

Full Length Research Paper

Effect of quadratic residue diffuser (QRD) microwave energy on root-lesion nematode, *Prathlenchus penetrans*

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Accepted 11 April, 2013

In this study, quadratic residue diffuser (QRD) microwave energy was used to control nematode *Pratylenchus penetrans* in soil. Microwave energy is a physical method that has been used to manage nematodes. This approach provides rapid heat transfer to soil with no lingering residual effects. QRD microwave radiation at a frequency of 2450 MHz was used to irradiate sandy clay loam soil containing a nematode layer. The pot dimensions were 17 cm high, 10 cm diameter and exposure times used were 10, 20, 30, 40, 50, 60, and 120 s. The soil water content was set at 0, 10, 20, 30, and 40%, respectively, based on dry mass. Total mortality was calculated at soil depths of 5, 10 and 15 cm. Microwave treatment time and soil water content significantly affected nematode mortality; also, longer exposure time and decreased soil moisture content resulted in a greater total mortality. However, 120 s radiation was demonstrated to be the most effective for killing nematodes at all soil water contents and soil depths.

Key words: Microwave energy, nematodes, pepper, *Pratylenchus penetrans*, physical control, quadratic residue diffuser.

INTRODUCTION

Plant-parasitic nematodes are serious pathogens to crop plants. Crop production has been severely hampered due to nematodes in several countries. Their effects are commonly underestimated by farmers, agronomists and pest management consultants, but it has been reported that 10% of world crop production is lost as a result of plant nematode damage (Whitehead, 1998). Several methods have been suggested to improve the economic production and growth of plants to compensate for damage caused by nematodes.

In regards to chemical control, there are several nematicides that can be used to effectively eliminate

nematode pests from annual crops (van Berkum and Hoestra, 1979). However, these chemical treatments often control nematodes only over a limited period of time and the nematodes tend to resume normal activities when the chemical dissipates. In addition, there have been problems associated with the persistence of toxic residues in food grains, the development of insect resistance and adverse environmental impact (Shahina and Salma, 2011). Biological control methods have been used to control nematode and have a reduced negative impact on the environment (Brower et al., 1996).

One natural biological control method involves the use

of an antagonistic organism to control nematodes. Some fungi and bacteria can suppress nematode populations. Various nematode antagonistic fungi have been studied for their use as potential bio-control agents including *Trichoderma harzianum*, *Paecilomyces*, *lilacinus* and *Arthrobotrys oligospora* (Sharma and Pandey, 2009). Although, a number of parasitic fungi and bacteria appear to have considerable biological control potential, these agents are only effective against specific nematodes and it is difficult to obtain a commercial agent for use in large farms.

Physical control strategies aim to prevent or reduce nematode spread and generally involve sterilization of the growing medium or use of uninfected planting material. Control of nematodes through physical methods typically involves heat treatment including steam, hot water, and solarization (Giblin-Davis and Verkade, 1988). Another physical method to control nematodes is the use of non-ionizing radio-frequency waves, which is a non-thermal method to treat soil. In addition, the use of non-ionized radio-frequency waves, which is also non-thermal, has been suggested (Reyffuss and Chipley, 1980). Microwave energy has been frequently proposed as an alternative method for controlling pests, and microorganisms, such as weed seeds (Bebawi et al., 2007), insects, nematodes, and soil-borne plant pathogens (Nelson, 1996).

The principle of microwave heating is based on tuning the frequency to the oscillation resonance range of molecules, such as water. This leads to strong molecular shaking (resonant critical frequency), and consequently to the production of heat within the material (Krasewski and Nelson, 1995). Another advantage of thermal treatment using microwaves is that, this approach produces no chemical residues (Mavrogianopoulos and Frangoudakis, 2000). Barker et al. (1972) concluded that treatment of nematode infested soil samples with 2,450 MHz had poor prospects for becoming a practical means to control nematodes. The root-knot nematode was controlled in small samples of potting soil exposed to 2,450 MHz energy in a microwave oven when temperatures lethal to the nematodes were achieved (O'Bannon and Good, 1971).

Since known microwave energy can be applied in controlling soil, insect and nematode. The microwave energy needs to be developed to obtain optimum results to be applied in general application. Microwave dispersed system Quadratic Residue Diffuser (QRD) is a new type of microwave energy. Quadratic residue diffuser was described in other recent publication (Monazzam and Nassiri, 2009) as a diffuser consisting of a series of wells of the same width and different depths, and the wells are separated by thin fins. The depths of the wells are controlled by a quadratic residue sequence, the phase differences and the structure will produce a significant scattering of the reflected wave. One of the companies in Korea (SAMICKTECH. Co Ltd) have noted that microwave with

quadratic residue diffuser system has a different phase induced from the reflected wave to eliminate the microwave mode heating up phenomenon and spark phenomenon. Studies on the effect of high energy microwave are ongoing in Korea and outstanding reactions have been demonstrated in the sterilization field. The objectives of this study were to identify the effects of alternative commercial QRD microwave energy on root-lesion nematode, *Pratylenchus penetrans* at different soil water content and on nematode mortality in deeper soil.

MATERIALS AND METHODS

Nematode culture

P. penetrans, collected from pepper (*Capsicum annum*) plant field (Youngyang Pepper Experiment Station, Gyeongbuk Agricultural Research and Extension Services) in Korea was stored in polyethylene bags and kept in a greenhouse. The following generation of nematodes was extracted from soil and nematode species in the pepper plant were identified. Extracted nematodes were used for culture in greenhouse with control management. The soil used to culture nematodes was autoclaved at 121°C for 30 min and then placed in polyethylene bags with a 15% soil water content. Nematodes were inoculated in each experimental bag. Pepper plant seedlings were transplanted to each bag and kept in a greenhouse at temperatures ranging from 16 to 25°C. Every eight weeks of culture, the total population of nematodes was measured until population reached 500 nematodes/100cc soil. This population level was used for all experiments.

Application procedures microwave treatment

The soil water content used in this study was 10, 20, 30, and 40% (Weight water/dry weight soil). Soils sandy clay loam at these different water contents were then placed in cylindrical plastic pots that were 17 cm in height and 10 cm in diameter. Each pot was inoculated with a nematode culture in the soil (Approximately 500 nematodes/100 cc) at the depth of 5, 10, and 15 cm. The thickness of the nematode layer was 1 cm. The samples were then exposed to (Quadratic Residue Diffuser (QRD) microwave energy and soil temperature and mortality of nematodes were measured. A microwave oven model QRD was used for all treatments. The volume and frequency of 95.740 cm³ (40 cm width, 42 long, and 57 cm high) and 2,450±30 MHz (16 KW) was used respectively to generate microwave radiation. The following parameters were varied during soil treatment time of microwave exposure, water content, and depth of soil. Packed soil pots were placed in the central cavity of the microwave oven and exposed to full power radio frequency for 10, 20, 30, 40, 50, 60, and 120 s. After removal from the microwave oven, an electric thermometer (Thermo Recorder TR-71S, T&D, Co. Japan) was inserted into the center of the pot to measure the soil temperature at the depth of 5, 10, and 15 cm. The soil temperatures were recorded at approximately 1 min intervals for 6 min after samples were taken from the microwave oven. After measuring the temperature, pots were closed and the soil was allowed to cool to room temperature for 60 min. The death rate of nematodes at soil depths of 5, 10, and 15 cm water then determined.

Nematode extraction

Sucrose floating (Byrd et al., 1966) and Baermann funnel methods

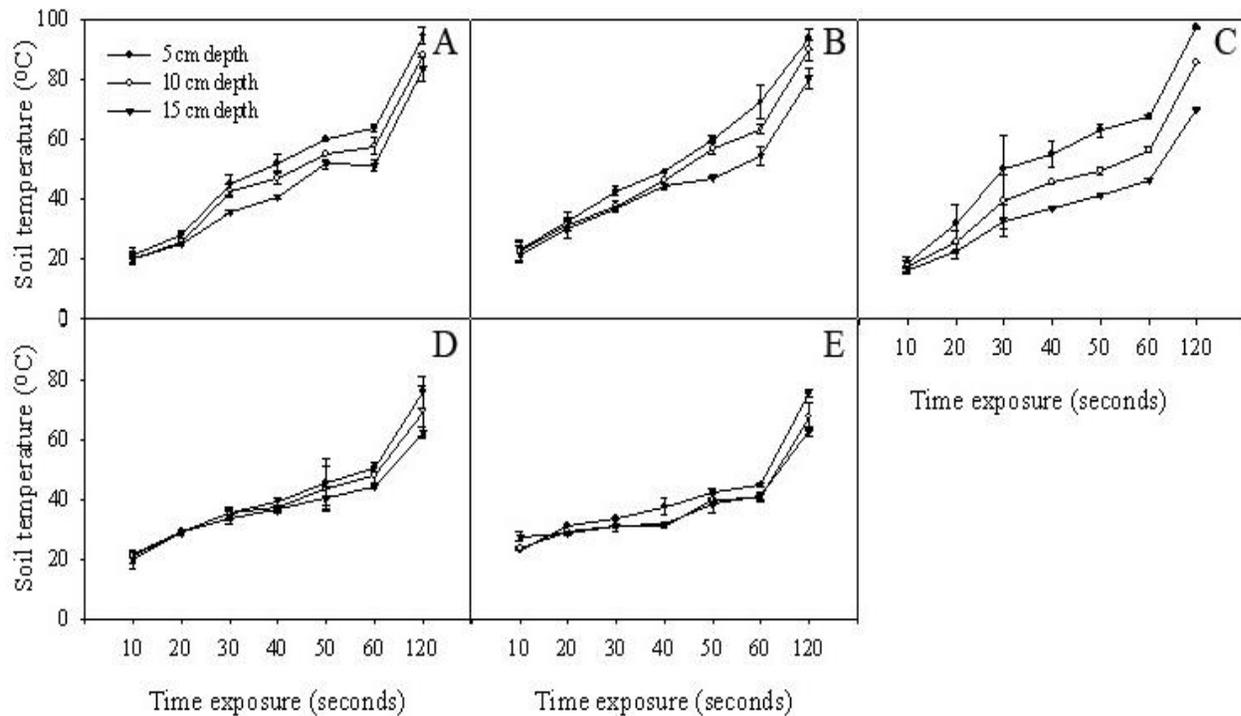


Figure 1. Effects of microwave exposure time and soil water content on soil temperature. **A.** % soil water content. **B.** 10% soil water content. **C.** 20% soil water content. **D.** 30% soil water content. **E.** 40% soil water content. Values are mean of three replica samples and significantly differences ($P < 0.05$) by Duncan Multiple Range Test.

(Baermann, 1917) were used to isolate nematodes. After each microwave treatment, 200 cc of soil were used to extract the nematodes. In the first step, nematodes were extracted from soil using the Baermann funnel method for as long as 3 days. The soil sample (50 cc soil each funnel) after remove from other contaminant soil (rocks, roots, etc.) placed on funnel. Soil spread evenly on Kimtech tissue, and completed filling funnel with water till level 5 mm above wire-mesh. After 3 days (with constant temperature 25°C) nematodes extracted were recovered by releasing water from the stem of funnel into a counting dish. During this process, live nematodes moved down from soil to the water and were directly calculated to determine the number of live nematodes. The sucrose floating method was used to determine the number of dead nematodes death still in the soil. Sucrose floating method was used by adding soil with 45% sucrose solution and centrifuged to separate nematode from soil particle. Dead nematodes collected with mesh and moved to Petri disc were counted using a stereoscopic microscope. If there was any live nematode, it was accumulated with the total survival nematode from the extraction Baermann funnel method. The total number of dead and live nematodes was used to calculate nematode mortality.

The statistical analysis

The statistical design of the experiment was completely randomized with three replicates. Treatment effects were analyzed by multifactor analysis of variance (ANOVA); if the ANOVA was significant, differences in treatments were determined through Duncan's multiple range tests ($P < 0.05$) using SPSS statistical software.

RESULTS

Highest soil temperatures a related to radiation time exposure in all treatments

The temperatures at different soil moisture content and soil depths (5, 10, and 15 cm) are shown in Figure 1. Soil temperature increased by duration of microwave treatment at all water contents and the temperature was lower than that of drier soil when treated for the same length of time. In addition, the soil temperature decreased with soil depth. Analysis of variance indicated that the effect of time, soil water content and soil depth on soil temperature was significant ($P < 0.05$). Soil temperature by microwave treatment at different water contents show that 120 s produced the highest temperature, whereas a treatment time of 10 s produced the lowest soil temperature at all soil water content. The water content was also shown to affect the soil temperature rise under all treatment conditions. A soil water content of 10% resulted in the highest temperature relative to other treatment conditions. The maximum soil temperature was observed at a depth of 5 cm at all exposure times and soil water content. The effect of soil depth on temperature was significantly lower at a depths of 10 and 15 cm (Figure 2).

The results from the analysis of variance for microwave

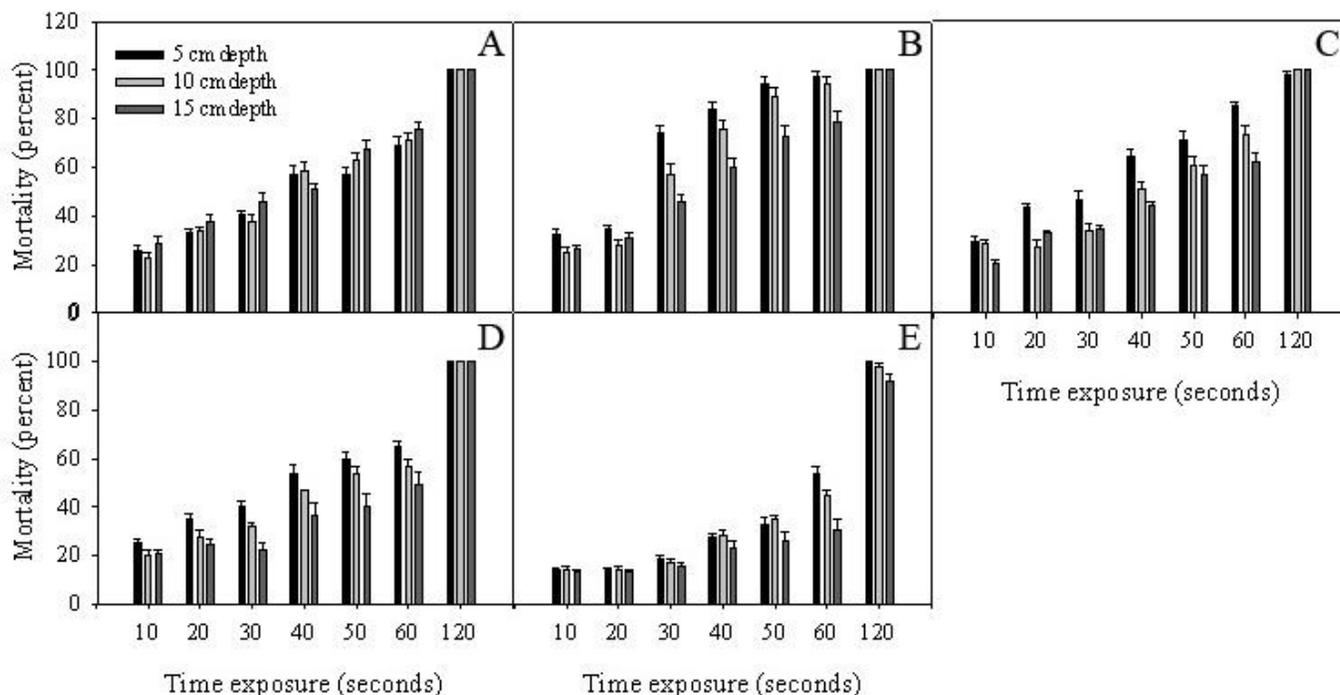


Figure 2. Effects of microwave exposure time and soil water content on mortality nematodes. **A.** 0% soil water content. **B.** 10% soil water content. **C.** 20% soil water content. **D.** 30% soil water content. **E.** 40% soil water content. Values are mean of three replica samples and significantly differences ($P < 0.05$) by Duncan Multiple Range Test.

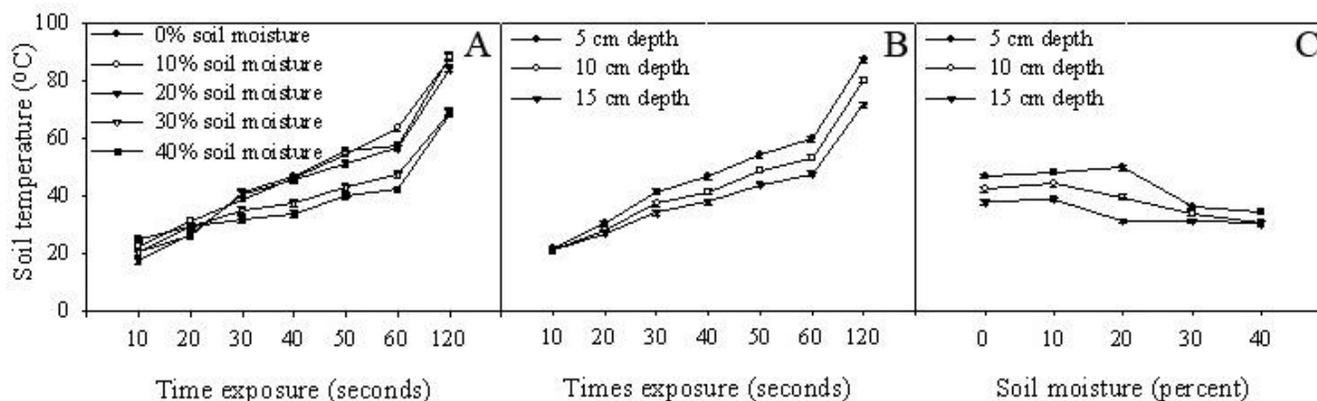


Figure 3. Two way interaction effects between microwave exposure time, soil water content, and soil depth on soil temperature. **A.** Microwave exposure time - soil water content. **B.** Microwave exposure time - soil depth. **C.** Soil water content-soil depth.

exposure time, soil water content, and soil depth on the mortality of nematodes is shown in Figure 3. This analysis indicated that the interactions between time exposure and different soil water content, interactions between exposure time and soil depth and interactions between soil water content and soil depth were significant ($P < 0.05$). The interactions between exposure time, soil water content, and soil depth with soil temperature was not significant. The soil temperature immediately after treatment was positively correlated with treatment time

and negatively correlated with soil depth and water content.

Highest mortality nematode related to radiation exposure time, soil water content, and depth soil

The mortality of nematodes was recorded at every exposure time, soil moisture content, and soil depth (5, 10, and 15 cm). Analysis of variance indicated that the

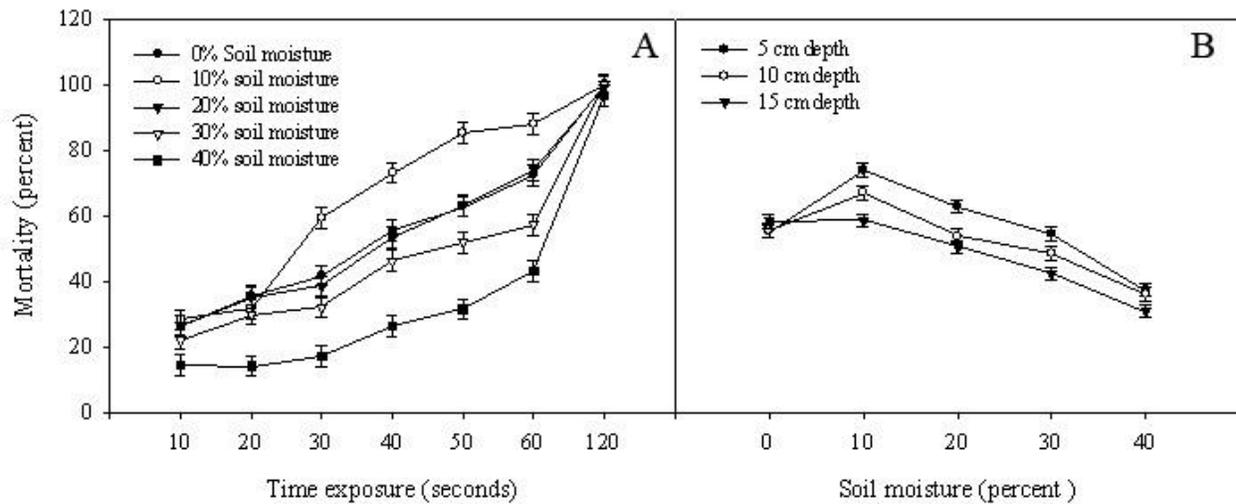


Figure 4. Two way interaction effects between microwave exposure time, soil water content, and soil depth on mortality of nematodes. **A.** Soil water content - soil depth. **B.** Microwave exposure time - soil water content. No significance was observed for the interaction between microwave exposure time and soil depth.

effect of exposure time, soil water content and soil depth on the mortality nematodes was significant ($P < 0.05$). The mortality of nematodes decreased with an increase in soil water contents at all exposure times. When the soil water content was 10%, the mortality of nematodes was the highest when compared with the higher soil water content (20, 30, and 40%) and these results were correlated with the soil temperature patterns. Differences in soil depth also affected the nematode death rate.

The significance of the interaction between microwave exposure time, soil water content, and soil depth on the mortality nematodes are shown in Figure 4. Significant ($P < 0.05$) interactions were observed between exposure time and soil water content, time exposure and soil depth; however, no significant interactions were observed between exposure time and soil depth and exposure time and soil water content.

Marginal mean of time exposure, soil water content, and soil depth on the soil temperature and mortality nematodes

The marginal mean (Table 1) of microwave exposure time of 120 s produced the highest temperature increase of 79.6°C, followed by exposure for 60, 50, 40, 30, 20, and 10 s. The same trend was observed regarding the nematode death rate, where 120 s exposure time produced the highest nematode death rate of 99.2% followed by exposure times of 60, 50, 40, 30, 20 and 10 s. The marginal mean depth shows that the soil depth of 5 cm produced the highest soil temperature (48.8°C) and death rate (56.8%), which was followed by soil depths of

10 and 15 cm.

DISCUSSION

The effect of QRD microwave treatment of soil on nematode was related to treatment time, depth of soil, and soil water content. The soil temperature immediately after treatment correlated positively with treatment time and negatively with soil water content and soil depth. Ferris, (1984) also reported that microwave oven treatment correlated positively with exposure time and negatively with soil water content. Higher water content in the soil may be needed to increase the temperature by microwave energy. In this study, 10% soil water content was shown to result in the highest temperature at all microwave exposure times and an increase in the soil water content resulted in a decrease in the soil temperature. In addition, the soil temperature decreased when the soil water increased. A water content of 30 and 40% significantly reduced the soil temperature at the same time exposure.

Soil temperature decreased with soil depth and the water content affected the range of soil temperature at each soil depth. Water content of 0% had the highest soil temperature at each depth when compared to the other soil water contents tested at the same exposure time. At a soil water content of 30 and 40%, the range in temperature at depths of 5, 10, and 15 cm was smaller. Soil temperature was increased by microwave treatment at all water contents and the soil temperature was higher in drier soil when treated at the same exposure time. In addition, the soil temperatures decreased with an

Table 1. The effect of microwave exposure time, soil water content, and soil depth on soil temperature and mortality of *Pratylenchus penetrans* from Quadratic Residue Diffuser (QRD) microwave energy.

Parameter	Treatment	Soil temperature (°C)	Mortality nematodes (%)
Time exposure (s)	10	21.3 ^g	23.6 ^g
	20	28.7 ^f	29.2 ^f
	30	37.6 ^e	37.8 ^e
	40	42.0 ^d	50.9 ^d
	50	49.0 ^c	58.9 ^c
	60	53.5 ^b	67.0 ^b
	120	79.6 ^a	99.2 ^a
Soil water content (%)	0	48.0 ^b	56.3 ^b
	10	49.3 ^a	65.9 ^a
	20	46.1 ^c	56.4 ^b
	30	40.4 ^d	48.5 ^c
	40	38.7 ^e	34.8 ^d
Soil depth (cm)	5	48.8 ^a	56.8 ^a
	10	44.3 ^b	52.2 ^b
	15	40.5 ^c	48.1 ^c

^aSuperscripts marginal mean in the same columns indicates significant differences at $P < 0.05$ by Duncan Multiple Range Test.

increase in soil depth.

The increase in soil temperature due to irradiation was dependent on the soil water content. Microwave energy is intercepted and dissipated by water molecules from the top of the profile as it penetrates downward, because of the very high specific heat capacity of water when compared to soil, much of the energy is intercepted by water resulting in a delay and smaller increase in soil temperature (Rahi and Rich, 2008). In the analysis of soil temperature as a function of soil depth, an increase in soil depth was shown to result in a decrease in soil temperature. This occurred because the microwave energy on the top intercepted and absorbed most of the energy. This energy is released quite rapidly to the soil, which resulted in higher temperatures at the lower soil depths.

An exposure time of 120 s and soil water content of 10% produced the highest temperature when compared to the other treatments with a maximum increase in temperature of 87.7°C. In contrast, an exposure time of 10 s and soil water content of 10% produced the lowest temperature with a maximum increase in temperature of 20.5°C. The interaction between an exposure time of 120 s and soil depth of 5 cm had a stronger effect on soil temperature, where the maximum increase in temperature was observed at a soil depth of 5cm (87.2°C). An exposure time of 10 s produced the lowest soil temperature at all soil water contents. At an exposure time of 10 s, no differences in temperature as a function

of soil depth (5, 10 and 15 cm) were observed. Under these conditions, the increase in temperature was 21°C. The increase in temperature at a soil water content of 20% and soil depth of 5cm was 54.7°C. In contrast, the increase in temperature at a soil water content and soil depth of 40 %, 5 cm, respectively, was only 37.4°C. An exposure time of 120 s killed nematodes under all treatment conditions. At this exposure time, the increase in temperature was greater than 70°C at all soil water content and soil depths. The mortality of nematodes closely followed the soil temperature pattern, and a soil temperature greater than 70°C was sufficient to kill nematodes.

The effect of exposure time, soil water content, and depth soil on soil temperature was shown to be correlated to the effect of these parameters on nematode mortality (O'Bannon and Good, 1971). An exposure time of 120 s at all different soil water content and soil depths resulted in the highest soil temperature with temperature increases greater than 70°C. Interestingly, the mortality of nematodes followed this soil temperature pattern and a soil temperature greater than 70°C was shown to be high enough to kill 99% of all nematodes. O'Bannon and Good (1971) showed that microwaves could control root-knot nematodes (*Meloidogyne* spp.) in small soil samples when a temperature of 72°C was achieved. Changes in both exposure time and soil depth resulted in different soil temperatures and death rates. Heald et al. (1974) reported that nematodes were controlled in a fine sandy

loam soil infested with the reniform nematode at depths of 5 cm, but that the nematodes survived at depths of 10 and 15 cm. Nematode mortality was closely correlated to soil temperature. For example, the 120 s exposure time produced the highest temperature and highest death rate. In addition, a soil water content of 10% produced the highest soil temperature and the highest death rate. These combined results indicate that nematode mortality was correlated with temperature.

Based on the results obtained, it can be concluded that 60 s of exposure to 2,450 Hz microwave irradiation of sandy loam soil between a soil water content of 0–10% (dry mass basis) was effective in controlling nematodes *P. penetrans* at a death rate of 80%. The estimated marginal mean for the soil water content showed that a water content of 10% produced the highest temperature with an estimated marginal mean 49.3°C, which was followed by a soil water content of 0, 20, 30, and 40%. A soil water content of 10% also produced the highest death rate with a value of 65.9%. The second highest death rate was observed at soil water contents of 0 and 20 % (both produced a 53% mortality rate), which was followed by 30 and 40%.

Overall QRD microwave system can be used for practical application to control soil organism soil, sterilizations, etc. Application of QRD microwave to control soil nematode needs to consider the character of soil, and soil water content. This application appears to be easy, rapid, low cost, and effective to control nematode on soil, although it is still difficult to apply in large-scale. Treatment with QRD microwave is more effective than the general microwave, and might be used to removed nematode in small amount of potting soil.

ACKNOWLEDGEMENTS

This research was supported by the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea. We would like to give our sincere thanks to the Kyungpook National University for the donation for this experiment.

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