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The potential role of vesicular-arbuscular mycorrhizal (VAM) fungi in the bio-control of the root-knot nematode *Meloidogyne incognita* and increase growth in two grapevine cultivars

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Abstract Two grapevine cultivars, namely, Thompson Seedless and Crimson Seedless (*Vitis vinifera*) were selected to study the potential of the vesicular-arbuscular mycorrhizal (VAM) fungi, *Glomus intraradices*, for the bio-control of infection and reproduction of sedentary endoparasitic nematode, *Meloidogyne incognita* and to evaluate the interaction effect between VAM fungi and nematode on seedling growth. VAM increasing plant resistance to the nematode where number of root galling and egg-masses is significantly suppressed. The number of root galling and egg-masses were significantly higher in Thompson seedless cultivar than Crimson cultivar in the second season. However, in the first season, there were no significant differences between the two cultivars. Mycorrhization with *G. intraradices* resulted in significantly better seedling growth, which was evident in higher shoot and root fresh and dry weight, even in the presence of M. incognita. Compared to the control, *G. intraradices* alone or in combination with M. incognita increased the leaf chlorophyll content. No differences in mycorrhizal colonization percentage were observed between the two cultivars. Although grapevine seedlings infected with nematode showed a lower percentage of mycorrhizal colonization, no significant differences of the presence of nematode were observed on percentage of root colonized by VAM.

Keywords nematode, root galling, *Glomus intraradices*, plant growth, chlorophll content

1. Introduction

Grapes (*Vitis. spp*) are important fruit crops of high economic value in the world. Amongst pathogens that can influence grapevines, the commonly occurring soil- borne nematode *M. incognitacan* cause significant damage to root systems [1]. The most complex plant-parasitic nematode feeding strategy is shown by endoparasitic sedentary nematodes (*Meloidogyne incognita*), which pick cells in the vascular cylinder to be transformed into a feeding site and then become sedentary with the onset of feeding. The acute toxicity of nematicides and fumigants has made their use unacceptable in some countries, due to possible adverse environmental consequence [2]. Due to their minimal effects on the environment, biological management methods are preferable and the land can be used extensively for economic development. As a possible alternative to chemical regulation, beneficial soil microorganisms such as arbuscular mycorrhizal fungi have been suggested [3]. In plants, VAM fungi are common root symbionts, colonizing species belonging to more than 80% of all plant families in the world [4]. They have been reported increases in plant uptake by grapevine of phosphate and other mineral nutrients under certain conditions [5]. Root colonization by VAM fungi in

various plants, including grapevine, is also known to increase tolerance or induce resistance to fungal pathogens and decrease nematode growth [6-7]. There is evidence that AM-induced defenses against root pathogens includes not only local but also systemic resistance, with a decrease in root infection in mycorrhizal and non-mycorrhizal sections of mycorrhizal root systems, indicating the presence of signal-mediated phenomena at a distance [8-9]. Both vesicular-arbuscular mycorrhizal (VAM) fungi and root knot nematodes are domestic soil organisms which share plant roots as resources of food. Consequently, due to the possibility of increased resistance or tolerance of VAM colonized plants to nematodes, there is interest in VAM-nematode interactions [10]. The mechanism suggested to explain this protective effect comprises: direct competition; plant growth, nutrition and morphology alteration-mediated mechanism; molecular and biochemical changes in mycorrhizal colonized plants that induce pathogen resistance; and soil microbiota alterations; and pathogen antagonism development [11-12]. The interactions between VAM and endoparasitic nematodes are strong, because it reaches the cortical cells colonized by VAM. Two types of nematodes are sedentary endoparasites, cyst and root-knot nematodes. Both cause changes in plant cells and feed on transformed cells. The feeding cells induced by these endoparasites within the vascular cylinder can proliferate into the cortex and invade the endodermis, where the cells colonized by VAM can be found, and creating space competition in the root cells between these two indigenous organisms [10]. On the other hand, VAM may alter root morphology with implications for nematode penetration and movement. VAM colonizes much faster than nematode, leading to modification in root physiology of host such as altering the chemical composition of roots exudates. Much work has been done on VAM-nematode interactions especially on root-knot (*Meloidogyne spp*.) nematodes given its economic significance. VAM have with root nematodes of several crop plant species including fruit trees [13] and banana [9]. Jaizme-Vega *et al* [13] noted that *Glomusmosseae* inoculation favours banana plant growth by improving plant nutrition, and by restricting the reproduction and galling of incognita during the early stages of plant development. Also, Elsen *et al* [9] showed that VAM has the potential to cause systemic resistance in the banana root system to plant parasitic nematodes, as VAM decreased the populations of nematodes by more than 50 percent. Interactions between VAM fungi and nematodes, especially in grapevines, have received relatively little attention. Therefore, the aim of this study was to assess the potential of the vesicular-arbuscular mycorrhizal (VAM) fungi, *Glomus intraradices*, for the bio-control of infection and reproduction of *Meloidogyne incognita*, the dominant pathogenic nematode species in grapevines of Egypt. In addition, to research the interaction effects between mycorrhizae and nematode on the growth of two grapevine cultivars.

2. Material and Methods

2.1. Plant material

The study was conducted for two consecutive years (2018 and 2019) at experimental plot located in Faculty of Agriculture, Alexandria University, Egypt. One- year- old seedlings of two grape cultivars namely Thompson Seedless and Crimson Seedless (*Vitis vinifera*) were used. The seedlings were uniform in size and grafted on the own rooted. The experimental seedlings were individually planted in black bags of polyethylene filled with around six kilograms of sandy soil.

2.2. Vesicular-arbuscular mycorrhizal (VAM) fungi and root-knot nematodes *M. incognita* inoculums application

On the 28th March 2018 and 2019, the experimental seedlings were evenly divided into two groups of 20 seedlings each. Mycorrhizae, *Glomus intraradices*, was inoculated in the first group of plants, while the second group was left without mycorrhizal inoculation as control; Inoculation was accomplished by applying 5 g of inoculum to the soil below the seedlings per plant. The strain of mycorrhizal *G. intraradices* was used in both experimental seasons isolated from the experimental Station of the University of Alexandria at Abies [14]. The VAM fungus was propagated in pot culture on onion (*Allium cepa* L.) plants in the clay–loam soil for about 10 weeks. At a rate of 1:7 (v:v) in the growth medium for mycorrhizal treatments, inoculum from pot cultures (spores, mycelium, root fragments and soil) was used. In non-mycorrhizal treatment, the inoculum was substituted by a heat sterilized inoculum. Two months later, half of each seedlings group (10 plants), with or without mycorrhizae were inoculated separately with 4000 nematode eggs and J2/pot of *M. incognita* by adding the nematode suspension in three holes around the root zone. Inoculum of the root-knot nematodes, *M. incognita* was collected from the culture collection of

the Nematology Research Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University.

2.3. Experimental design

The experimental seedlings were arranged in Randomized Complete Block Design (RCBD), with five replicates in each treatment, with a single plant for each replicate. The experiment include four treatments: an un-inoculated control (C), inoculation with vesicular-arbuscular mycorrhizal (VAM) fungi, inoculation with M. incognita (Mi), simultaneous inoculation with VAM and M. incognita (VAM + Mi), (4 treatments, 2 cultivars x 5 replicates = 40 seedlings in each experimental season).

2.4. Plant growth and assessment of mycorrhiza and nematode development

Experimental seedlings were harvested 90 days after inoculation of nematode. The following specific physical and chemical parameters were assessed: plant height, shoot and root fresh weight, and leaf chlorophyll content. After collecting the root fraction to determine VAM colonization percentage (5% in fresh weight), the remaining seedling material, the leaves, stem and roots were separated and dried at 70° C for 48 h for estimating plant biomass (shoot and root dry weight). For assessing the percentage of mycorrhizal root colonization at the end of the experiment, a portion of fresh roots (2 cm in length) were washed with distilled water and cut into root pieces of 1 cm. Root pieces were cleared for 20 minutes in 10 % KOH and were kept at 90 ° C. Subsequently they were rinse with water 3 times. Staining was done with 0.05% (w/v) Trypan blue lactoglycerol for 15 min at 90° C. the segments were examined under a research microscope [15]. Root segments that contained arbuscules, vesicles or hyphae were graded as mycorrhizal.

Nematode development was estimated by counting gall numbers and egg-masses on roots. Roots were stained for 15 minutes in an aqueous solution of phloxin B stain (0.15g/l) then washed with distilled water to stain the egg-masses of nematode [16-17].

2.5. Statistical Analysis

The data were statistically analyzed using the analysis of variance (ANOVA) according to Snedecor and Cochron [18]. Differences between treatments were determined using LSD at probability level of 0.05. VAM colonization and nematode reproduction data were transformed by arcsine (x/100) [19] to minimize the variance in the data.

3. Results

3.1. Mycorrhizal colonization

Mycorrhizal colonization percentage was detected 12 weeks after inoculation with VAM. In both season, the average percentage of VAM colonization ranged between 60 % and 70% at the end of experiments in treatments with simultaneous inoculation with VAM and nematode, and in treatments inoculated with VAM respectively (Fig. 1).

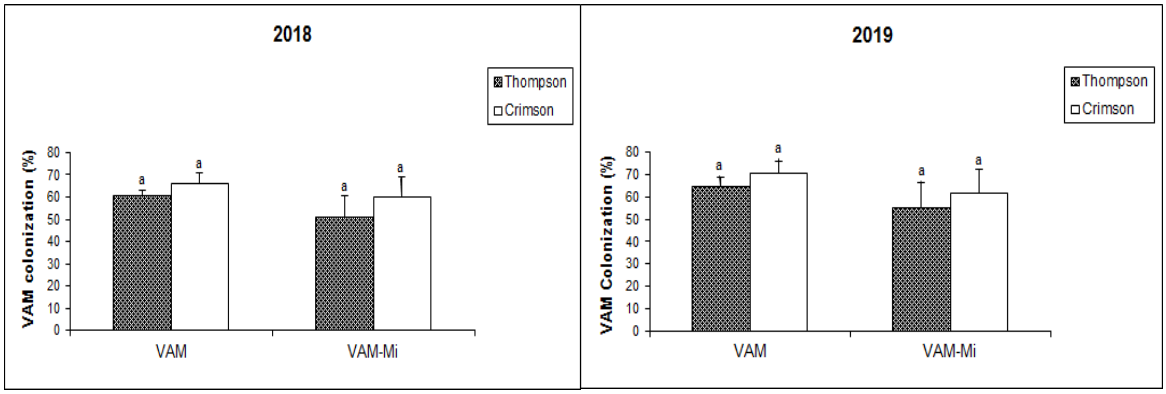

Figure 1: Percentage of grapevine roots infected by vesicular-arbuscular mycorrhizal (VAM) fungi and percentage of "Thompsn" and "Crimson" grapevine roots infected by vesicular-arbuscular mycorrhizal and M. incognita (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed.

However, although grapevine seedlings infected with nematode showed a lower mycorrhizal colonization percentage, no significant differences of the presence of nematode were recorded on percentage of root colonized by VAM.

Regarding the variation in the effect of the two cultivars on mycorrhizal root colonization percentage, the data in Figure 1 showed that, in both seasons, there were no significant differences were found between the two cultivars.

3.2. Effect of VAM on nematode

Concerning to the effect of VAM on the number of galls in root per plant of "Flame seedless" and "Crimson seedless" seedlings. Results showed there were significant differences between the mycorrhizal plants and the plants infected only by nematode (Fig. 2, a & b). In fact, the average of the number of galls in root per plant was drastically reduced by the presence of VAM than in its absence, in other words, the VAM presence caused a decreased of about 54.6 and 48.8% in the number of galls in root per plant of "Flame seedless" and "Crimson seedless" seedlings in the first and second season, respectively.

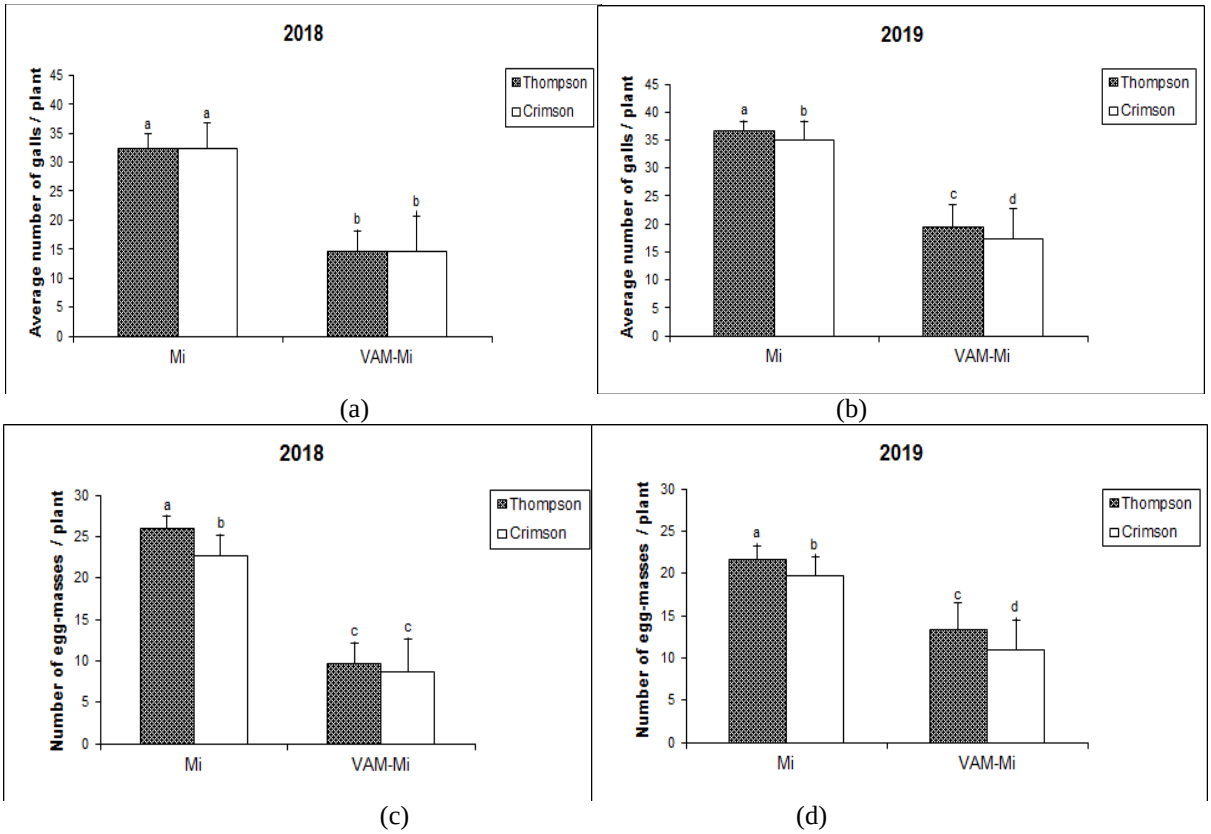

Figure 2 (a-d): Average number of galls/plant (a and b) and number of egg-masses/plant (c and d) of "Thompsn" and "Crimson" grapevines from two different treatments: inoculation with M. incognita (Mi) and simultaneous inoculation with VAM and nematode (VAM-Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed.

The same result was obtained in relation to the number of egg-masses in root per plant of "Flame seedless" and "Crimson seedless" seedlings which also varied among the two treatments (Fig. 2, c & d) with the micorrhizal plants had the lower average number of egg-masses on root than seedlings infected only by nematode, i.e, the number of egg-masses was less than about 62.3 and 41.1% in the first and second season, respectively in seedlings infect by nematode and VAM.

Regarding the variation in the effect of the two cultivars, the data in Figure 2 indicated that there were no significant differences were found in the number of galls in roots and the number of egg-masses, in the first season. Whereas, "Crimson seedless" seedlings had the highest number of galls and egg-masses in the second season.

3.3. Effect of VAM on seedlings development

Table 1: Plant height of "Thompsn" and "Crimson" grapevines from four different treatments: non-mycorrizal plants (control); mycorrizal plants (VAM); inoculation with *M. incognita* (Mi) and simultaneous inoculation with VAM and nematode (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed. Different letters above the bars indicate significant differences between treatments after two-way ANOVA and L.S.D test (P≤ 0.05).

Treatment	Cultivars (CV.)		Treatment means	Cultivars (CV.)		Treatment means
	Thompson seedless	Crimson seedless		Thompson seedless	Crimson seedless	
	2018			2019		
Control	50.67 cd	50.00 cd	50.33 b	70.00 a	50.67 bcd	60.33 a
Mi	53.33 bcd	45.33 d	49.33 b	40.67 d	44.00 cd	42.33 b
VAM	70.00 a	61.67 ab	65.83 a	53.33 abcd	63.33 ab	58.33 a
Mi + VAM	59.67 bc	60.67 ab	60.17 a	61.33 abc	55.00 abcd	58.17 a
CV. means	58.42 a	54.42 a		56.33 a	53.25 a	
L.S.D. at 0.05	Tr. = 6.70	Cv. = 4.88	Tr. × Cv. = 9.75	Tr. =12.97	Cv. =9.17	Tr. × Cv. =18.34

Table 2: Shoot fresh weight of "Thompsn" and "Crimson" grapevines from four different treatments: non-mycorrizal plants (control); mycorrizal plants (VAM); inoculation with M. incognita (Mi) and simultaneous inoculation with VAM and nematode (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed. Different letters above the bars indicate significant differences between treatments after two-way ANOVA and L.S.D test (P≤ 0.05).

Treatment	Cultivars (CV.)		Treatment means	Cultivars (CV.)		Treatment means
	Thompson seedless	Crimson seedless		Thompson seedless	Crimson seedless	
	2018			2019		
Control	50.33 bc	50.33 bc	50.33 b	51.33 c	52.00 c	51.67 b
Mi	44.33 d	48.33 c	46.33 c	44.00 f	46.33 e	45.17 c
VAM	53.67 a	55.67 a	54.67 a	54.33 b	57.00 a	55.67 a
Mi + VAM	48.67 bc	51.00 b	49.83 b	48.67 d	52.33 c	50.50 b
CV. means	49.25 b	51.33 a		49.58 b	51.92 a	
L.S.D. at 0.05	Tr. = 1.85	Cv. = 1.31	Tr. × Cv. = 2.62	Tr. = 1.20	Cv. = 0.85	Tr. × Cv. = 1.70

Table 3: Root fresh weight of "Thompsn" and "Crimson" grapevines from four different treatments: non-mycorrizal plants (control); mycorrizal plants (VAM); inoculation with M. incognita (Mi) and simultaneous inoculation with VAM and nematode (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed. Different letters above the bars indicate significant differences between treatments after two-way ANOVA and L.S.D test (P≤ 0.05).

Treatment	Cultivars (CV.)		Treatment means	Cultivars (CV.)		Treatment means
	Thompson seedless	Crimson seedless		Thompson seedless	Crimson seedless	
	2018			2019		
Control	42.33 cd	44.67 ab	43.50 bc	44.33 de	45.67 cd	45.00 b
Mi	40.67 d	43.33 bc	42.00 c	39.33 f	42.67 e	41.00 c
VAM	46.67 a	44.33 bc	45.50 a	47.67 bc	50.67 a	49.17 a
Mi + VAM	45.00 ab	44.00 bc	44.50 ab	44.33 de	48.33 ab	46.33 b
CV. means	43.67 a	44.08 a		43.92 b	46.83 a	
L.S.D. at 0.05	Tr. = 1.62	Cv. = 1.15	Tr. × Cv. = 2.29	Tr. = 1.82	Cv. = 1.29	Tr. × Cv. = 2.57

Table 4. Chlorophyll content of "Thompsn" and "Crimson" grapevines from four different treatments: non-mycorrizal plants (control); mycorrizal plants (VAM); inoculation with M. incognita (Mi) and simultaneous inoculation with VAM and nematode (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed. Different letters above the bars indicate significant differences between treatments after two-way ANOVA and L.S.D test (P≤ 0.05).

Treatment	Cultivars (CV.)		Treatment means	Cultivars (CV.)		Treatment means
	Thompson seedless	Crimson seedless		Thompson seedless	Crimson seedless	
	2018			2019		
Control	23.30 bc	17.27 c	20.28 bc	27.68 ab	16.17 d	21.93 a
Mi	20.33 c	18.62 c	19.48 c	19.13 cd	16.63 d	17.88 b
VAM	32.07 a	27.86 ab	29.97 a	31.63 a	18.62 d	25.13 a
Mi + VAM	27.47 ab	21.57 bc	24.52 b	25.17 b	23.27 bc	24.22 a
CV. means	25.79 a	21.33 b		25.90 a	18.67 b	
L.S.D. at 0.05	Tr. = 4.78	Cv. = 3.38	Tr. × Cv. = 6.76	Tr. = 3.28	Cv. = 2.32	Tr. × Cv. = 4.64

Table 5: Root fresh weight of "Thompsn" and "Crimson" grapevines from four different treatments: non-mycorrizal plants (control); mycorrizal plants (VAM); inoculation with *M. incognita* (Mi) and simultaneous inoculation with VAM and nematode (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed. Different letters above the bars indicate significant differences between treatments after two-way ANOVA and L.S.D test (P≤ 0.05).

Treatment	Cultivars (CV.)		Treatment means	Cultivars (CV.)		Treatment means
	Thompson seedless	Crimson seedless		Thompson seedless	Crimson seedless	
	2018			2019		
Control	32.33 b	32.33 b	32.33 b	33.67 c	33.00 cd	33.33 b
Mi	27.33 d	29.33 c	28.33 c	28.00 e	31.33 d	29.67 c
VAM	35.67 a	37.00 a	36.33 a	37.00 b	39.33 a	38.17 a
Mi + VAM	33.00 b	31.67 b	32.33 b	32.00 cd	35.67 b	33.83 b
CV. means	32.08 a	32.58 a		32.67 b	34.83 a	
L.S.D. at 0.05	Tr. = 0.97	Cv. = 0.68	Tr. × Cv. = 1.37	Tr. = 1.32	Cv. = 0.94	Tr. × Cv.= 1.87

Table 6. Root dry weight of "Thompsn" and "Crimson" grapevines from four different treatments: non-mycorrizal plants (control); mycorrizal plants (VAM); inoculation with M. incognita (Mi) and simultaneous inoculation with VAM and nematode (VAM+Mi) during 2018 and 2019 seasons. Standard errors of the mean (n= 5) are shown. Values of percentage are arcsine (x/100) transformed. Different letters above the bars indicate significant differences between treatments after two-way ANOVA and L.S.D test (P≤ 0.05).

Treatment	Cultivars (CV.)		Treatment means	Cultivars (CV.)		Treatment means
	Thompson seedless	Crimson seedless		Thompson seedless	Crimson seedless	
	2018			2019		
Control	26.33 bcd	27.67 bc	27.00 a	29.00 bc	27.67 cd	28.33 b
Mi	25.00 cd	23.67 d	24.33 b	24.33 e	27.33 d	25.83 c
VAM	29.67 ab	26.67 bcd	28.17 a	29.67 b	31.67 a	30.67 a
Mi + VAM	31.67 a	26.00 cd	28.83 a	27.67 cd	29.00 bc	28.33 b
CV. means	28.17 a	26.00 b		27.67 b	28.92 a	
L.S.D. at 0.05	Tr. = 2.56	Cv. = 1.81	Tr. × Cv. = 3.62	Tr. = 0.97	Cv. = 0.68	Tr. × Cv. = 1.37

In both seasons, there were significant differences between treatments in seedlings height of "Thompson seedless" and "Crimson seedless" cultivars (Table 1). The results showed that average of seedling height was greater in seedlings inoculated with VAM, but, there were not significant differences between heights of seedlings inoculated with VAM and nematode and non-mycorrhizal seedlings (control), as well as, between mycorrhizal seedlings. Root infection only by nematode had the lowest seedling height value.

The importance of shoot growth and biomass production on grapevine seedlings inoculated with VAM compared to the non-inoculated control seedlings and seedlings inoculated with *M. incognita* are showed in Tables 2 and 3. In both seasons, there were significant differences in average of shoot and root fresh weight of "Thompson seedless" and "Crimson seedless" cultivars among treatments. Data showed a highly significant effect of VAM in fresh biomass of grapevine plants. In fact, average of shoot and root fresh weight of mycorrhizal seedlings was higher than in the other all, and the seedlings inoculated with nematode alone had the lowest average of shoot and root fresh weight. By other side, there were not significant differences between shoot and root fresh weight of seedlings simultaneously inoculated with VAM and nematode and non-mycorrhizal seedlings (control).

The leaf chlorophyll content of "Thompson seedless" and "Crimson seedless" seedlings also varied significantly between treatments (Table 4). In the first season, data showed that average of leaf chlorophyll content was significantly higher in seedlings inoculated with VAM compared to un-inoculated ones, but seedlings infected only by nematode had significantly the lowest chlorophyll content values. There were not significant differences between leaf chlorophyll content of seedlings inoculated with VAM and nematode and non-mycorrhizal seedlings (control). In the meanwhile, mycorrhizal treatment resulted in significantly higher leaf chlorophyll content value than other treatments, which did not significantly differ among each other in the second season.

The shoot and root dry weight was similar to previous, i.e. there were significant differences in average of shoot and root dry weight of "Thompson seedless" and "Crimson seedless" seedlings among treatments (Table 5 and 6). The results showed that averages of shoot and root dry weight of mycorrhizal seedlings were higher than in the other all, and the seedlings infected with nematode alone had the lowest average of shoot and root dry weight. In the meanwhile, there were not significant differences between shoot and root dry weight of seedlings simultaneously inoculated with VAM and nematode and non-mycorrhizal seedlings (control), except, in the second season, mycorrhizal treatment resulted in significantly higher root dry weight than other treatments, which did not significantly differ among each other.

As for cultivar effect, the data obtained during both experimental seasons cleared that the shoot fresh weight and shoot and root dry weight of "Crimson seedless" cultivar was higher than that of "Thompson seedless" cultivar. In the meanwhile, leaf chlorophyll content of "Thompson seedless" cultivar was greater than that of "Crimson seedless". Moreover, no significant differences were found in plant height and fresh root weight between two cultivars, in both seasons.

Considering the interaction effect between cultivars and treatments, the data revealed that "Crimson seedless" inoculated seedlings had the highest plant height, shoot and root fresh, and shoot dry weight in both season and root dry weight in the second season. While, the highest leaf chlorophyll content values were recorded with "Thompson seedless" inoculated seedlings in both seasons and the highest root dry weight in the first season.

4. Discussion

In general native VAM reduced *M. incognita* population in grapevines. This confirmed previous researches on a wide variety of crops. Among them, Kesba and Al-sayedal [20] reported that the association between VAM and the grapevines increased the host tolerance to *M. incognita*, compensating for damaged caused by nematode, mainly by enhancing plant nutrition. Jaizme-Vega *et al*. [13] in a study with papaya also showed that *M. incognita* infection was significantly reduced in mycorrhizal plants. Ceustermans *et al* [21] found that the number of nematodes in the soil was decreased by at least 50% for apple seedlings inoculated with VAM, as compared to the seedlings only inoculated with nematodes. Kesba and Al-sayed [20] also reported in a study to investigate the tolerance of grapevines to root-knot nematode *M. incognita*, that the number of root galls and the number of eggs per root were significantly lower on plants inoculated with *Glomus intradices*, indicating that Mycorrhizae has the potential for improved grapevine tolerance for root-knot nematode infestation. Several

hyposis have been suggested on the mechanism of the VAM-induced resistance against pathogens from plants [12 22-24]. Based on our findings we can suggest the mycorrhizal association may cause the host plant to be more robust and therefore more resistant to or tolerant to pathogen attacks [11].

Furthermore, our observations can also be clarified by competition for photosynthates and host colonization infection sites. Indeed, the lack of significant differences between mycorrhizal seedlings and seedlings with combined inoculation in VAM root colonization suggests that there are a competitive interaction between VAM and *M. incognita* on grapevines by space. VAM may occupy infection sites on the root surface required by incognita to penetrate the root, or the root-knot may not further colonize cells in the root already occupied by the AMF. This result was established by other authors. Among them, de la Peña *et al* [25] stated that when both VAM and nematodes were together in the same root compartment of *A. arenaria*, Competition between these species occurred, while root colonization by VAM did not affect the nematode.

Competition for nutrients, with focus on competition for carbon, was suggested as an VAM-mediated mechanism biocontrol [26]. Adding like VAM, *M. Incognita* growth relies on carbon from photosynthesis. As the symbiotic VAM requires a lot of carbon from plants colonized by VAM, less carbon may be available for nematode colonization [12, 22]. It is estimated that the carbon transfer from the host plant to the AMF is between 4 and 20 percent of the total assimilated carbon in the host plant [27].

In fact, root biomass of grapevine seedlings simultaneously inoculated with VAM and *M. incognita* was significantly higher than for the others treatments. The presence of the VAM may have encouraged this increase in root biomass to the pathogen and the degradation of the root parts compensate for tissue damage and decay. Consequently, disease symptoms decrease dramatically. This result has been consistent with Cofcewicz *et al* [28] who found that there was an increase in the number of galls in simultaneous inoculation of plant roots with VAM and *M. Javanica* may be related to promoting VAM with the growth of root systems, which put a nematode at the disposal of greater number of infection sites. In addition, our findings can also be clarified by competition for host colonization sites and photosynthates. The growth of all nematodes-coping VAM-inoculated seedlings was comparable to or even better than the seedlings grown in a nematode-free and mycorrhiza-free substrate, suggesting that the seedlings were able to grow withstand the nematodes' damage. Thebiocontrol effect of VAM against plant-parasitic nematodes involving increased plant tolerance (by increased indirect nutrient uptake or altered root morphology facilitating direct nutrient uptake) may be influenced by different mechanisms, direct competition for nutrients and space, induced systemic resistance, and altered rhizospheric interactions [24]. The various mechanisms cannot be considered completely independent of each other, and biocontrol is likely to result from a combination of different mechanisms [29]. Although this was not the scope of our research, we hypothesize that it may indeed be a mixture of increased plant tolerance and space rivalry. We recorded an increased fresh weight of the roots of the VAM-inoculated seedlings compared to non-VAM-inoculated seedlings all infecting with nematodes. In addition, the presence of nematodes in mycorrhizal seedlings did not appear to have affected plant growth. Actually, either shoot and root fresh weight or shoot and root dry weight of grapevines simultaneously inoculated with VAM and nematode were higher than in non-mycorrhizal seedlings or in seedlings only inoculated with nematode. Other studies have shown that mycorrhizal inoculated plants often show increased roots growth and branching [30-31]. Hosseini *et al* [32] found that mycorrhizal inoculated apple rootstocks had the higher root fresh weight than un-inoculated ones. Also, Wu *et al* [33] found that the fresh root weight of peach seedlings was also significantly increased after inoculation with mycorrhizae compared with the non-VAM control. In addition, it has been suggested that increased root branching observed in mycorrhizal plants has implications for pathogen infection and may also counteract the suppressed roots growth caused by plant-parasitic nematode [24], which is in line with our research. Consequently, in VAM-inoculated plants, the direct absorption of the nutrients is promoted by enhancing root system structure [34]. The same result was reported by Shreenivasa *et al* [35] who found that maximum plant height, shoot and root dry weight , as well as, total dry weight were higher in plants inoculated with *Glomusspp* and *M. incognita* than in control or in plant only infected by nematode.

This study concluded that inoculation with VAM may be a new approach to nematode management. By inducing plant resistance to the nematode, where root galling and egg masses are negatively impacted and plant growth enhanced by the mycorhizae, VAM can provide a sustainable solution for nematodes in grapevines.

However, much more work is needed on various associations of cultivar-nematode-VAM fungi, grown under a variety of environmental conditions, before accurate generalizations or predictions can be made.

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