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Management of *Macrophomina phaseolina* on Tomato using some Plant Extracts, Plant Oils, and some Biocontrol Agents

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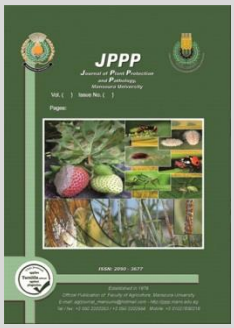

ABSTRACT

This study aims to control Charcoal root rot disease of tomato caused by *Macrophomina phaseolina* by using biocontrol agents and ecofriendly compounds. The results indicated that all tested plant water extracts reduced the linear growth and sporulation of *Macrophomina Phaseolina* both under laboratory and greenhouse conditions. Complete growth inhibition (100%) was observed in *Macrophomina Phaseolina* when cactus or clove extracts were applied at all tested concentrations (5, 10 and 15%). The best effective plant extract in reducing sclerotia population was nigella followed by clove. They resulted 40.0 and 34.2% reduction of sclerotia population respectively, compared to control. Also, the application of essential oils to soil previously infested with the pathogen at different tested concentrations (5, 10, and 15) significantly reduced disease incidence and sclerotia population of *Macrophomina Phaseolina.* The best results were obtained when mint oil was applied; followed by clove oil. *Trichoderma asperellum and Trichoderma koningii* were the best tested *Trichoderma* spp. isolates in reducing the incidence and severity of disease *Macrophomina phaseolina*. All the above-mentioned treatments reduced the charcoal rot incidence and improved the growth of tomato plants; significantly under green house and artificial soil infestation conditions.

Keywords: *Macrophomina Phaseolina . Trichoderma asperellum. T.koningii* . nigella. clove . mint oil.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetable crops in the world Mersha,2008. It is widely cultivated in all parts of the world and Egypt , it is the largest in density of production after potato ; Dorjee, 2000 and Hafez *et al*., 2012. Charcoal root rot of tomato caused by *Macrophomina phaseolina* is one of the most destructive diseases, resulting significant yield losses. *Macrophomina phaseolina* is a soil and seed- borne pathogen infects plants from seedling stage to maturity Purkayastha *et al*., 2006. It mainly produces either microsclerotia or pycnidia. *Macrophomina phaseolina* is a necrotrophic phytopathogen with a wide host range including more than 75 families of 500 cultivated and wild plant species Khan 2007; and Salik 2007. Most of species are economically important crops, as tomato , cotton, bean, melon and sunflower ; Amrita and Bhattacharyya 2008 ; Su *et al*. 2001; Purkayastha *et al*. 2006 and Singh *et al*. 2008 .The fungus causes many diseases such as charcoal root rot, stem rot, collar rot and seedling blight diseases of various crop plants; Sidawi *et al*., 2010. Plant extracts such as Menthe *arvensis* and *Allium sativum* affect the population, mycelial growth and microconidia germination of *Fusarium oxysporum* and the growth of *Macrophomina phaseolina* Moussa *et al*., 2010 and Sidawi *et al*., 2010. However, Kahkashan Arzoo *et al.*, 2012 reported that the extracts reduced infection percentage of charcoal root-rot and wilt diseases; significantly. Kzl *et al.*, 2005 mentioned that mint oil at the concentrations of 5, 10 and 15% exhibit antifungal activity against *Macrophomina phaseolina* fungus. Farrukh Aqil *et al*., 2001 detected that the lowest concentration of clove oil (0.05%) was found to be fungistatic and lysis occurred at 0.07% conc. and they also mentioned that maximum antifungal activity of an essential oil of clove followed by peppermint and eucalyptus was observed. Using the essential plant oils could be alternative to the chemical fungicides for controlling soil-borne pathogens. This needs specific experiments to achieve the sensitivity of each pathogen to the plant oil(s). Application of clove oil as a soil drench reduced the severity of tomato fusarium wilt incidence up to 70.6%; Abhishek Sharma *et al*., 2018. The bioagents activate pathogenesis-related protein synthesis before the pathogen invade the host plant which has a direct impact on decreasing the ability of pathogen to cause wilt and root-rot diseases Hafaz *et al*., 2012. *Trichoderma* spp. are effective biocontrol agents for several soil-borne fungal plant pathogens including *Macrophomina phaseolina* Howell 2003. *Trichoderma asperellum* as biocontrol agent against some soil borne diseases showed significant increase in vegetative parameters like root length, shoot length, plant weight and chlorophyll content 60 days after sowing. There was reduction in the incidence of fusarium wilt in tomato up to 85% Stuti and Saraf 2017.

The aim of the present study was to find out some ecofriendly methods as plant extracts, essential oils and *Trichoderma* spp. isolates agents to management charcoal root rot of tomato caused by *Macrophomina phaseolina*

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MATERIALS AND METHODS

I- Isolation of the tested fungi:

Isolation of the pathogenic fungi:

Diseased tomato plants showing clear wilt and \ or root-rot symptoms (charcol- rot disease) were collected from Sadat City, Menoufia governorate. Stem bases and roots of such plants were gently washed by running tap water to remove soil adhesive particles. The samples were surface sterilized by 70% ethanol, rinsed several times with sterilized distilled water, dried between sterilized filter papers, cut into small pieces and then planted on potato dextrose agar (PDA) medium contained antibacterial antibiotic (300 mg/l Streptomycin sulphate) (Akaeze and Modupe, 2017). Petri dishes were incubated at 25°C for 7 days and examined daily for the fungal growth (Jahanshir and Dzhalilov 2010).

Isolation of the antagonistic microorganisms:

Healthy tomato plants were collected from the same fields and the rhizosphere soil was used for isolating the associated microorganisms. Warcup soil plate (Ammar, 2003) and dilution plate methods were conducted using PDA medium. The plates were also incubated at 25°C for 7 days and examined daily (Sundaramoorthy and Balabaskar 2013).

II- Purification and Identification of the isolated microorganisms:

Streak and/or dilute/plate methods were followed in order to achieve pure culture(s) abundant from single propagule unit (SPU). Pure cultures were kept at 5°C until further studies have been carried out. According to the morphological and physiological aspects of the obtained isolates (Akaeze and Modupe, 2017); they were primarily identified (Gerlach and Nirenberg 1982) at Botany Department, Faculty of Agriculture, Menoufia University. Verification of identification was carried out at the Department of Mycological Researches, Plant Pathology Institute (ARC), Giza, Egypt.

III - Pathogenicity test experiments:

Under greenhouse conditions pathogenicity test experiments were carried out at the farm of Faculty of Agriculture, Menoufia University, Shebin El-Komi, Egypt at 2018 growing season.

Clay loam soil was autoclaved at 121°C for an hour. Pots (15 cm in diameter) were sterilized using 5% formalin for 5 minutes and left for a week until formalin was evaporated. The isolated fungi were individually grown on Barley medium (75 g barley grains + 25 g sand + 100 ml water); using 500 ml conical flasks. The flasks were incubated for 14 days at 25°C which were shacked every second day to allow the fungal growth. Sterilized soil was infested separately with each isolate at the rate of 3% of soil weight. The infested soil was irrigated every second day for 7 days to allow the fungus distribution into the soil. Cultivar K-186 of tomato seedlings 24 days old were planted after root sterilization by dipping in 5% formalin solution for 5 minutes, rinsing by sterilized distilled water and left to dry before sowing in the infested soil. Control treatment had the sterilized soil with the same percentage of sterilized Barley medium. Six replicates were used for each treatment and the pots were irrigated as needed. The plants were examined every week for disease incidence determination.

IV - Laboratory experiments: A complete randomized design (CRD) with three replicates was followed in these experiments.

Effect of plant extracts on *Macrophmina phaseolina* growth: Two hundred grams of each tested plant (Table 1) were soaked in 1000 ml sterilized distilled water for 24h. The obtained extracts were separately heated at 90°C for 30 m, then filtered through filter paper, completed to be 1L and autoclaved at 90°C for 60m (Metwally *et al*., 2010). Extracts were prepared and evaluated for bioactivity by agar dilution method (De Rodrigues *et al*., 2005; Akaeze and Modupe, 2017).

Table 1. Medicinal and ornamental plants used for extraction.

English name	Scientific name	Used part
Clove	*Syzygium aromaticum*	Fruits
Cactus	*Aloe vera*	Stem and leaves
Nigella	*Nigella sativa*	Seeds
Garlic	*Allium sativum*	Cloves
Mint	*Mentha arvensis*	Leaves

The concentrations of 5, 10 and 15% were obtained into PDA medium according to the formula

C1 x V1 = C2 x V2

C1 → More concentrated solution
V1 → Volume needed for a more concentrated solution
C2 → Final concentrated solution
V2 → Desired volume for the final solution

However, control treatment had PDA medium only.

Effect of some essential oils on *Macrophmina phaseolina* growth:

Some crude oils such as cactus, garlic, clove, mint, and nigella were obtained from El-Gomhouria company for oils, Cairo, Egypt. The oils were emulsified with 3% (v: v) tween 20. The emulsified oils were separately mixed with PDA medium to obtain the concentrations of 5, 10 and 15% while control treatment received tween 20 at the some used concentration (Fontes *et al*., 2018). Different volumes of either essential oils or plant extracts were mixed with the sterile PDA to obtain various concentrations. The supplemented PDA were inoculated with agar disc (5 mm in diameter) of *Macrophomina phaseolina* pathogen (from 7-day-old PDA cultures). They were incubated at 25 °C for 7 days. Then the fungal development was calculated (Ylar and Kadoglu 2016).

Effect of some biocontrol agents on *Macrophmina phaseolina* fungal growth:

The obtained five Trichoderma isolates (*T. harzianum, T. koningii, T. hamatum, T. asperellum and T. viride*) were tested for their antagonistic effect against *Macrophomina phaseolina*. Dual culture method was followed where the bioagent was inoculated on side and the pathogen on the opposite side of the petri plate (Sundaramoorthy and Balabaskar 2013; Devi *et al*., 2015). Control treatment had the pathogen only; in the middle of Petri dish. Three replicated plates for each treatment were maintained and incubated at 25 °C for 7 days. The results were recorded when control plate was full with the fungal growth; as the average of growth diameter (mm) and reduction of the growth (%); in comparison with control (Ghutukade *et al.,* 2015).

Growth diameter: The average diameter of the fungal growth (mm) was recorded when a Petri dish of the experiment showed full growth. Percent inhibition over control was calculated as the formula of (Sundaramoorthy and Balabaskar 2013):

$$PI\ \% = \frac{C-T}{C} \times 100$$

Where,
PI= Percent inhibition over control
C: Mycelial radial growth in control
T: Mycelial radial growth in treatment

Greenhouse experiments: Greenhouse experiments were carried out at the farm of Faculty of Agriculture, Menoufia University, Shebin El-Komi, Egypt; during 2019 and 2020 growing seasons. Pots of soil sterilization and soil infestation were conducted as mentioned in Pathogenicity test experiments.

Effect of plant water extracts and essential oils on the pathogen population in the soil. Tomato seedlings cultivar K-186 (24 days old) were sown in the pots previously infested with *Macrophomina phaseolina* (3%) of soil weight as a seedling/pot. The pots were irrigated by different water plant extracts at the rate of 75 ml/pot; using the concentrations of 5, 10 and 15%. However, control treatment pots received the same amount of sterilized distilled water instead of the extracts every week. Ten days after planting the seedlings; one gram of the middle of potted soil was picked up and added to 99 ml sterilized distilled water. Of this 1: 100 dilution; sclerotia of *Macrophomina phaseolina* was counted using x60 light microscope.

The same methods were followed for the efficacy of tested oils on the pathogen population into the soil. Emulsified oils were tested at 5, 10 and 15% concentrations (Abhishek Sharma *et al*., 2018).

Effect of plant water extracts and essential oils on charcoal- rot disease incidence: Both percentage and severity of infection with the disease under study were estimated after 55 days of sowing. charcoal- rot disease percentage of infection (PI) was determined from the six replicates according to this formula:

$$PI = \frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \times 100$$

However, the severity of infection (SI) was estimated using 0-4 scale and the formula:

$$SI = \frac{\text{Sum of (disease grade x No. of plants in grade)}}{\text{No. of total plants x Max. grade infection}} \times 100$$

Effect of plant water extracts and essential oils on plant growth parameters: The average of plant height, number of branches and number of leaves/ plants were determined at the end of the experiments.

Effect of some biocontrol agents on the disease incidence and tomato growth parameters: *Trichoderma* spp. isolates were mixed with the soil at the rate of 3% (w: w) at the same time of soil infestation with the pathogen (Sundaramoorthy and Balabaskar 2013). The same above methods of the disease determinations and tomato growth parameters were estimated to find out the effect of each tested *Trichoderma* spp. isolate (Al-Ameiri, 2015).

VI- Statistical analysis:

All experiments were conducted in completely randomized design. Mean values were compared by the least significant difference (LSD) testing at p = 0.05. Duncan's multiple Range test at p = 0.05 was used to compare means. All statistical analyses were performed using Costate, Statistical Software.

RESULTS AND DISCUSSION

Three isolates of *Macrophomina phaseolina* were obtained from the old tomato plants showed charcoal-rot disease symptoms. However isolate No. 3 was used for the rest studies where it showed more sclerotia formation Fig (1) in comparison with the other two isolates. In addition to five Trichoderma isolates which obtained from the rhizosphere of healthy tomato plants. These isolates were identified as *T. harzianum, T. asperellum, T. viride, T. hamatum* and *T. koningii*.

I- Laboratory experiments:

Effect of some plant water extracts on the growth of *Macrophomina phaseolina*:

Results given in Table (2) clear that all tested plant water extracts reduced the linear growth of *M. phaseolina* in comparison with control, significantly. *vitro.*

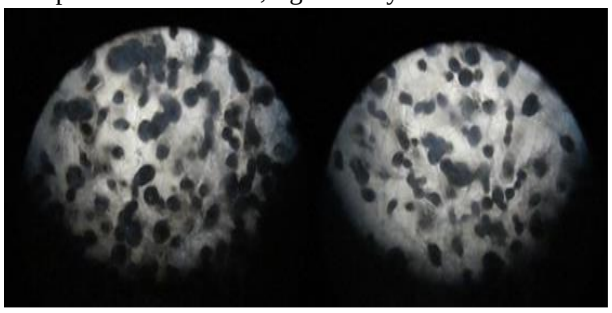

Figure 1. *Macrophomina phaseolina* sclerotia shape as shown under light microscope (X100).

Table 2. Effect of some plant water extracts on the growth of *Macrophomina phaseolina*:

Plant extract	Conc. (%)	Linear growth (mm)	Growth reduction (%)
Cactus	5	00.00J*	100.00
	10	00.00J	100.00
	15	00.00J	100.00
Clove	5	00.00J	100.00
	10	00.00J	100.00
	15	00.00J	100.00
Garlic	5	35.67e	59.62
	10	23.67gh	73.20
	15	7.33i	91.50
Mint	5	70.00b	20.75
	10	65.67c	25.65
	15	53.33d	37.36
Nigella	5	27.67f	68.67
	10	25.00g	71.70
	15	22.33h	74.72
Control		88.33a	00.00
LSD 0.05		1.56	

*: **Means with the same letter(s) are insignificant.**

The best results were obtained when either cactus or clove extracts were applied. Both extracts at all tested concentrations inhibited the fungal growth completely (100% growth reduction) while garlic plant extract (15%) reduced the fungal growth by 91.5%. However, the least

effective extract was mint which resulted only 37.36 % growth reduction, when used at 15% concentration. Nigella extract showed moderately efficiency in reducing the growth of *Macrophomina pha*seolina fungus. Such results were also obtained by Taiga *et al.,* 2008 who mentioned that 75 and 100% concentrations of *Aloe vera* (cactus) extract completely inhibited radial growth of some soilborne pathogens, *in*

Effect of some plant essential oils on the growth of *Macrophomina Phaseolina*: Results tabulated in table (3) clear that clove oil was the most effective one in reducing growth of *M. phaseolina* fungus. such oil reduced the fungal growth by 69.81, 100.00 and 100% when used at 5,10 and 15% concentrations; respectively. Mint oil came in the second rank and garlic oil was the third effective one. However, nigella and cactus oils were the least effect tested ones. These results are in harmony with Ugulino *et al.,* 2018 who reported that using essential oils could inhibite the mycelial growth of *Macrophomina phaseolina*. Beg and Ahmed 2002, reported that *Fusarium chlamydosporum* and *Macrophomina phaseolina* were found to be highly sensitive to clove oil.

Table 3. Effect of some plant oils on the growth of *Macrophomina Phaseolina*:

Plant oil	Conc. (%)	Linear growth (mm)	Growth reduction (%)
Cactus	5	67.67c*	23.39
	10	65.00d	26.41
	15	59.33e	32.83
Clove	5	26.67g	69.81
	10	00.00L	100.00
	15	00.00L	100.00
Garlic	5	32.00F	63.77
	10	25.67g	70.94
	15	20.67h	76.60
Mint	5	16.33I	81.51
	10	16.33J	83.78
	15	11.00K	87.55
Nigella	5	70.33b	20.39
	10	70.00b	20.75
	15	60.33e	31.70
Control(Tween)		88.00a	00.37
Control		88.33a	00.00
LSD 0.05		1.28	

*: Means with the same letter(s) are insignificant

Effect of some *Trichoderma spp*. isolates on the growth of *Macrophomina Phaseolina*:

Results present in table (4) clear that all *Trichoderma spp*. isolates had significant effects in reducing the growth of *Macrophomina phaseolina* fungus. *Trichoderma koningii* and *T. asperellum* caused the most growth reduction. However, *T. harzianum and T. hamatum* showed the least efficacy. Inhibition zones were noticed

between *T. koningii* (22.67mm) and *T. viride* (21.67mm) in side and *M. phaseolina* in the other side; While *T. hamatum, T. harzianum and T. asperellum* showed overgrowth on *M. phaseolina* as bio action. Such results were also observed by El komy *et al.*,2016 ; Akhtar *et al.*,2017 and Abdel-lateif and Bakr 2018.They observed mycoparasitism, antibiosis and lysis actions of *Trichoderma* spp. in addition to exhibited coiling around the hyphae of pathogen; Lakshman Prasad *et al*., 2016 .

Table 4. Effect of different *Trichoderma spp.* isolates on the growth of *Macrophomina Phaseolina* in vitro:

Trichoderma sp.	Linear growth (mm)	Growth reduction (%)	Mode of action O.G ×	I.Z -
T. asperellum	39.33e*	55.47	+	-
T. hamatum	50.67C	42.64	+	-
T. harzianum	55.00b	37.73	+	-
T. koningii	38.00e	56.98	_	22.67
T. viride	42.67d	51.69	_	21.67
Control	88.33a	-	-	
LSD 0.05	1.45	1.28		

*: Means with the same letter(s) are insignificant
× O.G: over growth (mm)
- I.Z: Inhibition zone (mm)

II--Greenhouse experiments:

Effect of some plant extracts on *Macrophomina Phaseolina* sclerotia population in the soil:

Sclerotia of *Macrophomina Phaseolina* were estimated every 10 days after soil infestation (3% of soil weight). Results illustrated in (table5) indicate that all tested plant extracts significantly decreased the number of sclerotia / 1 g soil; even at the low tested concentration (5%). Increasing the concentration of the tested plant extracts showed more efficiency in reducing the sclerotia population.

Table 5. Effect of some plant extracts on *Macrophomina phaseolina* sclerotia population per 1g soil:

Plant extract	Conc. (%)	10^	R+%	20^	R+%	30^	R+%	40^	R+%	50^	R+%
Cactus	5	9.3de*	20.0	8.0c	38.5	7.7cd	49.9	6.0de	66.6	5.3cd	75.0
	10	8.0fg	31.5	7.3cd	43.6	6.0efg	60.9	4.7efgh	74.4	3.3ef	84.4
	15	8.0fg	31.5	5.7ef	56.4	4.7hi	69.5	3.0	83.3	3.0fg	85.9
Clove	5	9.3de	20.0	7.7c	41.0	6.7ef	56.5	5.7def	68.5	5.0cd	76.6
	10	7.7gh	34.3	6.0e	53.9	5.3gh	65.2	4.3fghi	75.9	3.0fg	85.9
	15	7.3gh	34.2	5.3ef	59.0	4.3i	71.8	3.0ij	83.3	2.0gh	90.6
Garlic	5	10.3c	14.3	8.3c	35.9	8.0c	47.8	7.3bc	59.3	6.0bc	71.8
	10	9.3de	20.0	7.7c	41.0	6.0efg	60.9	5.7def	68.5	4.3de	79.2
	15	8.7ef	25.7	6.3de	51.3	5.7fg	65.0	4.0ghi	77.8	3.3e	84.4
Mint	5	11.3ab	0.02	9.7b	25.6	9.3b	39.1	7.7b	57.4	7.0b	67.2
	10	10.7bc	0.09	8.3c	35.9	7.0de	54.3	6.3cd	64.8	5.3cd	75.0
	15	10.0cd	0.14	7.3cd	43.6	6.3efg	58.7	5.0defg	72.2	4.3de	79.0
Nigella	5	9.0e	22.9	7.7c	41.0	6.3efg	58.7	4.7efgh	74.4	3.0fg	85.9
	10	7.3gh	37.2	5.3ef	59.0	4.0ig	73.9	3.3hig	81.5	2.0gh	90.6
	15	7.0h	40.0	4.7f	64.1	3.3g	78.3	2.3j	87.1	1.33h	93.8
Control		11.7a		13.0a		15.3a		18.0a		21.3a	
LSD 0.05		0.7		1.1		0.9		1.3		1.1	

*: Means with the same letter(s) are insignificant
^days after soil infestation
R+% Reduction of sclerotia population %

On the other hand, the nontreated pots showed more significant increase of sclerotia population counted periodically up to 50 days from soil infestation. The best effective plant extract in reducing sclerotia population was nigella followed by clove. They resulted 40.0 and 34.2% reduction of sclerotia population respectively, compared to control; after 10 days of soil infestation. These were 93.8 and 90.6% when estimated after 50 days from soil infestation. On the other hand, mint and garlic plant extracts showed the least efficiency on reducing the fungal sclerotia population in the soil. These results are in harmony with those obtained by Taiga *et al*., 2008; Ali *et al*., 2013 and Yeole *et al*., 2016.

Effect of some plant extracts on charcoal-rot incidence caused by *M. phaseolina*:

Results present in table (6) indicate that all tested plant extracts decreased percentage and severity of infection with *M. phaseolina* in comparison with control, significantly. Nigella plant extract showed superior effect in reducing the disease incidence; even at the lower concentration (5%). *Aloe vera* and clove extracts also gave good results of the disease reduction. However; mint and garlic extracts showed the least efficiency. In general, increasing any used plant extract (5-10-15%) resulted more efficiency in reducing both percentage and severity of infection; insignificantly except with garlic concentrations. such results are in logic and were also noticed by Torre *et al*., 2013.

Table 6. Effect of some plant extracts on the percentage and severity of infection with *M. phaseolina*:

Plant extract	Conc. (%)	Percentage of infection (%)	Severity of infection (%)
Cactus	5	33.3efg*	12.3ef
	10	22.2fgh	6.2efg
	15	00.0h	00.0g
Clove	5	22.2fgh	9.9efg
	10	11.1gh	3.7eg
	15	00.0h	00.0g
Garlic	5	66.6bcd	44.4c
	10	44.4def	24.7e
	15	33.3efg	13.6f
Mint	5	88.8ab	58.0b
	10	77.7abc	50.6bc
	15	55.5cde	32.1d
Nigella	5	11.1gh	1.2g
	10	11.1gh	1.2g
	15	00.0h	00.0g
Control		100.0a	86.4a
LSD 0.05		24.2	8.7

*: Means with the same letter(s) are insignificant

Effect of some plant extracts on tomato growth parameters grown in infested soil with *M. phaseolina*:

Application of the plant extracts on tomato plants grown in the infested soil with *M. phaseolina* (3% of soil weight) improved the growth of tomato plants in comparison with control ones (Table 7). Plant height was about tow folds of control in response to most of these applications. Nigella plant extract was the best for increasing plant height followed by clove and *Aloe vera* plant extracts. The average number of the abundant branches and leaves per plant were also increased, significantly, in response to the application of plant extracts to the soil. Nigella plant extract also showed the best results of increasing the number of branches and leaves / plant and this was followed by clove and *Aloe vera* and *clove* extracts. Generally, increasing the concentration of any tested plant extract gave more growth improvement and vice versa. Such results are confirmed by Pattnaik *et al*., 2012.

Table 7. Effect of some plant extracts on tomato growth parameters grown in infested soil with *M. phaseolina:*

Plant extract	Conc. (%)	Plant height / (cm)	No. of branches (per plant)	No. of leaves (per plant)
Cactus	5	32.3g*	6.0ef	30.0de
	10	35.5ef	6.7cde	32.7cd
	15	38.0cd	7.3abc	34.7bc
Clove	5	32.7g	6.0ef	30.0de
	10	36.0ef	6.7cde	33.3c
	15	38.5c	7.3abc	35.0bc
Garlic	5	30.3hi	5.7fg	28.3e
	10	31.0gh	5.7fg	29.3e
	15	34.3f	6.3def	31.7cde
Mint	5	25.7j	5.0g	24.0f
	10	26.3j	5.0g	24.7f
	15	29.0i	5.7fg	29.0e
Nigella	5	36.7de	7.0bcd	34.0bc
	10	40.3b	7.7ab	36.7b
	15	42.5a	8.0a	42.3a
Control		16.3k	3.67h	15.0g
LSD 0.05		1.6	0.7	3.0

*: Means with the same letter(s) are insignificant

Effect of some plant oils on *Macrophomina phaseolina* sclerotia population in the soil:

Results present in Table (8) clear that all concentrations of all tested oils had significant effect in reducing the number of *M. phaseolina* sclerotia in the soil previously infested with the pathogen.

Table 8. Effect of some plant oils on sclerotia population per 1g soil infested *with Macrophomina phaseolina:*

Plant oils	Conc. (%)	10^	R+%	20^	R+%	30^	R+%	40^	R+%	50^	R+%
Clove	5	11.3c*	37.2	9.0d	54.3	7.3d	65.2	6.7d	70.0	5.3d	77.0
	10	9.0d	50.0	7.7e	60.9	6.0e	71.4	5.0e	77.6	4.0e	82.7
	15	7.0ef	61.1	6.0f	69.5	4.7fg	77.6	4.0f	82.1	2.0g	91.3
Garlic	5	16.0b	11.1	14.3b	27.4	13.0b	38.1	10.7b	52.0	9.0b	60.9
	10	12.0c	33.3	11.0c	44.2	10.0c	52.4	8.7c	61.0	7.0c	69.6
	15	11.0c	38.9	8.7de	55.8	7.0d	66.7	6.0d	73.1	4.7de	79.6
Mint	5	9.0d	50.0	6.3f	68.0	5.3ef	74.8	4.3ef	80.7	3.0f	87.0
	10	8.0de	55.6	4.7g	76.1	4.0g	81.0	3.0g	86.5	2.0g	91.3
	15	6.0f	66.7	3.7gh	81.2	3.0h	85.7	2.0h	91.0	0.7h	97.0
Control (+)		6.0f	66.7	3.0h	84.8	2.0i	90.5	1.0i	95.5	0.0h	100
Control (-)		18.0a		19.7a		21.0a		22.3a		23.0a	
LSD 0.05		1.1		1.2		0.8		0.7		0.7	

*Means with the same letter(s) are insignificant

^days after soil infestation

R+% Reduction of sclerotia population %

Mint oil followed by clove one; at 15% concentration; gave the best effects in reducing sclerotia population estimated after 10-50 days from soil infestation. However, garlic oil showed the least efficiency. As the used oil concentration was increased; more efficiency was observed and vice versa. The number of sclerotia in the soil was decreased by time; in response to the oil application and this was increased by time in the non treated control pots (control -). Farrukh Aqil *et al*., 2001 detected that the maximum antifungal activity due mainly to clove and mint oil and low concentration of oil can Lysis conidia at a higher concentration within 18 h of incubation

Effect of some plant oils on charcoal-rot incidence caused by *M. phaseolina*:

Results given in table (9) indicate that mint oil followed by clove oil gave the best results of reducing both percentage and severity of infection with *M. phaseolina* fungus. All the tested oils affected the disease incidence; significantly; when compared to control- treatment (soil infested with *M. phaseolina* only). Essential oils are usually rich in various compounds, comprising 20 to 60 active substances .The major components found in it are often responsible for their biological properties. Nazzaro and Coppola 2017 indicated that essential oils inhibiting the fungi cell wall formation; disrupting the cell membrane by inhibiting ergosterol synthesis; affecting the fungal mitochondria by inhibiting the mitochondrial electron transport; inhibiting cell division Interfering with either RNA or DNA synthesis and/or inhibiting protein synthesis. All these factors leading to cell death in fungi and inhibiting disease incidence.

Table 9. Effect of some plant oils on the percentage and severity of infection with *M. phaseolina:*

Plant oil	Conc. (%)	Percentage of infection (%)	Severity of infection (%)
Clove	5	66.7abc*	50.0c
	10	33.3cd	13.0e
	15	16.7d	7.4e
Garlic	5	83.3ab	72.2b
	10	66.7abc	46.3c
	15	50.0bcd	35.2cd
Mint	5	50.0bcd	38.9cd
	10	33.3cd	20.4de
	15	16.7d	5.6e
Control (+)		16.7d	1.9e
Control (-)		100a	96.3a
LSD 0.05		33.6	20.5

*Means with the same letter(s) are insignificant

Effect of some plant oils on tomato growth parameters grown in infested soil with *M. phaseolina*:

Individual application of clove, garlic and mint oils at 5, 10 and 15% concentrations; improved the estimated growth characters of tomato plants sown in artificially infested soil with *M. phaseolina* (Table 10). The best effects were noticed when mint oil and clove oil were individually applied, while garlic oil gave the least efficiency. In general; increasing the concentration of tested oils showed much better effect on growth parameters. Such results were recommended by the abovementioned authors

Table 10. Effect of some plant oils on tomato growth parameters grown in infested soil with *M. phaseolina:*

Plant oil	Conc. (%)	Plant height / (cm)	No. of branches (per plant)	No. of leaves (per plant)
Clove	5	20.7g*	4.0efg	20.0g
	10	31.5c	5.3bcd	28.3d
	15	34.3b	6.0bc	30.0c
Garlic	5	17.5h	3.7fg	15.3h
	10	23.7f	4.7def	21.3f
	15	28.3d	5.0cde	25.0e
Mint	5	25.3e	4.7def	24.3e
	10	30.0c	5.3bcd	28.0d
	15	35.7b	6.3b	31.7b
Control (+)		45.0a	9.3a	48.0a
Control (-)		13.3i	3.0g	10.0i
LSD 0.05		1.5	1.0	1.3

*Means with the same letter(s) are insignificant

Effect of different *Trichoderma* spp. isolates on disease incidence and tomato growth parameters grown in infested soil with *M. phaseolina:*

Results present in Tables (11) clear that both percentage and severity of infection with *M. phaseolina* on tomato plants were significantly decreased in response to the application of any tested *Trichoderma* spp. isolates in comparison with the nontreated control. *Trichoderma asperellum and Trichdema Koningii* gave the best results of reducing the percentage of infection. In the meantime; *T.asperellum*, *T.koningii* and *T.viride* showed the best efficiency in reducing the severity of infection with *M. phaseolina*. Plant height was positively responded by the application of *T. asperellum* followed by *T. koningii*. They also increased the average number of abundant branches /plant. However *T.asperellum* showed superior effect in improving the average number of leaves per plant. In comparison with control (-) treatment; all the tested biocontrol agents improved tomato growth parameters; significantly (Table 12). Such results were recommended by Abdel-lateif and Bakr 2018. Beside the antifungal activity of *Trichoderma* spp, it activate pathogenesis-related protein synthesis before the pathogen invade the host plant which has a direct impact on decreasing the ability of pathogen to cause root-rot diseases Hafaz *et al*.,2012.

Table 11. Effect of different *Trichoderma* spp. isolates on the percentage and severity of infection with *M. phaseolina*:

Trichoderma sp.	Percentage of infection (%)	Severity of infection (%)
T. asperellum	22.2c*	4.9c
T. hamatum	55.5abc	27.1b
T. harzianum	88.8ab	75.3a
T. koningii	22.2c	8.6bc
T. viride	44.4bc	16.0bc
Control	100.0a	92.6a
LSD 0.05	42.9	19.1

*Means with the same letter(s) are insignificant

Table 12. Effect of different *Trichoderma* spp. isolates on tomato growth parameters grown in infested soil with *M. phaseolina:*

Trichoderma sp.	Plant height / (cm)	No. of branches (per plant)	No. of leaves (per plant)
T. asperellum	44.0a*	8.3a	45.0a
T. hamatum	33.3d	6.0bc	30.3c
T. harzianum	29.5e	5.0c	25.7d
T. koningii	40.5b	7.7a	36.7b
T. viride	37.0c	7.0ab	35.0b
Control	14.5f	3.3d	12.0e
LSD 0.05	1.9	1.4	2.5

*Means with the same letter(s) are insignificant

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مكافحة فطر ماكروفومينا فاسيولاي علي الطماطم باستخدام بعض المستخلصات والزيوت النباتية وكذلك بعض عوامل المكافحة الحيوية

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تهدف هذه الدراسة الي مكافحة مرض العفن الفحمي علي الطماطم والمتسبب عن الفطر *Macrophomina phaseolina* باستخدام كائنات تضاد حيوي ومركبات صديقة للبيئة . وأوضحت النتائج أن إستخدام المستخلصات النباتية المائية أدت الي تناقص نمو وأعداد الأجسام الحجرية للفطر *Macrophomina phaseolina* (Tassi.) تحت ظروف المعمل والصوبة. ولوحظ أن إستخدام كلا من مستخلص الصباروالقرنفل بتركيزاتهما المختلفه (٥ و ١٠ و١٥%) أدي الي تثبيط نمو الفطر تثبيطا كاملا (١٠٠%) . بينما ادي استخدام مستخلص حبة البركة يلية مستخلص القرنفل الي أفضل النتائج في تقليل أعداد الأجسام الحجرية للفطر (٤٠ و ٣٤,٢%) علي التوالي بالمقارنة بالكنترول. كما أدت معاملة التربة بالتركيزات المختلفة من الزيوت النباتية المختبره (٥ و ١٠ و١٥%) الي نقص حدوث المرض بالفطر الممرض بصورة معنوية وكذلك تعداد الوحدات التكاثرية للفطر في التربة .وتم الحصول علي أفضل النتائج عند استخدام زيت القرنفل أو زيت النعناع . وكان الفطرين *Trichoderma asperellum and Trichoderma Koningii* هما أفضل أنواع جنس *Trichoderma* المستخدمه في الدراسة حيث أظهرا النوعان أفضل النتائج في تقليل نسبة وشدة الاصابة بالمرض . كما أدت كل المعاملات المستخدمه إلي تحسن واضح في مواصفات النمو لنبات الطماطم وذلك تحت ظروف الصوبة والعدوي الصناعية بالتربة.