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Using some fungicide-alternatives to control late wilt of maize and improve its growth.

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Abstract

Maize (*Zea mays* L.) is suffering from late wilt which consider the most destructive disease in Egypt. Pot experiments were carried out to evaluate the potentiality of some biocontrol agents (*Bacillus subtilis* and *Trichoderma harzianum*), natural essential oils (camphor, marjoram and sesame oil) and mineral nutrition substances (potassium sulphate and calcium nitrate) to control late wilt disease on maize caused by *Magnaporthiopsis maydis* and improve its growth. All the tested treatments were capable to cause significant reduction of infection percent and number of infected internodes in the three tested maize cultivars when used as soil treatments. Also, they significantly increased stem fresh weight/plant (g), stem dry weight/plant (g), leaves fresh weight/plant (g) and leaves dry weight/plant (g) compared with control treatment. The biocontrol agent *Trichoderma harzianum*, marjoram oil and calcium nitrate proved to be the most effective treatments in this regard.

Keywords: Late wilt, *Magnaporthiopsis maydis*, biocontrol agents, natural essential oils, mineral nutrition substances and maize.

Introduction

Maize (*Zea mays* L.) is one of the most popular oldest and powerful cereals crops, which is popularly used for food, fodder and also for medical purpose in the world. Maize grains have great nutritional value as they contain 72% starch, 10% protein, 4.8% oil, 8.5% fiber, 3.0% sugar and 1.7% ash (Huma *et al.*, 2019). Late wilt of maize, caused by *Magnaporthiopsis maydis*, is the most destructive fungal disease of maize in Egypt (El-Shafey and Claflin, 1999; Klaubauf *et al.*, 2014 and Agag *et al.*, 2021). Successful control of such disease has been obtained by using a wide array of fungicides, but the application of chemical fungicides is extensive, harmful to human, living organisms and the environment, development of fungicidal resistance populations of the pathogen (Pimentel *et al.*, 1992 and Chen *et al.*, 2007). Thus, several non-chemical methods using mineral nutrition substances, natural essential oils and bio-control agents offer an effective way to replace the use of synthetic fungicides (Whipps, 2001 and Mancini and Romanazzi, 2014). Biological control received most of the attention because of their multiple modes of action to protect plants and their potential to be incorporated in integrated programs of management (Shoda, 2000 and Paulitz & Bélanger, 2001). *Bacillus subtilis* and *T. harzianum* are a promising biocontrol agents provide protection or prevention against plant pathogens by competition for nutrients and space, antibiosis, production of lytic enzymes and induced host resistance through increased activity of many enzymes such as peroxidase and polyphenoloxidase which play a defense role against invading pathogens (Vannacci and Gullino, 2000; Elshahawy and El-Sayed, 2018). In addition, they can produce some compounds which may act as plant growth promoters (Compant *et al.*, 2005). Essential oils are volatile hydrophobic liquids extracted from different parts of the aromatic plants. The antifungal activity of essential oils against phytopathogens and their major active compounds responsible of their antifungal properties have been reported by many studies in the world (Silva *et al.*, 2011; Tserennadmid *et al.*, 2011 and Dhaouadi *et al.*, 2018). Volatile compounds responsible of this antifungal activity are mostly molecules of terpenes, terpenoids and phenol, derived aromatic and aliphatic compounds, which have not only fungicidal activities but also bactericidal and viricidal properties as well (Rao *et al.*, 2010). Indeed, many authors (Panizzi *et al.*, 1993 and Sivropoulou *et al.*, 1996) reported that essential oils extracted from Lamiaceae plants contain phenolic compounds, which are well known for their antimicrobial activities. Managing crop disease through mineral nutrition de-emphasizes pesticide usage with their attendant hazards and is cost effective in sustainable agriculture (Dorcas, 2008). Calcium fertilization has been reported to enhance resistance to disease (Marshner, 1995). Combination of soil application of Ca $(NO_3)_2$ and foliar spray of fertilizer containing Ca^{2+} and Mg^{2+} resulted in significant reductions in severity and incidence of *Phytophthora infestans*, *Septoria lycopersici* and *Alternaia solani* on tomato (Aghofack-Nguemezi *et al.*, 2014). Therefore, the present study aim was to determine the effect of some biocontrol agents,

natural essential oils and mineral nutrition against late wilt disease caused by *M. maydis* in maize and their role on plant growth under greenhouse conditions.

Materials and Methods

The present study was carried out in the greenhouse of Sids Agricultural Research Station, Agric. Res. Center, Beni-Sweif governorate to evaluate the potentiality of some biocontrol agents (*Bacillus subtilis* and *Trichoderma harzianum*), natural essential oils (camphor, marjoram and sesame) at concentration of 25, 50 and 97 % (V/V) and mineral nutrition substances (calcium nitrate and potassium sulphate) at concentration of 15, 20 and 25 % (W/V) as soil drench on controlling late wilt disease of maize as well as improving plants growth parameters using three maize cultivars (SC. 10, SC. 124 and DC. 614) as a highly resistance, moderately resistance and susceptible, respectively and four *Magnaporthiopsis. maydis* isolates No. 1, 2, 3 and 4. The seeds of the three tested maize cultivars and the four tested isolates of *M. maydis* were kindly obtained from Maize Res. Dept., Field Crops Res. Inst., Agric. Res. Center at Giza, Egypt where *B. subtilis* and *T. harzianum* were kindly obtained from Department of Microbiology, Soil, Water & Environment Res. Inst., ARC, Giza, Egypt.

***In vitro* assay: Effect of *T. harzianum* and *B. subtilis* on linear growth of *M. maydis* isolates:**

Dual culture technique was used in these experiments. PDA medium was poured into Petri dishes (9 cm in diam.), after solidification, a disc (5 mm) of each one of the tested *M. maydis* isolates obtained from 7 days old culture was placed in one side of each plate. At the same time, a disc (5 mm) of *T. harzianum* isolate was placed in the opposite side of the plate. *B. subtilis* was inoculated by streaking onto one side of the prepared Petri dishes and at the same time another disc (5 mm) of the pathogen was placed in the opposite side. Each Treatment was replicated three times. On the other hand, PDA plates inoculated with *M. maydis* isolates agar disc only on the center of the plate served as un-treated control. The plates were incubated at 25 ± 2°C. When mycelial growth covers all medium surfaces in control, the reduction percentage of *M. maydis* mycelial growth of different isolates were calculated using the formula (Sirirat *et al.*, 2009): I = C-T/C x 100, Where: I = percentage of reduction of fungal growth, C = fungal growth of control, T = fungal growth of treatment.

Greenhouse experiments:

Inocula of the four tested *M. maydis* isolates were grown individually on sterilized sand corn medium (SCM) according to Ziedan (2003) for 15 days at 27°C. Clay pots (40 cm in diameter)) were soaked in 5% formalin solution for 10 min and left to dry in open air for two weeks. While clay soil was sterilized by adding 5% formalin solution and covered with polyethylene sheets for 7 days, then left uncovered for 10 days in order to be free from formaldehyde. Formalin-sterilized pots were filled with the formalin-sterilized soil. The inoculum of the desired tested isolates at the rate of 3.5 % (w/w) was mixed with the soil in each pot. The infested potted soil was kept moist for 5 days before planting to stimulate fungal growth and ensure its homogeneous distribution in the soil (Imara *et al.*, 2021).

Trichoderma harzianum was grown on PD broth medium in a conical flask, incubated at 27^{o}C for 15 days where *B. subtilis* was multiplied for 48h on a shaker. Suspension of *Trichoderma* isolate ($1x10^{6}$ cfu /ml) and *B. subtilis* (1×10^{8} cfu) as well as natural essential oils (camphor, marjoram and sesame) at concentration of 25, 50 and 97 % (V/V) and mineral nutrition substances (calcium nitrate and potassium sulphate) at concentration of 15, 20 and 25 % (W/V) were added separately to the infested soil, five days before sowing as a rate of 100 ml/pot. Untreated infested pots were used as control. Apparently healthy seeds of maize cultivars were superficially sterilized with 1% sodium hypochlorite for 2 min. and washed several times with sterilized water, then left to dry for 6 hours, and then planted in the infested soil (10 seeds /pot). The experiments were set in a randomized complete blocks design with three replications for each treatment. Plants watered when necessary. Percentage of infection, number of infected internodes as well as stem fresh weights/plant (g) were recorded after 90 days from sowing (Cavaglieri *et al.*, 2005).

Data were statistically analyzed for computing L.S.D. test at 5 % probability according to the procedure outlined by Snedecor and Cochran (1989).

Results

Biological control:

Results in Table, 1 and Figs. 1 & 2 show that reduction in linear growth of *M. maydis* isolates was significantly noticed with two bioagents tested and the highest reduction was exhibited in case *T. harzianum* treatment, being 72.2, 66.7, 83.3 and 88.9 % reduction in linear growth of *M. maydis* 1, *M. maydis* 2, *M. maydis* 3 and *M. maydis* 4, respectively followed by *B. subtilis* treatment with averages of 58.9, 55.6, 61.1 and 63.3 %, respectively for the four tested isolates. Moreover, the isolates tested significantly varied in their sensitivity to biocontrol agents tested. *M. Maydis* 4 isolate was the highest sensitive fungus to the tested bioagents which recorded the lowest linear growth (44.3 mm) followed by *M. Maydis* 3 (46.7 mm) and *M. maydis* 1 (50.7 mm), meanwhile *M. maydis* 2 was the least sensitive in this regard (53.3 mm).

Data presented in Tables (2 and 3) demonstrate that all the tested bioagents significantly

reduced percent of infection with late wilt and number of infected internodes in the three tested maize cultivars compared to the untreated control under greenhouse conditions. *T. harzianum* gave the lowest percent of infection and number of infected internodes which recorded 2.0 % & 1.2 in SC. 10, 13.5 % & 6.0 in SC. 124 and 14.3 % & 7.2 in DC. 614 maize cultivars, respectively. While *B. subtilis* treatment reduced percent of infection to 3.0, 14.1, 15.5 % and number of infected internodes to 2.2, 7.1, 8.9, respectively for the three maize cultivars compared to 10.3, 22.1, 26.3 % and 8.1, 15.7, 20.8 in control treatment.

Table 1. Effect of *Bacillus subtilis* and *Trichoderma harzianum* on linear growth of *M. maydis* isolates *in vitro*.

Treatments	*M. maydis* 1		*M. maydis* 2		*M. maydis* 3		*M. maydis* 4		Mean (T)
	Colony diameter (mm)	Reduction* %	Colony diameter (mm)	Reduction* %	Colony diameter (mm)	Reduction* %	Colony diameter (mm)	Reduction* %	
B. subtilis	37.0	58.9	40.0	55.6	35.0	61.1	33.0	63.3	**36.3**
T. harzianum	25.0	72.2	30.0	66.7	15.0	83.3	10.0	88.9	**20.0**
Control (untreated)	90.0	0.0	90.0	0.0	90.0	0.0	90.0	0.0	**90.0**
Mean (I)	**50.7**	**0.0**	**53.3**	**0.0**	**46.7**	**0.0**	**44.3**	**0.0**	**0.0**
L.S.D. at 0.05 %	T = 1.43; I = 1.99; TI = 3.44								

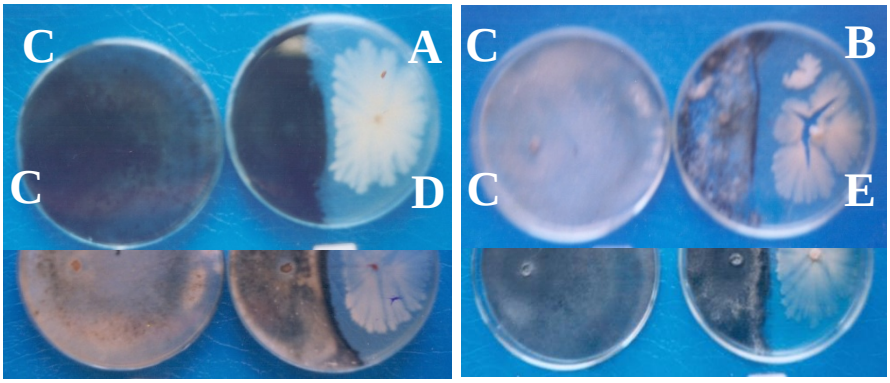

Fig. 1: Effect of *B. subtilis* (A, B, D, E) on linear growth of *M. maydis* 1, 2, 3 and 4 isolates compared with the untreated control (C).

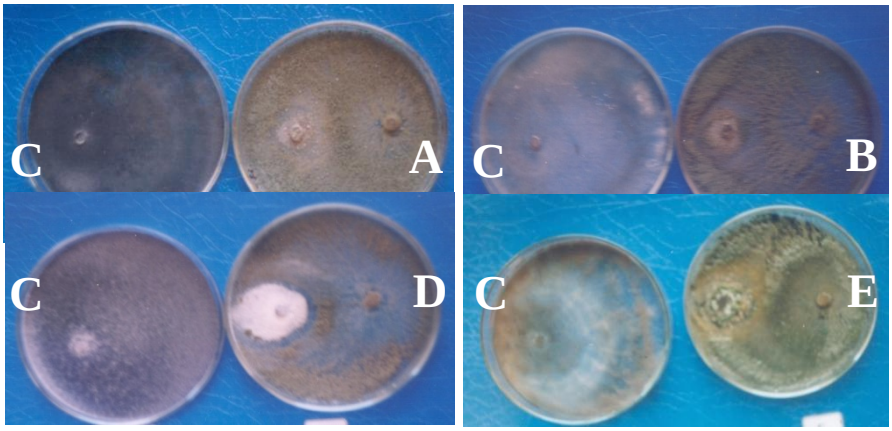

Fig. 2: Effect of *T. harzianum* (A, B, D, E) on linear growth of *M. maydis* 1, 2, 3 and 4 isolates compared with the untreated control (C).

Table (2): Effect of *Bacillus subtilis* and *Trichoderma harzianum* on infection percent of late wilt in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Treatments	Percent of infection (%)														
	SC. 10					SC. 124					DC. 614				
	M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T)**	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T)**	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T)**
B. subtilis	3.0	4.0	3.0	2.0	**3.0**	15.0	15.0	14.0	12.3	**14.1**	16.0	17.0	15.0	14.0	**15.5**
T. Harzianum	2.0	3.0	2.0	1.0	**2.0**	13.0	15.0	13.8	12.0	**13.5**	15.0	15.0	14.0	13.0	**14.3**
Control (untreated)	10.0	14.0	9.0	8.0	**10.3**	23.0	25.5	21.0	19.0	**22.1**	27.0	33.0	24.0	21.0	**26.3**
Mean (I)	**5.0**	**7.0**	**4.7**	**3.7**	-	**17.0**	**18.5**	**16.3**	**14.4**	-	**19.3**	**21.7**	**17.7**	**16.0**	-
L.S.D. at 0.05 %	T = 0.57 I = 0.63 T X I = 1.10					T = 0.57 I = 0.85 T X I = 0.47					T = 0.75 I = 0.64 T X I = 1.12				

Table 3. Effect of *Bacillus subtilis* and *Trichoderma harzianum* on number of infected internodes in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Treatments	No. of infected internodes														
	SC. 10					SC. 124					DC. 614				
	M. maydis ١	*M. maydis* ٢	*M. maydis* ٣	*M. maydis* ٤	**Mean (T)**	*M. maydis* ١	*M. maydis* ٢	*M. maydis* ٣	*M. maydis* ٤	**Mean (T)**	*M. maydis* ١	*M. maydis* ٢	*M. maydis* ٣	*M. maydis* ٤	**Mean (T)**
B. subtilis	2.3	3.0	2.3	1.3	**2.2**	7.7	8.0	7.0	5.7	**7.1**	9.7	11.0	7.7	7.0	**8.9**
T. harzianum	1.3	2.0	1.3	0.3	**1.2**	6.3	7.0	5.7	5.0	**6.0**	7.3	8.0	7.0	6.3	**7.2**
Control (untreated)	7.7	11.0	7.0	6.7	**8.1**	16.0	18.0	15.7	13.0	**15.7**	20.0	27.3	19.0	16.7	**20.8**
Mean (I)	**3.8**	**5.3**	**3.5**	**2.8**	-	**10.0**	**11.0**	**9.5**	**7.9**	-	**12.3**	**15.4**	**11.2**	**10.0**	-
L.S.D. at 0.05 %	T = 1.48 I = 0.66 T X I = 1.14					T = 1.08 I = 1.2 T X I = ns					T = 0.80 I = 0.47 T X I = 2.73				

In general, the effectiveness of the tested bioagents significantly varied according to the isolates tested. *M. maydis* 4 was the highest sensitive fungus to the tested bioagents which recorded the lowest percent of infection (3.7, 14.4 & 16.0 %) and number of infected internodes (2.8, 7.9 & 10.0), respectively for the three tested cultivars followed by *M. maydis* 3 and *M. maydis* 1, meanwhile *M. maydis* 2 was the least sensitive in this regard.The interaction between bioagents and the tested fungi had significant effect on percent of infection and number of infected internodes for the three tested maize cultivars. The lowest percent of infection (1.0, 12.0 & 13.0 %) and number of infected internodes (0.3, 5.0 & 6.3) were obtained from plants grown in soil infested with *M. maydis* 4 isolate and treated with *T. harzianum* treatment, where the maximum values were obtained from plants resulted from *B. subtilis* treatment and grown in soil infested with *M. maydis* 2 isolate that recorded 4.0, 15.0 & 17.0 % infection and 3.0, 8.0 & 11.0 infected internodes, respectively for the three tested maize cultivars.

In general, all tested bioagents promoted the growth of maize plants with significant increase of stem fresh weights/plant (g) (Table, 4). The highest values were recorded with *T. harzianum* treatment, being 371.4, 341.3, 254.3 g for fresh weight of stem, respectively for the three tested maize cultivars. Overall, improvement in growth parameters was significantly varied regarding the tested isolates.

Table 4. Effect of *Bacillus subtilis* and *Trichoderma harzianum* on stem fresh weight in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Treatments	Stem fresh weight (g)														
	SC. 10					SC. 124					DC. 614				
	M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T)**	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T)**	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T)**
B. subtilis	330.5	307.0	377.1	384.2	**349.7**	316.0	287.3	333.8	361.2	**324.6**	228.0	220.3	233.8	264.2	**236.6**
T. harzianum	360.9	343.7	384.0	397.0	**371.4**	336.4	311.9	341.6	375.2	**341.3**	254.7	237.0	257.4	268.0	**254.3**
Control (untreated)	220.3	214.6	220.6	233.7	**222.3**	164.1	145.4	174.5	187.6	**167.9**	110.5	101.9	130.5	135.7	**119.7**
Mean (I)	**303.9**	**288.4**	**327.2**	**338.3**	-	**272.2**	**248.2**	**283.3**	**308.0**	-	**197.7**	**186.3**	**207.2**	**222.6**	-
L.S.D. at 0.05 %	T = 13.3 I = 11.5 TI = 19.9					T = 8.8 I = 10.7 TI = ns					T = 9.8 I = 17.5 TI = ns				

Effect of essential oils on disese incedence:

According to the results obtained in Tables (5 and 6) it was found that the three tested essential oils (camphor, marjoram and sesame) significantly provided protection against late wilt and affected percent of infection and No. of infected internodes at an important level at the three tested concentration compared to the untreated control for the three maize cultivars. In general, the high efficacy values of the tested essential oils were observed when used at the 50 % concentration. The highest reduction in percent of infection and No. of infected internodes were resulted from using marjoram treatment followed by camphor treatment with significant differences between them at the three concentrations used. The reduction in infection percent and No. of infected internodes obtained with the marjoram oil reached to 4.4 & 2.8% for the SC. 10, 15.9 & 9.5% for the SC. 124 and 15.9 & 9.2% for the DC. 614, respectively at the 50 % concentration. While, reduction reached 5.2 % & 3.5, 16.3 % & 10.0 and 16.9 % & 10.9 due to camphor treatment when used at 50 % concentration for the three maize strains, respectively. Sesame treatment showed relatively less effect in this concern.

Results presented in Table 7 exhibit that the three essential oils tested improved plant growth as shown by the significant increments in fresh weights of stem (g/plant) compared to the untreated control. Significant differences among treatments were found for stem weights. In general, the weights were significantly affected by the concentration of essential oils added.

Among essential oils tested, marjoram was the most effective treatment in this respect. It yielded the highest mean of fresh weight of stem in the three maize cultivars, being 278.8, 234.2, 191.9, respectively. Increasing concentration to 50 % caused significant increases in the above mentioned growth parameters with all essential oils tested. The highest means of fresh weights of stem were recorded from marjoram treatment. The corresponding means were 307.2, 47.9g for the SC. 10, 260.8, 43.5 for the SC. 124 and 222.2, 32.2 g for the DC. 614, respectively. On the other hand, camphor treatment showed moderate effect whereas; Sesame treatment gave the lowest increase in fresh weights of stem in the three tested maize cultivars.

Effect of mineral nutrition substances on late wilt incedence:

Data shown in Tables (8 and 9) indicate that using any of the tested mineral nutrition substances, *i.e.* calcium nitrate and potassium sulphate at three concentrations caused a significant reduction in the percent of infection by late wilt and No. of infected internodes in the three tested maize strains compared to the untreated control. It is worthy to note that treatment with calcium nitrate gave the highest protection against late wilt of maize when applied at concentration 20%. The corresponding mean values of infection percent and No. of infected internodes were 1.2 % & 0.4, respectively for Sc. 10, 13.0 % & 6.1 for SC. 124 and being, 14.3 % & 7.0 for DC. 614, respectively. While, it reached 2.0 % & 1.3, 13.5 % & 6.9 and 16.0 % & 7.4 at the high concentration rate (30 %), respectively for the three tested maize cultivars. Moderate disease suppression was provided with potassium sulphate. The efficacy of the tested mineral nutrition substances in disease control was reflected on the plant growth, causing significant increase in the fresh weights of stem (Table 10). The pronounced increase in this respect was observed with calcium nitrate treatment which resulted in the highest mean values of stem fresh weight (393.3, 345.2, 222.2 g/plant), when applied at concentration 20%, respectively for the three maize cultivars and being, 368.3, 58.1, 169.1, 110.5 g/plant, respectively for Sc. 10, 337.6, 54.7, 159.6, 101.5 g/plant, respectively for SC. 124 and 216.3, 36.8, 150.2, 93.9 g/plant, respectively for DC. 614 when applied at concentration 30% with significant differences with potassium sulphate treatment.

Table 5. Effect of different concentrations of some plants essential oils on late wilt incdence in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Essential oils	Concentrations (%)	Percent of infection (%)																
		SC. 10						SC. 124						DC. 614				
		M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)
Camphor	0	10.0	14.0	9.0	8.0	**9.8**	7.5	23.0	25.5	21.0	19.0	**21.9**	18.3	27.0	33.0	24.0	21.0	**26.3**	19.2
	25	8.0	8.3	7.8	7.5	**7.9**		19.0	19.0	18.0	18.0	**18.5**		19.8	20.5	19.8	18.0	**17.7**	
	50	6.0	6.0	4.8	4.0	**5.2**		16.8	16.8	15.8	15.8	**16.3**		17.3	18.0	17.0	16.8	**16.9**	
	97	7.8	8.0	6.5	5.8	**7.1**		18.0	19.0	17.0	17.0	**17.8**		18.0	18.0	17.8	17.8	**18.8**	
Mean (T x I)		**6.9**	**7.6**	**7.2**	**8.3**	**---**		**18.0**	**18.2**	**18.3**	**18.5**	**---**		**18.8**	**18.9**	**19.3**	**20.0**	**---**	
Marjoram	0	10.0	14.0	9.0	8.0	**9.8**	6.4	23.0	25.5	21.0	19.0	**21.9**	17.4	27.0	33.0	24.0	21.0	**26.3**	18.7
	25	6.0	6.8	6.0	5.8	**6.2**		17.3	17.5	17.0	16.0	**17.0**		18.0	18.0	17.8	17.0	**17.9**	
	50	4.8	5.0	4.0	3.8	**4.4**		16.0	16.3	15.8	15.5	**15.9**		15.8	16.8	15.5	15.3	**15.9**	
	97	5.5	5.8	4.8	4.8	**5.2**		16.8	16.8	15.8	15.0	**16.1**		17.0	17.0	16.8	16.8	**17.3**	
Mean (T x I)		**5.2**	**6.1**	**6.4**	**7.9**	**---**		**16.5**	**17.0**	**17.9**	**18.0**	**---**		**18.3**	**18.3**	**18.5**	**19.4**	**---**	
Sesame	0	10.0	14.0	9.0	8.0	**9.8**	8.3	23.0	25.5	21.0	19.0	**21.9**	19.3	27.0	33.0	24.0	21.0	**26.3**	20.4
	25	8.5	8.8	7.5	7.5	**8.1**		20.3	21.0	20.0	18.0	**19.8**		20.0	20.8	20.0	18.0	**19.7**	
	50	7.5	8.5	7.3	7.0	**7.6**		19.0	19.0	17.3	16.8	**18.0**		19.0	19.3	19.0	18.0	**19.0**	
	97	8.0	8.0	7.3	6.8	**7.5**		19.3	20.0	18.3	17.8	**18.9**		19.5	20.0	18.3	18.3	**19.5**	
Mean (T x I)		**6.8**	**8.0**	**8.6**	**9.5**	**---**		**17.7**	**18.9**	**19.8**	**20.8**	**---**		**19.1**	**20.0**	**20.5**	**22.1**	**---**	
Mean of concentrations (C x I)	0	10.0	14.0	9.0	8.0	**9.8**	---	23.0	25.5	21.0	19.0	**21.9**	---	27.0	33.0	24.0	21.0	**26.3**	---
	25	7.0	7.2	7.5	7.8	**7.4**		18.2	18.3	18.5	18.7	**18.4**		18.5	16.0	17.7	18.7	**17.7**	
	50	5.7	3.8	6.5	5.7	**5.4**		16.4	16.5	17.2	16.9	**16.8**		17.3	17.4	17.4	17.9	**17.5**	
	97	6.5	6.7	6.4	6.8	**6.6**		16.9	18.4	17.4	17.6	**17.6**		18.1	18.9	18.7	18.4	**18.5**	
Overall mean (I)		6.3	6.9	7.4	8.6	---		17.4	18.1	18.7	19.1	---		18.7	18.6	19.5	20.5	---	
L.S.D. at 0.05 % for:				**T x C**	**= 0.66**					T x C	= 0.63					T x C	= 0.62		
Essential oils (T)			**= 0.38**	**T x I**	**= 0.76**			T	= 0.36	T x I	= 0.72			T	= 0.36	T x I	= 0.71		
Concentrations (C)			**= 0.38**	**C x I**	**= ns**			C	= 0.36	C x I	= 0.72			C	= 0.36	C x I	= 0.71		
Isolates (I)			**= 0.44**	**T x C x I**	**= ns**			I	= 0.42	T x C x I	= 1.25			I	= 0.41	T x C x I	= 1.23		

Table 6. Effect of different concentrations of some plant's essential oils on number of infected internodes in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Essential oils	Concentrations (%)	No. of infected internodes																	
		SC. 10						SC. 124						DC. 614					
		M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)
Camphor	0	7.7	11.0	7.0	6.7	**8.1**	5.5	16.0	18.0	15.7	13.0	**15.7**	12.6	20.0	27.3	19.0	16.7	**20.8**	14.3
	25	5.7	6.0	5.7	5.3	**5.7**		13.0	13.0	12.7	11.3	**12.5**		13.3	14.7	13.0	12.7	**13.4**	
	50	4.0	4.0	3.3	2.7	**3.5**		10.7	11.0	9.3	9.0	**10.0**		11.0	11.7	10.7	10.3	**10.9**	
	97	5.3	5.7	4.3	3.7	**4.8**		12.7	13.0	12.0	10.7	**12.1**		12.3	12.7	11.7	11.3	**12.0**	
Mean (T x I)		**5.7**	**6.7**	**5.1**	**4.6**	**---**		**13.1**	**13.8**	**12.4**	**11.0**	**---**		**14.2**	**16.6**	**13.6**	**12.8**	**---**	
Marjoram	0	7.7	11.0	7.0	6.7	**8.1**	4.7	16.0	18.0	15.7	13.0	**15.7**	11.5	20.0	27.3	19.0	16.7	**20.8**	13.1
	25	4.3	5.3	4.3	4.0	**4.5**		10.7	11.0	10.3	9.7	**10.4**		12.3	12.7	11.7	11.0	**11.9**	
	50	3.0	3.3	2.7	2.3	**2.8**		9.7	10.0	9.3	9.0	**9.5**		9.3	10.3	9.0	8.3	**9.2**	
	97	3.7	4.0	3.0	3.0	**3.4**		11.3	11.3	9.3	9.0	**10.2**		10.7	11.0	10.3	10.0	**10.5**	
Mean (T x I)		**4.7**	**5.9**	**4.3**	**4.0**	**---**		**11.9**	**12.6**	**11.2**	**10.2**	**---**		**13.1**	**15.3**	**12.5**	**11.5**	**---**	
Sesame	0	7.7	11.0	7.0	6.7	**8.1**	6.3	16.0	18.0	15.7	13.0	**15.7**	13.8	20.0	27.3	19.0	16.7	**20.8**	15.3
	25	6.7	7.0	5.3	5.3	**6.1**		15.0	15.7	14.7	12.3	**14.4**		14.0	15.0	14.0	13.0	**14.0**	
	50	5.3	6.7	5.0	4.7	**5.4**		13.0	13.0	10.7	10.0	**11.7**		13.0	13.7	13.0	12.3	**13.0**	
	97	5.7	5.7	5.3	5.0	**5.4**		13.3	15.0	12.7	11.7	**13.2**		13.7	14.3	12.7	12.3	**13.3**	
Mean (T x I)		**6.4**	**7.6**	**5.7**	**5.4**	**---**		**14.3**	**15.4**	**13.5**	**11.8**	**---**		**15.2**	**17.5**	**14.7**	**13.6**	**---**	
Mean of oncentrations (C x I)	0	7.7	11.0	7.0	6.7	**8.1**	---	16.0	18.0	15.7	13.0	**15.7**	---	20.0	27.3	19.0	16.7	**20.8**	---
	25	5.6	6.1	5.1	4.9	**5.4**		12.9	13.2	12.6	11.1	**12.5**		13.2	14.1	12.9	12.2	**13.1**	
	50	4.1	4.7	3.7	3.2	**3.9**		11.1	11.3	9.8	9.3	**10.4**		11.1	11.9	10.9	10.3	**11.1**	
	97	4.9	5.1	4.2	3.9	**4.5**		12.4	13.1	11.3	10.5	**11.8**		12.2	12.7	11.6	11.2	**11.9**	
Overall mean (I)		5.6	6.7	5.0	4.7	**---**		13.1	13.9	12.3	11.0	**---**		14.1	16.5	13.6	12.6	**---**	
L.S.D. at 0.05 % for:				**T x C**	**= 0.86**					T x C	= 1.15					T x C	= 1.08		
Essential oils (T)		**= 0.21**		**T x I**	**= ns**			T	= 0.71	T x I	= ns			T	= 0.91	T x I	= ns		
Concentrations (C)		**= 0.50**		**C x I**	**= 1.00**			C	= 0.66	C x I	= 1.20			C	= 0.62	C x I	= 1.11		
Isolates (I)		**= 0.50**		**T x C x I = ns**				I	= 0.60	T x C x I = ns				I	= 0.58	T x C x I = ns			

Table 7. Effect of different concentrations of some plant's essential oils on stem fresh weight in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Essential oils	Concentrations (%)	Stem fresh weight (g)																	
		SC. 10						SC. 124						DC. 614					
		M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)
Camphor	0	220.3	214.6	220.6	233.7	**222.3**	264.4	164.1	145.4	174.5	187.6	**167.9**	226.1	110.5	101.9	130.5	135.7	**119.7**	185.7
	25	261.2	258.4	264.9	268.2	**263.2**		233.4	233.0	236.5	249.8	**238.2**		201.6	192.0	202.0	205.1	**200.2**	
	50	290.2	290.0	300.8	307.0	**297.0**		252.4	250.1	262.5	264.0	**257.3**		212.0	210.6	214.9	218.7	**214.1**	
	97	266.6	261.0	278.9	294.3	**275.2**		239.6	233.2	244.6	247.0	**241.1**		207.1	206.0	211.0	212.1	**209.1**	
Mean (T x I)		**259.6**	**256.0**	**266.3**	**275.8**	**---**		**222.4**	**215.4**	**229.5**	**237.1**	**---**		**182.8**	**177.6**	**189.6**	**192.9**	**---**	
Marjoram	0	220.3	214.6	220.6	233.7	**222.3**	278.8	164.1	145.4	174.5	187.6	**167.9**	234.2	110.5	101.9	130.5	135.7	**119.7**	191.9
	25	289.6	282.0	290.0	292.1	**288.4**		248.8	245.0	249.7	261.5	**251.3**		207.5	206.9	211.0	212.7	**209.5**	
	50	301.7	300.0	306.9	320.0	**307.2**		261.4	255.7	262.6	263.5	**260.8**		221.0	218.3	222.6	227.0	**222.2**	
	97	296.0	291.2	300.3	301.0	**297.1**		250.0	249.3	262.3	265.7	**256.8**		214.8	212.0	218.4	220.0	**216.3**	
Mean (T x I)		**276.9**	**272.0**	**279.5**	**286.7**	**---**		**231.1**	**223.9**	**237.3**	**244.6**	**---**		**188.5**	**184.8**	**195.6**	**198.9**	**---**	
Sesame	0	220.3	214.6	220.6	233.7	**222.3**	254.5	164.1	145.4	174.5	187.6	**167.9**	218.2	110.5	101.9	130.5	135.7	**119.7**	180.2
	25	258.4	253.0	268.5	269.0	**262.2**		223.4	222.0	226.8	240.0	**228.1**		195.5	190.0	195.9	201.7	**195.8**	
	50	266.0	258.1	269.5	271.8	**266.4**		234.8	233.0	246.8	253.0	**241.9**		202.8	200.6	203.1	207.0	**203.4**	
	97	261.5	261.0	267.7	278.0	**267.1**		231.2	223.8	236.2	248.0	**234.8**		200.0	194.7	205.5	207.0	**201.8**	
Mean (T x I)		**251.6**	**246.7**	**256.6**	**263.1**	**---**		**213.4**	**206.1**	**221.1**	**232.2**	**---**		**177.2**	**171.8**	**183.8**	**187.9**	**---**	
Mean of oncentrations (C x I)	0	220.3	214.6	220.6	233.7	**222.3**	---	164.1	145.4	174.5	187.6	**167.9**	---	110.5	101.9	130.5	135.7	**119.7**	---
	25	269.7	264.5	274.5	276.4	**271.3**		235.2	233.3	237.7	250.4	**239.2**		201.5	196.3	203.0	206.5	**201.8**	
	50	286.0	282.7	292.4	299.6	**290.2**		249.5	246.3	257.3	260.2	**253.3**		211.9	209.8	213.5	217.6	**213.2**	
	97	274.7	271.1	282.3	291.1	**279.8**		240.3	235.4	247.7	253.6	**244.2**		207.3	204.2	211.6	213.0	**209.1**	
Overall mean (I)		262.7	258.2	267.4	275.2	**---**		222.3	215.1	229.3	237.9	**---**		182.8	178.1	189.7	193.2	**---**	
L.S.D. at 0.05 % for:				**T x C = 2.1**							T x C = 3.1						T x C = 1.7		
Essential oils (T)			**= 1.7**	**T x I = ns**					T = 1.6		T x I = ns				T = 1.4		T x I = ns		
Concentrations (C)			**= 1.2**	**C x I = 3.6**					C = 1.8		C x I = 3.1				C = 1.0		C x I = 2.3		
Isolates (I)			**= 1.8**	**T x C x I = 6.3**					I = 1.6		T x C x I = ns				I = 1.2		T x C x I = ns		

Table 8. Effect of different concentrations of potassium sulphate and calcium nitrate on infection percent of late wilt in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Treatments	Concentrations (%)	Percent of infection (%)																	
		SC. 10						SC. 124						DC. 614					
		M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)
Potassium sulphate	0	10.0	14.0	9.0	8.0	**9.8**	4.6	23.0	25.5	21.0	19.0	**21.9**	15.3	27.0	33.0	24.0	21.0	**26.3**	17.5
	15	3.0	5.0	3.0	2.5	**3.4**		14.0	16.0	13.0	13.0	**14.0**		17.0	17.0	16.0	15.0	**16.5**	
	20	2.0	3.0	2.0	1.5	**2.1**		13.0	14.0	13.0	12.0	**13.0**		15.0	16.0	15.0	14.0	**14.8**	
	30	3.0	4.0	3.0	2.0	**3.0**		14.0	15.0	14.0	12.0	**13.8**		16.0	17.0	16.0	15.0	**15.0**	
Mean (T x I)		**3.0**	**4.8**	**4.3**	**6.3**	**---**		**14.8**	**14.3**	**15.4**	**16.8**	**---**		**16.8**	**16.8**	**17.5**	**18.8**	**---**	
Calcium nitrate	0	10.0	14.0	9.0	8.0	**9.8**	4.4	23.0	25.5	21.0	19.0	**21.9**	15.5	27.0	33.0	24.0	21.0	**26.3**	17.5
	15	4.0	6.8	4.0	2.8	**4.4**		16.0	16.0	14.3	13.8	**15.0**		17.0	17.0	16.0	16.0	**16.3**	
	20	1.3	1.3	1.0	1.0	**1.2**		13.0	14.8	12.8	11.3	**13.0**		15.0	15.0	14.0	13.0	**14.3**	
	30	2.0	3.0	1.5	1.5	**2.0**		14.0	14.0	13.0	12.8	**13.5**		15.0	15.0	15.0	14.0	**16.0**	
Mean (T x I)		**2.8**	**5.0**	**4.3**	**5.2**	**---**		**14.3**	**15.2**	**15.4**	**17.0**	**---**		**16.5**	**16.5**	**18.0**	**19.0**	**---**	
Mean of concentrations (C x I)	0	10.0	14.0	9.0	8.0	**9.8**	---	23.0	25.5	21.0	19.0	**21.9**	---	27.0	33.0	24.0	21.0	**26.3**	---
	15	2.7	4.9	3.5	4.5	**3.9**		13.4	14.5	14.2	16.0	**14.5**		15.5	16.0	17.0	17.0	**16.4**	
	20	1.3	2.0	1.7	1.7	**1.7**		12.7	12.9	12.5	13.9	**13.0**		14.5	13.5	15.0	15.0	**14.5**	
	30	1.8	2.5	3.0	2.8	**2.5**		14.0	12.5	13.4	14.5	**13.6**		15.5	15.0	15.0	16.5	**15.5**	
Overall mean (I)		3.0	4.9	4.3	5.8	**---**		14.5	14.7	15.4	16.9	**---**		16.6	16.6	17.8	21.8	**---**	
L.S.D. at 0.05 % for:				**T x C**	**= 0.58**					T x C	= 0.67					T x C	= 0.57		
Treatments (T)		**= ns**		**T x I**	**= 0.58**			T	= ns	T x I	= 0.67			T	= ns	T x I	= ns		
Concentrations (C)		**= 0.41**		**C x I**	**= 0.83**			C	= 0.47	C x I	= 0.94			C	= 0.40	C x I	= 0.81		
Isolates (I)		**= 0.41**		**T x C x I**	**= 1.17**			I	= 0.47	T x C x I	= 1.33			I	= 0.40	T x C x I	= 1.15		

Table 9. Effect of different concentrations of potassium sulphate and calcium nitrate on number of infected internodes in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Treatments	Concentrations (%)	No. of infected internodes																
		SC. 10						SC. 124						DC. 614					
		M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)
Potassium sulphate	0	7.7	11.0	7.0	6.7	**8.1**	3.5	16.0	18.0	15.7	13.0	**15.7**	9.2	20.0	27.3	19.0	16.7	**20.8**	11.9
	15	2.3	3.3	2.3	1.7	**2.4**		7.7	9.7	6.3	6.3	**7.5**		10.7	11.0	9.7	7.7	**9.8**	
	20	1.3	2.0	1.3	0.7	**1.3**		6.3	7.7	6.3	5.7	**6.5**		7.7	9.7	7.3	7.0	**7.9**	
	30	2.3	3.0	2.3	1.3	**2.2**		7.7	8.0	7.7	5.7	**7.3**		9.3	10.7	9.0	7.3	**9.1**	
Mean (T x I)		**3.4**	**4.8**	**3.2**	**2.6**	---		**9.4**	**10.9**	**9.0**	**7.7**	---		**11.9**	**14.7**	**11.3**	**9.7**	---	
Calcium nitrate	0	7.7	11.0	7.0	6.7	**8.1**	3.3	16.0	18.0	15.7	13.0	**15.7**	9.3	20.0	27.3	19.0	16.7	**20.8**	11.4
	15	3.0	5.3	3.0	1.7	**3.3**		9.7	9.7	7.7	6.7	**8.5**		10.7	11.0	9.7	9.7	**10.3**	
	20	0.3	0.7	0.3	0.3	**0.4**		6.0	7.7	5.7	5.0	**6.1**		7.3	7.7	7.0	6.0	**7.0**	
	30	1.3	2.3	0.7	0.7	**1.3**		7.7	7.7	6.3	5.7	**6.9**		7.7	7.7	7.3	7.0	**7.4**	
Mean (T x I)		**3.1**	**4.8**	**2.8**	**2.4**	---		**9.9**	**10.8**	**8.9**	**7.6**	---		**11.4**	**13.4**	**10.8**	**9.9**	---	
Mean of concentrations (C x I)	0	7.7	11.0	7.0	6.7	**8.1**	---	16.0	18.0	15.7	13.0	**15.7**	---	20.0	27.3	19.0	16.7	**20.8**	---
	15	2.7	4.3	2.7	1.8	**2.8**		8.7	9.7	7.0	6.5	**8.0**		10.7	11.0	9.7	8.7	**10.0**	
	20	0.8	1.4	0.8	0.5	**0.9**		6.2	7.7	6.0	5.4	**6.3**		7.5	8.7	7.2	6.5	**7.5**	
	30	1.8	2.7	1.5	1.0	**1.7**		7.7	7.9	7.0	5.7	**7.1**		8.5	9.2	8.2	7.2	**8.3**	
Overall mean (I)		3.2	4.8	3.0	2.5	---		9.6	10.8	8.9	7.6	---		11.7	14.1	11.0	9.8	---	
L.S.D. at 0.05 % for:				**T x C = ns**						T x C = 0.62						T x C = ns			
Treatments (T)		**= ns**		**T x I = ns**				T = ns		T x I = ns				T = ns		T x I = ns			
Concentrations (C)		**= 0.73**		**C x I = 0.77**				C = 0.44		C x I = 1.21				C = 0.85		C x I = 1.10			
Isolates (I)		**= 0.38**		**T x C x I = ns**				I = 0.60		T x C x I = ns				I = 0.55		T x C x I = ns			

Table 10. Effect of different concentrations of potassium sulphate and calcium nitrate on stem fresh weight in three maize strains inoculated by four isolates of *M. maydis* under greenhouse condition.

Treatments	Concentrations (%)	Stem fresh weight (g)																	
		SC. 10						SC. 124						DC. 614					
		M. maydis 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)	*M. maydis* 1	*M. maydis* 2	*M. maydis* 3	*M. maydis* 4	**Mean (T x C)**	Overall mean (T)
Potassium sulphate	0	220.3	214.6	220.6	233.7	**222.3**	314.9	164.1	145.4	174.5	187.6	**167.9**	287.6	110.5	101.9	130.5	135.7	**119.7**	185.7
	15	330.0	301.9	356.4	359.2	**336.9**		328.1	260.6	334.4	334.8	**314.5**		215.0	213.0	220.0	229.2	**200.2**	
	20	359.6	337.2	360.0	390.8	**361.9**		333.8	328.0	335.0	366.6	**340.9**		230.4	220.9	250.0	261.7	**214.1**	
	30	330.4	305.8	355.1	362.3	**338.4**		328.3	285.0	330.6	365.0	**327.2**		223.2	215.9	225.5	248.6	**209.1**	
Mean (T x I)		**310.1**	**289.9**	**323.0**	**336.5**	**---**		**288.6**	**254.8**	**293.6**	**313.5**	**---**		**182.8**	**177.6**	**189.6**	**192.9**	**---**	
Calcium nitrate	0	220.3	214.6	220.6	233.7	**222.3**		164.1	145.4	174.5	187.6	**167.9**	286.1	110.5	101.9	130.5	135.7	**119.7**	191.9
	15	305.2	282.6	306.6	346.7	**310.3**	323.5	261.0	260.2	323.2	330.0	**293.6**		215.0	212.4	220.1	220.6	**209.5**	
	20	393.2	388.2	395.8	395.9	**393.3**		335.8	320.1	358.2	366.6	**345.2**		250.0	232.0	262.8	266.6	**222.2**	
	30	358.7	333.0	390.5	391.0	**368.3**		329.0	328.6	334.2	358.7	**337.6**		231.3	230.6	250.0	262.0	**216.3**	
Mean (T x I)		**319.4**	**304.6**	**328.4**	**341.8**	**---**		**272.5**	**263.6**	**297.5**	**310.7**	**---**		**188.5**	**184.8**	**195.6**	**198.9**	**---**	
Mean of concentrations (C x I)	0	220.3	214.6	220.6	233.7	**222.3**	---	164.1	145.4	174.5	187.6	**167.9**	---	110.5	101.9	130.5	135.7	**119.7**	---
	15	317.6	292.3	331.5	353.0	**323.6**		294.6	260.4	328.8	332.4	**304.0**		204.6	199.5	206.5	208.9	**204.9**	
	20	376.4	362.7	377.9	393.4	**377.6**		334.8	324.1	346.6	366.6	**343.0**		216.5	214.5	218.8	222.9	**218.1**	
	30	344.6	319.4	372.8	376.7	**353.4**		328.7	306.8	332.4	361.9	**332.4**		211.0	209.0	214.7	216.1	**212.7**	
Overall mean (I)		314.7	297.2	325.7	339.2	**---**		280.5	259.2	295.6	312.1	**---**		185.6	181.2	192.6	195.9	**---**	
L.S.D. at 0.05 % for:				**T x C = 8.16**						T x C = 14.67							T x C = 4.87		
Treatments (T)			**= ns**	**T x I = ns**				T = ns		T x I = ns				T = 4.10			T x I = ns		
Concentrations (C)			**= 5.77**	**C x I = 9.95**				C = 10.37		C x I = ns				C = 3.44			C x I = 7.09		
Isolates (I)			**= 4.97**	**T x C x I = 14.07**				I = 13.72		T x C x I = ns				I = 3.45			T x C x I = ns		

The corresponding mean values for potassium sulphate treatment in this respect were 361.9, 58.4, 168.7, 110.4 g/plant, respectively for Sc. 10, 340.9, 55.8, 161.0, 102.3 g/plant, respectively for SC. 124 and 214.1, 35.5, 149.8, 93.5 g/plant, respectively for DC. 614 when applied at concentration 20%, respectively for the three maize cultivars and being, 338.4, 54.1, 161.5, 104.9 g/plant, respectively for Sc. 10, 327.2, 53.3, 158.1, 100.1 g/plant, respectively for SC. 124 and 209.1, 33.0, 147.5, 92.2 g/plant, respectively for DC. 614 when applied at concentration 30% with significant differences between them. The lowest values of these plant growth parameters were found in concentration 15 % for the two mineral nutrition substances

Discussion

The strategy of pest management depends on using alternative safe methods rather than chemical pesticides. Plants respond to pathogen attack or elicitor treatments by activating a wide variety of protective mechanisms designed to prevent pathogen replication and spreading. The defense mechanisms include the fast production of reactive oxygen species (De Gara *et al.*, 2003), alterations in the cell wall constitution, accumulation of antimicrobial secondary metabolites known as phytoalexins (Agrios, 2005), activation and/or synthesis of defense peptides and proteins (Castro and Fontes, 2005). Mineral nutrition substances, natural oils and bio-control agents can be applied successfully in plant production by enhancing natural resistance against plant diseases and as a plant growth stimulant (Amein *et al.*, 2011 and O'Brien, 2017). In the present study, it is worthy to note that the tested bioagents applied significantly reduced losses caused by late wilt disease on maize plants and increased its productivity. These treatments significantly reduced percent of infection and No. of infected internodes at a satisfactory level and increased fresh, dry weights of stem and leaves. *T. harzianum* treatment showed the highest efficacy in this respect. This result is in line with the report of Elshahawy and El-Sayed (2018) who found that application of *Trichoderma* spp. as seed + soil treatment significantly reduced the infection percentage with late wilt disease and increased the grain yield as well as ear parameters compared to check treatment under greenhouse and field conditions. *Trichoderma* species show strong antagonistic activity against many soilborne fungi (Ayed *et al.*, 2006). The success of *Trichoderma* as a biocontrol agent is believed to involve various modes of action, including antibiotic production, secretion of lytic enzymes and direct penetration of the host hyphae. Also, *Trichoderma* spp., caused colonization of lateral roots which may acts as a barrier for the invasion and colonization by the fungus. The hydrolytic enzymes, such as chitinase, glucanase and protease, produced by *Trichoderma* may play a key role in its ability to penetrate and kill a host fungus. Furthermore, Contreras-Cornejo *et al.* (2016) reported that *Trichoderma* induces root branching and increases shoot biomass as a consequence of cell division, expansion and differentiation by the presence of fungal auxin-like compounds. This plant growth promotion due to its role in plant hormone production, vitamin production or conversion of materials to a form useful to the plant, nutrient release from soil or organic matter, increased uptake and translocation of minerals in addition inhibited the pathogen through parasitism, predator, antibiosis, competition for space and nutrition as well as by inducing the resistant in plants against pathogens (Suarez *et al.*, 2005). From essential oils tested, marjoram effectively reduced percent of infection and No. of infected internodes caused by *M. maydis* isolates to an acceptable level. Marjoram is known for the production of essential oils rich in phenolic compounds, like thymol and its isomer carvacrol which have strong antifungal and antimicrobial properties (Dhaouadi *et al.*, 2018). On the other hand, the present results demonstrated that application of calcium nitrate significantly affected the percent of late wilt infection in three tested maize cultivars, reduced No. of infected internodes and consequently increased fresh and dry weights of stem and leaves of maize plants and that agrees with the findings of Aghofack-Nguemezi *et al.* (2014) and Otusanya (2018) who indicated that leaf lignin, tannin and flavonoid content were higher in the calcium nitrate soil-amended plants than the control. Preformed secondary metabolites or phytoanticipins such as lignin, flavonoid and tannin, which are formed on leaf surface cell layers or within leaf cells, have antimicrobial properties as they provide a chemical barrier which resists pathogen enzymatic degradation, or release toxic substances within the cells, conferring chemical protection against pathogenic metabolites (Dube, 2014).

Conclusions

The biocontrol agents, essential oils and mineral nutrition used have potential in crop management and can be used in an integrated management programs to reduce the deleterious impact of late wilt disease in maize plants**.**

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استخدام بعض بدائل المبيدات لمكافحة الذبول المتأخر فى الذرة وتحسين انتاجيتها

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تعانى الذرة (*Zea mays* L.) من الذبول المتأخر الذى يعتبر من اخطر الامراض التى تصيب الذرة فى مصر. تم اجراء تجارب فى الصوبة من اجل تقييم فعالية بكتريا *Bacillus subtilis* ، فطر *Trichoderma harzianum* وبعض الزيوت الطبيعية مثل زيت الكافور والبردقوش والسمسم بالأضافة الى بعض مواد التغذية المعدنية مثل كبريتات البوتاسيوم ونترات الكالسيوم فى مقاومة الذبول المتأخر فى الذرة والذى يسببه فطر *Magnaporthiopsis maydis* وتحسين الانتاجية. عموما، كل المعاملات كان لها تأثير ايجابى على الحد من نسبة الأصابة بالمرض وعدد السلاميات المصابة فى الثلاث اصناف الذرة المختبرة عند استخدامها كمعاملات للتربة مع زيادة معنوية فى الوزن الطازج والجاف للساق والأوراق بالمقارنة بالكنترول. وكانت المعاملة بفطر *Trichoderma harzianu* *m* وزيت البردقوش ونترات الكالسيوم هى الأكثر فعالية فى هذا المجال.