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INFLUENCE OF RIZOLEX-T-60 WP AND FURADAN-10G ON *GLOMUS CLARUM* COLONIZATION AND GROWTH OF WATERMELON

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ABSTRACT

Glomus clarum symbiosis of watermelon plants gave positive results in their tested growth parameters of shoot and root systems lengths and dry weights, as well as, leaf pigmentation of chlorophyll a,b and carotenoids and the elements contents of N, P, Ca, Mg, K and Na, compared to non-mycorrhizal plants. The mycorrhizal infection and spore population were markedly influenced by the pesticide level. Half of the recommended concentration of the nematocide (Furadan-10) was responsible for about 39 and 59% increase of mycorrhizal colonization and spore production. While the lower fungicide level increased mycorrhizal infection by 6%. The recommended levels and the duplicates of both pesticides were conducive to mycorrhizal activities. At the latter levels, nematocide was obviously more drastic to *Glomus clarum* than the fungicide. Growth of watermelon responded differently to the applied pesticide level and mycorrhizal inoculation. At half of the recommended levels, mycorrhizal plants have higher values of growth parameters comparing to pesticides free plants, while *G.clarum* free plants show lower values. Increased pesticides levels were deleterious to watermelon growth parameters. However, plant pigments appeared to less susceptible to pesticides stress. The nematocide Furadan-10 was more drastic to the growth parameters of watermelon, either mycorrhizal or non-mycorrhizal colonized, than do the fungicide (Rizolex-T-60wg) instead of the applied nematocide levels were half that of the fungicide.

Key words : *Glomus clarum* , Vesicular arbuscular mycorrhizal(VAM), Rizolex-T-60wg, Watermelon plant (*Citrullus lanatus*).

1. INTRODUCTION

Vesicular arbuscular mycorrhizal (VAM) fungi are common in arable soils and can enhance plant growth and nutrients uptake (Sreenivasa and Bagyarag, 1989; Al-Garni and Daft, 1990; Caris *et al*, 1998; Liu *et al*, 2002; Ryan and Angus, 2003).VAM symbiosis can protect host plants against detrimental effects (Ruiz-Lozano *et al*, 1999; Quilanbo, 2000). They enhance water uptake through improved hydraulic conductivity and increasing leaf conductance and photosynthetic activity (Dell-Amico *et al*, 2002).In watermelon (*Citrullus lanatus* Thunb.) mycorrhizal colonization was found to improve not only the plant yield and water use efficiency, but also the quality of the fruits (Kaya *et al*, 2003). Hence the value of applying pesticides to field crops might be partially counter balanced by side effects on the VAM fungal population (Ocampo and Hayman, 1980). Many pesticides have been used so far to study their effect on VAM fungi. Most of these pesticides were found deleterious, but some are quite compatible with VAM fungi (Trappe *et al*, 1984; Udaiyan *et al*, 1995; Laatikainen and Heinonen-Tansk, 2002; Salem *et al*, 2003). In a previous work, the author studied the influence of the insecticide Malathion-57% and the fungicide Flonex-M400 (both are water soluble) on mycorrhizal colonization and growth of *Zea mays* and *Vicia faba* (Al-Garni, under consideration).The objective of this study is to investigate the effects of two powdered pesticides; Rizolex-T-60WP (fungicide) and Furadan-10G (nematocide) on mycorrhizal colonization and growth of watermelon (*Citrullus lanatus*) plants.

in chlorophylls were poorly influenced by mycorrhizal inoculations. The tested elements content of watermelon responded differently to *G.clarum* inoculations. P and N showed the highest increase (about 155 and 144%, respectively), while Ca and Mg were moderately increased (about 69 and 67%, respectively). On the other hand, Na and K showed the lowest increase values, as the plant was *G.clarum* inoculated. These findings indicated that generally the growth parameters of watermelon increased as the plant inoculated with the spores of *G.clarum.* In accordance with these findings it was reported that VAM fungi are associated with increased growth of many plant species due to increased nutrients uptake (Sreenivasa and Bagyaraj, 1989; Brundrett *et al*, 1996; Rao and Tak, 2002; Quilambo, 2003). In watermelon, mycorrhizal colonization was found to improve not only the plant yield and water use efficiency, but also the quality of the fruit (Kaya *et al*, 2003). VAM colonization increased photosynthetic rate (Ruiz-Lozano *et al*, 1996a), direct hyphal water uptake from the soil and transfer to the host plant (Ruiz-Lozano *et al*, 1996b), enhanced water uptake and photosynthetic activity (Dell-Amico *et al*, 2002). Mycorrhizae are described as improving the absorption of several nutrient elements as P, N, K, Mg, and Ca (Al-Karaki and Al-Radad, 1997; Liu *et al*, 2002).

Table-1: Effect of *Glomus clarum* inoculation on some growth parameters of *watermelon* plants grown for weeks.

Growth parameter	Non-mycorrhiza	Mycorrhiza	Efficiency of mycorrhizal inoculum
Shoot length(cm)	20.4	33.2	62.75
Root length(cm)	4.3	6.3	46.51
Shoot dry weight(g)	0.26	0.51	96.15
Root dry weight(g)	0.025	0.043	79.17
Leaf pigments(mg/g):			
Chlorophyll a	3.51	4.85	38.18
Chlorophyll b	2.96	3.20	8.11
Carotenoids	2.01	2.95	46.77
Element content(mg/g): N	5.7	13.9	143.86
P	0.75	1.91	154.67
Ca	6.2	10.5	69.00
K	0.9	1.02	13.3
Mg	3.95	6.6	67.09
Na	1.1	1.46	32.7

3. 3. Effect of Rizolex-T-60wg and Furadan-10G on growth of mycorrhizal and non-mycorrhizal watermelon

Table-3: Effect of different concentrations of the fungicide (Rizolex-T-60wg) and the nematocide(Furadan-10) on some growth parameters of watermelon plants infected or non-infected with *Glomus clarum* and grown for 6 weeks.

Growth parameters	Rizolex-T-60wg (g/m^2 soil)	+VAM	-VAM	Furadan-10G	+VAM	-VAM
Shoot length(cm)	0.5 1.0 2.0	31.1 25.4 20.1	16.5 13.2 12.0	0.25 0.50 1.00	40.8 20.1 10.9	17.9 14.5 9.3
Root length(cm)	0.5 1.0 2.0	6.4 4.0 3.6	3.1 2.3 2.0	0.25 0.50 1.00	6.6 4.0 3.0	4.4 3.6 2.7
Shoot dry weight(g)	0.5 1.0 2.0	0.57 0.47 0.22	0.19 0.17 0.05	0.25 0.50 1.00	0.63 0.16 0.07	0.12 0.06 0.02
Root dry weight(g)	0.5 1.0 2.0	0.06 0.03 0.02	0.02 0.01 0.004	0.25 0.50 1.00	0.09 0.03 0.02	0.004 0.003 0.003
Chlorophyll a(mg/g)	0.5 1.0 2.0	5.26 4.21 3.71	3.60 3.40 2.90	0.25 0.50 1.00	5.94 4.08 3.52	3.51 3.11 2.85
Chlorophyll b(mg/g)	0.5 1.0 2.0	3.48 2.61 2.22	2.56 2.11 2.01	0.25 0.50 1.00	4.15 2.63 2.22	2.42 2.30 1.21
Carotenoids(mg/g)	0.5 1.0 2.0	3.05 2.78 2.51	1.90 1.51 1.27	0.25 0.50 1.00	3.27 2.58 2.22	1.70 1.51 1.21
N content(mg/g)	0.5 1.0 2.0	15.3 11.2 6.1	3.0 2.0 1.0	0.25 0.50 1.00	16.4 10.0 5.9	2.8 1.90 0.4
P content(mg/g)	0.5 1.0 2.0	2.1 1.5 0.3	0.4 0.3 0.03	0.25 0.50 1.00	2.4 1.3 0.3	0.4 0.2 0.01
Ca content(mg/g)	0.5 1.0 2.0	13.0 10.26 8.10	4.2 3.01 1.75	0.25 0.50 1.00	14.20 8.43 5.96	3.75 2.60 1.40
K content(mg/g)	0.5 1.0 2.0	1.06 0.90 0.53	0.60 0.31 0.12	0.25 0.50 1.00	1.20 0.61 0.40	0.51 0.20 0.07
Mg content(mg/g)	0.5 1.0 2.0	8.32 6.31 2.25	2.63 1.21 1.00	0.25 0.50 1.00	8.35 4.21 1.93	2.61 1.11 0.86
Na content(mg/g)	0.5 1.0 2.0	1.61 1.22 1.04	1.01 0.82 0.39	0.25 0.50 1.00	1.64 1.00 0.91	1.00 0.68 0.10

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Data in table 3 indicated that using half the recommended levels (lowest concentrations) resulted in detectable decreases in all tested growth parameters, while drastic effects were recorded upon using the recommended levels and their duplicates in *G.clarum* free plants. However, in *G.clarum* colonized plants positive results in growth parameters were estimated at the lowest pesticides levels, but the higher concentrations lead to decreased in growth parameters, and noticeably still lower than that of *G.clarum* free plants. These indicate that mycorrhizal inoculation supported their host plant to survive inhibitory effects of the pesticides. The results revealed that the nematocide at its lower concentration ($0.25 g/m^2$) was more positive for *G.clarum* colonized watermelon growth parameters than do the lower ($0.5 g/m^2$) fungicide level. However, recommended concentration or its duplicate of the nematocide showed drastic effects on growth parameters than those attained with the fungicide recommended level or its duplicate, i.e. the Furadan-10 at its recommended concentration or higher has drastic influence in the tested parameters than d Rizolex-T60wg(Fungicide), under the tested conditions. The plant leaves pigmentation were the least growth parameters that responded to the higher levels of both pesticides, where the decrease not exceeded about 31% in mycorrhizal inoculated plant and not more than 40% for *G.clarum* free plants, at the highest concentrations of both pesticides. These may indicate that the photosynthetic system of watermelon can resist the pesticides stress.

Mycorrhizal inoculation assisted watermelon roots to absorb more nutrient elements still in lower or higher pesticides concentrations, noticeably than *G.clarum* free plants. Generally, this role was most active for absorption of P and N and moderately for Ca and Mg and lower assist for K and Na.

CONCLUSION

In accordance with these findings it was reported that mycorrhiza can survive to pesticide concentrations and enhance plant productivity (Marin *et al*, 2002). Pesticides have negative effect on plant growth and actively of mycorrhizal symbiosis, as well as elements content (Bushan *et al*, 1990; Khaliel and Sohaibani, 1994; Schweiger and Jakobsen, 1998). Pesticides have positive influence in plant growth at low levels, but disturb the symbiotic relationship between VAM and the host plant (Menendez *et al*, 1999). Pesticides lower doses have positive influence in plant pigments of chlorophyll a, b and carotenoids, while higher concentrations have adverse effect (Shilling *et al*, 1994; Shimizu *et al*, 1994).

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3.2. *Glomus clarum* as influenced by the pesticides

The influence of different levels of the fungicide (Rizolex-T-60wg) and nematocide (Furadan-10) on mycorrhizal root infection of watermelon plants and the spores population of *Glomus clarum* in the rhizospheric soil (Table 2), indicated that half of the recommended fungicidal level slightly stimulated mycorrhizal infection of about 6%. However, the nematocide at its lower concentration was accompanied by increased root infection of about 39% and rhizospheric spore numbers of about 59%. While the recommended or doubled levels of both pesticides have negative influences on both mycorrhizal infection and spore population. This drastic effect was more obvious with the nematocide than the fungicide, instead of all the tested nematocide levels were half that of the fungicide. This indicates that Furadan-10 was more toxic to nematode and other soil microorganisms especially fungi including *G.clarum* than the tested fungicide (Rizolex-T-60wg). At its lower concentration (half the recommend) may be lethal to some microorganisms that compete *G.clarum* that resulted in favourable conditions for mycorrhizal infection and sporulation. In this field, it was reported that populations of VAM are affected, not only by fungicides and general biocides, but also by nematocides and insecticides. Sometimes their effects were slight or even positive and may enhance them, but usually they decreased mycorrhizal infections and spore numbers, especially at high doses (Ocampo and Hayman, 1980; Kurle and Pfleger, 1004; Trappe *et al*, 1984; Menendez *et al*, 1999).

Table-2: Effect of different concentrations of the fungicide (Rizolex-T-60WP) and the nematocide (Furadan-10G) on *G.clarum* infection (%) and spore numbers (spore / 100g dry soil) of watermelon, after 6 weeks of growth.

Pesticide concentration (g / m^2 soil)	***G.clarum* infectivity (%)**	**No of Spores /100g dry soil**
Control	46.2	22
Rizolex-T-60wg		
0.75	49.0	22
1.00	29.3	17
2.00	23.5	10
Furadan-10		
0.75	64.1	35
1.12	31.2	14
2.24	17.1	9
L.S.D.	11.02	09.28

2. MATERIALS AND METHODS

Plastic pots (18cm wide and 13cm deep) were filled with 2 kg of autoclaved sandy loam soil passed through a 2-mm sieve and mixed with rock phosphate at the rate of 0.2g per kg^{-1}soil. The soil used contains 87% sand, 7% silt and 6% clay and had a pH of 7.4. The total soluble salts were 1.28 %, organic content of about 2.36%, total nitrogen of 1.05mg, and total phosphorus of 0.061mg per kg.

The pesticides used included Rizolex-T60WP {o-(2,6-dichloro-4-methyl-phenyl) o,o di-methyl-phosoro-thioate (Tolcotos-methyl) and Bis (di-methyl-thio-carbameyl)disulfide or Tetra-methyl-thiuram disulfide (Thiram)} and Furadan-10G (Carbofuran: 2,3-dihydro-2,3-dimethyl-7-benzofuranyl methyl carbamate). Three levels of each pesticide were used: the half recommended rate, the recommended rate and double the recommended rate. They were applied as powders by mixing them with a layer of moistened soil 5cm down each pot only once prior to direct sowing.

Immediately prior to sowing, Pots were inoculated with *Glomus clarum*(300 spores per pot) produced in pot cultures of maize. The mycorrhizal spore suspension was distributed third-way down each inoculated pot. Inoculated controls were maintained without pesticides, while non-inoculated controls received equivalent amount of filtered suspension free of mycorrhizal propagules.

Seeds of watermelon (*Citrullus lanatus*) were kindly provided from the agricultural department, Faculty of Metrology, King Abdulaziz University, Jeddah, Saudi Arabia. Seeds sizes and weights were homogenous and surface sterilized (0.1 % $HgCl_2$ + 0.2 % HCL for 5 min), followed by repeated washes with sterile distilled water (Vincent, 1976). Two seeds were planted in each pot and irrigated twice a week with 60 ml, one of which is containing Hoagland's solution, minus phosphorus (Hoagland and Arnon, 1950 ; Downs and Hellmers, 1975).

Watermelon plants (*Citrullus lanatus*) were grown in a greenhouse with a day / night cycle of 28 / 22° C and illumination period of 13 h a day for eight weeks. At the end of the tested growth period, plants height, dry weight of roots and shoots, plant pigments, mycorrhizal colonization and nutrient content were determined. Roots were carefully washed from adhering soil using tap water. A sample of approximately 0.5g fresh roots from each pot was removed and cut into small segments to estimate the mycorrhizal density, using the grid-line intersect method (Giovannetti and Mosse, 1980), after clearing with 10 % KOH and staining with trypan blue (Phillips and Hayman, 1970). Chlorophyll a,b and carotenoids were estimated spectro-photometrically (Metzner *et al*, 1965), after acetone extraction of the pigments from fresh leaves. Nitrogen content was estimated using Nessler reagent (Delory, 1949; Humphries, 1956). Other nutrient content were estimated by the method of Fernandez *et al* (1985).

Data were tested statically using the one way analysis of variance and the means were separated by the least significant difference, LSD ($P \leq 0.05$ for N= 5).

3. RESULTS AND DISCUSSION

3.1. Response of watermelon plants to *Glomus clarum* infection:

Inoculation of watermelon plants with *Glomus clarum* spores has a positive effect on the tested growth parameters of foliar and root systems dry weights (Table1). The increase in shoot system dry weight was always concomitant with the increase in shoot length(about 43%), but not so with root system which showed only about 6.25%, under *G.clarum* infection. This indicates that *G.clarum* hyphae play an important role in increased water and nutrients absorption by watermelon roots. However, chlorophyll a and b showed small increases(about 11, 8%, respectively) as the plant was *G.clarum* inoculated, while the carotenoids have about 47% increase under the same conditions. These indicated that photo-systems I and II which depends

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