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Macrophomina phaseolina (Tassi) Goid Culture Filtrates is a Lucrative Source for Weed Control

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Abstract. The mycoherbicides have been recognized as an effective approach for weed control as being eco-friendly, low cost, and of long-lasting. This research was carried out to evaluate the herbicidal effect of *Macrophomina phaseolina*, the causal of sesame charcoal rot. The fungal culture was extracted with water and the extract toxicity was evaluated by seed germination, detached leaf, and whole plant bioassays on several weed species of Iraq (*Cynanchum actum*, *Malva parviflora*, *Sorghum halepense*, *Lolium rgidum*, and *Silybum marianum*). The results revealed that the extract of the fungal culture was significantly inhibited the germination of the examined weeds seeds by more than 86.7 %. The detached leaf bioassay also indicated that the culture extract was of potent toxic effect to all examined weed species, regardless their types (broad or narrow and mono or dicotyledonous). The phytotoxicity symptoms appeared on the detached leaves were as tissue and chlorophyll disintegration, yellowing, necrosis, and death. The same toxic symptoms were also recorded on the whole plant bioassay in vivo but the weed species of the narrow leaves however, showed significantly much less effect. The phytotoxicity on the examined weed species ranged from 66 to 83%. Moreover, the fungal culture extract was also found to be of potent toxic effect to milk thistle (*Silybum marianum*) despite the application method as foliar spraying or root immersing with 100% and 20% of the extract respectively. The results of this research showed for the first time the effect of *M. phaseolina* culture filtrates as a potent herbicide potential on different weed species.

1. Introduction

Weeds are an ever-present critical challenge to the agribusiness and the globe. The weeds cause potential economic yearly losses in agriculture that reach up to 10% of the world production. The weeds impose costs on agriculture production by reducing the crop quantities and qualities or increasing its production costs [1]; [2]. Hence, having effective weed control programs in agriculture is very important to reduce losses in production and assure food security. Several methods for weed control have been recommended and the chemical herbicides alone account for almost 50% of the agrochemical markets [3].

Herbicides usage has been criticized due to their usage restrictions as environmental hazards, affect non-target organisms, and induce herbicide resistance [4]. These negative consequences of the synthetic herbicides ensure the necessity of a viable alternative for weed control. Weeds biologically controlled by using insects, fungi, bacteria, or their metabolites are extensively studied and the effective microorganisms become a vital part of weeds control [5]; [6]; [7]; [8]; [9]. Mycoherbicide's strategy has been recognized as effective weed management as being eco-friendly, relatively low cost, and long-

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lasting. Many plant pathogens were proved to be of herbicidal properties, such specie of *Alternaria*, *Fusarium*, *Derechslera*, *Trichoderma*, *Aspergillus*, *Colletotrichum* [2]; [3]; [5]; [7]; [8]; [9]. The herbicidal efficacy of fungi, however, is affected by the environment. Therefore, to overcome this constrain the toxic fungal metabolites have been suggested to be used as herbicides instead of the living fungi [2]; [10]; [11]; [12] [13]; [14]; [15].

The charcoal rot pathogen (*Macrophomina phaseolina*) of sesame has been reported to attack a wide host range. It causes a plant disease also named summer wilt, or black rot [16]; [17]. Moreover, the *Macrophomina phaseolina* was reported to elaborate several phototoxic metabolites named aspertin, isoaspertin, phomalacton, phaseolinic acid, phonenon, phaseolinone. In addition to (-) - botryodiphodin, and phaseolinone [16]; [18]; [19]. No previous attempts have been mentioned to evaluate the herbicidal potentiality of *M. phaseolina* metabolites for weeds control. The present study was therefore conducted to explore and high light the effect of *M. phaseolina* culture extract as a bio-herbicide.

2. Materials and Methods

2.1. Isolation of Fungus

The fungus used in this research was isolated by aseptically culturing of little bits of surface-sterilized sesame plant tissue of charcoal rot symptoms on potato dextrose agar (PDA) culture. The plates were incubated at 25± 2. º C. The associated fungus was affirmed morphologically as *M. phaseolina* (5, 8). The fungus was purified and sub cultured into PDA slants and kept in the refrigerator at 4ºC for further use.

2.2. Preparation of aqueous extract of M. phaseolina culture filtrates (AEMCF)

The AEMCF was prepared according to the technique described by [4]. Potato sucrose broth PSB was prepared (200 gram of sliced potato tuber and 20-gram sucrose/liter distilled water) and distributed equally into Erlenmeyer flasks (100 ml per 250ml flask) and autoclaved at 121 °C and 15 psi for 15 minutes, cooled and each flask aseptically inoculated with three discs of a 5mm taken from the *M. phaseolina* culture margin on PDA. The flasks were incubated at 25 ±2 º C for 21 days with daily handshaking. The cultures of each three flasks were blended by electric blender and aseptically filtrated through the Whatman No.1 under section pressure. The extract filtrates about 70% of the total used broth media were divided into three portions and treated as follows:

1. The first portion was filtrated aseptically through 0.45 µm Millipore membrane under suction pressure condition.
2. The second portion was autoclaved for 10 minutes (121 º C and 15psi).
3. The third portion was autoclaved for 20 minutes (121 º C and 15psi).

To check for the viability of the fungal cells in the extracts of the above treatments a sample of each portion (0.25 ml) was aseptically cultured on the surface of the PDA plate and incubated for a week at 25 ±2 º C.

Each portion was evaluated for its toxicity on the sesame detached leaf bioassay as previously described (23). Given the bioassay results, the first and second portions showed relatively the highest toxicity. Hence the *M. phaseolina* culture cell-free filtrates were prepared by a similar method of the second portion for every further investigation to assess the effect of the AEMCF as herbicide. The effect of the AEMCF was assessed according to the following bioassays:

2.2.1. Seed germination. The seed germination bioassay was carried out according to the procedure described before [20]. Seeds of Mallow, Bindweed, and Johnson grass were washed separately and surface sterilized by soaking in a 5 % bleach solution (17% NaOCl) for three minutes and washed thoroughly with sterile water. Enough surface-sterilized seeds of each weed were taken aseptically and divided into two portions. One portion was soaked overnight in sterilized water and the other was soaked in the AEMCF. Five seeds of each soaked weed were placed on a circle at the age of pre-wetted sterilized

filter paper on the Petri plat replicated three times. The other three replicates of the sterile water-soaked weed seeds (5 seeds per plat of each weed) were served as control. Plats were labelled and randomly distributed according to completely randomized design (CRD) and incubated in the growth room at 25±2 ºC and 10 hours daily light for 10 days. The germination % was calculated by the following formula:

$$\text{Seed germination (\%)} = \frac{\text{No. of germinated Seed}}{\text{15 (total No. of the examined seeds)}} \times 100$$

2.2.2. Detached leaf (DL) bioassay. The detached leaf (DL) procedure described by Sharma et al. (24) was adopted. Leaves of relatively the same size and age of each of the examined weeds (Mallow, Bindweed, and Johnson grass) were washed and surface sterilized by soaking in a 5 % bleach solution (17% NaOCl) for three minutes and washed thoroughly with sterile water. Three leaves of each weed were soaked for five minutes in the AEMCF and another three were soaked in sterile water served as control. Each leaf was placed on the sterilized Petri plate, the plates were labelled and randomly distributed according to CRD and incubated in the growth chamber at 25± 2 ºC and daily 10 hours light for 5 days. The results were recorded by measuring the phytotoxic damaged leaf tissue according to the formula reported before [6].

$$\text{Damaged leaf area (\%)} = \frac{\text{Width of damaged area}}{\text{Length of damaged area}} \times \frac{\text{Width of total leaf area}}{\text{Length of total leaf area}} \times 100$$

2.3. Foliar Spray

The herbicide effect of the AEMCF was examined on the whole plants as follows:

2.3.1. In vitro. Young plants of 4-5 leaves of the relatively same size of milk thistle (Silybum marianum) were gently taken off with their whole root system as much as it could be from the field and washed thoroughly with tap water. The roots of each plant were immersed in 50 ml tap water in a 100ml glass vial. The vials with the plants were incubated in a growth room at 25 ± 2 º C and daily hours light for 72 hours before they were used. The incubated milk thistle plants were treated as follows:

2.3.2. Experiment 1. The foliar of three replicated plants were thoroughly sprayed with 100 % the AEMCF. Alternatively, the foliar of the other three replicated plants were thoroughly sprayed with sterile water served as control.

2.3.3. Experiment 2. The root system of the other three replicated plants was immersed in 20 % AEMCF. Alternatively, the root system of the other three replicated plants was immersed in sterile water served as control.

The vials with plants of various treatments were randomly distributed in the growth room according to the CRD at 25º ± 2 º C and daily light for 10 hours and the results were collected after 10 days. The herbicide potential of the fungal extract was recorded as the percentage of tissue damaged according to the procedure described before [21].

2.3.4. In vivo

2.3.5. Experiment 1. Four different species of weeds (Mallow, Bindweed, Johnsongrass, and Bluegrass) of three high-density patches were selected in this experiment after all other weeds were hand removed from each patch. The three batches of each weed plant were gently sprayed with the AEMCF. The herbicidal potential of the fungal extract was recorded as a percentage of the damage per plant according to the procedure described before [21].

2.3.6. Experiment 2. Weed plants of the mallow, bindweed, Johnson grass were grown from seeds and transplanted seedling of milk thistle were maintained in sterile field soil in plastic pots inside the plastic house. Three replicates of each weed species were sprayed thoroughly with % 100 of the AEMCF and the other three were left without treatment served as control after four weeks from the sowing of mallow, Bindweed, Johnson grass or transplanted of the milk thistle. The pots were distributed in the plastic house

according to CRD. The herbicide potential of the fungal extract was recorded as the percentage of tissue damaged following the procedure described before [21].

2.4. Statistical Analyses

This research was carried out at the laboratory and field of the Department of Plant Protection, College of Agriculture University of Anbar statistically. All data from laboratory and field bioassays were analyzed using analysis of variance and the LSD test to differentiate the treatment means at the 5% level of significance.

3. Results and Discussion

The fungus associated with the sesame infected plant was identified as *M. phaseolina* according to its morphology as previously described in several research papers [21]; [22]; [23]. The fungus showed growth of dens black mycelium mat after 21 days of incubation in the potato sucrose broth PSB. The sterilized extracted culture filtrates by filtration and autoclaving showed no growth on PDA after 10 days of incubation. This indicated that both sterilization methods completely got rid of any fungal propagules in the final filtrates. The preliminary examination for the phytotoxicity of the culture filtrates on detached sesame leaves revealed that the highest toxicity, as indicated by fast and clear chlorosis and necrosis of the sesame leaf tissue was that of the filtrating through the Millipore membrane and 10 minutes autoclaving.

The culture autoclaved for 20 minutes showed relatively low and slow toxicity. Accordingly, it could be concluded that the phototoxic constituents of the culture filtrates of the *M. phaseolina* were water-soluble and relatively heat stable and it most likely was not of a protein entity (enzymatic).

The results revealed that the AEMCF was highly inhibited the seed germination of examining weeds. The filtrates caused more than 86.7 % inhibition of all the examined weed seed species with highly significant differences in the germination of the treated and non-treated seeds (Figure 1) (Table 1).

Table (1) The effect of the aqueous extract of *Macrophomina phaseolina* culture on seed germination of various weeds *In vitro*.

Weed Species	% Seed Germination	
	Control (non-treated)	Treated
Bindweed Convolvulus arvensis	100	6.7
Mallow Malva neglecta	100	3.3
Johnsongrass Sorghum halepense	93.3	13.3
LSD α at 5%	8.39	

Notes: Results taken after ten days and each value is an average of three replicates (five seeds per each replicate).

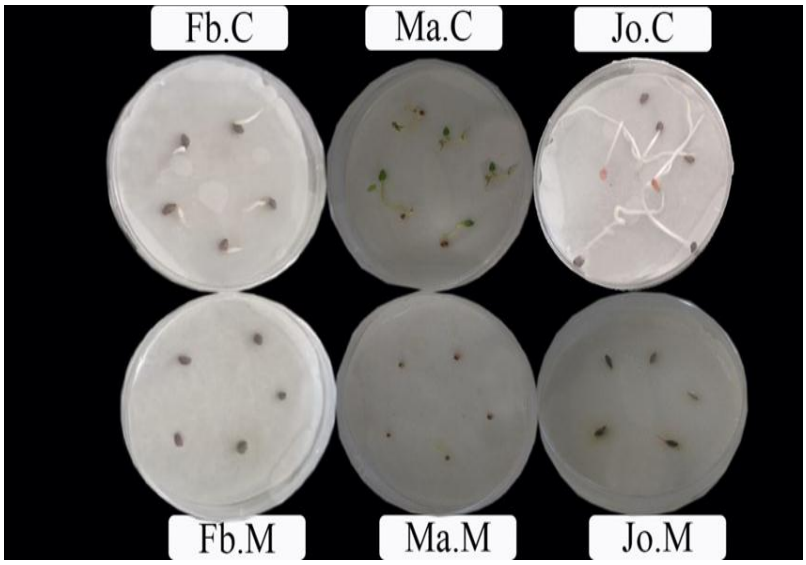


Figure (1) The effect of the aqueous extract of *Macrophomina phaseolina* culture filtrates on seed germination of various weeds. (C = Control without treatment, M= Treatment with culture filtrates, Fb= Field big weed, Ma= Mallow, and Jo= Johnsongrass).

These results agreed with the results of several other types of research on the effect of fungal metabolites on weeds seed germination in vitro [9]; [15]. The detached leaf bioassay also indicated a potential toxic effect of the AEMCF on both broad or narrow weed leaf types or that of mono and dicotyledonous (that included mallow, bindweeds, Johnsongrass, and bluegrass). The phytotoxicity of the AEMCF appeared as tissue and chlorophyll disintegration, yellowing, necrosis, and death on all examined weed species (Figure 2). The phytotoxicity damage of the AEMCF was ranged between 75 % on Johnsongrass to 95.5% on Mallow (Table 2). These findings supported the previous research as many fungi filtrates of toxic effects on detached leaf bioassay [4];[21].

Table (2) Toxicity of the AEMCF on various weeds with different bioassay techniques

Weed Species	Detached Leaf Bioassay Technique (in-vitro)		Foliar Spray Technique (in vivo)	
	%Phytotoxic Damage		%Phytotoxic Damage	
	Control	Treated	Control	Treated
Filed Bindweed *Convolvulus arvensis*	3.3	86.7	4.0	81.6
Bluegrass *Poa pratensis*	1.7	83.3	-*	*
Johnsongrass *Sorghum halepense*	0.0	75.0	3.3	66.6
Mallow *Malva neglecta*	0.0	95.5	0.0	83.3
Milk Thistle *Silybum marianum*	-*	-*	1.7	78.3
LSD α at 5%	14.69		12.42	

Notes: Results taken after ten days and each value is an average of three replicates.
* Not tested.

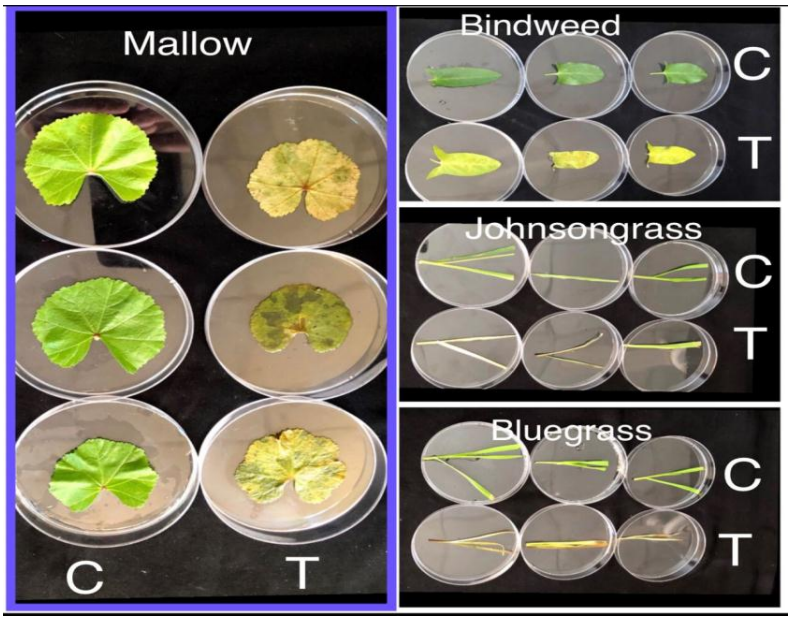


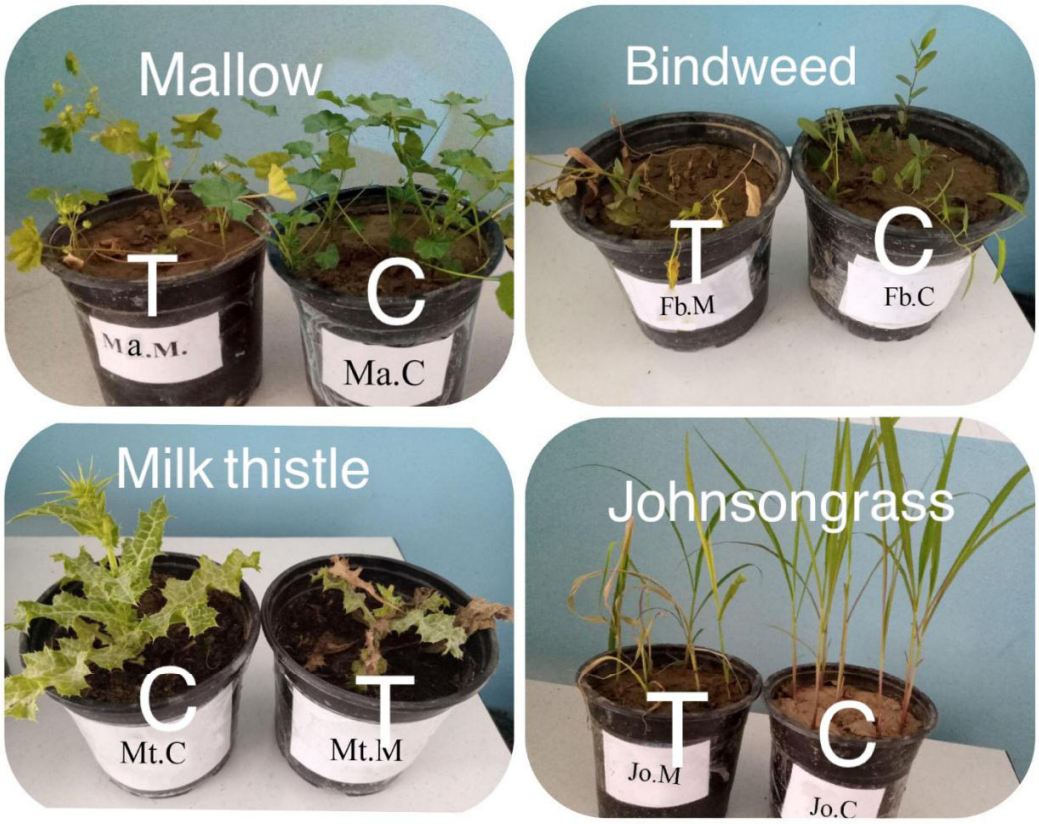
Figure (2) Toxicity of the AEMC on detached leaf bioassay of various weeds (C = Control without treatment, M= Treatment with culture filtrates).

Similar phytotoxic symptoms of the AEMCF were also shown on the whole plant bioassay in vivo at the plastic house (Table 2) and the field as well (Table 3) on the Milk Thistle, Mallow, Bindweeds, Johnsongrass, and Bluegrass. The phytotoxic symptoms also appeared as chlorosis, necrosis, withering, and drying (Figures 4 and 5). The highest phytotoxicity was recorded on Mallow (83- 87% damaged in open field and green house respectively) followed by Bindweed (81- 75 % damaged in open field and green house respectively) and Milk Thistle (78 % damaged in green house). On contrary to the results of the detached leaves and that of the plastic house the weed species of narrow leaves showed significantly no response to the fungal filtrates on the field. This contradiction on the results most likely to be attributed to the weather conditions or the nature of the narrow leaves.

Table (3) Phytotoxicity of the AEMCF on various weeds as foliar spray technique in vivo

Weed Species	Foliar Spray Technique (in vivo)	
	%Phytotoxic Damage	
	Control	Treated
Filed Bindweed *Convolvulus arvensis*	0.0	75.0
Bluegrass *Poa pratensis*	0.0	6.0
Johnsongrass *Sorghum halepense*	0.0	5.0
Mallow *Malva neglecta*	0.0	87.0
LSD α at 5%	14.87	

Notes: Results taken af



ter ten days

and each value is an average of three replicates

Figure (3). Phytotoxicity of the AEMCF as foliar spray on various weeds grown in the plastic house (C = Control without treatment, M= Treatment with culture filtrates).

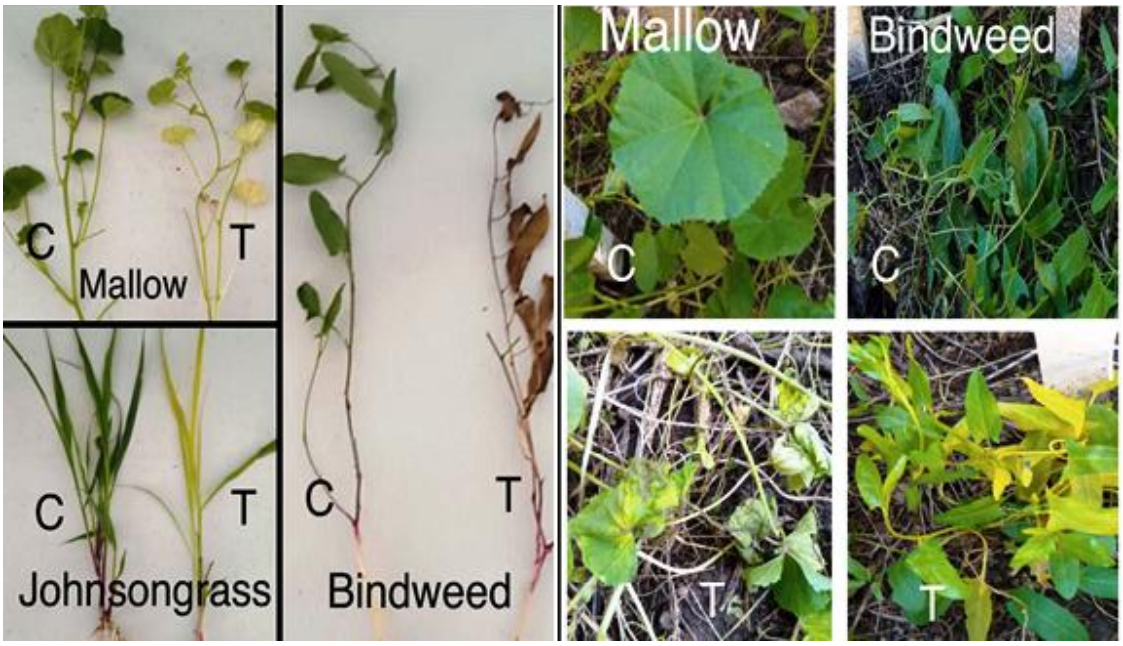


Figure (4) Phytotoxicity of the AEMCF on various weeds as a foliar spray in the field (C = Control without treatment, M= Treatment with culture filtrates).

Besides, the results of the bioassay of the AEMCF on the Milk Thistle plants (in vitro) clearly showed positive toxicity results despite the application methods as foliar sprayed or root immersed of 100 and 20% culture filtrates respectively. The toxic symptoms also appeared as the withering of the leaves, with chlorosis, necrosis, and drying (Table 4) (Figure 5).

Table (4) Effect of the AEMCF as foliar Spray and root immersed on Milk Thistle plants in vitro

Treatment	% Aqueous Extract Concentration	% Phototoxic Damage
Foliar spray	0.0	3.3
	100	88.3
Root immersion	0.0	0.0
	20	86.7
LSD α at 5%	13.3	

Notes: Results taken after seven days and each value is an average of three replicates

The results of this experiment indicated that the toxicity of the AEMCF was of contact and systemic mechanism effect.

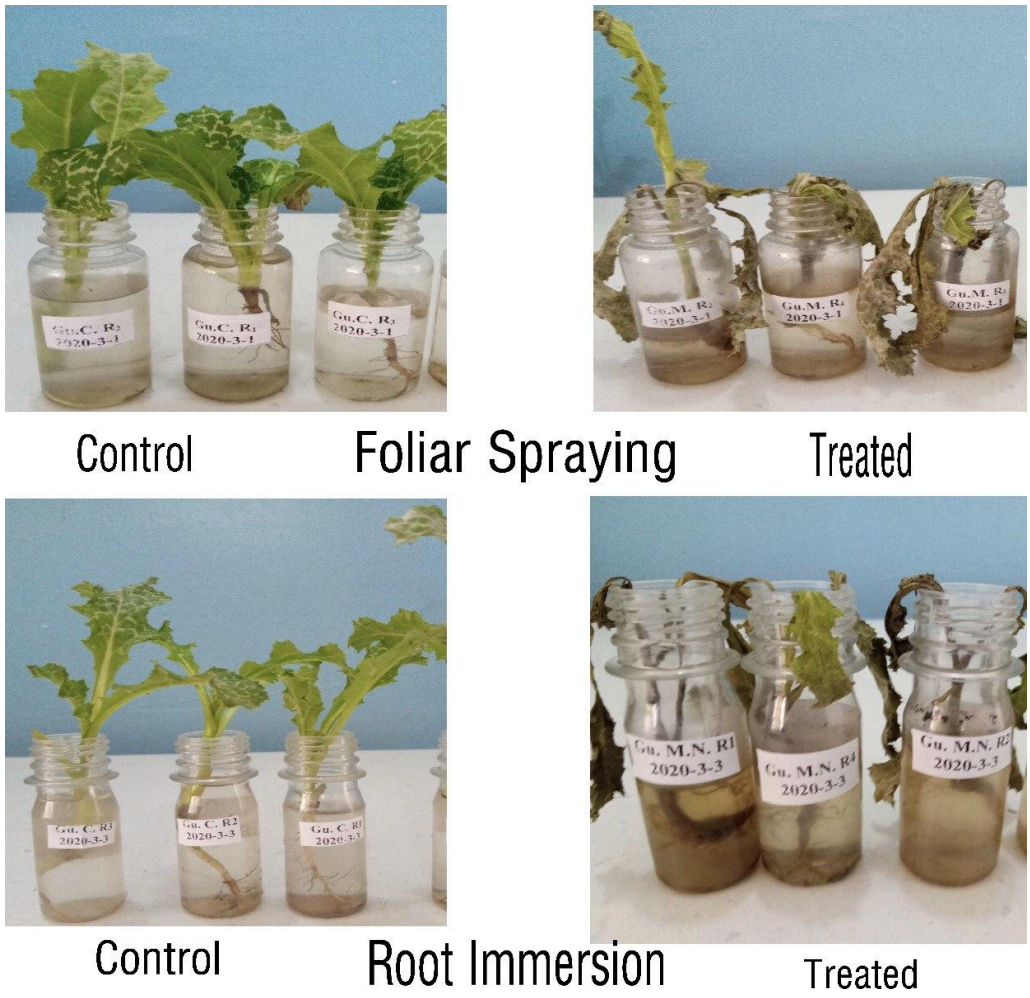


Figure (5) Phytotoxicity of the AEMCF as foliar spray and root immersion on Milk Thistle *in vitro*.

The results of all the bioassays in this research revealed for the first time a potent herbicidal effect of *M. phaseolina* culture filtrates against several winter weeds that widely spread in the middle of Iraq. Further researches are still needed to examine the effect of the AEMCF on the summer weeds as well. The toxic constituents of the culture filtrates against weeds were water-soluble, heat-stable, and relatively highly produced at an effective concentration. In this research, the herbicidal potential of *M. phaseolina* culture filtrates is highlighted for the first time and supports the previously reported findings that fungi of an attractive proposition for controlling weeds biologically [3]; [6]; [7]; [8]; [9]; [24]. The large application scale of the culture filtrates of *M. phaseolina* however, needs to be further studied to identify the active constituent (s) that may be used as analogs for the synthesis herbicide (s) as a natural product for weed management.

4. Conclusions

The current findings revealed for the first time a powerful herbicide potential of *Macrophomina phaseolina* culture filtrate on various weed species. The toxic constituents of the culture filtrates against weeds were water-soluble, heat-stable, and produced at evidently effected concentration.

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