	138.84	143.49
Si	4.77	4.93	108.83	114.61
Cont	4.27	4.57	98.33	104.4
L.S.D. 5%	0.15	0.18	1.74	1.96
A x S.g	5.3	5.5	117.7	119.6
Ax T.h	5.5	5.7	132.4	134.2
Ax B.s	5.8	6.0	155.7	157.2
A x MPK	6.1	6.3	156.3	159.9
Ax Si	5.0	5.2	116.6	117.1
Ax cont	4.4	4.8	112.7	114.7
B x S.g	4.6	4.8	108.6	116.3
B x T.h	4.9	5.0	108.9	117.6
B x B.s	5.0	5.0	114.4	123.8
B x MKP	5.6	5.7	121.3	127.1
B x Si	4.5	4.7	101.1	112.1
B x cont	4.1	4.4	84.0	94.1
L.S.D. 5%	0.21	0.26	2.46	2.77

Pd o.r. (A)* = Planting density One row (A), Two row (B)** = T. r. (B), S. g= *Streptomyces griseus*, T.h= *Trichoderma harzianum* and B.s = *Bacillus subtilis*

Conclusion

As a conclusion, one row of plants in the ridge led to decrease powdery mildew disease incidence and severity, increase the vegetative growth characteristics, fruit quality and yield compared with two rows. The highest reduction in disease incidence was obtained when *B. subtilis* was used. Maximum values of the vegetative growth characteristics, fruit quality and yield were recorded when foliar application with MKP followed by *B. subtilis* in one row application.

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تأثيراستخدام بعض عوامل المقاومة الحيوية، الأملاح الطبيعية و كثافة النباتات على مقاومة مرض البياض الدقيقى فى الفلفل الحلو وتأثير ذلك علي بعض الصفات البستانية تحت ظروف الصوبـــة

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يهدف هذا البحث إلى دراسة تأثير ثلاثة من عوامل المقاومة الحيوية المختلفة، وهـــــي *Trichoderma harzianum, Bacillus subtilis* and *Streptomyces griseus*، بالإضافة إلى اثنين من الأملاح الطبيعية وهى سيليكات البوتاسيوم والفوسفات أحادي البوتاسيوم مع اختلاف كثافة نباتات الفلفل الحلو المنزرعة على مسافتين (زراعة في صف واحد أو صفين على مصطبة الزراعة) وذلك على نسبة الإصابة بمرض البياض الدقيقى وشدته، بعض الصفات البستانية و المحصول فى الفلفل الحلو. وقد أجريت التجربة تحت ظروف صوبة بلاستيكية مرتفعة، محطة أبحاث الخضر في قها، محافظة القليوبية، خلال موسمين متتاليين ٢٠١٥ و ٢٠١٦. وقد أوضحت النتائج أن الزراعة في صف واحد من النباتات على المصطبة أدى إلى انخفاض فى نسبة و شدة الإصابة بالبياض الدقيقي عند مقارنتها بالزراعة فى صفين. ووجد أنه عند زراعة النباتات فى صف واحد زيادة فى معدلات نمو الفلفل من حيث ارتفاع النبات، عدد الأوراق، وعدد الأفرع، مساحة الورقة، والوزن الطازج والجاف للنبات مقارنة بالزراعة فى صفين. سجلت زيادة فى جودة الثمار (متوسط وزن الثمرة، طول وقطر الثمرة وسمك اللحم) وأيضا بعض الصفات الكيميائية والمحصول (المحصول المبكر والمحصول الكلي / نبات) عند الزراعة فى صف واحد. وفيما يتعلق بالمقاومة الحيوية والأملاح الطبيعية، أظهرت النتائج أن أعلى انخفاض في نسبة الاصابة بالمرض تم الحصول عليــها عنــد استخــدام *B. subtilis*. كذلك تــم تقديــر الشدة المرضية للمسبب.

و سجلـت المعاملة *T. harzianum* أعلى نسبة كفاءة فى خفض الشدة المرضية. أيضاً سجلت أعلى القيم لصفات النمو الخضري وجودة الثمار والمحصول الكلي عند الرش الورقي بالبوتاسيوم أحادي الفوسفات، يليــه استخــدام *B. subtilis*. أظهرت التحاليل الكيميائية للنباتات المعاملة ان مقاومة المرض مرتبطة بشكل ايجابى بكمية الفينولات والسكريات. تأثير التفاعل بين الزراعة فى صف واحد مع الرش بالبكتيريا *B. subtilis* أو البوتاسيوم أحادى الفوسفات الذي أعطى أعلى خفض فى شدة ونسبة الاصابة بالبياض الدقيقى مصحوباً بزيادة معنوية فى المحصول المبكر وعدد الثمار والمحصول النهائى مقارنة بالتفاعلات الاخرى.