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Research Article

Role of Arbuscular Mycorrhizae Fungi and Humic Acid in Controlling Root and Crown Rot of Strawberry

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Abstract

Background and Objective: Strawberry plants are threatened by root and crown rot diseases during nursery stage and under field conditions leading to substantial losses in production. This investigation aimed to control root and crown rot of strawberry plants in nursery and field using Arbuscular Mycorrhizal Fungi (AMF) and humic acid as alternative means to fungicides. The experiments were carried out for two seasons in naturally infested nursery and field in Al-Qalyubia governorate, Egypt. **Methodology:** Humic acid and Arbuscular Mycorrhizal Fungi (AMF) (a mixture of *Claroideoglomus etunicatum*, *Rhizophagus diaphanous* and *R. intraradices*), singly and in combination were used to control root and crown rot in nursery and field. The effect of treatments on disease severity and different growth parameters was investigated in addition to the effect of treatments on sugar content, phenols and firmness of the fruits. Occupation of roots by AMF was also examined in the different treatments. **Results:** The AMF and humic acid significantly reduced the disease severity in both nursery and field. The combined treatment of AMF and humic acid in the nursery did not improve their efficiency in reducing disease severity compared to the single treatments with either one, while in the field the combined treatment of AMF and humic acid improved their efficiency in reducing disease severity compared to single treatments. However, results revealed that the root occupation with AMF was reduced by adding humic acid to treatments. The dual effect of humic acid and AMF was clear in increasing both early, total yield and healthy seedlings production in the nursery. The AMF was the most effective treatment in increasing sugar content of the fruits. Data also revealed the lack of correlation between disease severity and each of total and free phenols. **Conclusion:** The AMF and humic acid can be recommended for controlling root and crown rot of strawberry plants in nursery and field. The use of these two treatments in disease control during nursery stage led to the production of healthy and vigorous seedlings to be used commercially in the fields.

Key words: Strawberry, root rot, crown rot, arbuscular mycorrhizal fungi (AMF), humic acid, fungicides, total sugars, phenols, fruit firmness, anthocyanin

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Strawberry plants (*Fragaria*×*ananassa* Duchesne) are subjected to various kinds of diseases attacking different parts of the plant resulting in significant reduction in yield[1]. Root and crown rot diseases are considered major threats of strawberry plants and can lead to great losses either in fruit yield or transplants production in nurseries[2]. Different soil-borne pathogenic fungi e.g., *Rhizoctonia solani*, *R. fragariae*, *Phytophthora cactorum*, *Pythium ultimum*, *Macrophomina phaselina* and *Fusarium* spp., cause such diseases either individually or in combinations[1,3-7]. Generally, it is suggested that using healthy, strong and good quality strawberry transplants will greatly improve the growth and yield resulting from strawberry production fields. Therefore, using disease management measurements is necessary, not only to avoid fungal infection and reduce crop losses in the field during production, but also to produce a healthy and strong transplants in nurseries. Due to the complexity of the root and crown rot diseases, different means could be adopted together to achieve a significant reduction in losses caused by such diseases[2]. Fumigation of the soil by methyl bromide was widely applied by strawberry farmers to control soil borne pathogens[8]. However, the prohibition of methyl bromide and also the scrutiny regulations of using fungicides have led strawberry growers to adopt alternative means to control diseases[2,9].

Arbuscular mycorrhizal fungi (AMF) are beneficial symbionts living with about 80% of plant species and ubiquitously present in most terrestrial ecosystems[10,11]. The well-known beneficial effect of AM fungi is promoting plant growth by supplying plants with nutrients, mainly phosphorus and also by improving the establishment of plant roots in soil[10,12]. Moreover, AMF have also great impacts on the relations between plants and the different soil borne organisms[12,13]. The AM fungi play a potential role in protecting plants from soil borne pathogens[11,14,15]. Therefore, their use in plant disease control has been widely studied and applied during recent years.

In addition, the use of different kinds of organic amendments, such as humic substances is common means to improve soil structure and water uptake by plants as well as promoting plant growth via facilitating the absorption of soil nutrients[16-19]. The dual effect of AMF and humic acid in plant disease management was previously studied and they were found to work synergistically in reducing disease severity caused by soil borne pathogens[16,20]. However, this dual effect in disease control, as far as we know, has not been evaluated in the case of strawberry plants under the Egyptian edaphic factors. Therefore, the objectives of this study were; (i) To evaluate the potential use of AMF and humic acid (singly and in combination) in the control of root and crown rot diseases of strawberry plants in both nursery and field; (ii) To investigate the effect of humic acid on AMF and its efficiency in disease control and (iii) To evaluate the effect of AMF and/or humic acid on sugars content, phenols and anthocyanin pigment as well as strawberry fruit firmness. Experiments were conducted during 2015-2016 and 2016-2017 growing seasons under nursery and field conditions in Al Qalyubia governorate, Egypt, where strawberry is being widely grown and root and crown rot is an endemic disease of strawberry in this area.

MATERIALS AND METHODS

Field and nursery sites: All experiments were carried out under field and nursery conditions at Kafr Al Sohby, Al Qalyubia governorate, where the soil is naturally infested with root and crown rot fungi based on previous investigation by Shehata[21] on root and crown rot of strawberry plants. The strawberry cultivar festival was used in this investigation during the two seasons.

AMF inocula: The AMF used in this study were obtained from Mycology Research and Disease Survey Dept. Plant Pathology Research Institute where mycorrhizae fungi are maintained on Sudan grass (*Sorghum sudanensis* Pers.) roots grown in a greenhouse. The used AMF was a mixture of *Claroideoglomus etunicatum* (former name is *Glomus etunicatum*); *Rhizophagus diaphanous* (former name is *G. diaphanum*) and *Rhizophagus intraradices* (former name is *G. intraradices*) which were previously isolated from the Egyptian soil.

Nursery treatments: The treatments in the nursery were as following; (1) AMF (approximately 17,000 spore/transplant); (2) Humic acid (Humi Power, OMRI, Canada) 1 g (dissolved in 1 L water)/transplant; (3) AMF+Humic acid; (4) Fungicides (Rizolex T 1 g L^{-1}+Tachgarin 1 mL L^{-1}+Topsin M 1 g L^{-1}) mixed at ratio of 1:1:1v (1 L $transplant^{-1}$) and (5) Control (untreated). One month old strawberry transplants were used in this experiment. Transplants were grown in the nursery in mid-April (each season) in rows at 1.5×1.5 m space. The above mentioned treatments were applied five times during nursery stage, with 30 days intervals. The plants were then collected to be transferred to the field and used as transplants. These transplants were appraised with the naked eye and categorized as healthy and diseased during the transplanting process. Disease severity was estimated as the ratio of infected area of the roots and crowns to the whole root or crown using disease index of 11 categories according to Horsfall and Barratt[22] and disease severity was measured according to the following equation:

$$\text{Disease severity (\%)} = \frac{\text{Sum (No. each infection category} \times \text{Numerical value of infection category)}}{\text{Total no. of roots or crowns} \times \text{Highest numerical value of infection category}} \times 100$$

AMF colonization of roots: Strawberry seedlings were examined to evaluate the colonization by AMF fungi using the method described by Phillips and Hayman[23]. Percentage of infection was estimated by examining 20 root pieces and counting the number of roots colonized with AMF and the following Eq. was applied:

$$\text{Root infection (\%)} = \frac{\text{Number of AMF positive pieces}}{\text{Total number of pieces scored}} \times 100$$

Number of AMF spores in rhizosphere soil: The number of AMF spores in the rhizosphere was estimated using the wet sieving technique as described by Gerdemann and Nicolson[24].

Field treatments: Healthy strawberry transplants produced from the treated nursery were selected for the field trial. The transplants were planted in rows, 50 cm apart and in 30 cm distance in the row. Transplants were then treated after planting with the same treatments mentioned earlier. Fruits were harvested after 60 days of planting to evaluate the early yield. Later on, after 30 days, berries were harvested and the total yield was evaluated. Berries samples from each treatment were collected for the chemical analysis step.

Chemical analysis: Ten grams of strawberry fruits from each sample were cut into small pieces and grounded in 95% boiling ethanol. Extraction was carried out in Soxhlet units by placing the macerated tissues in the units and using 75% ethanol in the extraction process. This process lasted for 12 h then the ethanol extractions were filtrated and evaporated until the complete removal of the ethanol. The remaining extract was then dissolved in 5 mL of 50% isopropanol and kept in -20°C until use.

Total sugar: Total sugar content in the strawberry fruits was determined using the method described by Thomas and Dutcher[25]. The analysis was performed in three replicates for each treatment.

Total and free phenols: Total phenol content was determined according to the method described by Simons and Ross[26]. The analysis was performed in three replicates for each treatment.

Anthocyanin: For anthocyanin pigments extraction from the fruits, the method described by Fuleki and Francis[27] was used. The analysis was performed in three replicates for each treatment.

Firmness: Fruit firmness was measured on each fruit by using fruit pressure tester (hand held penetrometer), plunger size (1/2 cm^2) diameter and measures firmness as Newton (N).

Statistical analysis: The experiment design used in the present study was a randomized block with three replicates. All means were compared at 5% and Least Significant Difference (LSD) was used to compare treatment means. Linear correlation coefficient (r) was used to evaluate the relationship between symptoms and biochemical variables. Statistical analysis was performed with the software package[28] SPSS10.0.

RESULTS

Effect of AMF and/or humic acid treatments on disease severity: The influence of AM fungi and humic acid treatments singly or in combination on disease severity in the nursery was presented in Table 1. All treatments caused significant reduction in disease severity on the root in the first season. Fungicides were the most effective treatment in reducing the disease severity. The AM fungi and humic acid showed the same level of effectiveness since the difference in disease severity was insignificant. The combined treatment of AM fungi and humic acid did not improve their efficiency compared to the single treatments with either one. On crown,

Table 1: Effect of AMF and/or humic acid treatments on disease severity on root and crown rot of strawberry (Festival cv.) in nursery during 2015/2016 and 2016/2017 growing seasons

	2015/2016 season		2016/2017 season	
Treatments	Root	Crown	Root	Crown
AMF	8.20	6.17	7.10	5.03
Humic acid	9.10	7.80	7.50	7.10
AMF+humic acid	6.70	3.13	3.47	2.80
[a]Fungicides	2.80	1.50	1.87	1.40
Control	36.27	33.00	40.00	35.00
LSD (p = 0.05)	3.60	1.47	1.09	1.62

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

the results followed the same trend however, the combined treatment with AM fungi and humic acid was better than the single treatments ($p\leq0.05$).

In the second season, the level of reduction in disease severity caused by different treatments showed almost the same pattern of the first season however, the combined treatment of AM fungi and humic acid was better than the single treatments on both crown and root.

Data presented in Table 2 showed the effect of humic acid and/or AMF on disease severity of strawberry plants in the field. Generally, the pattern of the results from the field was almost similar to the results of the nursery. Fungicides treatment gave the highest reduction in disease severity compared to the control. The combined treatment of AM fungi and humic acid improved their efficiency in reducing disease severity compared to single treatments.

Disease severity on root and crown were not correlated in the nursery (r = -0.059, p = 0.872), while they were highly correlated with disease severity on root in the field (r = 0.986, p = 0.000) (Table 3). However disease severity on crown in the nursery was not correlated with disease severity in the field (r = 0.061, p = 0.867) (Table3).

Effect of AM fungi and/or humic acid on different growth parameters: In this study, the different treatments affected plants during growth in the nursery and this was obvious in the different growth parameters shown in Table 4.

Fungicides treatments improved the number of lateral roots. Data also showed that the single treatment with AMF or humic acid significantly improved root system growth (root length and number of lateral roots). The number of lateral roots increased almost 7-10 folds and the root length increased about 6 folds compared to the control, when plants were treated with AMF and humic acid in combination. Moreover, the increase in crown diameter was highly significant in all treatments. The best treatment in improving crown diameter was fungicides followed the dual treatment with AMF and humic acid.

Data also revealed that number of lateral roots, root length and crown diameter were increased almost similarly and consistently in the two successive seasons.

Presence of AM fungi in roots and soil: Data in Table 5 showed that plants treated with AMF were highly colonized by AMF. Also the highest number of spores present in rhizosphere soil was in case of the single treatment with AMF (Table 6). Combining AMF and humic acid in one treatment also significantly increased the root occupation and spores density in the soil but was still lower than the single treatment

Table 2: Effect of AMF and/or humic acid treatments on disease severity on root and crown rot of strawberry plants (Festival cv.) in the field during 2015/2016 and 2016/2017 growing seasons

	2015/2016 season		2016/2017 season	
Treatments	Root	Crown	Root	Crown
AMF	6.60	4.10	4.93	3.47
Humic acid	8.40	4.93	6.17	3.70
AMF+humic acid	3.47	2.80	2.00	1.50
[a]Fungicides	1.87	1.50	0.90	0.20
Control	30.30	25.00	37.00	32.00
LSD ($p\leq0.05$)	0.93	1.33	1.39	0.49

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

Table 3: Correlation between disease severity on root and crown of strawberry in the nursery and the field (combined data of the two seasons of 2015/2016 and 2016/2017)

	Variables		
Variables	Disease severity on root (nursery)	Disease severity on crown (nursery)	Disease severity on root (field)
Disease severity root/nursery	-	-	-
Disease severity crown/nursery	-0.059[a] (0.872)[b]	-	-
Disease severity root/field	0.986[a] (0.000)[b]	-0.062[a] (0.858)[b]	-
Disease severity crown/field	0.979[a] (0.000)[b]	-0.061[a] (0.867)[b]	0.996[a] (0.000)[b]

[a]Linear correlation coefficient, no = 10, [b]Probability level

Table 4: Effect of AMF and humic acid on some growth parameters and characteristics of transplants produced in nurseries during 2015/2016 and 2016/2017 growing seasons

	2015/2016 season			2016/2017 season		
Treatments	No. of lateral roots	Root length (cm)	Crown diameter (mm)	No. of lateral roots	Root length (cm)	Crown diameter (mm)
AMF	30.00	22.5	12.00	33.00	24.00	13.00
Humic acid	25.00	20.5	10.00	27.00	22.50	11.00
AMF+humic acid	37.00	28.0	14.00	40.00	30.00	15.00
[a]Fungicides	45.00	35.6	18.00	48.00	38.50	19.00
Control	5.00	4.50	4.00	4.00	5.50	3.00
LSD ($p\leq0.05$)	6.20	3.95	1.84	2.15	1.93	1.52

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

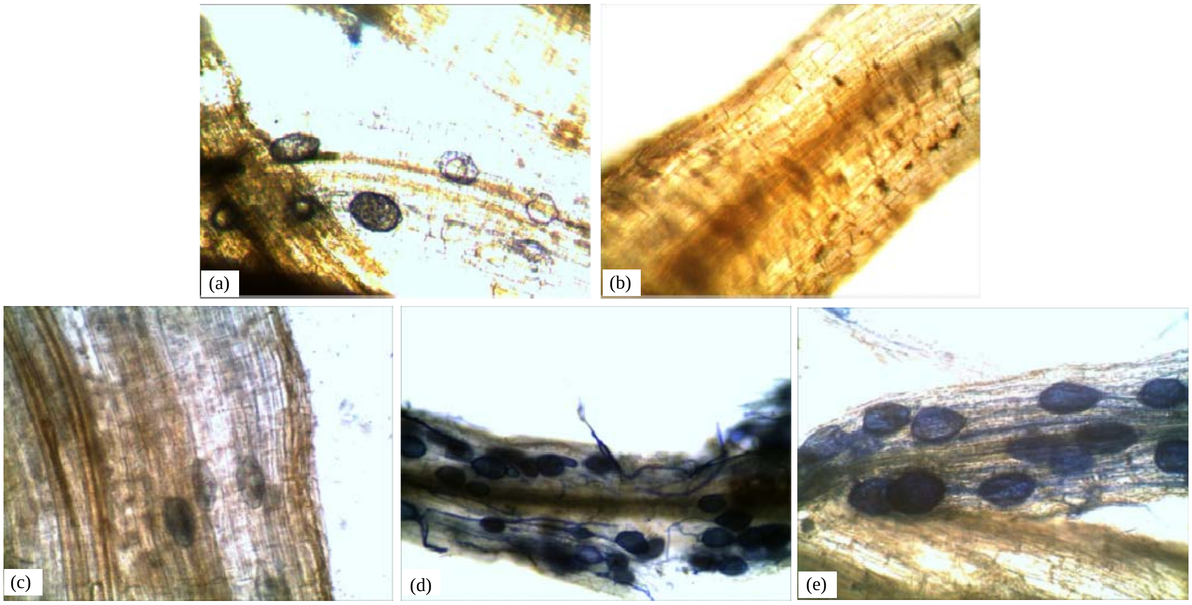

Fig. 1(a-e): Strawberry roots showing different levels of occupation by arbuscular mycorrhizal fungi, (a) Control, (b) Fungicides, (c) Humic acid treatment, (d) AM fungi and (e) Humic acid+AM fungi

Table 5: Percentage of occupied strawberry roots by AM fungi

	Root occupation by AMF (%)	
Treatments	2015/2016 season	2016/2017 season
AMF	83.00	70.76
Humic acid	59.00	49.33
AMF+humic acid	74.33	74.00
[a]Fungicides	11.67	3.33
Control	38.33	42.67
LSD ($p \leq 0.05$)	22.30	23.75

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M, AMF: Arbuscular mycorrhizae fungi

Table 6: Spore number of AM fungi in the rhizosphere of strawberry plants

	Number of spores in rhizosphere	
Treatments	2015/2016 season	2016/2017 season
AMF	1391.00	1117.0
Humic acid	308.00	368.7
AMF+humic acid	1378.00	1261.0
[a]Fungicides	28.00	51.0
Control	170.33	226.0
LSD ($p \leq 0.05$)	369.90	500.0

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

with AMF. Therefore it was obvious from the results that adding humic acid to AMF in one treatment reduced the efficiency of AMF in root occupation and soil colonization. Also this treatment affected the shape of the spores of mycorrhizae making it looking slightly irregular (Fig. 1a-e).

Data revealed that adding humic acid singly to the soil did not affect the colonization of strawberry roots with mycorrhizae (that is naturally present in soil).

It was also noticed from the data that the fungicide treatment was deleterious to the mycorrhizae as it caused dramatic reduction in colonization of the roots by AMF compared to the untreated control.

Effect of treatments on losses of transplants and early and total yield of strawberry: Prior to growing plants in the field, transplants produced in the nursery were examined to choose the healthy ones for planting. Data revealed that the most effective treatment in reducing the disease severity was the highest in producing healthy transplants (visually healthy) (Fig. 2). Data presented in Fig. 2 showed that the treatment with AMF and humic acid reduced the losses in transplants compared with the control. It also showed that humic acid alone was the least effective treatment in reducing losses in both seasons while fungicides were the most effective treatment.

The influence of the tested treatments on fruits yield is given in Table 7. Generally, both early and total yield were significantly increased compared to the control. Fungicides treatment was the most effective in increasing both early and total yield. The effect of the single treatments with AMF and humic acid was very close in both early and total yield. However, the combined treatment of AMF and humic acid improved their effects and significantly enhanced the yield ($p \leq 0.05$).

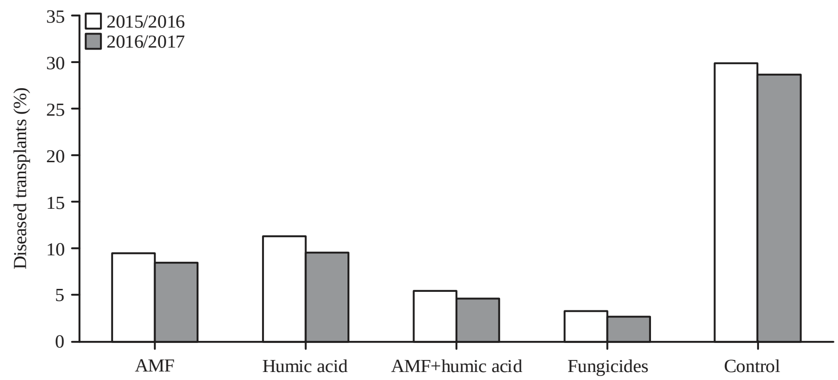

Fig. 2: Effect of different treatments in losses (%) in number of strawberry transplants produced in the nurseries

Table 7: Effect of AMF and humic acid on early and total yield of strawberry plants (festival cv.) under field conditions during two successive seasons 2015/2016 and 2016/2017

	2015/2016 season		2016/2017 season	
Treatments	Early yield (kg/21 m^2)	Total yield (kg/21 m^2)	Early yield (kg/21 m^2)	Total yield (kg/21 m^2)
AMF	12.50	62.50	14.50	68.00
Humic acid	10.00	50.00	12.50	57.50
AMF+humic acid	17.00	85.00	18.00	90.50
[a]Fungicides	23.00	110.00	25.00	120.00
Control	4.50	22.00	4.00	20.00
LSD ($p\leq 0.05$)	2.13	6.87	3.16	8.25

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

Table 8: Effect of different treatments on total sugar contents (mg g^{-1}), anthocyanin (mg g^{-1}) and fruit firmness (N)

	2015/2016 season			2016/2017 season		
Treatments	Sugars (mg g^{-1})	Fruit firmness (N)	Anthocyanin (mg g^{-1})	Sugars (mg g^{-1})	Fruit firmness (N)	Anthocyanin (mg g^{-1})
AMF	9.88	21.26	9.00	1.17	11.75	5.01
Humic acid	7.12	24.20	8.13	1.35	10.45	6.57
AMF+humic acid	7.32	22.00	10.20	1.62	11.92	6.69
[a]Fungicides	6.09	22.14	6.57	2.54	14.71	6.85
Control	4.72	22.60	6.34	3.58	10.94	4.34
LSD ($p\leq 0.05$)	1.67	NS	NS	1.12	NS	NS

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

Table 9: Effect of different treatments on total and free phenol content

	2015/2016 season		2016/2017 season	
Treatments	Total phenol	Free phenols	Total phenols	Free phenols
AMF	2.90	2.04	13.34	7.45
Humic acid	4.45	3.20	13.08	7.74
AMF+humic acid	3.46	3.21	12.99	9.74
[a]Fungicides	4.46	3.33	15.86	9.31
Control	2.45	1.95	16.52	6.88
LSD ($p\leq 0.05$)	1.05	2.32	3.12	2.06

[a]Fungicides mixture of Rizolex T, Tachgarin and Topsin M

Effect of treatments on total sugar content, anthocyanin, firmness of berries and total and free phenols: Treatments varied in their effect on fruits content of total sugars. In the first year all treatments significantly increased the sugar content of the fruits compared to control. The most effective treatment in improving the sugar content was AMF treatment followed by the combination of AMF and humic acid treatment. However, in the 2nd year the total content of sugar was decreased significantly in all treatments (Table 8). Treatments varied in their effect on the anthocyanin content of the fruits. Generally, all treatments increased the content of the anthocyanin but this improvement was insignificant. Similarly, the effect of the different treatments in fruit firmness was generally insignificant (Table 8).

Effect of treatments on total and free phenols: The effect of the different treatments on the total and free phenols varied between the two seasons (Table 9). In the first season, all treatments significantly increased the total phenols content, while the increase in free phenols was insignificant in all treatments compared to the control. On

Table 10: Correlation between phenols content and disease severity

	Variables		
Variables	Total phenol	Free phenols	Disease severity on root
Total phenols	-	-	-
Free phenols	0.930[a] 0.000[b]	-	-
Disease severity on roots	0.065[a] 0.859[b]	0.207[a] 0.566[b]	-
Disease severity on crown	0.105[a] 0.773[b]	0.163[a] 0.652[b]	0.990[a] 0.000[b]

[a]Linear correlation coefficient (r), [b]Probability level

the other hand, both total and free phenols were generally decreased in the second year compared to the control treatment although in some cases this decrease was insignificant (Table 9).

Linear correlation coefficient (r) was used to evaluate the association between phenols and disease severity. No correlation was observed neither between total phenols and disease severity nor between free phenols and disease severity (Table 10).

DISCUSSION

Different types of practices are being applied in plant protection area towards achieving sustainable agriculture and reducing the usage of chemicals and deleterious substances[29,30]. Accordingly this study aimed to use AM fungi and humic acid to control strawberry root and crown rot disease complex.

In the present study, treating plants with AM fungi significantly reduced the disease severity on both root and crown of strawberry under the nursery and field conditions in two successive seasons. This reduction in disease severity varied from 77-85% compared to the untreated control. In addition, AM fungi greatly affected the production of healthy transplants resulting from the nursery stage in addition to improving the different growth characteristics of these transplants.

The role of AM fungi in protecting plants from soil borne pathogens was widely studied and several mechanisms were suggested to be involved[11,12]. It was found that AM fungi directly compete with the various soil borne pathogens for root colonization and infection sites on the root[13,31,32]. Cordier *et al*.[33] studied the localized competition between the AM fungus *Glomus mosseae* and *Phytophthora nicotianae* var. *parasitica* on colonizing tomato roots. They found that tomato roots that were previously colonized with *G. mossae* were less damaged by the infection with *P. nicotianae* var *parasitica* since the former occupied the infection sites on the roots.

The AM fungi can also provoke plants to develop systemic resistance after their invasion to the roots which provides plants with some protection against different pathogens and pests[14,15,34]. Jasmonic acid (JA), glucanases, chitinases and different pathogenesis related proteins (PRs) were found to be involved in biochemical changes in plants due to induced resistance after application with mycohorrhiza[14,15,34,35]. Moreover, the improvement of plant nutrition is a well-established effect of AM fungi on plant growth and it is indeed suggested to be involved in bio protection of plants[36]. The AM fungi uptake minerals, especially phosphorus, from rhizosphere and transport them via their hyphae into the plant root and then to the whole plant.

In the present investigation, AM fungi were found to enhance root development since the root length and the number of lateral roots was higher in AM treated plants. These results were in consistent with previous findings of Norman *et al*.[37] where the architecture of strawberry plants treated with *Glomus fasciculatum* and *G. etunicatum* was changed and the root developing and branching were greatly enhanced. Moreover, these plants were less affected by *Phytophthora fragariae*.

In addition, current data also revealed that using humic acid significantly reduced disease severity of root and crown rot of strawberry in both nursery and field which was confirmed in two successive seasons. These results were in agreement with the findings of Sahni *et al*.[38] where treatment with vermicompost (consisting of humic substances) combined with growth promoting strain of *Pseudomonas syringae* reduced the infection with *Sclerotium rolfsii* in chickpea plants.

However, present findings differed from those of Yigit and Dikilitas[39] who found that tomato plants treated with humic substances were more susceptible to root rot caused by *Fusarium* spp. although the root growth was enhanced by the treatments.

Humic acid is a type of organic substances that are being introduced to the soil to improve its properties and increase the plant uptake of water and mineral elements by plant roots.

This improvement could indirectly affect the infection with plant diseases and reduces disease severity. However, humic substances could also directly affect the soil borne plant pathogens. Humic substances were found also to suppress the growth and conidial germination of *Alternaria alternata*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *melonis* and *F. oxysporum* f. sp. *lycopersici in vitro*[40,41]. However, the effect of humic acid in reducing disease severity on strawberry plants could be due to the enhancement of root growth of the plants and thus improving nutrients and water uptake. Moreover, data from the present investigation revealed that the combined treatment of both AM and humic acid generally gave better results than the single treatments of either one, although, in some cases, the results were not significantly different from the single treatment of AM.

There are limited investigations on the dual effect of AMF and humic acid on plant diseases. However, our findings were in consistent with those of Demir *et al*.[20] which revealed that combing AMF with humic acid reduced the severity of *Verticillium dahlia* wilt on tomato, pepper and eggplant in addition to enhancing the plants growth and their nutritional conditions.

In the present investigation the effect of humic acid on the efficiency of AM fungi was clear, but not significant. Humic acid reduced the occupation of roots by AM fungi however this effect was not reflected on the disease severity or plant growth parameters. In addition, some biochemical responses of plants due to treating with AM fungi and humic acid were investigated. Some of the applied treatments increased total and free phenols content; however, the correlation between phenol content and disease severity was always non-significant. It was found in previous investigations that the levels of total phenols increased after inoculating plants with mycorrhizae[14,42,43]. In the present study, mycorrhizae singly increased total phenols in only one season. On the other hand, AM fungi and/or humic acid application did not affect anthocyanin pigment or fruits firmness.

CONCLUSION

According to the obtained results of this investigation, it may recommend AM fungi and humic acid as means for controlling root and crown rot diseases of strawberry in the nursery and the field also to improve different plant growth parameters and fruits yield.

SIGNIFICANCE STATEMENT

This study confirmed that AMF are able to protect strawberry plants against root and crown rot disease and improve the yield and different growth parameters. Combing AMF and humic acid was more effective in reducing root and crown rot disease of strawberry and improving growth parameters compared to the single treatments of either.

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