

Using of Magnetic Water Technology for the Management of Brown Rot Disease of Potato

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ABSTRACT

This study aims at the investigation of using magnetic water (MW) to manage brown rot (bacterial wilt) disease of potato. The effect of magnetic field (MF) on the viability of *Ralstonia solanacearum* (the bacterial causal pathogen of this disease) was investigated by passing the suspension of *R. solanacearum* through a magnetic liquid modifier tube. The viable count of the bacterial pathogen increased with the increasing of the incubation time (1, 2, 3 and 4 h) as well as with increasing the number of passes through the magnetic tube. In the pots experiment (carried out at Tag El-Ezz Agricultural Research Station, Dakahlia Governorate, Egypt - during the period of 17 February to 12 May 2016), the irrigation with MW led to significant increases in total phenols, polyphenol oxidase, peroxidase, total chlorophylls, chlorophyll a chlorophyll b, and carotenoids. Significant increase in plant height after 50 and 70 days of planting and decrease in disease rating were noticed. The viable count of *R. solanacearum* in rhizosphere of the plant irrigated with MW was significantly decreased after 70 and 108 days of planting when compared with the plants irrigated with tap water (TW). The irrigation with MW led to significant increase in potato yield (25.64 %) and a significant decrease in the percentage of infected tubers (40.22 %) in comparing with the plants irrigated with TW. These studies encourage more studies for the use of MW to manage brown rot disease of potato as well as other plant diseases.

Keywords: *Ralstonia solanacearum*, rhizosphere, yield, infected tubers, magnetic field.

INTRODUCTION

Potato is one of the most vital crops in the world, it is the fourth consumed food after wheat, maize and rice (Mahgoub *et al.*, 2015). In 2016, the total cultivated area of potato in the world was 19,246,462 hectares (47,559,068.63 feddans) producing 376,826,967 tons of tubers with an average of 7.92 tones/feddan. Compared with in Egypt, potato is an important crop in Egypt for local consumption and exportation with an average yield of 11.025 tons/feddan (FAO, 2016).

Brown rot disease of potato is a very important disease that threatens potato plantation in the world. The causal bacterial pathogen; *Ralstonia solanacearum* has a wide host range. It infects more than 450 plant species belonging to 54 families. It has high persistence. There is no available resistant of crop varieties (Patil *et al.*, 2017).

The earth's geomagnetic field affect plants and other living organisms on the earth. External application of a magnetic field (MF) alters the growth and development of the organisms (Maffei, 2014).

The MF may alter the viability of bacteria. Fojt *et al.* (2004) showed that low-frequency MF ($t < 30$ min, $B_m = 10$ mT, $f = 50$ Hz) led to decrease the viability of *Staphylococcus aureus*, *Leclercia adecarboxylata* and *Escherichia coli*. On the other hand, Fijałkowski *et al.* (2015) revealed that the rotating magnetic field (RMF) ($B \frac{1}{4} 25-34$ mT, RMF frequency $f \frac{1}{4} 5-50$ Hz, time of exposure $t \frac{1}{4} 60$ min, temperature of incubation 37 °C) encouraged the growth of *E. coli*, *K. oxytoca*, *S. aureus*, *S. marcescens*, *S. mutans*, *C. sakazakii*, and *S. xylosum*. Ren *et al.* (2018) indicated that low-intensity MF (15–35 mT) enhanced the capacity of *Acinetobacter* sp. B11 to eradicate oil by 11.9% at 25 mT comparing with bacteria without magnetic field. Li *et al.* (2015) showed that the intensity of MF and the time of treatment play an important role in the bacterial growth. The treatment of 300 mT for 10 minutes improved *Paenibacillus* sp. growth, while, high magnetic field intensity (500mT) caused inhibition of

Paenibacillus sp. growth. Mahdi *et al.* (2017) indicated that magnetic water had bactericidal effect against *Pseudomonas aeruginosa* having multidrug resistance.

An external application of MF on water causes changing in the electronic, atomic and molecular structure leading to differences in viscosity, boiling and solidifying point, and other properties (Pang and Deng 2008). Irrigation with MW caused an increase in soil moisture in comparing with the control treatments for different solutions of saline and hard water. Therefore, irrigation with MW plays an important role in decreasing the quantity of irrigation water (Surendran *et al.*, 2016).

Irrigation with MW can improve quantitatively and qualitatively the growth and development of plants by enhancing the germination of seeds, the early vegetative growth of seedlings and can also adjust the mineral content of seeds or fruits. Therefore, MW could be considered as one of the most promising tools in the future to improve the crop productivity in an eco-friendly way (Teixeira da Silva and Dobranszki, 2014). Hozayn and Abdul Qados (2010) revealed that irrigation with MW improved the chemical constituent, growth and yield of chickpea in pots experiment. El Sayed (2014) showed that irrigation with MW improved the chemical composition, growth and yield of broad bean plant in pots experiment. Moreover, Ghanati *et al.* (2015) indicated that irrigation with MW enhanced the growth, metabolism, yield quality and quantity of maize plants. Ahmed and Abd El-Kader (2016) showed that the use of MW in irrigation of potato (*var.* Diamont) in field experiment improved the vegetative growth, tuber yield, component of potato plants, decreased soil salinity and enhanced soil fertility.

This study aims to investigate the effect of MW on the viability of *R. solanacearum* and the effect of irrigation with MW on the management of brown rot disease of potato through the effect on physiological properties of potato plant, yield of potato tubers, percentage of infected

potato tubers and the viability of *R. solanacearum* in the rhizosphere of potato plant.

MATERIALS AND METHODS

Bacterial pathogen:

A virulent isolate of *R. solanacearum* (race 3 biovar 2) was obtained from Culture Collection of Bacterial Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Potato tubers:

Potato tubers cultivar (Spunta) was obtained from Nubaria district, Bahaira Government, Egypt.

Magnetic Tube:

Magnetic liquid modifier tube (Russian technology) with a total length of 10.5 cm, the length of magnetic part 8.5 cm., the magnetic field intensity was 35 mT, the flow rate of water was 1 L in 38 seconds (0.026 L/sec. 0.095 m³/h.)

Effect of MF on viability of *R. solanacearum*:

R. solanacearum grown on Nutrient Glucose Agar (NGA) slants for 48 h at 28 °C, was harvested into tape water (TW) in a flask and adjusted to a concentration about 10⁸ CFU/ ml. The bacterial suspension was passed through the magnetic tube (MT) for one pass then one-third of the bacterial suspension was taken in another flask. The two third remaining bacterial suspension was passed 9 times through the MT for achieving 10 passes and half of this suspension was separated in a third flask. The remaining part of the bacterial suspension was passed through the MT additional 10 passes to achieve 20 passes. Then, there were three flasks of the bacterial suspension passed through the MT 1, 10 and 20 times. The bacterial suspensions were incubated in a shaking incubator (28 °C and 120 RPM). After 1, 2, 3 and 4 h serial dilutions were done for each bacterial suspension in each flask and plated onto SMSA plates (Elphinstone *et al.* 1996). The plates were incubated at 28 °C for 48 h. The colony forming unit/ ml (CFU/ ml) was counted and log CFU/ ml was calculated. For each dilution, five replicates were prepared.

Preparation of bacterial pathogen for pots experiment:

R. solanacearum was inoculated in Nutrient Glucose Broth (NGB) and incubated in an incubator shaker (28 °C and 120 RPM) for 48 h. Bacterial growth was harvested by centrifugation (10000 rpm/ min for 20 min.). The precipitate was re-suspended in sterilized TW. The bacterial suspension was adjusted to 10⁸ CFU/ ml concentration.

Pots experiment

This experiment was carried out at Tag El-Ezz Agricultural Research Station, Dakahlia Governorate, Egypt during the period of 17 February to 12 May 2016. The plastic sacks (pots) of 35 cm diameter were filled with 20 kg non-sterilized soil. This soil composed of a mixture of clay and sand 1:1 (w: w). Each pot was irrigated with water, left for 48 h and infested by the 100 ml of *R. solanacearum* suspension (10⁸ CFU/ ml). After 48 h., two slices were planted in each pot, one plant was harvested after 40 days and the other plant was harvested at the end of the experiment. The pots were divided into two groups; the control plants were irrigated with TW and the treated plants were irrigated with MW.

Biochemical assessment of potato plants in pot experiment

The following biochemical assessments were carried out in plants harvested after 40 days of planting. The total phenol content was determined using Folin Ciocalteu reagent according to the method described by Blainski *et al.* (2013). Assay of polyphenoloxidase (PPO) and peroxidase (POD) were carried out according to the methods described by Seleim *et al.* (2014). Total chlorophyll, chlorophyll a, chlorophyll b and carotenoids in potato leaves were determined according to Mackinney (1941).

Disease rating:

Wilt symptoms were recorded daily according to the scale of Kempe and Sequeira (1983) where, (0 = no symptoms, 1= up to 25 % wilt, 2 = 26-50 % wilt, 3 = 51-75 % wilt, 4 = 76-99% of the foliage wilted and 5 = dead plants. Each replicate was examined and disease rating was recorded. Mean value for each treatment was calculated.

Tubers yield:

At harvesting time, tubers of each pot were weighted. The mean weight and the percentage in the increase of tubers yield was calculated.

The percentage of the increase in tubers yield = [(Tubers weight of treatment – tubers weight of control) / tubers weight of control] X100

Infected potato tubers:

tubers were stored for 60 days at room temperature to encourage the development of latent infection and facilitate symptoms detection (Graham *et al.* 1979). After storage period, potato tubers were cut to detect the number of infected tubers and the mean value of percentage of infected tubers was calculated for each treatment.

The decrease in infected tubers % =

[(Infected tubers % of control - Infected tubers % of treatment) / infected tubers % of control] X100

Count of *R. solanacearum* in plant rhizosphere:

Soil samples were collected from rhizosphere of plants of each pot. The soil samples from five replicates of each treatment were mixed well and left to be air dried then grounded after plant debris were removed.

The bacterial count of *R. solanacearum* was carried out on SMSA plates as described by Larkin (2016).

Statistical analysis:

Preliminary experiment was analyzed using Analysis of Variance and Duncan's Mutable Range test was used to compare the means at probability (*p*) value of ≤0.05). Unpaired t-test was used to statistically compare between magnetic and normal water treatments using SPSS statistics software package, Version 16. All values are means of five replicates.

RESULTS AND DISCUSSION

Data in Table (1) show that the increase in the number of passes through the magnetic tube (MT) and the increase in the incubation time in MW led to significant increase in the viability of *R. solanacearum* while the viability of *R. solanacearum* did not significantly change by incubation in TW. The highest recorded value of log CFU/ ml (8.272) was recorded when the bacterial suspension was passed through the magnetic tube 20 times

and incubated for 4 h. After 1 h. of incubation time, the value of log CFU/ ml of *R. solanacearum* was 7.788 after one pass while it increased to 8.083 and 8.206 after 10 and 20 passes, respectively. The same manner was observed with the incubation periods 2, 3 and 4 h. Moreover, the bacterial count increased significantly by increasing the number of passes with incubation time 1 h., the values were 7.788, 8.083 and 8.206 in case of one, 10 and 20 passes, respectively.

Table 1. Effect of magnetic water on Bacterial count of *R. solanacearum*.

Incubation time (h)	Log CFU/ ml of <i>R. solanacearum</i>				Mean
	Control	1 Pass	10 Pass	20 Pass	
1h	7.774 ^{gh}	7.788 ^{gh}	8.083 ^{ef}	8.206 ^c	7.962B
2 h	7.762 ^h	7.811 ^g	8.111 ^e	8.220 ^{bc}	7.976B
3 h	7.766 ^h	8.068 ^f	8.165 ^d	8.251 ^{ab}	8.063A
4 h	7.763 ^h	8.086 ^{ef}	8.195 ^{cd}	8.272 ^a	8.075A
Mean	7.766 ^D	7.938 ^C	8.139 ^B	8.237 ^A	

Means followed by different superscript small letter (s), capital letter or superscript capital letter are differed significantly according to Duncan multiple range test (p value of ≤ 0.05)

In case of 4 h. incubation time, log CFU/ ml increased significantly from 8.086 for one pass to 8.195 and 8.272 for 10 and 20 passes, respectively. These results are in contrary with the results of Fojt *et al.* (2004) who reported that low-frequency MF led to decrease of the viability of *S. aureus*, *L. adedecarboxylata* and *E. coli*. Moreover Mahdi *et al.* (2017) indicated that MW had bactericidal effect against *P. aeruginosa*. Whereas, the findings of this investigation had the approval of Fijałkowski *et al.* (2015) who revealed that the RMF encouraged the growth of *E. coli*, *K. oxytoca*, *S. aureus*, *S. marcescens*, *S. mutans*, *C. sakazakii*, and *S. xylosum*. Furthermore, obtained results had the approval of Ren *et al.* (2018) who indicated that low-intensity MF enhanced the activity of *Acinetobacter* sp. B11. In addition, Li *et al.* (2015) indicated that the treatment of 300 mT for 10 minutes improved *Paenibacillus* sp. growth. (Fijałkowski *et al.*, 2015) mentioned that the enhancement of bacterial viability may be due to the activation of bacterial metabolic activity that stimulates the growth rate and biofilm formation. Li *et al.* (2015) and Ren *et al.* (2018) revealed that the stimulation of bacterial growth may also due to the increasing of bacterial enzymatic activities.

Data in Fig. (1) illustrate that, in the pots experiment, the irrigation with MW led to significant increase in the total phenols, polyphenol oxidase (PPO) and peroxidase (POD). The total phenols increased from 13.61 mg GAE/g FW in the plants irrigated with TW to 18.79 mgGAE/g FW in the plants irrigated with MW and PPO increased from 8.22 to 13.57 Unit/min. g F.W., POD increased from 15.83 Unit / min./ g FW in plants irrigated with TW to 24.64 Unit/ min./ g FW in plants irrigated with MW. Therefore, the irrigation with MW significantly increased the systemic induced resistance of potato plants. These results are confirmed by the results of Ghanati *et al.* (2015) who indicated that irrigation with MW enhanced the metabolism of maize plants and increased the activity of ascorbate peroxidase and superoxide dismutase. Phenols act as antimicrobial, antioxidant, and photoreceptor. Phenols rapid accumulate at the infection site as the first

step of the defense strategy that hinders the pathogens' growth. There are probable roles for defense role of POD such as the toxicity to pathogens, quickening the infected cells death that decreases the availability of cellular proteins to the pathogen, causing a physical barrier to pathogens in the cell wall, and quinone redox cycling leading to H₂O₂ and other reactive oxygen species that are known as defense signaling. The PPO play a role in the creation of toxic secondary metabolites and its simultaneous oxidant and antioxidant activity that can help in the defense response of plants in response to stress conditions (Ezzat *et al.*, 2015)

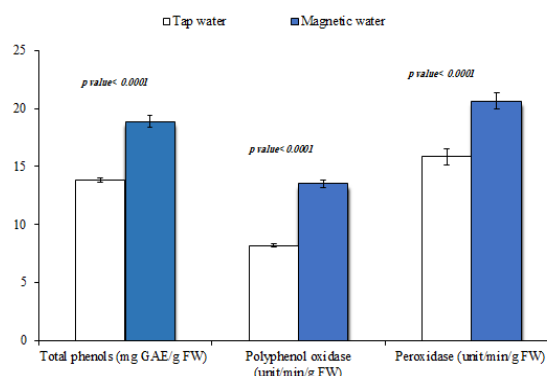


Fig. 1. Effect of irrigation with MW on phenolic compounds and defense related enzymes of potato plants after 40 days of planting. p value ≤ 0.05 level is significant according to unpaired t -test.

Data in Fig. (2) show that chlorophylls were significantly increased in potato plants irrigated with MW and carotenoids were significantly increased. Total chlorophyll increased from 2.013 mg/g FW in the plants irrigated with TW to 3.109 mg/ g FW in plants irrigated with MW. Chlorophyll a increased from 1.375 to 2.235 mg/g FW in the plants irrigated with TW and plants treated with MW respectively. Also, Chlorophyll b increased from 0.638 in the plants irrigated with TW to 0.874 mg/g FW in plants irrigated with MW and carotenoids increased from 0.692 in the plants irrigated with TW to 0.788 in plants irrigated with MW. These results revealed that the irrigation with MW significantly enhanced the physiological characters of potato plants. These results are in harmony with findings of Hozayn and Abdul Qados (2010) who indicated that irrigation with MW led to increments of chlorophylls a and b, total chlorophylls and carotenoids of chickpea in pots experiment. Furthermore, these findings are confirmed with results of Ghanati *et al.* (2015) who reported that the photosynthetic pigments levels (chlorophyll a and b, flavonoids and the of anthocyanin's contents) of the leaves of maize plant increased in treatment irrigated with MW in comparing with the plants irrigated with TW. Photosynthetic pigments are indicators of plant health and immunity. The chloroplasts have an important role in plant immunity by hosting biosynthesis of many key defense-related molecules, including secondary messengers and hormones. The chloroplast is a main direct target for many pathogen effectors to destroy accumulation and signaling of pro-defense molecules (Serrano *et al.* 2016)

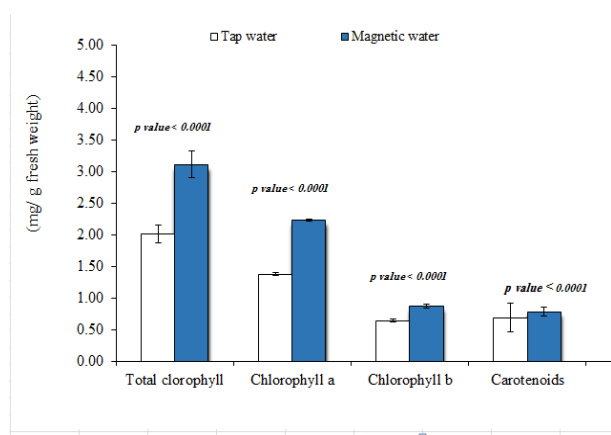


Fig. 2. Effect of irrigation with MW on photosynthetic pigments after 70 days of planting
 p value ≤ 0.05 level is significant according to unpaired t -test.

Data in Fig. (3) show that the irrigation with MW caused a significant increase in plant height after 50 days from 15.6 cm in the plants irrigated with TW to 17.4 cm in plants irrigated with MW. Furthermore, the plant height after 70 days of planting significantly increased from 23.6 to 27.8 cm in the plants irrigated with TW and plants irrigated with MW respectively. Ahmed and Abd El-Kader (2016) indicated that the irrigation with MW enhanced the vegetative growth, plant height and leaf area of potato plants in a field experiment conducted at El-Gharbiah Governorate, Egypt. This improvement in vegetative growth of plants irrigated with MW may be due to the enhancement of physiological characters.

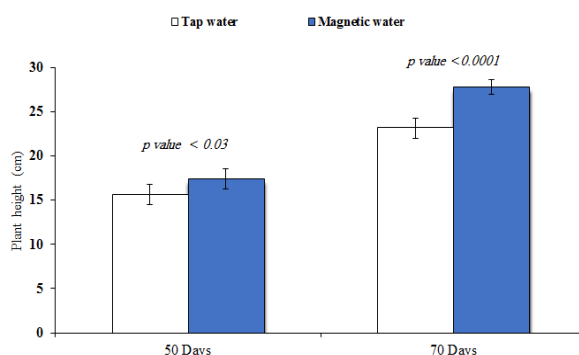


Fig. 3. Effect of irrigation with MW on plant height after 50 and 70 days of planting.
 p value ≤ 0.05 level is significant according to unpaired t -test.

Data in Fig. (4) indicate that the disease rate of bacterial wilt disease significantly decreased from 4.2 in case of plants irrigated with TW to 3.4 in plants irrigated with MW. These results may be due to the enhancement of physiological characters and systemic resistance of potato plants due to irrigation with MW.

Data in Fig. (5) reveal that the irrigation with MW led to a significant decrease of the viable bacterial count of *R. solanacearum* in the rhizosphere of potato plants after 70 days of the planting and this decrease was significantly after 108 days of the planting (harvesting time).

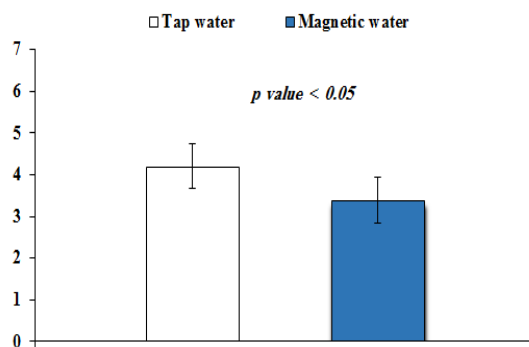


Fig. 4. Effect of irrigation with MW on disease rating of brown rot disease of potato.

Disease rating scale: 0 = no symptoms, 1= up to 25 % wilt, 2 = 26-50 % wilt, 3 = 51-75 % wilt, 4 = 76-99% of the foliage wilted and 5 = dead plants, p value ≤ 0.05 level is significant according to unpaired t -test.

After 70 days of planting, log CFU/ ml of *R. solanacearum* decreased from 6.387 for plants irrigated with TW to 6.283 for plants irrigated with MW, while, the value after 108 days of the planting decreased from 4.460 for the plants irrigated with TW to 4.327 for plants irrigated with MW.

The decreases of *R. solanacearum* viability may be due to the enhancement of induced systemic resistance of potato plants that reflecting on the root exudates that might inhibit *R. solanacearum*. This suggestion had a confirmation of Baetz and Martinoia (2014) who showed that root exudates play an important role in the plant defense. In addition, the irrigation with MW changes the soil characters (Surendran *et al.*, 2016) that may inhibit *R. solanacearum*. Furthermore, the microflora of rhizosphere may directly be affected by the irrigation with MW and/ or may be affected by the variation of soil characters resulting from irrigation by MW that may encourage one or more microorganism that antagonized *R. solanacearum*, leading to inhibition of the pathogen's growth.

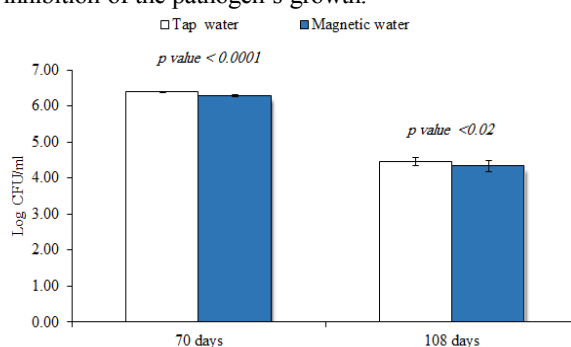


Fig. 5. Effect of irrigation with MW on viable count of *R. solanacearum* in rhizosphere after 70 and 108 days of planting
 p value ≤ 0.05 level is significant according to unpaired t -test.

Data in Fig. (6) illustrate that the irrigation with MW led to a significant increment in yield of potato from 82.02 g in plants irrigated with TW to 103.05 g in plants irrigated with MW causing an increment in the yield by 25.64 %. This increasing of potato yield had the same

harmony of Ahmed and Abd El-Kader (2016) who indicated that the irrigation with MW increased the potato tubers yield and enhanced the tubers quality. Furthermore, Hozayn and Abdul Qados (2010), El Sayed (2014) and Ghanati *et al.* (2015) indicated that irrigation with MW increased the yield of chickpea, broad bean and maize plants. This enhancement of potato tubers yield is expected results and may back to the improvement of physiological characters and vegetative growth of potato plants.

Also, results in Fig. (6) show that the infected potato tubers percentage after 60 days of storage decreased from 92 % in plants irrigated with TW to 55 % in the plants irrigated with MW causing 40.22 % decrement in infected potato tubers percentage. This decrement in the infected tubers percentage may be due to the enhancement of the induced systemic resistance of potato plants irrigated with MW. This effect also may be due to the decrement of the viability of *R. solanacearum* in the rhizosphere of these plants.

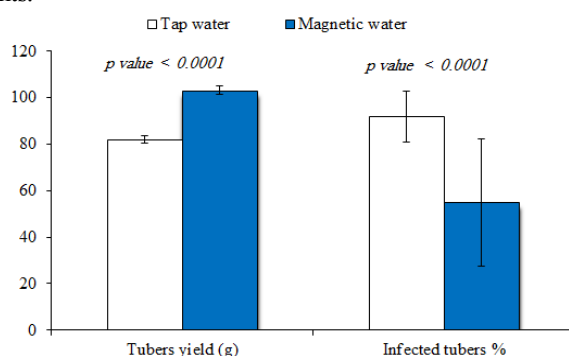


Fig. 6. Effect of irrigation with MW on tubers yield and infected tubers percentage
 p value ≤ 0.05 level is significant according to unpaired t -test.

CONCLUSION

The viability of *R. solanacearum* increased due to its passing through MF. The irrigation of potato plants with MW caused significant enhancement in the physiological characters, vegetative growth, decreasing the disease rating of bacterial wilt disease of potato plants, increasing the potato tubers yield and decreasing the percentage of infected potato tubers. These findings encourage the use of MW for irrigation of potato plants as environmentally friend tool to manage brown rot disease of potato, enhance potato yield and encourage the investigation the possibility of the using the irrigation with MW to manage other plant diseases.

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استخدام تكنولوجيا الماء الممغنط لمكافحة مرض العفن البني في البطاطس

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^١ قسم بحوث الأمراض البكتيرية – معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر
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تهدف هذه الدراسة إلى بحث إمكانية استخدام الماء الممغنط لمكافحة مرض العفن البني (الذبول البكتيري) في البطاطس، تم دراسة تأثير المجال المغناطيسي على حيوية بكتيريا *Ralstonia solanacearum* (المسبب البكتيري لهذا المرض) عن طريق تمرير معلق بكتيريا *R. solanacearum* (حوالي 10^8 CFU/ml) من خلال أنبوبة ممغنطة معدلة للسوائل، زادت حيوية الممرض البكتيري بزيادة فترة التحضين بعد ١، ٢، ٣ و ٤ ساعات، وكذلك بزيادة عدد مرات المرور خلال الأنبوبة الممغنطة، في تجربة الأصص (نفذت بمحطة البحوث الزراعية - تاج العز - دقهلية - مصر، خلال الفترة من ١٧ فبراير حتى ١٢ مايو ٢٠١٦)، وجد أن الري بالماء الممغنط أدى إلى زيادة معنوية في الفينول الكلي، والبوليفينول أوكسيديز، والبيروكسيداز، والكلوروفيل الكلي، والكلوروفيل أ، والكلوروفيل ب، والكاروتينات، وكذلك تم ملاحظة زيادة معنوية في طول النبات بعد ٥٠ و ٧٠ يومًا من الزراعة وانخفاض معدل المرض. انخفضت حيوية بكتيريا *R. solanacearum* في التربة المحيطة بجذور النباتات التي تم ريها بالماء الممغنط انخفاض معنوي بعد ٧٠ و ١٠٨ يومًا من الزراعة بالمقارنة مع النباتات التي تم ريها بماء الصنبور. أدى الري بالماء الممغنط إلى زيادة معنوية في محصول البطاطس (٢٥.٦٤٪) وانخفاض معنوي في نسبة الدرنات المصابة (٤٠.٢٢٪) مقارنة بمعاملة الري بماء الصنبور. هذه النتائج تشجع على المزيد من الدراسات لاستخدام الماء الممغنط لمكافحة مرض العفن البني في البطاطس والأمراض النباتية الأخرى.