

Evaluation of the Antifungal Effect of Nanoparticles (Magnesium Oxide, Silver) and Chemical (Phylax) on *Fusarium Solani f.sp Cucurbitae*, Pathogenic Agent of Melon

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Abstract – In this study, forty isolates identified as *Fusarium solani* based on morphological characterizes, *F. solani* was one of the most frequently isolated species. Also, *Fusarium solani* pathogenesis over the roots with negative seedling growth and positive with the occurrence of root diseases. In this study, we analyzed the inhibition effect of different NPs (AgNPs, MgNPs, and chemical (Phylax)) against plant pathogenic fungi in vitro. And in vivo In vitro, the results indicated that NPs (AgNPs, MgNPs) and chemical (Phylax) posses the antifungal properties against at various level. The positive control had the lowest height and weight and was significantly different from all other treatments in all of the growth parameters, while Treatment with (AgNPs150 ppm, MgNPs 2%,3% ppm) and 3% Phylax resulted in maximum inhibitions of *F.solani*. In vivo, five characters (heigh plant, shoot and root fresh and dry weight were measured based on the greenhouse, field experimental results. Treatment with (AgNPs, MgNPs) and Phylax had the heighest measured parameters than positive control.

Keywords – *Fusarium Solani f. sp. Cucurbitae Race 1 and 2*, Crown and Root Rot of melon, Antifungal Effect, AgNPs, MgNPs, Phylax.

I. INTRODUCTION

Fusarium solani is one of the important phytopathogenic causing serious losses on cucurbit plant in Iraq, which are responsible for crown and Root rots of melon, all cucurbit crops are susceptible to *Fusarium solani* in the seedling stage. This pathogenic has two race,race1 is pathogenic on root, crown and fruit but race 2 is pathogen only on fruit, Race 1 attacks the hypocotyls, causing a cortical stem rot, it is also attack mature fruit. Race2 of this pathogenic is reported only in limited areas in the world and has less significance. [1]. [2] .Nanotechnology is the new era of technology which deals with matter at atomic or molecular level. It helps to modify biomarkers, imaging, cell labeling, antimicrobial agents and drug delivery for treatment of diseases. [6] [Nanosilver is one of the most thoroughly investigated nanomaterials and owes its popularity to its biocidal properties. [17] Its antimicrobial activity is associated with the characteristic structure of nanoparticles. It exhibits a high antifungal activity. [4] [MgO is an important inorganic oxide and has been widely used in many fields. Many studies have shown that MgO nanoparticles have good antibacterial activity.[10]. The present work entitled," Evaluation of the antifungal effect of

nanoparticles (magnesium oxide,silver) and chemical (Phylax) on *Fusarium solani F.sp cucurbitae*, pathogenic agent of melon)"is attempt to study some of these aspects with the following objectives.

Objectives

1. Testing the efficiency of some nanomaterials (silver, magnesium oxide) and chemical (phylax) inhibition in fungus destroy or reduced toxin in vitro.
2. Evaluate the efficiency of nanoparticles and chemicals for Suppression fungus under plastic-house condition.

II. MATERIALS AND METHODS

Analysis of Effect of Magnesium Oxide Nano particles on the Respective Fungus

Different concentrations of magnesium oxide nanoparticles from PDA media were used for analyzing the antifungal properties. To do so 1%, 2%, and 3% concentrations of magnesium oxide nanoparticles prepared from deionized water The mixing each of them individually to the homogenizer for half an hour to ensure the mixing well then the exposure of the each them to ultrasonic frequency of 22-24 HZ for two minutes to preserve the nanoscale particle size distribution and to ensure homogeneous distribution in the mixture, add all of the concentration separately to the flask 100 ml container of 2.4 g (PDA) and 30 ml of distilled water were added to autoclaved growth medium after its temperature reached approximately 40-50 °C, agar plugs of uniform size (diameter, 8 mm) containing fungi were inoculated simultaneously at the center of each Petri dish containing Magnesium oxides nanoparticles, followed by incubation at 28 ± 2°C for 14 days . Growth inhibition percent was calculated using the radial growth of mycelium according. The acquired results were compared with those of control group to which no nanoparticles have been added. All tests were conducted in three replications.

Silver Nanoparticles

In vitro assay was performed on of growth medium (PDA) treated with different concentrations (50,100,150) of silver nanoparticles, the mixing each of them individually to the homogenizer for half an hour to ensure the mixing well then the exposure of the each them to ultrasonic frequency of 22-24 HZ for two minutes to preserve the nanoscale particle size distribution and to ensure homogeneous distribution in the mixture . Five mL

of AgNPs having different concentrations were poured into growth media prior to plating in a Petri dish (90 × 15 mm). Media containing silver nanoparticles were incubated at room temperature. After 48 hr of incubation, agar plugs of uniform size (diameter, 8 mm) containing fungi were inoculated simultaneously at the center of each Petri dish containing silver nanoparticles, followed by incubation at 28 ± 2°C for 14 days. Growth inhibition percent was calculated using the radial growth of mycelium according to the following equation;

Growth inhibition (%) = $\frac{R-r}{R}$. where R is the radial growth of the fungal mycelia on the control plate and r is the radial growth of fungal mycelia on the plate treated with nanoparticles and chemical.

Characterization of Phylax

Dutch selko company experts to efficiency of phylax in preventing fungal growth and mycotoxin. It's a mixture of organic acids and their salts if it consists of ascorbic acid, formic acid, propanoic acid mono and diglycerides of edible fatty acid, lactic acid, citric ammonia, 1,2,propandiol, materials publisher and water. It is non-toxic extract.

Isolation and Identification of the Pathogen

Fourth samples from infected plants were taken from root and stem rot naturally diseased cucurbit plant during 2015. These samples were obtained from four provinces (Babylon. Baghdad. Najaf, Diwaniyah). Samples were cut into 0.5-1cm and washed under running tap water for 30 minute, then surface sterilized in 1% sodium hypochlorite for 2 minute and cultured on Potato dextrose agar (PDA) supplemented with 200 mg/l Tetracycline and incubated at 25±1C° for 7 days, single spore technique was made for each isolate. Isolates were identified to the species level according to their cultural and morphological characteristic [8] [3]

III. FIELD EXPERIMENT

The effects of Nanosilver, Nano magnesium oxide and phylax were evaluated on melon growth parameters. Pathogen was prepared by growing them on millet grains in 100 ml flask as follow, the grain were soaked overnight in distilled water, autoclaved twice (121 c for 45 min. and then inoculated with agar discs of 5 days-old fungal culture . flask were incubated at 28 c in dark for 21 days, the fungal inocula were then mixed with soil .

Surface sterilization was accomplished by immersing the seeds in 5% sodium hypochlorite solution for 5 min and followed by washing by sterile distilled water, all treatment (nano- silver, nano- magnesium oxide, phylax) was accomplished by soaking in with different concentrations for 2 h .There were thirteen groups of treatments with three replications for each treatment. Treatments included; 1- control 2- silver without 3- silver with pathogen 4- magnesium oxide nanoparticles without

5- magnesium oxide nanoparticles with pathogen 6- Phylax without 7- Phylax with pathogen 8-nanosilver + magnesium oxide with pathogen 9- nanosilver + magnesium oxide without pathogen 10- nanosilver +Phylax with 11- nanosilver +Phylax without 12- nanosilver + magnesium oxide + Phylax with pathogen 13- nanosilver + magnesium oxide + Phylax without

Plant Growth Parameters

Plant Height (H)

The height of the plant was measured from the base of the hypocotyls to the seedling's apex. Ruler is used to measure the plant height of each seedling with unit of centimeter (cm).

Fresh and Dry Weight of the Plant

Scale is used to measure fresh and dry weight of the plant: shoot (SFW) and root (RFW) fresh weight plus shoot (SDW) and root (RDW) dry weight. The samples needed to put in

Oven at 70±1 °C for 72 hours in order to take the dry weight of seedlings

Growth Measures

The growth measures (plant height, fresh and dry weight of plants and roots) were assessed following the same methods described in the previous section. The plant and root parameters were recorded separately after 45 days of the experiment, where the plants were uprooted and washed under running tap water.

Disease Severities (DS)

Symptom severity of the areal parts of the plants was assessed (8, 10, 12, 14 days after pathogen inoculation) using the following index 0, no symptoms; 1, yellowing of the cotyledons or the first leaf; 2, yellowing of two leaves; 3, yellowing of three or more leaves; 4, died plant. The disease severity index was calculated using the formula (Soriano- Martin *et al.*, 2006): Disease severity index (%) = $\sum \frac{ni si}{4n} \times 100$

4N Where ni is the number of plants affected by each degree of severity, si the degree of severity of the attack (0 – 4) and N the total number of plants used for each energy level applied.

IV. RESULTS

Growth of the Fungal Colony

The results showed that *Fusarium solani* was predominant in all simple with 100% frequency. *Fusarium solani* forms white to cream color mycelium on the PDA. Microscopic examination was showed three type of spores, macroconidia were spindle to cylindrical shape containing between three to five septa with distinctly or barely notched basal cells, microconidia formed on false head on long monophilids, growth laterally on the aerial mycelium. Chlamydo spores terminal and intercalary single or in chains.



Fig. 1. Cultural characteristics of *F. solani* the causal agent of the crown and root rot disease of melon. (A) Growth of the fungal colony on the PDA

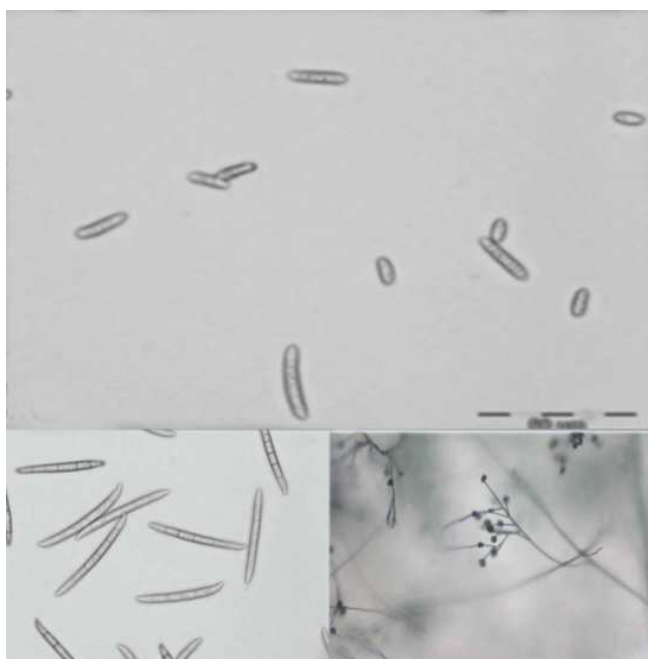


Fig. 2. (B) microscopic characteristics of *F. solani* the causal agent of the crown and root rot disease of melon on carnation leaf- piece agar medium (1) Microconidia (2) Macroconidia (3) Long monophialides conidiospher

Pathogenicity Test

All isolates of the *Fusarium solani* were tested for their pathogenicity on apparently healthy and uniform 7 days-old seedling melon in the greenhouse. Roots and stems of the melon seedling were washed in running tap water before inoculation. Conidial suspension of each individual isolate was prepared by pouring sterile distilled water and gently scraping the conidia of 7 days-old cultures on PDA plates grown under the standard grown condition. The concentration of the pooled suspension was adjusted to $2 \cdot 10^6$ conidia/ml by using a haemocytometer.

The roots of seedling were soaking in 20 ml conidial suspension for 20 min for root inoculation technique for stem inoculation technique, 20 ml of the conidial suspension of each *Fusarium solani* was sprayed on the stems. The control plants were inoculated by both techniques with ml of sterile distilled water. Three replicates were performed for each isolate and the experiment was repeated twice. Results of the pathogenicity test showed that all isolates of *Fusarium solani* were highly pathogenic to melon plants. The first

wilting occurred 7 days after inoculation in melon plants. 100% percentage of melon plants inoculated with the forty isolates died, inoculated plants exhibited a cortical rot at the base of the stem and upper part of the root system caused discoloration and necrosis then brown rot of the stems. While all uninoculated plants remained asymptomatic, the pathogen was recovered from symptomatic plants, fulfilling Koch's postulates.

In Vitro Antifungal Assays

Results presented in this study confirm that nanoparticles have significant inhibitory effects and antifungal activity on colony formation from mycelia of *Fusarium solani* in vitro. The growth rate of strain in the presence of the tested nanoparticles and other compound chemical are summarized in Table

NO.	Treatment	Concentration	Growth fungi	Growth inhibition %
1	Silvernanoparticle without AgNPs		9.0	-
2	AgNPs	50%	1.0	88.9
3	AgNPs	100%	0.18	98
4	AgNPs	150%	0.0	100

5	MgoNPs	1%	0.719	92.1
6	MgoNPs	2%	0.0	100
7	MgoNPs	3%	0.0	100
8	MgoNPs without		9.0	-
9	phylax	0.1	1.7	81.1
10	phylax	0.2	0.75	91.66
11	Phylax	0.3	0.0	100
12	Phylax without		9.0	-

There were significant differences between different concentrations of nanoparticles on growth inhibition of *Fusarium solani*, while there was no growth inhibition in the negative control. The higher inhibition of fungal growth was recorded at a concentration of 150% ppm from silver nanoparticle was observed on PDA medium. Concentration of 10 0%, 50% ppm inhibition the fungal growth by 98% and 88.9% respectively.

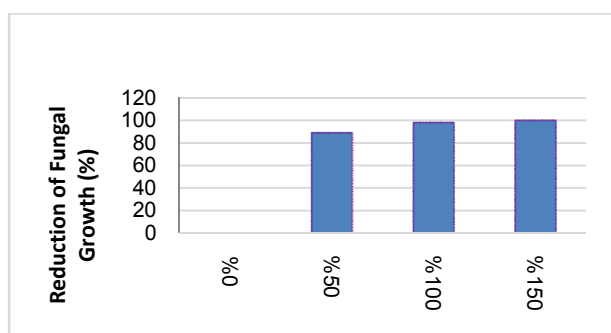


Fig. 2. AgNps concentration (ppm)

The different concentration of nanoparticles of Mgo caused inhibition the fungal growth, higher inhibition at 3%, 2% by 100% meanwhile at concentration of 1% by 92.1%.

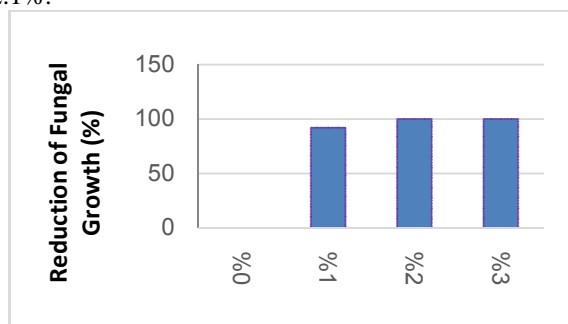


Fig. 3. MgoNps concentration (ppm)

The higher inhibition of fungal growth was recorded at a concentration of 0.3 from PHYLAX by 100% and, 91.66%, 81.1% with a concentration 0.2, 0.1.

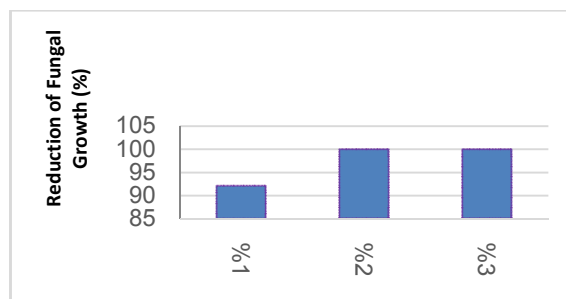


Fig. 4. Phylax concentration.

The results in the current study are confirmed by the finding of [6] reported that the synthesized nanoparticles can significantly inhibit (87.1%, 86.5% and 83.5%) to growth of phytopathogens *Colletotricum coccodes*. [20] reported that the controlling effect increases in the administered dosage of nanoparticle and there exists a direct correlation between the administered dosage and controlling effect, the concentration of 2% MgoNps had the greatest effect in both liquid and solid media [18], reported that the when roots were drenched with MgoNps suspension prior to inoculation with pathogen, the incidence of disease was significantly reduced. Also, these results agree with selko Dutch company research regarding item phylax inhibitory immune substance for the growth of many fungi. The results are close conformity with finding of [4] reported that the inhibitory effect of nanoparticle may be due to the directly attach to and penetrate the cell membrane of the spore and killed them. Also [19] reported that antimicrobial activity of AgNps on microorganism was depended on the concentration of AgNps and was closely associated with the formation of pits in the cell wall of the microorganism than Ag accumulated in the membrane caused the permeability, resulting in the cell death. Inhibition increased at the concentration of AgNps increased. This could be due to high density at which the solution was able to saturate and cohere to fungal hypha and deactivate plant pathogenic fungi.

In Field Experiment

Plant Height

There were significant differences between thirteen studied treatments on plant height. The highest shoots were obtained for the plant grown in T13(87 cm), T9(75) Followed by T11(68), T12(67). The lowest plant height was given for these plant grown in T1(22 cm), T3(), T5(29). No significant difference of plant height were observed between T3, T5 observed between T3, T5.

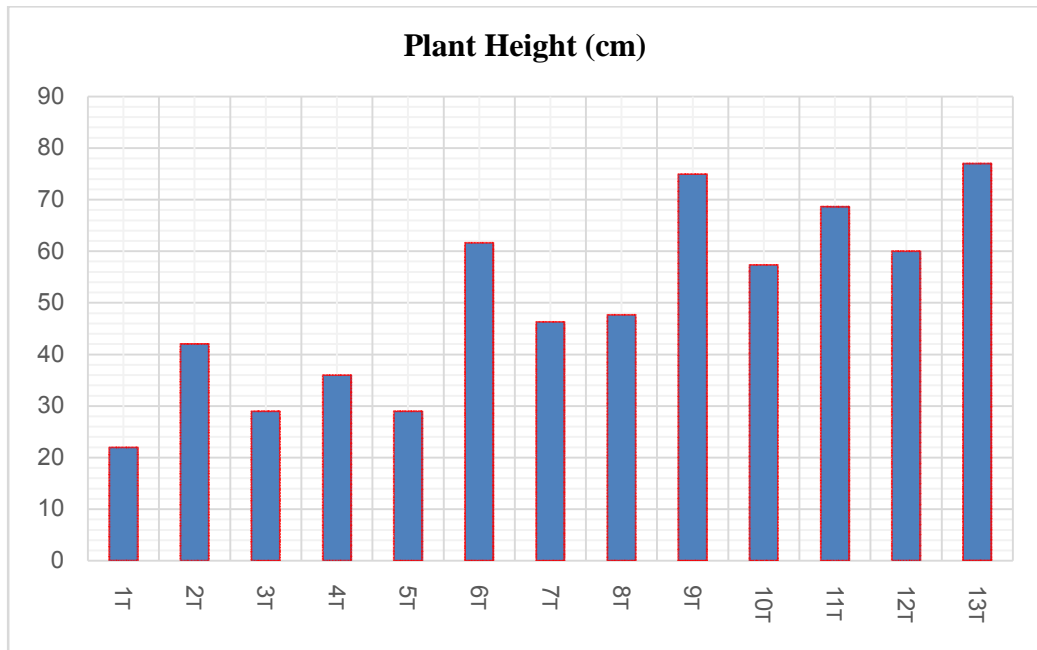


Fig. 5. Plant height of melon treated with thirteen treatment.

Fresh and Dry Shoot Weight

The results showed that the effect of different type of nanoparticles was significant on fresh and dry shoot. The highest fresh shoot and dry weight were obtained for the plant grown in T13 (6.403 g), (1.206 g). The lowest shoot and dry weight was given for these plant grown in T1,T3, T5. No significant difference of shoot and dry weight were

observed between T3,T5. The data obtained are in agreement with the previously published results by Razzaq at el 2015 reported that all level of silver nanoparticles (25, 50, 100, 150 ppm) applied to soil in pots significantly enhanced fresh weight, dry weight and chlorophyll content over control.

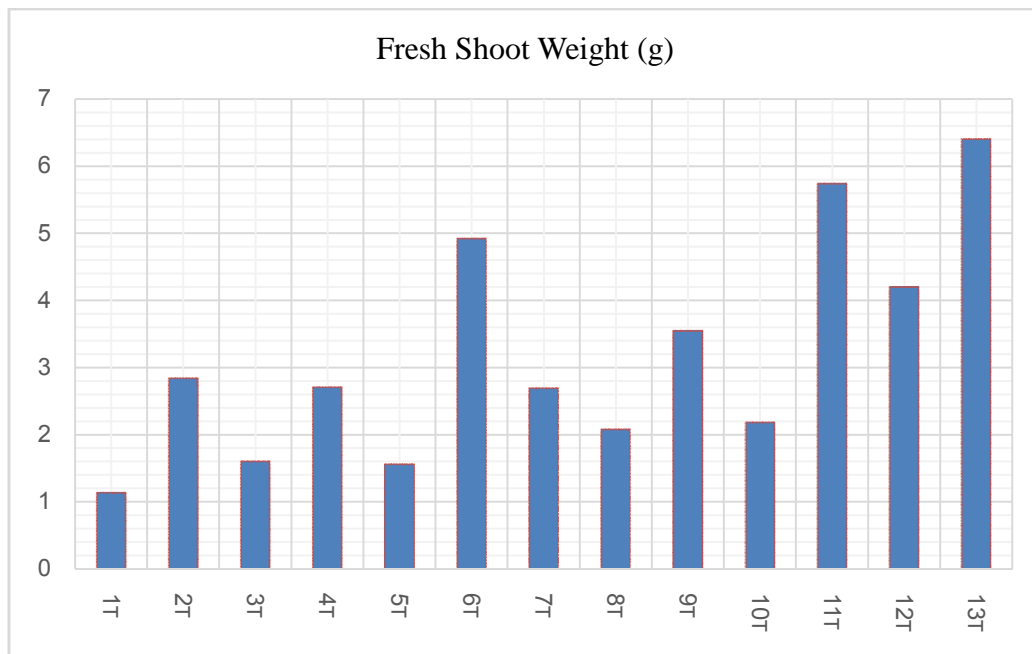


Fig. 6. Fresh shoot weight

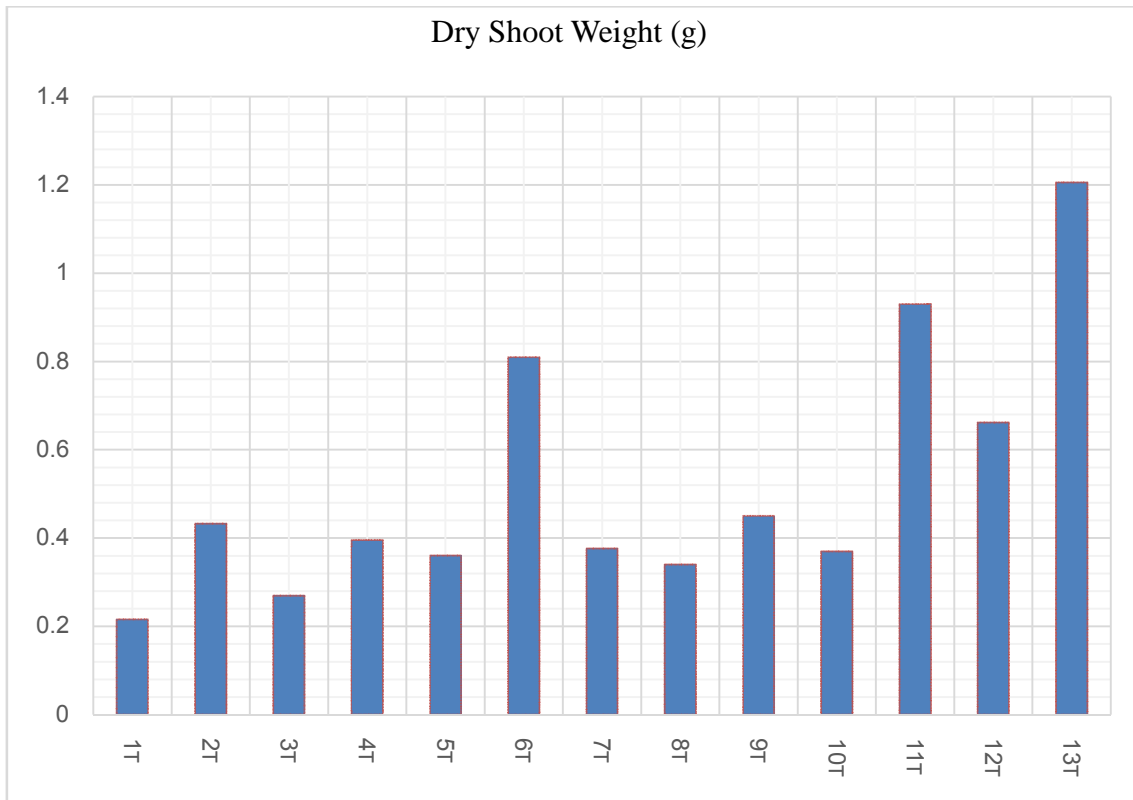


Fig. 7. Shoot dry weight of melon treated with thirteen treatment.

Fresh and Dry Root Weight

Results showed that fresh and dry weight of melon seedling root significantly. according to root fresh weight, treatment were grouped with significant differences in groups, T13,T11 had the highest weight (2.65 g), (1.09 g).

positive control T1 and T3 had lowest weight. Root dry weight, treatment T13,T11 had highest weight (1.206 g), (0.93 g), while treatment T1, T3,T5 had lowest weight

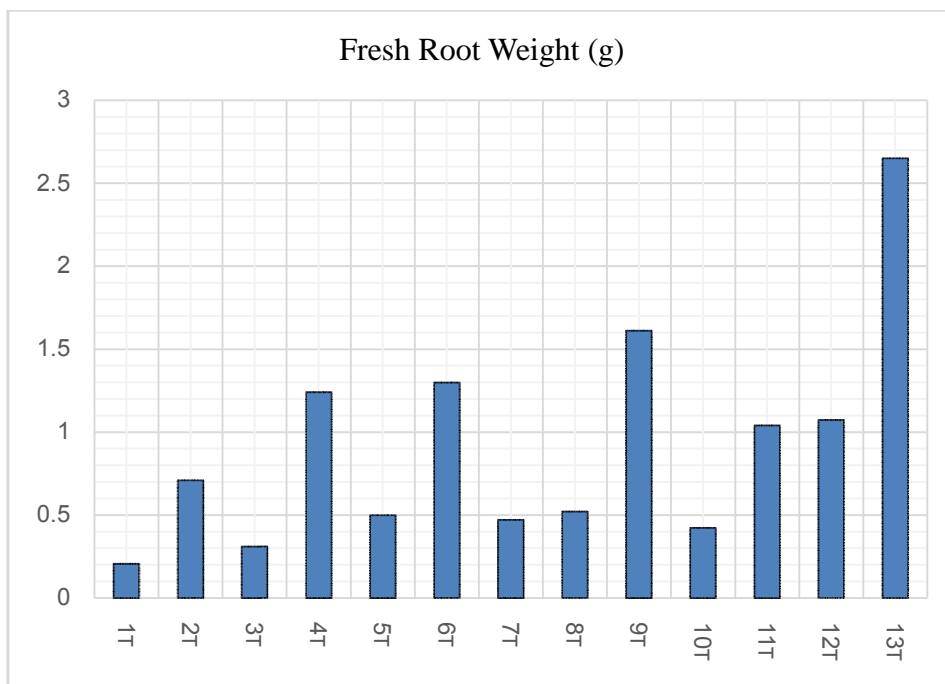


Fig. 8. Fresh root weight (g)

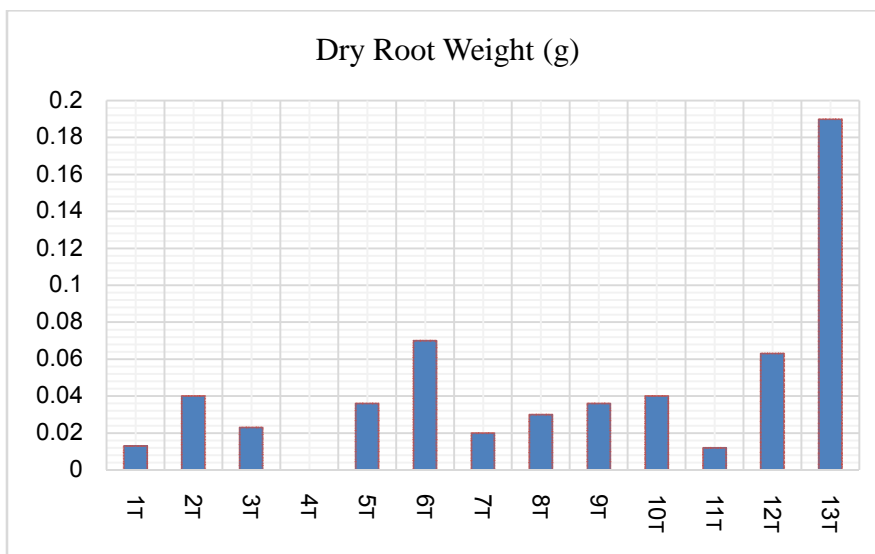


Fig. 9. Dry Root Weight (g)

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