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Growth, Physio-Biochemical Aspects, Productivity and Fungicide Residue in Tomato Plants Affected by Tolclofos-Methyl (Rizolex) Fungicide and some Remediating Agents

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ABSTRACT

Vegetative growth of Tomato was negatively influenced by Tolclofos-methyl addition, and its phytotoxic effect was more pronounced on plant height and leaf area than other growth parameters. Application of Tolclofos-methyl fungicide at high rate caused marked decline in leaf chloroplast pigments, and carotenoids were more influenced. Total water content and leaf water deficit increased but the relative water content decreased as a result of Tolclofos-methyl application. Transpiration rate tended to increase at the lower dose of Tolclofos-methyl but decrease at its higher rate. Membrane integrity was increased with increasing the fungicide rate. Mineral elements (N, P, K, Ca, Mg, Fe, Zn, Mn), plant growth promoter substances (IAA, GA_3, Cytokinins), and total free amino acids were reduced, whereas ABA, proline content, total phenol and the activity of phenoloxidase and peroxidase were increased in the fungicide exposed plants. Using Tolclofos-methyl fungicide at the high rate significantly reduced the number of flowers and fruit, vitamin C content of the fruits, yield, and water use efficiency of tomato plants. Total soluble solids and acidity in tomato fruits were increased, whereas lycopene content was decreased. Tolclofos-methyl fungicide residue in tomato fruits was dramatically enhanced above the maximum level of residue of Tolclofos-methyl fungicide (100 µg kg^{-1} in tomato fruits) and reached 176.8 and 423.2 µg kg^{-1} fresh weight at the rates of 2 and 4 g m^{-2} soil, respectively. Application of *Bacillus*, *Trichoderma*, GA_3 and BP caused marked enhancements in the growth, yield, plant water relations, biochemical constituents, photosynthetic pigments and chemical properties of tomato fruits under both of the normal and fungicide pollution conditions. In addition, using these agents reduced the fungicide residue in fruits by about 42, 73, 35 and 5 % at the fungicide rate of 2 g m^{-2} soil and by about 80, 38, 68 and 47% at the rate of 4 g m^{-2} soil, respectively. It could be concluded that *Trichoderma* followed by *Bacillus* at the recommended dose of fungicide or *Bacillus* followed by GA_3 at the exceeded dose of fungicide are useful tools to alleviate the phytotoxic effects and reduce the residue level of Tolclofos-methyl in tomato fruits below its maximum level.

Keywords: Tomato, Tolclofos-methyl, Agents, Physiological aspects, Biochemical constituents, Yield, Fungicide residue.

INTRODUCTION

Tomato (Solanum *Lycopersicun* L.) belong to Solanaceae family and its fruit has special nutritional value. Its fruits are rich in nutrient elements (iron, phosphorus, calcium) and vitamins, and contain proteins, fats and carbohydrate in appreciable amounts. In Egypt and all over the world, tomato fruits used for soup, salad, pickles, ketchup, puree, sauces and in many other ways, also used as a salad vegetable. Egypt occupies the 5th rank between the tomato producing countries in the world. The harvested area of tomatoes in Egypt is 199,792ha with a yield of 7,943,285 tons in 2016 (FAOSTAT, 2016). Tomato is susceptible to more than 200 diseases and this can lead to high reductions by 70 - 95% in its yield. There are many diseases can attack tomato plants in the root system (fusarium wilt, verticiliun wilt, bacterial wilt, rhizoctonia), shoot system (early blight, leaf spot, bacterial canker, late blight), and fruits (bacterial spot, bacterial speck, anthracnose). Fungal diseases of tomato are resulted from several pathogenic fungi (Wani, 2011) and can cause marked decreases (20-35%) in its yield (Gullino *et al.,* 2000; McGrath, 2004). Consequently, fungicides become crucial to control of fungal diseases. The abusive application of fungicides in agriculture leads to fungicide contamination in the environment, water and soil. The levels of fungicide contamination are found in high levels in soils and drainage water as well as in different plant organs such as leaves and fruits. Fungicides may produce stable residues that can absorb by the root system and then translocate to the leaves and fruits. This may be responsible for some plant phytotoxic symptoms and for many chronic diseases for animal or human (EFSA, 2009). Tolclofos-methyl (Rizolex) is an organophosphate with curative and slightly systemic action, and has high fungi toxicity to *Rhizoctoniasolani* (Virgen-Calleros *et al.,* 2000). Studies concerning the effect of fungicide Tolclofos-methyl "Rizolex" on the growth, yield and its components, as well as, the chemical and physiological aspects of plants especially tomatoes are scarce. Bal *et al*. (2006) found that fungicide Rizolex and peat combination was more favorable for length and stem diameter of seedling, fresh weight and number of leaves of the lettuce plants at harvest, while single use of fungicide retarded the growth and development of seedlings. Therefore, this work was aimed to study the changes induced in the physiological and biochemical aspects of the polluted-tomato plants with fungicide Tolclofos-methyl "Rizolex" and treated with some remediating agents as an ecological technique for counteracting its phytotoxic effect on tomato plants and its toxic residue in their fruits.

MATERIALS AND METHODS

1. Pot experiment

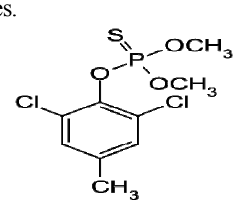
Greenhouse experiments were conducted in a glasshouse under natural light conditions at the Experimental Farm of the Faculty of Agriculture, Shibin El-Kom, Menoufia University. Tomato transplants cv. Floradade, age 30 days, obtained from the Horticulture Dept., Faculty of Agriculture, Menoufia University were grown in plastic pots with 30-cm inner diameter, on two consecutive seasons (April 5, 2015 and 2016). The experimental completely random design involved 15 treatments (4 replicates of each treatment) as follows:

1) Control.
2) T-MF2 (2 g Rizolex.m^{-2} soil).
3) T-MF4 (4 g Rizolex.m^{-2}soil).
4) Bacillus (24 g.m^{-2}soil).

5) Trichoderma (24 $g.m^{-2}$soil).
6) GA_3 (100 ppm).
7) Bp (0.04% w/w).
8) T-MF2+ Bacillus.
9) T-MF4+ Bacillus.
10) T-MF2+Trichoderma.
11) T-MF4+Trichoderma.
12) T-MF2+ GA_3.
13) T-MF4+ GA_3.
14) T-MF2+ Bp.
15) T-MF4+ Bp.

2. Fungicide and remediating agents

Pesticide used in this work was Tolclofos-methyl (50% SC) Fungicide, white crystalline solid, obtained from Pesticide Dept, Faculty of Agric., Shibin El-Kom. The common name of Tolclofos-methyl is Rizolex with a chemical name of *O*-(2,6-Dichlor-4-methylphenyl)-*O*,*O*-dimethylthiophosphate, a molecular formula of $C_9H_{11}Cl_2O_3PS$, a molecular weight of 301.1266 $gmol^{-1}$ and a structural formula of Bacillus bacteria and Trichoderma in powder form, obtained from Center of Agric. Res., Biological control Dept, Min. of Agric., Cairo. The Barbry Plant "BP", French product, contains NPK at rate 18% (NPK 18:18:18), oligoelements (B 150 mg/l; Cu 32 mg/l; Fe 420 mg/l; Mn 270 mg/l; Mo 13 mg/l, Zn 120 mg/l); especial Polymer Barbry Plant was allowed by Ministry of Agriculture, France under 9010133 at rate of 0.004%; transplantation 0.05%; cellulose fibers 10%; stimulative liquid 40% and other materials resistant to salinity and control of diseases.



3. Experimental work

Pots were filled with 8 kg clay loam soil taken from the Experimental Farm of Agric., Faculty of Agriculture (EC_e=2.8 $dS.m^{-1}$; pH=7.9; Soluble salts= 0.16%), treated with the different agents mentioned before then tomato transplants were carefully sitting in them. All pots were received the recommended fertilizers (calcium superphosphate (15.5% P_2O_5) at rate of 1.6 g P_2O_5 pot^{-1} before planting, nitrogen (N) in the form of ammonium nitrate (33% N) at a rate of 1.36 g N pot^{-1} and potassium (K) in the form of potassium sulphate (48% K_2O) at a rate 0.81 g K_2O pot^{-1} were added in two doses throughout the growth period). Pots were irrigated with tap water whenever required to keep the moisture in soil at about 65% of the total water holding capacity (WHC) of the soil during the experimental period. After eighty days from transplanting, from each replicate one plant sample was carefully and randomly taken from each treatment to determine the following:

4. Growth characters

Plant height (cm), leaves number per plant, dry weights of root, stem and leaves (g $plant^{-1}$) were recorded. These parts were dried at 70ºC for 72 hrs in an electric oven and then the root/shoot ratio was calculated. The total leaf area ($cm^2.plant^{-1}$) was determined using the disc method according to Bremner and Taha (1966), and leaf area index (LAI) was calculated according to the formula of Simone *et al*. (1993) (LAI= total leaf area, cm^2/ area of pot surface, cm^2).

5. Physiological and Biochemical characteristics

Photosynthetic pigments were estimated in fresh leaves extracted by acetone 80%, calculated as $mg.g^{-1}$ dry weight according to Wettestein (1957).Total water content (TWC, %), relative water content (RWC, %), leaf water deficit (LWD, %), sclerophylly degree (%) and transpiration rate ($mg.cm^{-2}.h^{-1}$); were measured according to Kalapos (1994) and Kreeb (1990).Succulence degree was calculated using the formula, succulence degree = leaf fresh weight/leaf dry weight. Water use efficiency was calculated as g. kg^{-1} H_2O [(total fruit yield, (g)/amount of water used, (kg H_2O)] according to Vites (1965). Membrane integrity (%) was measured according toYan *et al*. (1996) as follow: A portion of the excised fresh leaves was washed then put in a beaker containing double distilled water. The beakers were kept at 30 °C for 3 hrs, then conductivity (C_1) of the solution was measured by the conductivity meter (Model: CD-4301).

After boiling the samples for 2 min, their conductivity (C_2) was measured again when the solution was cooled to room temperature. The percentage of electrolyte leakage was calculated according to the formula:

$$[EC\ \% = (C_1/C_2) \times 100].$$

Proline content was extracted from a known weight of the fresh leaves by 3% aqueous sulphosalicylic acid, filtered through double rings filter paper then estimated (µmole. g^{-1} f.w.t.) according to the methods described by Bates *et al*. (1973). Total phenol content was estimated in plant leaves (mg. g^{-1} dwt.) according the methods described by Sadasivam and Manickam (1992). The phenoloxidase and peroxidase activities was determined according the methods described by Sadasivam and Manickam (1992). Nutrient elements (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and copper (Cu)) were determined in 0.2 gm of dried ground leaves of the tested plants after its digestion in H_2SO_4 (concentrated), H_2O_2 (5:1) according to A.O.A.C. (1995).

The concentration of the N, P, K, Ca and Mg elements were expressed in (%) of dry weight, whereas the elements Fe, Mn and Zn were expressed in mg kg^{-1}. Plant hormones in fresh shoots of tomato plants were extracted and determined according to Shindy and Smith (1975), hormone analysis was performed by using HPLC according to Crocier and Moritz (1999). Phytohormones were calculated as ng. g^{-1} fwt. The Chemical aspects of tomato fruits were estimated in the harvested tomato fruits. The total soluble solids (%) was determined using an Abe hand refractometer, acidity (%), vitamin C concentration (mg Ascorbic acid.100 g^{-1} fwt.) and lycopene concentration (mg. 100 g^{-1} fwt.) were estimated using the methods of A.O.A.C. (1995). Total carbohydrates (mg. g^{-1} dwt.) and total protein (%; Total N*6.25) were estimated in a known weight of tomato fruit powder according to the methods described by Sadasivam and Manickam (1992).

6. Flowering and Yield traits

Three plants from each treatment was tagged to measure the flowering and fruiting characters then the number of flowers per plant and fruit set (%) were recorded.

Red ripe tomato fruits for each treatment were carefully harvested two times weekly then the fruit diameter (cm), fruit yield (g. $plant^{-1}$) and total yield ($g.pot^{-1}$) were estimated, and calculated as $kg.m^{-2}$.

7. Fungicide residue determination

A sample from the harvested tomato fruits from the different treatments was taken to estimate the fungicide residue of Tolclofos-methyl (Rizolex) in tomato fruits using Gas Liquid according to the method described by Their and Zeumer (1987), the residue of fungicide as $\mu g.kg^{-1}$ fruits was calculated.

8. Statistical analysis

Data obtained were statistically analyzed using SAS software, version 9.2 (SAS Institute 2008) to conduct analysis of variance tests (ANOVA). Orthogonal comparisons between the means of the different treatments were performed using Duncan's New Multiple Range at a 5% significance level.

RESULTS AND DISCUSSION

Results

1. Growth parameters

Application of Tolclofos-methyl fungicide had significant adverse effect on plant height (PH) either using the recommended dose or the high rates (Table 1). Under the high rate (4 gm^{-2} soil), the reduction percentage in PH reached about 38% for the 1st season and 33% for the 2nd one compared with the control plants. However, using the remediating agents (Bacillus bacteria, Trichoderma, GA_3 and BP) in the non-polluted soil caused an increase in plant height. A relevant decrease in the number of leaves per plant was observed at 2g fungicide (16% for 1st season and 18% for 2nd season), while using the highest rate of fungicide (4 g m^{-2} soil) caused more decrease in number of leaves per plant (40 % for 1st season and 33% for 2nd season). A significant decrease in total leaf area (LA) and leaf area index (LAI) was noticed at both of the recommended dose of fungicide (38 and 56% for 1st season and 39 and 36% for 2nd season) and the high rate (47 and 72% for 1st season and 43 and 39% for 2nd season). Bacillus agent gave the highest increase in LA (99 and 144% for 1st season and 199 and 229% for 2nd season at 2 and 4 g fungicide m^{-2} soil, respectively) and LAI (99 and 258% for 1st season and 190 and 228% for 2nd season at 2 and 4 g fungicide m^{-2} soil, respectively) of the polluted tomato plants over the control fungicide treated plants without using the remediating agents.

Dry weights of all plant organs were significantly decreased with increasing the rate of fungicide up to 4g in soil. Treating the fungicide-polluted soils (2 g m^{-2} soil) with BP was not only useful in alleviation the inhibitory effect of fungicide on root, stem and whole plant dry matter compared with the other treatments, but also caused great increases in these traits (181, 91and 94 % for1st season; 197, 213, 168% for 2nd season, respectively). The obtained results showed an inconsistent trend in Shoot/Root ratio (S/R ratio) at the rate of 4g m^{-2}soil fungicide, whereas a significant reduction (42% and 32% for 1st and 2nd seasons, respectively) in S/R ratio at the low rate (2 g m^{-2} soil). In the polluted soils with fungicide at 2 g m^{-2} soil, using Bacillus bacteria led to a great increase in S/R ratio meanwhile at the high rate (4g m^{-2} soil) Trichoderma only was effective in this respect compared with the other agents

Table 1. Effect of Tolclofos-methyl fungicide, some agents and their combinations on some vegetative growth characters of tomato plants during the two growing season 2015 and 2106.

Characters / Treatments	Plant height (cm)	leaves No. per Plant	Dry weight (g/plant)				Shoot/ root ratio	Leaf area (cm^2/plant)	Leaf Area Index	Plant height (cm)	leaves No. per Plant	Dry weight (g/plant)				Shoot/ root ratio	Leaf area (cm^2/ plant)	Leaf Area Index
			Root	Stem	Leaves	whole						Root	Stem	Leaves	whole			
	Season 2015									Season 2016								
Control	41.55^{bcd}	12.50^{cde}	0.41^{de}	0.90^{def}	1.15^{de}	2.46^{gh}	5.00^{c}	339.02^{c}	2.70^{ab}	40.50^{bcd}	13.33^{cde}	0.44^{fg}	0.86^{def}	1.39^{fgh}	2.69^{def}	5.11^{bc}	395.83^{f}	1.58^{cde}
T-MF2	29.00^{gh}	10.50^{f}	0.47^{de}	0.82^{ef}	1.12^{de}	2.41^{gh}	2.90^{ef}	211.64^{f}	0.90^{c}	29.50^{fg}	11.00^{ef}	0.58^{efg}	0.78^{ef}	1.25^{gh}	2.61^{ef}	3.50^{cdef}	240.90^{h}	1.02^{e}
T-MF4	25.60^{h}	7.50^{g}	0.31^{e}	0.74^{f}	0.95^{e}	2.00^{h}	5.45^{bc}	180.05^{f}	0.76^{c}	27.00^{g}	9.00^{f}	0.38^{g}	0.71^{f}	1.01^{h}	2.10^{f}	4.53^{bcd}	225.53^{h}	0.96^{e}
Bacillus	50.75^{a}	13.75^{bcd}	0.55^{cde}	2.00^{a}	1.85^{bc}	4.40^{bcd}	7.00^{a}	447.43^{ab}	3.56^{a}	50.6^{a}	14.67^{cd}	0.72^{cdef}	1.73^{bc}	1.55^{efgh}	4.00^{cde}	4.56^{bcd}	595.15^{c}	2.37^{bc}
Trichoderma	42.00^{bcd}	14.75^{b}	0.43^{de}	1.39^{bcde}	1.23^{de}	3.05^{f}	6.09^{b}	339.85^{c}	2.71^{ab}	46.50^{ab}	16.67^{bc}	0.53^{efg}	1.48^{bcde}	1.38^{fgh}	3.39^{cdef}	5.40^{b}	431.25^{e}	1.72^{cde}
GA_3	47.55^{ab}	14.50^{b}	0.50^{cde}	1.70^{ab}	1.82^{bc}	3.82^{de}	7.04^{a}	435.03^{ab}	3.46^{a}	45.34^{abc}	15.33^{bcd}	0.49^{efg}	1.76^{bc}	2.22^{cde}	4.47^{bc}d	8.12^{a}	740.00^{a}	2.95^{ab}
Bp	47.00^{abc}	18.00^{a}	0.99^{abc}	1.72^{ab}	1.84^{bc}	4.55^{abc}	3.60^{d}	469.56^{a}	3.74^{a}	45.50^{abc}	20.00^{a}	0.71^{cde}	1.62^{bc}	1.93^{defg}	4.26^{bcde}	5.00^{bc}	588.42^{c}	2.34^{bc}
T-MF2 + Bacillus	42.70^{bcd}	11.50^{ef}	0.75^{bcde}	1.33^{bcde}	2.22^{ab}	4.30^{cde}	4.72^{c}	420.62^{b}	1.79^{bc}	45.90^{abc}	14.00^{cde}	0.64^{defg}	1.61^{bc}	3.58^{a}	5.83^{ab}	8.08^{a}	698.09^{b}	2.96^{ab}
T-MF2+ Trichoderma	31.20^{fgh}	12.50^{cde}	0.53^{cde}	0.90^{def}	1.05^{e}	2.48^{h}	3.66^{de}	279.14^{e}	1.19^{bc}	33.90^{abc}	13.00^{de}	0.65^{deg}	1.09^{cde}	1.47^{fgh}	3.21^{cdef}	3.92^{bcde}	299.21^{g}	1.27^{de}
T-MF2 + GA_3	37.35^{def}	12.50^{cde}	0.84^{bcd}	0.95^{def}	1.10^{de}	2.89^{fg}	2.43^{f}	363.97^{c}	1.55^{bc}	39.60^{ef}	14.00^{cde}	1.24^{b}	1.20^{cdef}	1.30^{fgh}	3.74^{cdef}	2.01^{f}	246.28^{h}	1.05^{e}
T-MF2 + Bp	40.50^{cde}	14.00^{bc}	1.32^{a}	1.56^{abc}	2.16^{abc}	5.04^{a}	3.80^{d}	357.51^{c}	1.52^{bc}	45.50^{cde}	18.00^{ab}	1.72^{a}	2.44^{a}	2.83^{bc}	6.99^{a}	3.07^{def}	575.31^{c}	1.59^{cde}
T-MF4 + Bacillus	46.40^{abc}	14.00^{bc}	1.06^{ab}	1.60^{abc}	2.27^{ab}	4.93^{ab}	3.66^{ed}	439.77^{ab}	2.72^{ab}	48.50^{a}	14.00^{cde}	0.99^{bc}	2.01^{ab}	3.02^{ab}	6.02^{ab}	5.06^{bc}	740.85^{a}	3.15^{a}
T-MF4+ Trichoderma	43.25^{bcd}	18.00^{a}	0.55^{cde}	1.45^{abcd}	2.45^{a}	4.45^{bc}	7.04^{a}	445.20^{ab}	1.89^{bc}	45.50^{abc}	21.00^{a}	0.74^{cde}	1.56^{bcd}	2.47^{bcd}	4.77^{bc}	5.44^{b}	450.37^{de}	1.91^{cd}
T-MF4 + GA_3	34.75^{efg}	12.00^{def}	0.68^{bcde}	1.09^{cdef}	2.25^{ab}	4.00^{cde}	4.90^{c}	332.27^{cd}	1.41^{bc}	37.50^{de}	13.00^{de}	0.78^{cde}	1.11^{cdef}	2.95^{ab}	4.84^{bc}	5.18^{bc}	459.82^{d}	1.95^{cd}
T-MF4 + Bp	28.75^{gh}	11.00^{ef}	0.99^{abc}	0.81^{ef}	1.63^{cd}	3.73^{e}	2.46^{f}	300.78^{de}	1.28^{bc}	29.50^{fg}	12.00^{def}	1.06^{bc}	0.81^{ef}	1.99^{def}	3.86^{cdef}	2.65^{ef}	377.60^{f}	1.60^{cde}

T-MF2: Tolclofos-methyl fungicide at rate 2 $g.m^{-2}$ soil, T-MF4: Tolclofos-methyl fungicide at rate 4 $g.m^{-2}$ soil, GA_3: Gibberellic acid, Bp: rBarby Plant.

2. Physiological and Biochemical characteristics

1. Photosynthetic pigments

A significant decrease in photosynthetic pigments (chlorophylls a, b and total and carotenoids content) was found in the leaves of fungicidal polluted plants with 4 g fungicide m^{-2} soil as shown in Table (2). The percentage of reduction in these pigments were 46, 3, 33 and 56% for the 1st season) and 43, 3, 31 and 53% for the 2nd season, respectively. Treating the fungicidal polluted soils with Bacillus, GA_3, Trichoderma and BP not only led to counteracting the inhibitory effect exerted by fungicide but also increased the concentration of photosynthetic pigments.

Generally, treatment with Bacillus bacteria caused an increase of 99, 74 and 93% for chl. a, total chl. and carotenoids, respectively at the 1st season.

2.Plant Water relations

Data illustrated in Table 3 indicate that the leaf total water content (TWC) tended to increase in the plants grown in the Tolclofos-methyl fungicidal polluted soils (14 % and 20% at the rate of 2 g fungicide. m^{-2} soil; 12and 16% at the rate of 4 g fungicide m^{-2}soil). Regarding the relative water content (RWC) and leaf water deficit (LWD) in relation with fungicide rates, remediating agents as well as their combinations, it is found that RWC sharply decreased in the polluted soils with 4 g fungicide m^{-2}soil by 25% at the 1st season and by 20% at the 2nd season, whereas LWD was increased by 31% at the 1st season and by 20% at the 2nd season. Succulence degree (SuD) significantly increased with the application of the fungicide. Under the normal conditions, treating with Bacillus and BP led to an increase in SuD, whereas treating with the other agents decreased it. In the fungicidal polluted soils, all remediating agents significantly decreased SuD at both two fungicide rates except for GA_3 under the low rate and Bacillus under the high rate which caused a marked increase by about 38% for GA_3 and 63% for Bacillus. Sclerophylly degree (ScD) decreased under the two levels of fungicide pollution. In the polluted soils with the recommended dose of fungicide, it is found that all remediating substances significantly increased ScD, the maximum increment was observed for Bacillus (109 and 145%, 1st and 2nd seasons, respectively) followed by Trichoderma (103 and 135%), then BP (94 and 120%) and GA_3 (41 and 50%). Leaf transpiration rate (TR) was inconstant under fungicide pollution conditions. At the recommended fungicide rate (2 g fungicide m^{-2} soil), TR increased and reached about 48 and 60%; but decreased at the rate of 4 g fungicide m^{-2}soil by 27 and 27% at the 1st and 2nd seasons, respectively.

Table 2. Effect of Tolclofos-methyl fungicide, some agents and their combinations on photosynthetic pigments of tomato leaves during the two growing seasons 2015 and 2016.

Characters Treatments	Chlorophyll a (mg/g D.W.)	Chlorophyll B (mg/g D.W.)	Total Chlorophyll (mg/g D.W.)	Carotenoids (mg/g D.W.)	Chlorophyl a/b	Total Chlorophyll/ Carotenoids	Chlorophyll a (mg/g D.W.)	Chlorophyll b (mg/g D.W.)	Total Chlorophyll (mg/g D.W.)	Carotenoids (mg/g D.W.)	Chlorophyl la/b	Total Chlorophyl / Carotenoids
	Season 2015						Season 2016					
Control	1.86^{cde}	0.84^{a}	2.70^{def}	1.42^{cde}	2.22^{abcd}	1.90^{bcd}	1.95^{cde}	0.90^{b}	2.85^{efg}	1.55^{cd}	2.17^{abc}	1.84^{bcd}
T-MF2	1.70^{cde}	0.96^{a}	2.67^{def}	0.95^{de}	1.77^{abcde}	2.81^{ab}	1.92^{cde}	0.96^{ab}	2.88^{efg}	1.09^{d}	2.00^{abcd}	2.64^{ab}
T-MF4	1.00^{e}	0.82^{a}	1.82^{f}	0.63^{e}	1.21^{def}	2.88^{ab}	1.11^{e}	0.87^{b}	1.98^{g}	0.73^{d}	1.28^{cd}	2.71^{ab}
Bacillus	2.70^{bc}	1.38^{ab}	4.08^{bc}	2.90^{ab}	1.96^{abcde}	1.41^{d}	2.75^{bc}	1.75^{ab}	4.50^{bc}	2.90^{b}	1.57^{bcd}	1.55^{cd}
Trichoderma	1.95^{cde}	0.85^{a}	2.80^{def}	1.48^{cde}	2.3^{abc}	1.89^{bcd}	2.00^{cde}	1.18^{ab}	3.18^{def}	1.75^{cd}	1.69^{abcd}	1.82^{bcd}
GA_3	3.99^{a}	1.65^{b}	5.63^{a}	3.43^{a}	2.41^{ab}	1.64^{cd}	4.00^{a}	1.90^{a}	5.90^{a}	4.55^{a}	2.11^{abcd}	1.30^{d}
Bp	3.03^{b}	1.15^{ab}	4.18^{b}	2.16^{bc}	2.64^{a}	1.94^{bcd}	3.45^{ab}	1.30^{ab}	4.75^{b}	2.30^{bc}	2.65^{a}	2.07^{bcd}
T-MF2 + Bacillus	2.02^{cde}	1.44^{b}	3.46^{bcd}	1.48^{cde}	1.41^{bcdef}	2.34^{abcd}	2.17^{ab}	1.44^{ab}	3.61^{cde}	1.64^{cd}	1.51^{bcd}	2.20^{abcd}
T-MF2+Trichoderma	2.16^{bcd}	1.76^{b}	3.91^{bc}	1.67^{cd}	1.23^{cdef}	2.34^{acde}	2.21^{cd}	1.76^{ab}	3.97^{bcd}	1.71^{cd}	1.26^{cd}	2.32^{abc}
T-MF2 + GA_3	2.71^{bc}	1.11^{ab}	3.82^{bc}	1.21^{de}	2.45^{ab}	3.15^{a}	2.75^{bc}	1.11^{ab}	3.86^{bcd}	1.24^{d}	2.48^{ab}	3.11^{a}
T-MF2 + Bp	1.30^{de}	1.00^{a}	2.30^{ef}	1.08^{de}	1.31^{cdef}	2.12^{abcd}	1.31^{de}	1.00^{ab}	2.31^{fg}	1.25^{d}	1.31^{cd}	1.85^{bcd}
T-MF4 + Bacillus	1.19^{cde}	1.18^{ab}	3.17^{cde}	1.22^{de}	1.69^{abcdef}	2.60^{abc}	1.70^{de}	1.20^{ab}	2.90^{efg}	1.15^{d}	1.42^{bcd}	2.52^{abc}
T-MF4+Trichoderma	1.18^{de}	1.60^{b}	2.77^{ef}	1.11^{de}	0.74^{f}	2.49^{abc}	1.20^{e}	1.15^{ab}	2.35^{fg}	1.20^{d}	1.04^{d}	1.96^{bcd}
T-MF4 + GA_3	1.19^{de}	0.99^{a}	2.18^{ef}	0.92^{de}	1.20^{def}	2.37^{abcd}	1.31^{de}	1.03^{ab}	2.34^{fg}	1.05^{d}	1.27^{cd}	2.23^{abcd}
T-MF4 + Bp	1.20^{de}	1.32^{ab}	2.52^{def}	0.96^{de}	0.91^{ef}	2.62^{abc}	1.52^{de}	1.02^{ab}	2.54^{fg}	1.06^{d}	1.49^{bcd}	2.40^{abc}

T-MF2: Tolclofos-methyl fungicide at rate 2 g.m^{-2} soil, T-MF4: Tolclofos-methyl fungicide at rate 4 g.m^{-2} soil, GA_3: Gibberellic acid, Bp: Barbry Plant.

3. Membrane integrity

Membrane integrity (MI) was enhanced as Tolclofos-methyl fungicide rate increased (Table 3). Under the low rate (2 g fungicide m^{-2} soil) the percentage of increment in MI reached about 19 and 27 %, while under the high rate (4 g fungicide m^{-2} soil) reached about 40 and 46% at the 1st and 2nd seasons, respectively. This indicates that Tolclofos-methyl fungicide at the recommended and exceeded rates caused a severely damage of cellular membranes of tomato plants.

4. Biochemical characteristics

The concentration of some macro-elements (N, P, K, Ca, Mg) and some micro-elements (Fe, Mn and Zn) were slightly decreased at the rate of 2 g fungicide m^{-2} soil and sharply decreased with increasing the fungicide level in soils by 4 g m^{-2} soil (Table 4). At the recommended dose of Tolclofos-methyl fungicide, treating the polluted soils with BP led to a great increase in N (20 %), P (43%), Ca (63%), Mg (27%), Fe (21%) and Zn (97%) whereas treating with GA_3 and Bacillus gave the greatest increase for K (46%) and Mn (44%), respectively. Under the high rate of fungicide, all remediating agents caused relevant increases in the determined minerals. Moreover, the maximum increase was recorded by using BP for N and P; GA_3 for K, Ca, Fe, Mn and Zn and by using Bacillus for Mg.

Remarkable increase in the activity of phenoloxidase (PPO) and peroxidase (PO) enzymes as a result of the application of Tolclofos-methyl fungicide was observed (Table 4). The percentage of increase in PPO and PO activities was about 19 and 96% at the recommended dose and about 37 and 50% at the+ high dose of fungicide, respectively. Under the rate of 2g m^{-2} soil, Bacillus gave the highest decrease in PPO activity followed by Trichoderma then GA_3, whereas under the rate of 4 gm^{-2} soil, Trichoderma was the best substance followed by GA_3 then Bacillus. The highest decrease in PO activity was achieved by using GA_3 followed by Bacillus then Trichoderma, under the rate of 2 g.m^{-2} soil.

Tolclofos-methyl fungicide at the rate of 2 and 4 g m^{-2} soil caused a noticed decrease in total free amino acids content (TFAA) by 26 and 47%, respectively compared with the untreated tomato plants. Under the recommended dose of fungicide (2 g m^{-2} soil), all agents caused marked increases in TFAA, the greatest increment was given by BP (49%) followed by GA_3 (47%), meanwhile under the exceeded level of fungicide (4 g m^{-2} soil), GA_3 (47%) followed by Bacillus (40%).

Application of fungicide at both rates caused a marked increase in leaf proline content; this increment was decreased with increasing the fungicide level (96% and 60% at 2 and 4 g fungicide m^{-2} soil, respectively). The maximum decrease was observed by using Bacillus (30%) followed by Trichoderma and GA_3 (17%), meanwhile BP agent caused a marked increase in proline content (65%). Under the exceeded dose of fungicide, the relationship between agents and leaf proline content has another trend. Proline content was increased by 149% (BP), 109% (Bacillus), and 36% (GA_3) and slightly decreased by using Trichoderma (1%). Leaf phenol content was increased as Tolclofos-methyl fungicide rate increased (16% under the rate of 2 g fungicide and 30% under the rate of 4 g m^{-2} soil). Additionally, in the polluted soil with 2 and 4 g fungicide m^{-2} soil, all remediating agents enhanced the leaf phenol content; BP agent gave the greatest increase (42% and 62%, respectively).

Table 4. Effect of Tolclofos-methyl fungicide, some agents and their combinations on mineral status, enzymes activity, total free amino acids and total phenolics in tomato leaves during the growing season 2016.

Characters Treatments	Mineral content								Enzymes activity O.D./g F.W.		Total Free Amino Acids (mg/g DW)	Proline (µmol/g F.W.)	Total Phenol (mg/ g DW)
	N	P	K	Ca	Mg	Fe	Mn	Zn	Phenoloxidase	Peroxidase			
			%				ppm						
Control	2.95^{b}	0.45^{ab}	3.20^{bcde}	1.95^{bc}	0.72^{ab}	213.49^{cdef}	62.51^{abc}	25.30^{def}	0.52^{ab}	0.22^{a}	34.55^{c}	1.86^{fg}	189.20^{g}
T-MF2	2.45^{a}	0.42^{ab}	2.43^{de}	1.30^{bc}	0.78^{ab}	185.75^{gh}	50.25^{cde}	23.00^{ef}	0.62^{ab}	0.43^{a}	25.51^{de}	3.65^{cd}	220.41^{f}
T-MF4	1.99^{a}	0.35^{b}	2.10^{e}	1.25^{c}	0.50^{b}	125.20^{i}	35.51^{e}	21.52^{f}	0.71^{a}	0.33^{a}	18.45^{e}	2.98^{de}	245.45^{de}
Bacillus	2.98^{b}	0.59^{ab}	3.93^{ab}	2.30^{abc}	0.82^{a}	253.40^{ab}	72.55^{ab}	45.53^{a}	0.50^{ab}	0.21^{a}	41.14^{bc}	2.21^{ef}	225.31^{ef}
Trichoderma	2.80^{ab}	0.57^{ab}	3.71^{bc}	2.12^{abc}	0.75^{ab}	233.90^{bc}	62.95^{abc}	40.20^{ab}	0.47^{ab}	0.18^{a}	41.50^{bc}	1.62^{fg}	227.04^{ef}
GA_3	3.03^{b}	0.55^{ab}	3.97^{ab}	2.55^{ab}	0.84^{a}	255.46^{a}	73.15^{a}	45.50^{a}	0.43^{b}	0.20^{a}	53.37^{a}	1.26^{g}	230.82^{ef}
Bp	3.00^{b}	0.65^{a}	3.53^{bcd}	2.37^{abc}	0.93^{a}	265.50^{a}	75.21^{a}	47.48^{a}	0.42^{b}	0.25^{a}	48.09^{ab}	1.97^{fg}	232.72^{ef}
T-MF2 + Bacillus	2.45^{a}	0.55^{ab}	3.31^{bcde}	1.90^{bc}	0.86^{a}	220.15^{cde}	72.51^{ab}	40.25^{ab}	0.52^{ab}	0.32^{a}	37.40^{c}	2.55^{ef}	259.58^{d}
T-MF2+Trichoderma	2.50^{a}	0.45^{ab}	3.20^{bcde}	1.87^{bc}	0.75^{ab}	201.65^{defg}	58.01^{abcd}	35.25^{bc}	0.55^{ab}	0.35^{a}	26.11^{d}	3.02^{de}	307.26^{b}
T-MF2 + GA_3	2.75^{a}	0.55^{ab}	3.55^{bcd}	2.12^{abc}	0.89^{a}	223.49^{cd}	62.50^{abc}	45.30^{a}	0.57^{ab}	0.30^{a}	36.11^{c}	3.02^{de}	307.26^{b}
T-MF2 + Bp	2.94^{b}	0.60^{ab}	3.13^{bcde}	2.12^{abc}	0.99^{a}	223.75^{cd}	60.11^{abc}	45.35^{a}	0.60^{ab}	0.40^{a}	38.08^{c}	6.03^{b}	312.18^{b}
T-MF4 + Bacillus	2.35^{a}	0.50^{ab}	3.00^{bcde}	1.89^{bc}	0.82^{a}	195.50^{fgh}	52.24^{cd}	35.50^{bc}	0.62^{ab}	0.30^{a}	25.75^{de}	6.23^{b}	283.80^{c}
T-MF4+Trichoderma	2.25^{a}	0.43^{ab}	2.58^{cde}	1.65^{bc}	0.70^{ab}	175.20^{h}	42.15^{de}	30.45^{cde}	0.59^{ab}	0.29^{a}	24.85^{de}	2.95^{de}	300.37^{bc}
T-MF4 + GA_3	2.50^{a}	0.50^{ab}	3.20^{bcde}	1.95^{bc}	0.78^{ab}	200.23^{efg}	55.45^{bcd}	40.25^{ab}	0.60^{ab}	0.35^{a}	27.15^{d}	4.05^{c}	302.72^{bc}
T-MF4 + Bp	2.80^{ab}	0.52^{ab}	3.11^{bcde}	1.90^{bc}	0.80^{ab}	197.95^{efg}	52.17^{cd}	32.25^{cde}	0.65^{ab}	0.37^{a}	24.07^{de}	7.43^{a}	397.32^{a}

T-MF2: Tolclofos-methyl fungicide at rate 2 g.m^{-2} soil, T-MF4: Tolclofos-methyl fungicide at rate 4 g.m^{-2} soil, GA_3: Gibberellic acid, Bp: Barbry Plant.

Figure 1 shows the effect of Tolclofos-methyl fungicide rates, and their combinations with some remediating agents on the concentration of endogenous phytohormones (Indole acetic acid (IAA), Gibberellic acids (GAs), Kinitin, Benzyl adenine (BA), Zeatin and Abscisic acid (ABA)) and the ratio of promoters to inhibitors in tomato shoots. Figure 1 shows that concentration of IAA in shoots of tomato plants was decreased at all fungicide levels (2 and 4 g fungicide m^{-2} soil) by 26% and 33%, respectively. It is important to clarify that under the low fungicide rate, BP gave the highest increase followed by Bacillus bacteria then GA_3 (74, 29 and 23%, respectively), whereas under the high rate of fungicide, treatments of Bacillus bacteria and Trichoderma had the best results followed by BP then GA_3 (54, 55, 31 and 28%, respectively). shoots of tomato plants showed a decrease in the GA_3 concentration with all fungicide treatments under the recommended rate by 24% and the exceeded rate by 30% compared with the control plants. In the polluted soil with 2 and 4 g fungicide m^{-2} soil, all remediating agents enhanced the shoots-GA_3 concentration; GA_3 agent gave the greatest increase in this respect (47% and 42%, respectively) as shown in Figure 1.

A marked decrease in the concentration of kinetin in shoots of tomato plants due to Tolclofos-methyl fungicide application at rates 2 and 4 g m^{-2} soil and the percentage of decrease reached about 28 and 36%, respectively compared with the untreated plants. The maximum increment in kinetin was induced by Bacillus bacteria (51%) followed by GA_3 (47%), then BP (37%) and Trichoderma (25%). However, with the high dose, kinetin concentration in tomato shoots was relevantly increased by Bacillus (85%), followed by Trichoderma (80%) then GA_3 (55%) and BP (24%) agents. The concentration of Benzyl adenine was decreased by about 28% and 33% under 2 and 4 g fungicide m^{-2}soil rates, respectively, compared with the untreated plants (Figure 1). The maximum increase in Benzyl adenine was achieved by using BP (36%) at the recommended rate; meanwhile Trichoderma (65%) gave the highest increase at the higher fungicide rate. There is a great increase in Zeatin content occurred by using all the remediating agents under the two fungicide rates. Trichoderma and Bacillus bacteria gave the best response followed by GA_3 then BP (104, 88, 67 and 63%, respectively). Using GA_3 or BP or

Trichoderma in the fungicide-polluted soils tended to be more effective in decreasing ABA than using Bacillus bacteria. A marked increase in the concentration of ABA as a result of contamination the soil with Tolclofos-methyl fungicide at rates of 2 (25%) and 4 (33%) g m^{-2} soil was observed (Figure 1). Under the low rate of fungicide, the highest decrease in ABA was induced by GA_3 or BP (44%) followed by Trichoderma (18%) then Bacillus (13%), whereas at the rate of 4 g, GA_3 (45%) gave the best response followed by Trichoderma (37%), BP (33%) then Bacillus (22%).

The ratio of promoters to inhibitors (P/I ratio) in tomato plants was decreased as the Tolclofos-methyl fungicide rate increased (Figure 1). The percentage of decrease in P/I ratio reached about 40 and 49%, under the two rates of fungicide, respectively. Additionally, the application of BP, GA_3, Bacillus and Trichoderma was very useful in changing P/I ratio from lower to higher values, under the fungicide pollution conditions. Under the higher rate of fungicide, the highest P/I ratio was observed by using GA_3 and Trichoderma, followed by Bacillus then BP, with an increase of 157, 148, 95 and 74 %, respectively compared with the untreated plants.

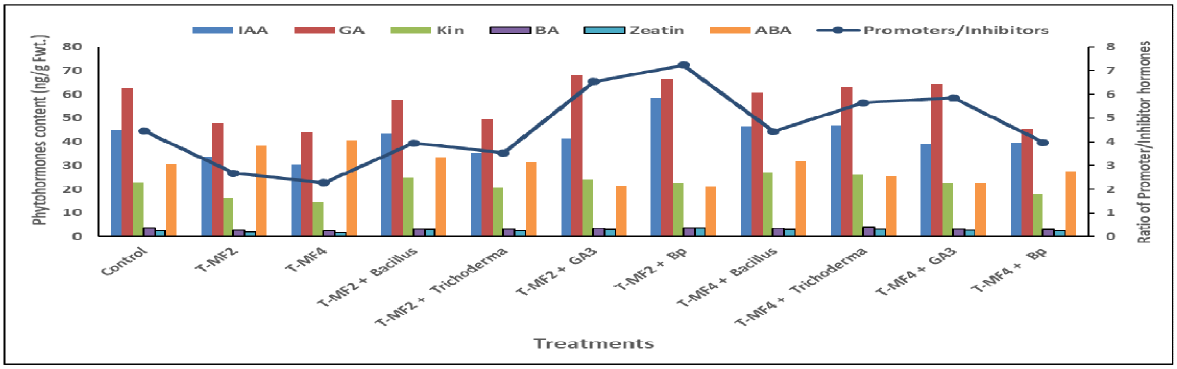


Figure 1. Effect of T-M fungicide and remediating agents on the phytohormones content and promoters/inhibitors ratio in shoots of tomato plants.

3.Flowering and fruit setting

Data presented in Table 5 show the effect of Tolclofos-methyl fungicide, some agents and their combinations on the flowering and fruit setting percentage of tomato plants. The number of flowers per plant was significantly increased by about 20 and 35% as a result of treating by Tolclofos-methyl fungicide at the recommended dose of fungicide 2 g m^{-2} soil, but significantly decreased by about 20 and 19% under the exceeded rate 4 g fungicide during both of the 1^{st} and 2^{nd} seasons, respectively. Under the recommended level of fungicide, Bacillus followed by Trichoderma, GA_3 and BP were effective in increasing the flowers number leading to a high increase by about 158, 144, 122 and 72%, respectively. Under the exceeded level of fungicide, Bacillus, Trichoderma, GA_3 and BP remedies are effective in alleviation of the adverse effects of fungicide on fruit setting percentage, additionally increased this percentage by about 254, 248, 231 and 202%, respectively, in the 1^{st} season. The same trend was observed in the 2^{nd} season.

Table 5. Effect of Tolclofos-methyl fungicide, some agents and their combinations on yield traits of tomato plants and water use efficiency during the growing seasons 2015 and 2016.

Characters Treatments	Season 2015							Season 2016						
	Flowers No/ plant	Fruits No./ plant	Fruit Set (%)	Fruit Diamete (cm)	Fruit Yield g/ plant	Fruit Yield kg/ m2	Water use efficiency (g/kg H2O)	Flowers No/ plant	Fruits No./ plant	Fruit Set (%)	Fruit Diameter (cm)	Fruit Yield g/ plant	Fruit Yield kg/ m2	Water use efficiency (g/kg H2O)
Control	7.50^{g}	1.75^{i}	23.33^{g}	3.04^{cde}	29.45^{h}	1.17^{f}	13.39^{g}	8.33f^{g}	2.50^{fg}	30.01^{e}	3.15^{bc}	54.58^{g}	2.17^{gh}	24.81^{fg}
T-MF2	9.00^{fg}	2.15^{i}	23.89^{g}	3.07^{cde}	30.26^{h}	1.21^{f}	13.98^{g}	11.25^{dfg}	3.50^{efg}	31.11^{e}	3.25^{bc}	63.80^{fg}	2.54^{fgh}	29.00^{fg}
T-MF4	6.00^{g}	1.15^{i}	19.17^{g}	2.25^{efg}	11.54^{i}	0.46^{f}	5.24^{g}	6.75^{g}	1.75^{g}	25.93^{e}	1.50^{e}	20.18^{h}	0.81^{h}	9.17^{h}
Bacillus	13.33^{cdf}	8.50^{df}	63.77^{abc}	4.85^{ab}	369.92^{b}	14.73^{b}	168.15^{b}	14.67c^{de}	10.25^{bc}	69.87^{ab}	4.98^{a}	430.71^{b}	17.15^{b}	195.78^{b}
Trichoderma	11.00^{cdfg}	5.00^{h}	45.46^{f}	3.50^{cd}	149.95^{e}	5.97^{de}	68.16^{d}	11.50^{dfg}	7.50^{bcd}	65.22^{abc}	3.60^{bc}	174.98^{d}	6.97^{de}	79.54^{d}
GA_3	13.50^{cdf}	6.50^{fgh}	48.15ef	3.80^{bcd}	160.03^{de}	6.37^{de}	72.74^{d}	12.35c^{df}	7.00^{bcde}	56.68^{cd}	3.35^{bc}	195.65^{d}	7.79^{cd}	88.93^{d}
Bp	15.00^{cd}	9.33^{cde}	62.20^{abcd}	3.95^{bc}	278.78^{c}	11.10^{c}	126.72^{c}	13.24c^{df}	7.67^{bcd}	57.93^{bcd}	3.75^{bc}	246.13^{c}	9.80^{c}	111.88^{c}
T-MF2 + Bacillus	23.25^{a}	12.50^{a}	53.76^{cdf}	5.08^{a}	567.25^{a}	22.58^{a}	257.84^{a}	20.25^{a}	14.00^{a}	69.14^{ab}	4.20^{ab}	681.94^{a}	27.15^{a}	309.97^{a}
T-MF2+Trichoderma	22.00^{a}	11.50^{ab}	52.27^{df}	2.90^{cde}	169.28^{d}	6.74^{d}	76.95^{d}	19.67^{ab}	10.67^{ab}	54.25^{cd}	3.90^{abc}	192.70^{d}	7.67^{cd}	87.59^{d}
T-MF2 + GA_3	20.00^{ab}	10.00^{bcd}	50.00^{f}	1.60^{fg}	51.00^{g}	2.03^{f}	23.18^{efg}	16.67^{abc}	9.50^{bcd}	56.99^{cd}	1.90^{de}	105.45^{e}	4.20^{fg}	47.93^{e}
T-MF2 + Bp	15.45^{bc}	11.00^{abc}	71.20^{a}	1.90^{efg}	39.49^{gh}	1.57^{f}	17.95f^{g}	15.33^{bcd}	8.25^{bcd}	53.82^{cd}	1.98^{de}	111.38^{e}	4.44f^{g}	50.63^{e}
T-MF4 + Bacillus	14.00^{cde}	9.50^{bcde}	67.86^{ab}	3.09^{cde}	88.16^{f}	3.51^{def}	40.07^{e}	12.00c^{df}	8.67^{bcd}	72.25^{a}	3.48^{bc}	123.81^{e}	4.93^{ef}	56.28^{e}
T-MF4+Trichoderma	10.00^{dfg}	6.67^{fgh}	66.70ab	2.70^{def}	78.31^{f}	3.12^{ef}	35.60^{ef}	10.00^{fg}	6.50c^{de}	65.00^{abc}	2.72^{cd}	108.81^{e}	4.33^{fg}	49.46^{e}
T-MF4 + GA_3	8.67^{fg}	7.50^{efg}	63.44^{abc}	1.20^{g}	32.18^{gh}	1.28^{f}	14.63^{g}	11.00^{dfg}	5.67^{def}	51.55^{d}	1.32^{e}	49.78^{g}	1.98^{gh}	22.63^{g}
T-MF4 + Bp	9.50^{efg}	5.50^{gh}	57.90^{bcde}	1.95^{efg}	38.50^{gh}	1.53^{f}	17.50^{fg}	11.00^{dfg}	6.57^{cde}	59.73^{bcd}	1.90^{de}	78.84^{f}	3.40^{fg}	35.84^{f}

T-MF2: Tolclofos-methyl fungicide at rate 2 $g.m^{-2}$ soil, T-MF4: Tolclofos-methyl fungicide at rate 4 $g.m^{-2}$ soil, GA_3: Gibberellic acid, Bp: Barbry Plant.

4.Yield quantity and quality traits

1.Yield quantity

Data presented in Table 5 indicate that tomato fruits number (FN) significantly increased with using Tolclofos-methyl fungicide on the recommended dose of 2 g by 23 and 40%, meanwhile FN was significantly decreased by about 34 and 30%using the high dose of 4 g m^{-2} soil in the 1^{st} and 2^{nd} season, respectively. Bacillus and BP treatments gave the highest FN under the normal conditions and reached about 3-4 times over the control soils. In the polluted soils with fungicide, at the recommended dose of fungicide, all remediating agents significantly increased the FN by 481 and 300% (Bacillus), 435 and 205% (Trichoderma), 365 and 171% (GA_3), 412 and 136% (BP) over the control in the 1st and 2nd seasons, respectively. At the high dose of fungicide, the remedies alleviated the adverse effect of fungicide and caused a great enhancement in FN by 726 and 395% (Bacillus); 480 and 271% (Trichoderma); 552 and 224% (GA_3); 378 and 275% (BP) over the control.

Concerning the effect of fungicide on fruit diameter (FD), results in Table 5 showed a slight increase in FD (1 and 3.2%) under the recommended dose (2 g) but it was sharply reduced under the higher dose (4 g) by 26 and 52% in the 1st and 2nd seasons, respectively. In the polluted soils and under 2g dose, Bacillus gave a significant increase in FD (66%), whereas the other agents had an inconsistent trend during the two seasons. Under the high dose of 4g, treating with Bacillus and Trichoderma led to an increase in FD by about 37 and 20% (1st season), 132 and 81% (2nd season). Tomato yield (TY) was slightly increased by Tolclofos-methyl fungicide at rate 2 g^{-2} soil (3 and 17%), but was significantly reduced at the high dose (4 g) by 61 and 63% during the 1st and 2nd seasons, respectively. The highest percentage of increase was recorded by using Bacillus (663 and 509%); followed by Trichoderma (578 and 435%), BP (233 and 320%); then GA_3 (178 and 144%) over the corresponding controls without during the 1st and 2nd seasons, respectively.

2.Yield quality (Some chemical constituents of tomato fruits)

Results in Table 6 showed the changes induced in some chemical constituents of tomato fruits *i.e.* total carbohydrates, crude protein, free amino acids, total soluble solids, lycopene, acidity and vitamin C as affected by Tolclofos-methyl fungicide, some remediating agents and their combinations during the two seasons. Total carbohydrates content (TCC) in tomato fruits was significantly decreased as the fungicide dose increased. The percent of decrease reached about 7 and 13% at fungicide dose of 2 g and about 17 and 22% at 4 g fungicide dose during the 1st and 2nd seasons, respectively. Application of all remediating agents in the polluted and non-polluted soils showed a significant enhancement in TCC. The total protein content (TPC) in tomato fruits followed the same trend as TCC in relation to fungicide effects. Additionally, all remediating agents added to the polluted and the non-polluted soils caused an increase in TPC, GA_3 gave the greatest increase under the normal (36 and 18%) and pollution (22 and 27% at the rate of 2 g; 36 and 36% at the rate of 4 g) conditions.

The fungicide at the rate of 2 and 4 g caused a marked decrease in total free amino acids content (TFAA) by 22 and 39% (1st season), 17 and 32% (2nd season) respectively compared with the control. At the recommended dose of fungicide (2 g m^{-2} soil), all agents caused marked increases in TFAA, the greatest one is given by GA_3 (51 and 44%) followed by BP (43 and 47%), meanwhile at the exceeded level of fungicide, BP (71 and 47%) followed by GA_3 (65 and 47%) were the best.

Total soluble solids (TSS) was positively affected by fungicide application more at rate of 4 g m^{-2} soil (60 and 62%) than at rate of 2 g m^{-2} soil (42 and 51%). TSS tended to be increased in the non-polluted soils and fungicide polluted soils treated with all agents used. At rate of 2 g fungicide, all agents significantly increased it, higher increases were observed by BP agent (69 and 63%) and GA_3 (56 and 57%). At rate of 4 g fungicide, GA_3 only has a positive effect in both two seasons, other agents showed inconsistent trends.

Treating the non-polluted soils with Bacillus bacteria gave the greatest increase in fruit lycopene arrived to 84 (1st season) and 81% (2nd season).In the polluted soils with fungicide at rate 2 g m^{-2} soil, Bacillus treatment not only overcome the slight deleterious effect of fungicide but also recorded the highest increase in lycopene (225 and 112%), followed by BP treatment (144 and 75%), then GA_3 (65 and16%) and Trichoderma (56 and 16%). At the high dose (4 g m^{-2} soil), Bacillus agent again gave the greatest increase in lycopene pigment (72 and 66%) followed by Trichoderma (54 and 40%) and BP (31 and 55%) then GA_3 (28 and 23%) relative to its owing fungicide control.

Fruit acidity was significantly increased when the tomato plants were grown in polluted soils with fungicide, reached the highest percentage at the high rate of fungicide (4 g m^{-2} soil). At rate of 2 g fungicide, BP and Bacillus treatments caused a decrease in acidity by 44 and 26% (BP) and 12 and 7% (Bacillus), Trichoderma agent had no effect; whereas GA_3caused a significant increase in acidity by 44 and 52%. At rate of 4 g fungicide, all agents exception of Trichoderma increased the fruit acidity, GA_3 treatment recorded the highest value, followed by BP, then Bacillus treatments.

Vit.C in tomato fruit was positively affected by fungicide at the lower dose (2 g) recorded an increase by 22 and 40% % but was decreased at the high rate (4 g) with reduction reached 10 and 13 % if compared with the control. Bacillus treatment was more effective in increasing vit.C in fruits of plants grown in the non-polluted soils, whereas GA_3 (at the rate of 2 g) and Bacillus (at the rate of 4 g) treatments gave the highest vit.C content ranged from 52.7 to 120.1 % under the fungicidal pollution conditions.

5.Water Use Efficiency (WUE)

Data presented in Table 5 indicate that WUE was slightly increased (4 and 17%) by Tolclofos-methyl fungicide at rate of 2 gm^{-2}soil but was significantly reduced by 61 and 63% at rate of 4 g m^{-2} soil, in the 1st and 2nd seasons, respectively. Under the non-pollution conditions, it is clearly that all agents used significantly increased WUE. Bacillus gave the highest WUE, followed by BP agents, reached 3-6 times of the control. At rate of 2 and 4 gm^{-2}, Bacillus and Trichoderma gave the greatest WUE followed by BP, if compared with the polluted plants without agents.

5.Fungicide residue

Table 6 shows that Tolclofos-methyl fungicide residue was dramatically increased with increasing the fungicide rate in soils. Fungicide residue in tomato fruits recorded about 177 and 423 µg/kg fresh weight at the rates of 2 and 4 g, respectively. Adding Trichoderma, Bacillus bacteria, GA_3 and BP as remediating agents to the fungicide polluted soils with the rates used, led to a reduction in fruit fungicide residue by 73, 42, 35 and 5 %, at the rate of 2 g fungicide, and by 38, 80, 68 and 47% at the rate of 4 g fungicide, respectively. These results indicate that application of Bacillus and GA_3 or Trichoderma or BP were more useful for remediation or at least reducing the toxic level of fungicide (at the recommended or higher doses) and consequently overcoming its toxicity effect on tomato plant.

Table 6. Effect of Tolclofos-methyl fungicide, some agents and their combinations on some chemical constituents and fungicide residue of tomato fruits in the two growing seasons 2015 and 2016.

Characters Treatments	Season 2015							Season 2016								
	Total Carbo-hydrates (mg /gDW)	Total protein (%)	Total Free Amino Acids (mg/ gDW)	Total Soluble Solids (%)	Lycopene concentration (mg/100g FW)	Acidity (%)	Vitamin C (mg AA/100 FW)	Total Carbo-hydrates (mg/ gDW)	Total protein (%)	Total Free Amino Acids (mg/ gDW)	Total Soluble Solids (%)	Lycopene concentration (mg/100g FW)	Acidity (%)	Vitamin C (mg AA/100 FW)	fungicide residue (µg/kg fruits)	Reduction of fungicide residue (%)
Control	151.43^{bcd}	19.06^{a}	30.56^{abc}	4.50^{e}	2.96^{g}	0.65^{b}	18.45^{ef}	167.65^{bcde}	17.44^{ab}	25.67^{cde}	4.45^{f}	3.75^{f}	0.75^{b}	16.75^{gh}	12.35i	-
T-MF2	140.70^{cd}	17.64^{a}	23.67^{cd}	6.40^{cde}	2.67^{g}	0.98^{ab}	22.47^{def}	145.25^{ef}	15.45^{ab}	21.38^{ef}	6.70^{cde}	3.50^{f}	1.02^{ab}	23.48^{def}	176.76^{d}	-
T-MF4	125.24^{d}	14.97a	18.56^{d}	7.20^{bcde}	5.78^{def}	1.11^{ab}	16.64^{f}	130.35^{f}	12.80^{b}	17.45^{f}	7.20^{bcde}	5.31^{de}	1.15^{ab}	14.65^{h}	423.21^{a}	-
Bacillus	175.45^{abc}	21.86^{a}	35.50^{a}	5.75^{cde}	5.45^{def}	1.35^{ab}	25.64^{cd}	190.64^{abcd}	19.25^{ab}	33.35^{ab}	6.30^{de}	6.80^{bc}	1.09^{ab}	22.50^{defg}	-	-
Trichoderma	167.67^{abc}	21.50^{a}	32.67^{ab}	5.87^{cde}	3.55fg	1.56^{ab}	20.67^{def}	197.65^{abc}	18.45^{ab}	35.50^{a}	5.80^{e}	4.25^{ef}	1.23^{ab}	21.78^{efg}	-	-
GA_3	190.67^{a}	23.53^{a}	37.75^{a}	5.98^{cde}	4.05^{fg}	1.25^{ab}	19.20^{def}	200.01^{ab}	20.55^{a}	35.50^{a}	6.63^{cde}	5.45^{cde}	1.10^{ab}	18.90^{efgh}	-	-
Bp	192.57^{a}	22.25^{a}	33.56^{ab}	4.87^{de}	4.30^{efg}	0.95^{b}	18.89^{ef}	210.25^{a}	18.95^{ab}	28.38^{bcd}	6.65^{cde}	4.46^{ef}	1.09^{ab}	20.75^{efgh}	-	-
T-MF2+Bacillus	161.67^{abc}	21.06^{a}	30.56^{abc}	8.90^{abc}	8.67^{abc}	0.86^{b}	31.97^{b}	187.35^{abcd}	17.60^{ab}	25.67^{cde}	7.80^{bc}	7.43^{ab}	0.95^{ab}	30.95^{abc}	102.02^{fg}	42.28
T-MF2+Trichodern	151.43^{bcd}	20.24^{a}	29.96^{abc}	7.20^{bcde}	4.17^{efg}	0.98^{ab}	24.90^{cde}	190.20^{abcd}	19.40^{ab}	28.67^{bcd}	7.50^{bcd}	4.06^{ef}	1.05^{ab}	25.70^{cde}	48.45^{h}	72.59
T-MF2+GA_3	183.47^{ab}	21.53^{a}	35.65^{a}	10.00^{ab}	4.40^{efg}	1.41^{ab}	37.80^{a}	180.60^{abcd}	19.67^{a}	30.67^{abc}	10.50^{a}	4.06^{ef}	1.55^{ab}	35.85^{a}	115.60^{fg}	34.60
T-MF2+Bp	174.40^{abc}	20.25^{a}	33.76^{ab}	10.80^{a}	6.52^{cde}	0.55^{b}	20.78^{def}	182.85^{abcd}	16.65^{ab}	31.38^{abc}	10.90^{a}	6.11^{bcd}	0.76^{b}	24.80^{cdef}	167.93^{de}	4.99
T-MF4+Bacillus	152.67^{bcd}	19.56^{a}	25.47^{bcd}	8.80^{abc}	9.91^{a}	1.29^{ab}	30.09^{bc}	157.30^{def}	15.25^{ab}	23.35^{def}	6.80^{cde}	8.79^{a}	1.35^{ab}	32.24^{ab}	85.44^{g}	79.81
T-MF4+Trichodern	143.57^{cd}	18.46^{a}	23.48^{cd}	7.00^{bcde}	8.89^{a}b	1.11^{ab}	19.60^{def}	165.45^{cde}	17.45^{ab}	20.38^{ef}	8.00^{bc}	7.43^{ab}	1.11^{ab}	20.65^{efgh}	262.03^{b}	38.09
T-MF4+GA_3	151.43^{bcd}	20.36^{a}	30.56^{abc}	8.00^{abcd}	7.39^{bcd}	2.03^{a}	21.00^{def}	175.50^{bcde}	17.45^{ab}	25.57^{cde}	8.50^{b}	6.55^{bcd}	2.10^{a}	25.25^{cdef}	134.98^{ef}	68.11
T-MF4+Bp	151.43^{bcd}	19.26^{a}	31.67^{abc}	7.00^{bcde}	7.59^{bcd}	1.35^{ab}	29.83^{bc}	160.68^{def}	15.75^{ab}	25.57^{cde}	8.00^{bc}	8.22^{a}	1.65^{ab}	28.40^{bcd}	226.88^{c}	46.39

T-MF2: Tolclofos-methyl fungicide at rate 2 $g.m^{-2}$ soil, T-MF4: Tolclofos-methyl fungicide at rate 4 $g.m^{-2}$ soil, GA_3: Gibberellic acid, Bp: Barbry Plant.

Discussion

1. Growth

It is clearly from the above-mentioned results that fungicide Tolclofos-methyl particularly the high rate (4 g m^{-2} soil) caused an adverse effect on growth of tomato plants. These results are in accordance with Mohamed and Akladious (2017), who found that, Rhizolex T, Mon-cut and Tondro fungicides caused significant decrease in plant height, dry weight of plant, as compared with untreated cotton. The adverse effect of fungicide Tolclofos-methyl on growth may be due to its inhibitory effect on the percentage of gibberellins and auxins in plant tissues and its inhibitory effect on the photosynthesis activity and photosynthesis rate (Figure 1 and Table 2) and/or it acts as inhibitors on the electron transport chain, inhibitors of enzymes, inhibitors of nucleic acid metabolism, protein synthesis and sterol synthesis (WHO, 1994) which reflect in a reduction in plant growth. The growth of tomato plants in Tolclofos-methyl treated soil was better in the presence of Bacillus, Trichoderma, GA_3 or BP agents. It was found by Ijaz *et al.* (2015) that application of IAA, GA_3 and kinetin alleviated the phytotoxic effect of some pesticides. Moreover, they evaluated the role of growth regulator trinexapac and fungicides on growth, yield, and quality of winter rapeseed (*Brassica napus* L.) and found that plant height and leaf area index were affected significantly by their applications. Other investigators found that macro-elements or micro-elements (Corlett *et al.,* 2014) or biocontrol agents (El-Morsi and Abdel-Monaim, 2015) overcome the inhibitory effects of pesticides. The stimulating effect of Bacillus on growth parameters of the fungicide polluted tomato plants might be ascribed to their action in improving nutritional status and producing growth regulators i.e. lAA, GA_3 and cytokinins or attributed to their ability to produce anti-fungal compounds, which could reduce diseases (Fayyad, 2006). Trichoderma had high ability to parasitize many soils borne pathogenic fungi or/and to produce inhibitory substances and antibiotic that inhibited the growth of the pathogenic fungi (Wojtkowiak-Gębarowska and Pietr, 2006). GA_3 had stimulating effect on the growth of polluted plants due to its promoting effect on cell division and elongation (Davies, 2010), also to its action in enhancement the photosynthetic system and consequently increase net assimilation rate (Vidhu and Murthy, 1985). BP played a vital role in alleviation the harmful effect of fungicide on growth and enhanced plant growth due to improving the nutrient uptake by plants (Sabrah *et al.,* 1993) and enhancing the available soil water (Corlett *et al.,* 2014).

2. Photosynthetic pigments

Tolclofos-methyl fungicide at higher concentration had a severely harmful effect on all photosynthetic pigments as shown the above-mentioned results. This damage in photosynthetic pigments and photosynthesis may be due to its inhibitory effect by destroying chloroplasts, affecting photosystem II activity and chlorophyll biosynthesis (Petit *et al.,* 2012). The obtained results are closed to Mohamed and Akladious (2017) who found that Rhizolex T, Mon-cut and Tondro fungicides caused a significant decrease in photosynthetic pigments as compared with untreated plants. The role of bacteria or Trichoderma in enhancing the photosynthetic pigments could be attributed to their beneficial effect on production growth regulators, i.e., lAA, GA and cytokinins (Lazarovits, 1995). The promoting effect of GA_3 on photosynthetic pigments in tomato plants grown under the pesticide pollution conditions might be resulted from its marked role in reducing the concentration of fungicide in the plants.

3. Plant water status and Membrane integrity

It is obvious from the results mentioned before, Tolclofos-methyl fungicide at the two rates had a harmful effect on plant water status and led to a decrease in relative water content, an increase in lea water deficit and transpiration rate. In this concern, a large number of pesticides have been found to reduce transpiration rate (Gopi *et al.,* 2005). Nasrabadi and Dhumal (2014) noticed that using the pesticidal/xenobiotic at high concentration adversely affect relative water content in tomato, and it decreased with increasing concentrations of pesticides. Tolclofos-methyl fungicide caused damage in cellular membrane leading to disruption in membrane permeability. These results are in accordance with Shoaib *et al*. (2014) who noted that membrane permeability index was significantly increased up to 3-fold by application of fungicides during early growth stages of the plant. Application of Bacillus and Trichoderma improved the leaf water status of polluted plants due to their beneficial effects on root growth and water uptake by plants. The role of GA_3 in improving the leaf water content of polluted plants ascribed to its role in overcoming or reducing the phytotoxic effect of pesticide. Improving the water status in leaves of polluted plants as a result of application of BP may be attributed to its role in increasing water holding capacity (Agafonov,1983).

4. Biochemical constituents

The concentrations of N, P, K, Ca, Mg, Fe, Mn and Zn were decreased with increasing the fungicide level in soils. The obtained results are in agreement with those reported by Jan *et al*. (2014) who revealed that nutrients uptake in wheat plants decreased due to the use of Chloroflorochlorine, Acrobat and Redimoldgold fungicides as compared with no fungicides. Application of Bacillus, Trichoderma, GA_3 ad BP overcame the adverse effect of fungicide on the plant mineral nutrient contents and increased them. In this respect, Darwesh and Mustafa (2012) noted that the nitrogen, phosphorus, magnesium and iron in the soya bean shoot tissues in the presence of mycorrhiza + lower concentration of fungicides were significantly greater than the control and higher concentration treatments.

Marked increases in the activity of phenoloxidase (PPO) and peroxidase (PO) enzymes as a result of Tolclofos-methyl fungicide treating were observed. The obtained results are in accordance with those reported by Mohamed and Akladious (2017) noticed that Rhizolex T, Mon-cut, Tondro, Maxim, Hemixet and Flosan fungicides caused significant increases in antioxidant enzymes as compared with untreated plants. The activity of these enzymes was decreased as a result of using the agents in the polluted and non-polluted soils. On the other hand, Abada and Ahmed (2014) stated that the three oxidative-reductive enzymes, *i.e.* PAL, PO and PPO were greatly increased in the tomato leaves of all sprayed treatments with the bioagents *B. thuringiensis* and *T. harzianum* and the IRC salicylic acid alone or in different combinations compared with control treatment.

Tolclofos-methyl fungicide caused a marked decrease in total free amino acids content, whereas application all remediating agents (Bacillus, Trichoderma, GA_3 and BP) had a positive effect on leaf TFAA content under the pollution and the non-pollution conditions. In this concern, Mohamed and Akladious (2017) revealed that Rhizolex T, Mon-cut and Tondro fungicides caused significant decrease in total free amino acids as compared with untreated plants. In contrast, Rabert *et al*. (2013) found that application of fungicides caused an increase in total amino acids in different crops.

The fungicide at both two rates caused a marked increase in leaf proline content. At the exceeded dose fungicide, proline content was increased by BP, Bacillus and GA_3 and slightly decreased by Trichoderma. The obtained results are in agreement with those reported by Rabert *et al*. (2013) who pointed out that triazole (hexaconazole, 15 mg l^{-1} and tebuconazole, 10 mg l^{-1}) treatments increased proline and glycine betaine contents when compared to control. On the other hand, Mohamadi and Rajaei (2013) found that Triamidefon (TDM) treatment decreased leaf proline content.

Leaf phenol content was increased as Tolclofos-methyl fungicide rate increased. In the polluted soil with 2 and 4 g fungicide m^{-2} soil, all remediating agents enhanced the leaf phenol content. Similar results were obtained by Mohamed and Akladious (2017) noted that Rhizolex T, Mon-cut, Tondro, Maxim, Hemixet and Flosan fungicides increased significantly total phenols compared with the untreated plants.

The concentrations of IAA, GAs and Cytokinins (Kinetin, Benzyl adenine (BA), Zeatin) as well as the ratio of P/I in shoots of tomato plants were decreased, meanwhile ABA was increased in the exposed tomato plants to Tolclofos-methyl fungicide (Figure 1).Adding BP, Bacillus bacteria, GA_3 and Trichoderma agents to the fungicide-polluted soils tended to be effective in increasing the concentration of IAA, GAs and Cytokinins as well as the ratio of P/I in shoots of the polluted tomato plants. These results are in line with those presented by Tort and Türkyilmaz (2003) who found that the fungicide Captan at a recommended concentration of 2.5 g. l^{-1} as well as 5.0 g. l^{-1} showed higher values of internal ABA contents of the leaf than the higher concentration 7.5 g. l^{-1} as well as control. Mohamed and Akladious (2017) observed that Rhizolex T, Mon-cut and Tondro fungicides caused significant decrease in phytohormones as compared with untreated cotton plants.

5.Flowering and Fruit setting

Number of flowers per plant and fruit set were significantly increased as a result of treating by Tolclofos-methyl fungicide at the recommended dose of fungicide, but significantly reduced at the exceeded rate. The toxic effect of Tolclofos-methyl fungicide on flowering and fruit set might be resulted from its inhibitory effect on the percentage and activity of gibberellins and auxins (as shown in Figure 1) and this decreased the number of flowers, abscission percentage and fruit set percentage. Also, the fungicide treatment induced pollen sterility as high as 10%, which could reflect in decreasing fruit set percentage as well as the final yield (Bharadwaj *et al*., 1993). Bacillus Trichoderma, GA_3 and BP were effective in increasing the flowers number and fruit set. Similar results were reported by Looney *et al*. (1998) on apple. The stimulating effect of Bacillus and Ttrichoderma organisms, BP and GA_3 on flowering and fruiting of tomato plants polluted with Tolclofos-methyl fungicide could be explained by the effective role of microorganisms in improving nutritional status of plants and production of growth substances, i.e., lAA, GA and Cytokinin (Lazarovits, 1995). GAs had a promoting effect on growth and metabolism that reflected in flowering and fruiting (Davies, 2010). BP also had a stimulating effect on growth, sufficient supply by macro- and microelements and other substances and this caused high increase in the number of flowers, fruit set and decreased the total shedding percentage (Selim, 1999).

6. Yield Attributes

Tomato fruits number, diameter and yield significantly increased with using Tolclofos-methyl fungicide rate of 2 g, but were significantly decreased at the rate 4 g. In this respect, Jan *et al*. (2014) revealed that wheat yield decreased with Chloroflorochlorine, Acrobat and Redimoldgold fungicides application as compared with the control. In the untreated and polluted soils with fungicide, application of Bacillus bacteria, Trichoderma, GA_3 and BP have highly significant effect in increasing fruits number, diameter and yield. Adding Bacillus bacteria or Trichoderma led to a marked increase in yield components (Cwalina-Ambroziak and Amarowicz, 2012) and that may be not only due to its role in removing the toxic effect of fungicide but also the high bacterial production of phytohormones and improving nutrition which play in promotion of strong root and shoot (Lazarovits, 1995). The stimulatory effect of BP on tomato yield of plants polluted with fungicide could be attributed to its promoting effect on growth, flowering and fruit set through its role in increasing the availability of water and nutrients that reflected on most metabolism processes which in turn to produce highly yield as reported by Selim (1999).

Total carbohydrates (TC), protein and free amino acids in tomato fruits were significantly decreased as the fungicide dose increased. In this concern, Mohamed and Akladious (2017) found that Rhizolex T, Mon-cut, Tondro, Maxim, Hemixet and Flosan fungicides caused significant decrease in soluble sugars and soluble protein as compared with untreated plants. Total soluble solids (TSS) were positively affected by fungicide application more at rate of 4 g m^{-2} soil than at rate of 2 g m^{-2} soil. TC, protein and TSS tended to be increased in the non-polluted soils and fungicide polluted soils treated with all agents used. Fruit acidity and lycopene content were significantly increased in the fruits of tomato plants grown in contaminated soils with fungicide. Domínguezam *et al.* (2012) noticed that application of pyraclostrobin + boscalid to Nereida tomatoes did not modify acidity and did not induce the accumulation of lycopene. Vit.C in tomato fruit was positively affected by fungicide at the lower dose but was decreased at the high rate. Domínguezam *et al*. (2012) observed that fenhexamid increased the accumulation of vitamin C in tomato fruits compared with fruit treated with pyraclostrobin + boscalid.

Adding Bacillus bacteria or GA_3 or BP and Trichoderma to the non-polluted and polluted soils with fungicide significantly improved the chemical properties of tomato fruits (quality of fruits) and that may be due to their promotion effect on growth and yield as well as vital roles in physiological and biochemical processes in plant. In this connection, Ali and Selim (1996) observed that inoculation of tomato plants with Azotobacter resulted in a rise in fruit sugars and vitamin C contents.

7.Water Use Efficiency (WUE)

Water use efficiency was slightly increased by Tolclofos-methyl fungicide at rate of 2 g, but was significantly reduced at rate of 4 g. In this respect, Diaz-Espejo *et al.,* (2012) found that the application of strobilurin fungicide had no clear effect on water use efficiency in leaves of grapevines after three fungicide applications. Under the pollution conditions, all agents used significantly increased WUE. Bacillus gave the highest WUE.

8. Fungicide residue

Tolclofos-methyl fungicide residue was dramatically increased with increasing the fungicide rate in soils. The residue levels in fungicide treated tomato fruits are above the maximum level of residue of Tolclofos-methyl fungicide (0.1 mg/kg in Tomato fruits). These results may be explained why the phytotoxic effect of fungicide (particularly the higher dose) on most characters measured was more extremely. These findings are in agreement with Sarmamy and Khidir (2013) who showed that after three months of pesticide treatments, residues of Metalaxyl (Ridomil) and chlorpyrifos were detected in samples of bean and wheat plants. Accumulation Tolclofos-methyl fungicide in fruits could be attributed to its curative and slightly systemic action, absorption by roots with translocation to the leaves and other organs (Worthing and Hance, 1991).

Application of Bacillus and GA_3 or Trichoderma or BP were more useful for remediation or at least reducing the toxic level of fungicide (at the recommended or higher doses) and consequently overcoming its toxicity effect on tomato plant (Table 6).These results are in accordance with those found by Maheshwari and Singh (1989), who reported that application of an optimum concentration of GA_3 (100 ppm) along with graded concentration of pesticide could significantly reduce the level of toxic residues in plant. The role of GA_3 in overcoming the toxic residue in plant tissues may be attributed to its stimulating action on enzymes activity in plants might directly or indirectly diminish the toxic effects of these organocarbametes pesticides (Sengupta *et al.,* 1988). The action of Bacillus and Trichoderma organisms in reducing or alleviation the phytotoxic residue of fungicide in tomato plants could be ascribed to their beneficial effects on improving nutritional status, producing growth regulators i.e. lAA, GA and cytokinins (Lazarovits, 1995) or to their ability to produce anti-bacterial and anti-fungal compounds that reduce diseases (Fayyad, 2006). Remediating the harmful residue level of fungicide in plants by BP may be due to its role in improving the nutrient uptake by plants (Sabrah *et al.,* 1993) or supplying by sufficient macro- and microelements and other substances that control diseases and alternate pollutants.

CONCLUSION

Tolclofos-methyl fungicide has phytotoxic effects on growth, yield quantity and quality as well as most physiological and biochemical aspects of tomato plant. Fungicide residue in tomato fruits dramatically increased to the toxic level with increasing the fungicide rate. Application of Bacillus bacteria, Trichoderma, GA_3 and BP agents are very useful substances in reversion the inhibitory effect of fungicide on growth, yield and other characters, alleviation or at least reducing the level of toxic residue of fungicide in tomato fruits below the maximum residue levels.

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النمو، الصفات الفسيولوجية والبيوكيميائية، والإنتاجية ومتبقي المبيد الفطري في نباتات الطماطم المتأثرة بالمبيد الفطري تولكلوفوس-ميثيل (ريزولكس) وبعض المواد العلاجية

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أشارت النتائج الي أن قياسات النمو لنبات الطماطم تأثرت سلبياً بإضافة التولكلوفوس-ميثيل، وكان تأثيره الضار أكثر وضوحاً في صفتي ارتفاع النبات ومساحة الأوراق مقارنة بصفات النمو الأخرى. أدي إستخدام المبيد الفطري بالجرعة الأعلي إلي إنخفاض معنوي في صبغات البناء الضوئي في الأوراق، وكانت الكاروتينات أكثر تأثراً. كما أدي إلى زيادة في محتوي الماء الكلي و نقص في محتوى الماء النسبي في الأوراق. لوحظت زيادة معنوية في معدل النتح الورقي عند إستخدام التركيز المنخفض من التولكلوفوس-ميثيل، بينما إنخفض معدل النتح عند إستخدام التركيز العالى. كما ازدادت نفاذية الأغشية مع زيادة تركيز المبيد الفطري. إنخفض تركيز العناصر المعدنية (النيتروجين، الفوسفور، البوتاسيوم، الكالسيوم، الماغنسيوم، الحديد، الزنك والمنجنيز)، الهرمونات النباتية الداخلية (إندول حمض الخليك ، حمض الجبريلك ، السيتوكينينات) والأحماض الأمينية الكلية الحر ، بينما ازداد حمض الأبسسيسك ، البرولين ، الفينولات الكلية و نشاط كلا من إنزيم الفينول أوكسيديز و البيروكسيديز في النباتات المعاملة بالمبيد. أدى إستخدام مبيد التولكلوفوس-ميثيل بالتركيز العالى إلى نقص ملحوظ في عدد الأزهار والثمار، محتوى الثمار من فيتامين ج، محصول الثمار وكفاءة إستهلاك الماء في نباتات الطماطم . كما ازداد تركيز المواد الصلبة الذائبة الكلية في ثمار الطماطم بينما إنخفض محتوى الثمار من الليكوبين. أظهرت النتائج زيادة ملحوظة في متبقي المبيد الفطري تولكلوفوس-ميثيل في ثمار الطماطم عن الحد الأقصى (١٠٠ ميكروجرام / كجم ثمار الطماطم)، ووصل إلى ١٧٦.٨ و ٤٢٣.٢ ميكروجرام/ كجم وزن طازج عند ٢ و ٤ جم مبيد / متر مربع تربة على التوالي. أدت المعاملة ببكتيريا الباسيلس ، فطرالتريكوديرما، حمض الجبريليك وبوليمر BP إلى تحسن ملحوظ في النمو، المحصول، العلاقات المائية للنبات ، المكونات البيوكيميائية، صبغات البناء الضوئي والخواص الكيميائية لثمار الطماطم تحت كلا من الظروف الطبيعية وظروف التلوث بالمبيد الفطري. كما أدى إستخدام هذه المواد إلى خفض تركيز متبقي المبيد في الثمار بنحو ٤٢ و ٧٣ و ٣٥ و ٥٪ عند تركيز المبيد الفطري ٢ جم / متر مربع تربة، وبنحو ٨٠ و ٣٨ و ٦٨ و ٤٧٪ عند معدل ٤ جم مبيد/ متر مربع تربة، على التوالي. يمكن التوصية بإستخدام فطر التريكوديرما يليه بكتيرياالباسيلس عند التركيز الموصي به من المبيد الفطري، وإستخدام بكتيريا الباسيلس يليه حمض الجبريليك عند التركيز العالي من المبيد الفطري كمواد مفيدة للحد من التأثير السام للمبيد وازالة أو خفض مستوى متبقي المبيد تولكلوفوس-ميثيل في ثمار الطماطم إلى أقل بكثير من الحد المسموح به .