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Integration of Soil Solarization with Chemical and Biological Control of Stem Rot Disease of Jerusalem Artichoke

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Soil solarization was reported that effective method to control stem rot disease caused by *Sclerotium rolfsii*, thereby the aim of this study was to improve soil solarization method by combining of biocontrol and chemical control. The experimental was done in the laboratory, pots, greenhouse and micro plot. The effect of high temperatures (45°C, 50°C and 60°C) on the survival of *S. rolfsii* sclerotia were conducted in the laboratory. At 45 °C, sclerotia treated for 6 hours showed germination of 36%. However, treated at 50°C and 60°C for 1 hours, the sclerotia could not germinate. The effect of soil solarization on sclerotial survival of *S. rolfsii* was carried under green-house and small plot conditions. The sclerotia were placed deeply under 2 soil levels of 5 and 10 cm and evaluated weekly on germination rate. After 1 week of soil solarization, sclerotia germinated 7.2% at 5 cm depth and 0% after 2 weeks. At 10 cm depth, sclerotia were could not germinate after 1 week of soil solarization. For small plot experiment of soil solarization, the germinated of sclerotia at 5 cm depth was 0% after 3 weeks of soil solarization while 35.2% germination for non solarization. Integrated control of stem rot using fungicide, biocontrol agents and soil solarization was conducted under green-house condition. The result was clearly that soil solarization alone or in combination with others were effective methods against the disease with disease index 0. Without soil solarization, *T. harzianium* T9 with carboxin caused low disease index of 0.2. The integrated control of stem rot was also undertaken in small plot (1.5×1.5m) experiment with comparison on disease incident and tuber yield of *H. tuberrosus*. Using soil solarization in combination with *T. harzianium* T9 and *Glomus clarum* was the best method that reduced disease with index of 0.33 and provided high tuber yield of 1.69 kg/m².

Keywords: *Helianthus tuberrosus*, *Sclerotium rolfsii*, *Trichoderma harzianum*, *Bacillus firmus*, carboxin.

Jerusalem artichoke (*Helianthus tuberosus* L.) is an inulin containing crop with a great potential for functional food products, bio-ethanol production and animal feed additive[1,2]. Jerusalem artichoke can serve human need as food, feed and fuel[3,4,5]. Maximum tuber yield can be up to 90 t/ha and carbohydrates yield can be as high as 5–14 t/ha. Inulin of Jerusalem artichoke tubers is beneficial to health as it can reduce the risk for diabetes or obesity[6]. As a tuber crop that produces carbohydrate, Jerusalem artichoke is a candidate for bio-ethanol production[7]. However, stem rot disease caused by *Sclerotium rolfsii* is a serious problem to Jerusalem artichoke production in temperate[8] and tropical regions[9].

Initial symptoms of Jerusalem artichoke stem rot consisted of wilting of new shoots and leaves followed by browning and collapse of all

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foliages. Crown and lower stem tissues were colonized internally and externally by white, cottony mycelium. Tan, spherical sclerotia that measured approximately 1 mm in diameter formed on surfaces of the affected crowns and stems[8].

Carboxin can be used as a fungicide and it is effective in controlling *S. rolfsii* on peanut and other vegetable crops[10]. *Trichoderma harzianum* was effectively used as a biocontrol agent against *S. rolfsii* under both green house and field conditions[11].Soil solarization is a nonchemical disease management technology for controlling weeds, nematodes and soilborne pathogens and the application of soil solarization is compatible with chemical and biological controls of soilborne disease[12,13].

Stem rot disease is rather new for Jerusalem artichoke grown in the tropics, and the reliable and cost-effective disease control is not available. For more effective control of stem rot disease in Jerusalem artichoke, integrated management control should be verified. The objective of this study was to verify the use of integrated management control of stem rot disease in Jerusalem artichoke including biological control, chemical control and soil solarization under greenhouse and small plot. The information obtained in this study will be used as basic knowledge for stem rot control under field conditions.

MATERIALS AND METHODS

Four experiments were conducted to investigate integrated management control of stem rot disease in Jerusalem artichoke. The experiments consisted of the study of heat tolerance of *S. rolfsii* and the effect of heat exposure time, the effect of mulching on persistence of *S. rolfsii* in clay pots, the effect of mulching on persistence of *S. rolfsii* in small plots under field conditions and integrated management control of *S. rolfsii* including soil mulching, chemical control and biological control.

Preparation of plant materials and inoculum

The isolates of *S. rolfsii* were collected from Jerusalem artichoke grown at the Agricultural Exhibition Center of Khon Kaen University. Tuber samples showing disease symptoms were incubated in moist chamber for 7 days until the fungus developed sclerotia. The sclerotia were maintained on potato dextrose agar (PDA) at room temperature for 5 days.

Jerusalem artichoke varieties were incubated in moist coconut coir dust for germination for a week and then transferred to plastic trays for a week until the seedlings had two leaves. The seedlings were later transferred to pots with 6 inches in diameter containing sterilized soil. There were five pots for each sampling unit with one plant in the pots.

S. rolfsii was maintained on potato dextrose agar (PDA) medium in Petri dishes and stored in test tube slants. The isolate was later transferred to autoclaved sorghum seeds in polyethylene bags containing 10 g of seeds per bag. After two weeks of incubation, the inoculum was ready for use.

Heat tolerance of *S. rolfsii*

Twenty five sclerotia of the *S. rolfsii* isolate were loaded in micro tubes and stored in a water bath at 45, 50 and 55 °C for 0, 1, 2, 3, 4, 5 and 6 hours. Temperatures were treated as different experiments and 7 heat exposure times were considered as treatments, which were arranged in a completely randomized design with five replications. After exposure to heat, the sclerotia were cultured on PDA in Petri dishes at room temperature (30±5 °C) for 24 hours. The data were recorded for germination percentage.

Effect of temperature on sclerotium germination in clay pots

One hundred sclerotia were loaded into each micro tube. The micro tubes were embedded in clay pots with 20 cm in diameter containing sterilized soil at 5 cm and 10 cm from surface. The pots were watered, covered with a transparent plastic sheet with thickness of 0.08 mm and wrapped the pots tightly with strings to increase soil temperature and promote growth of the fungus. Four treatments including no-mulching, mulching with dry soil, mulching with wet soil and room temperature (30±5 °C) control were arranged in a completely randomized design with three replications. Therefore, there were 18 tubes for each replications in which three tubes were sampled weekly. The pots were exposed to the sun for six weeks from July 14 to September 27, 2012.

The control treatment was stored in an opaque box at room temperature for 1, 2, 3, 4, 5 and 6 weeks. Daily temperatures were recorded at at 3

times 9.00 pm 1.00 am and 4.00 am at soil depths of 5 cm and 10 cm from the soil surface.

Every week, the sclerotia were placed on PDA in petri dishes (25 sclerotia/plate) and incubation at room temperature (30±5 °C) for 24 hours, and the data were recorded for germination percentage.

Soil solarization on survival of *S. rolfsii* in small plots

One hundred sclerotia were loaded into each micro tube. The loaded micro tubes were embedded in the small plots of 1x1.5 m at 5 cm and 10 cm from surface. The treatments consisting of mulching, no mulching and room temperature were arranged in a completely randomized design with three replications. Mulching was carried out during 14 July to 4 September, 2012. The plots were supplied with water to provide sufficient moisture and covered with transparent plastic sheet with the thickness of 0.08 mm. Soil temperatures were recorded daily at 9.00 am, 1.00 pm and 4.00 pm at 5 cm and 10 cm from soil surface. Sclerotium samples (three micro tubes for each replications) were taken weekly for germination test on PDA in Petri dishes (25 sclerotia/plate) and incubated at room temperature (30±5 °C) for 24 hours. The data were recorded for germination percentage.

Integrated management control

Integrated management control of *S. rolfsii* consisting of chemical control (the use of carboxin), soil mulching (transparent plastic sheet) and biological control (the use of *Trichoderma harzianum* T9 and Arbuscular mycorrhizal fungi (AMF) *Bacillus firmus* BSR 032) was investigated in Jerusalem artichoke. The combinations of the disease management methods were compared in this study.

Preparation of plant materials

JA38 variety of Jerusalem artichoke susceptible to stem rot disease was used in this study. The tubers were cut into small pieces, incubated in moist coconut coir dust for germination for a week and then grown in plastic trays for a week until the seedlings had two leaves. The single seedlings were later transferred to pots with 6 inches in diameter containing sterilized soil.

Preparation of *Trichoderma harzianum* T9 inoculum

Spore suspension of *T. harzianum* T9 was used for preparation of inoculum. The isolate was cultured on PDA incubated at room temperature (30±5 °C) for 5 days and the spore was washed spore from colony surface using sterile distilled water. Washed spores were counted by haemacytometer and diluted to obtain the concentration of 10^8 spores/ml.

Preparation of *Bacillus firmus* BSR 032 inoculum

Isolate BSR 032 of *B. firmus* kindly provided by the Department of Plant Pathology, Khon Kaen University, Khon Kaen, Thailand, was used in this study. The isolate was cultured on nutrient agar (NA) and incubated at room temperature (30±5 °C) for 48 hours. After incubation, colonies of *B. firmus* BSR 032 from NA in petri dishes were cut using a 0.5 cm diameter cork borer and transferred to nutrient broth (NB). After 48 hours of incubation, the OD suspension was fine to 0.1 at wave length 600 nm (1.62×10^9cfu/ml).

Treatments consisted of chemical control (carboxin), biological control (BSR032 and *T. harzianum* T9) and soil solarization (mulching), and the treatment combinations including Uninoculated control, Inoculated *S. rolfsii* control, Carboxin**,** *T. harzianum* T9 + carboxin, *B. firmus* BSR032 + carboxin, *T. harzianum* T9 + *B. firmus* BSR032, *T. harzianum* T9, *B. firmus* BSR032, Mulching, Mulching+*T. harzianum* T9, Mulching + *T. harzianum* T9+carboxin, Mulching+*T. harzianum* T9+*B. firmus* BSR032, mulching + BSR032**,** mulching + *B. firmus* BSR032+carboxin, mulching + carboxin, mulching+ *B. firmus* BSR032+*T. harzianum* T9+carboxin, there were 16 treatments totally. The treatments were arranged in a completely randomized design with five replications in greenhouse.

Sterilized soil was mixed with *S. rolfsii* (culture on sorghum grains) at the rate of 10 g inoculum per 1 kg soil. For the treatments with soil mulching, the soil in the pots was watered to obtain soil moisture at field capacity and the pots were covered with transparent plastic sheet with the thickness of 0.08 mm. The pots was wrapped with plastic sheet with strings. Most treatments except un-treated control and *S. rolfsii* treated control were exposed to the sun for six weeks. The data for soil temperature were recorded at soil depths of 5 cm and 10 cm at 9.00 am, 1.00 pm and 4.00 pm during May 23rd to July 4th, 2012. After six weeks, JA38 was treated with the aforementioned treatments.

Carboxin was applied to the crop at the concentration of 10 g per 20 liters of water and the amount of 20 ml/pot at two days after transplanting. After soil solarization Jerusalem artichoke were planted. Carboxin was applied to plant at the concentration of 10 g per 20 liters of water and the amount of 20 ml/pot around the crown of the Jerusalem artichoke at two days after transplanting. But the treatment that combined with biocontrol agent was applied after transplanting for 5 days. *T. harzianum* T9 and *B. firmus* BSR032 were applied to the crop at the concentration of 10^8 spores/ml and $1.62x10^9$cfu/ml, respectively. Suspension was applied on pots around the crowns of the Jerusalem artichoke at the amount of 20 ml/pots at two days after transplanting. In treatment 4, 5, 14 and 16, 20 ml of carboxin was applied on pots around the crown of the Jerusalem artichoke after transplanting for 5 days. At 20 days after transplanting, the crop was assessed for disease reactions using six levels of disease score including 0 = no symptom, 1 = small necrosis, 2 = small brown lesion, 3 = brown lesion, 4 = large lesion and plant wilt and 5 = sclerotia abundant and plant dead. At 30 days after transplanting, the data were recorded for plant height and shoot fresh weight. The samples were further oven-dried at 80 ºC for 12 hours and shoot dry weight was determined.

Integrated control of stem rot disease of Jerusalem artichoke in small plots

Five treatments including *S. rolfsii*, mulching+carboxin+*S. rolfsii*, mulching+*T. harzianum* T9+*S. rolfsii*, mulching+*G. clarum*+*T. harzianum* T9+*S. rolfsii* and mulching+*S. rolfsii* were arranged in a randomized complete block design with three replications. The plot size was 1.5×1 m.

Preparation of arbuscular mycorrhizal fungi (AMF)

AMF (*Glomus clarum* KKURA0305)-infested soil (10 spores g^{-1} soil) was obtained from the KKU Department of Microbiology. The AMF spores were multiplied by a pot culture technique using maize as a host plant[14]. AMF spores were inoculated on surface-sterilized maize seeds (10% sodium hypochlorite for 30 min) which were sown in plastic pots (30 cm diameter) containing twice-sterilized sandy loam soil; the Roi-et soils eries (Re; fine-loamy, mixed, subactive, isohyperthermic Aeric Kandiaquults) obtained from local farm fields.

The plants were grown in a greenhouse (30–35 °C) with a transparent plastic roof and open sides. Water was applied via saucers until about 2 weeks after planting in order to prevent leaking of *G. clarum* spores while watering, and after that applying on the soil surface was done normally. No fertilizer or any chemical was applied to the soil.

At 90 days after planting maize in the pot culture, the plants were cut at above the soil surface and the soil was allowed to dry out in the pot. After drying, the soil was crushed by hand and used as inoculums for the evaluation described in the sub-heading 2.5. Total number of AMF spores was measured by a sucrose centrifugation method to evaluate total among of spore and ensure that the produced spores were of *G. clarum*[15]. The percentage of root colonization was also determined by the method described by Trouvelot *et al*[16] after clarifying and staining of root by acetic glycerin solution[17]. After measuring of AMF on pot inoculum production, sandy loam containing spores of *G. clarum* was ready to use as a potting medium amendment.

Preparation of *Trichoderma harzianum* T9 inoculum

Isolate T9 of *T. harzianum* obtained from the Department of Plant Pathology, Khon Kaen University (KKU), Khon Kaen, Thailand, was used in this study. The isolate was cultured on potato dextrose agar (PDA) and incubated at room temperature (30±5 °C). After 3 days of incubation, mycelium of *T. harzianum* T9 from PDA in Petri dishes was cut using a 0.5-cm-diameter cork borer and transferred to sterilized sorghum grains in plastic bag containing 500 g of steam-sterilized sorghum grains. The sorghum grains in plastic bag were regularly shaken to facilitate thorough colonization by the fungus. After 10 days of incubation, the inoculum was ready to use.

***S. rolfsii* was cultured on to sorghum medium described in the above experiment**

S. rolfsii was inoculated into the plots on the soil surface at the rate of 1 kg/7.5 m^2, and the sorghum inoculum was mixed into the soil using a rake. Water was supplied to the plots until the plots were wet, and, for mulching treatments, the plots were covered with transparent plastic sheet. Bricks were placed on the edges of the plastic sheet, and the plots were exposed to the sun for six weeks.

Four seedlings of Jerusalem artichoke were transplanted into the plots with a spacing of 50×50 cm. For the treatment with *G. clarum*, *G. clarum* of 25 g/seedling (100 spores/plant) was mixed intopotting medium at the bottom of the hills prior to planting. For the treatment with *T. harzianum* T9, *T. harzianum* T9 at the rate of 1 kg/ 7.5 m^2 was inoculated into the soil by spreading the inoculum on the soil surface. For the treatment with carboxin, carboxin at the concentration of 10 g/20 liters of water and the rate of 10 liters/7.5 m^2 was applied on the soil surface. At 1 week after transplanting, the data were recorded for number of dead plant and the tubers were harvested at 120 days after transplanting.

Data analysis

The data for all experiments and parameters were analyzed statistically according to the experimental design for each experiment. Means were separated by Duncan's multiple range test at 0.05 probability level using the SAS program.

RESULTS AND DISCUSSION

Heat tolerance of *S. rolfsii*

Germination percentage of *S. rolfsii* reduced with time and high temperature (Table 1). At 45ºC, significant reduction in germination percentage was observed at five hours of exposure (42.5%) to heat and significant reduction continued to six hours of exposure (36%). At 50 ºC, significant reduction in germination percentage was observed as early as one hour of heat exposure (35.3%), and germination percentage was 0% at two hours of exposure until six hours of exposure. At 55 ºC, sclerotia of *S. rolfsii* did not germinate in all exposure times although the exposure time was 1 hour (Table 1).

Soil solarization in clay pots

The temperature of soil surface in a depth of 5 cm and 10 cm at 9:00 am, 1:00 pm and 4:00 pm were changed in both of depth when cover with transparent plastic and in control treatment that is not covered by plastic soil temperatures in clay pots lower than the outside temperature. Even if temperatures in dry soil and wet soil are close to outside temperature. At 1:00 pm temperatures in soil in the pots were higher than outside 6 °C, in wet soil has highest temperature (40 °C). On 4:00 pm the temperature inside the pot covered with a clear plastic was highest, the highest temperature in wet soil at a depth of 5 cm and 10 cm is 42 °C and 43 °C respectively, in dry soil has a maximum temperature of 41 °C and 42 °C. In the pot not covered the soil temperature at a depth of 5 cm was 39 °C and 37 °C in 10 cm of depth**.**

Exposure of *S. rolfsii* to the sun at 5 cm below the soil surface significantly (0.8-56.8 %) reduced germination of *S. rolfsii* (0.2-14.2) compared to room temperature control (100%) and germination reduced with time (Table 2). The reduction in germination of *S. rolfsii* occurred for no mulching (56.8 %), dry soil mulching (1.8) and wet soil mulching (0.8%) at one week after the initiation of exposure. It is important to note here that mulching treatments both in dry and wet soil greatly reduced germination.

At two week after initiation of mulching, no mulching had 25.6 % of germination and room temperature had 99.2 %, whereas *S. rolfsii* for mulching treatments both in wet and dry soils did not germinate. At three weeks and after three weeks, *S. rolfsii* that was exposed to the sun for both mulching and no mulching treatments did not germinate, whereas 95.2-96% of sclerotial germination under room temperature were detected.

At the depth of 10 cm below the soil surface, no mulching slowly reduced germination of *S. rolfsii*, and no significant difference between room temperature control and no mulching was observed before six weeks of mulching (Table 3). Mulching more rapidly reduce germination of *S.*

Table 1. The percentage of germination of *S. rolfsii* sclerotia at temperature 45, 50 and 60ºC for 0-6 hours

Time	Germination(%)		
(hours)	45 ˚C	50 °C	55 °C
0	100 a	100[1/] a	100
1	100 a	35.3 b	0
2	99.2 a	0 c	0
3	97.6 a	0 c	0
4	94.4 a	0 c	0
5	42.5 b	0 c	0
6	36 c	0 c	0
C.V. (%)	9.76	10.30	-

Means in the same column with the same letter are not significantly different (P≤0.05, DMRT).

[1/]The data were transformed by $Y = \sqrt{X + 0.5)}$

rolfsii, and significant difference between mulching and room temperature control was observed as early as four weeks of mulching, whereas room temperature control did not reduce germination of *S. rolfsii*.

Efficacy of soil solarization to germination of *S. rolfsii* sclerotia in small plots

The temperature of 3 period, 9.00 am 1.00 pm and 4.00 pm between 3 November 2012-17 December 2012, found that in the morning and afternoon (9:00 am and 1.00 pm), the temperature of the soil in a depth of 5 cm from the soil surface is higher than 10 cm, but in the evening (4.00 pm) the temperature at a depth of 10 cm higher than the soil temperature in morning and afternoon and higher than the plot is not mulched with clear plastic. The soil temperature at a depth of 5 cm maximum measured in plots covered plastic is 41 °C temperature minimum is 16 °C at a depth of 10 cm temperature up to a 41 °C temperature and minimum is 15 °C. The soil depth of 10 cm in no mulching plot maximum temperature is 39 °C minimum temperature is 15°C (data not shown).

In micro-plot experiment at 5 cm below soil surface, mulching and no mulching reduced germination of *S. rolfsii* (Table 4). However, significant reduction in germination was observed as early as two weeks for mulching treatment and

Table 2. Percentage of *S. rolfsii* sclerotia germination after buried in clay pot mulching by covered with transparent plastic at a depth of 5 cm from soil surface

Treatment	Percentage of sclerotia germination (%)					
	week 1	week 2	week 3	week 4	week 5	week 6
No mulching	56.8 b	25.6 b	0 b	0 b	0 b	0 b
Mulching (dry soil)	7.2 c	0 c	0 b	0 b	0 b	0 b
Mulching (wet soil)	0.8 c	0 c	0 b	0 b	0 b	0 b
Room temp.(30+5°C)	100 a	99.2 a	95.2 a	96 a	95.2 a	95.2 a
C.V. (%)	22.3	11.1	10.51	8.33	7.03	7.03

Means in the same column with the same letter are not significantly different (P > 0.05,DMRT)

Table 3. Percentage of *S. rolfsii* sclerotia germination after buried in clay pot mulching by covered with transparent plastic at a depth of 10 cm from soil surface

Treatment	Percentage of sclerotia germination(%)					
	week 1	week 2	week 3	week 4	week 5	week 6
No mulching	100 a	100 a	100 a	24.4 a	82.4 a	74.4 b
Mulching	98.4 a	95.2 a	82.4 a	71.2 b	40.8 b	16 c
Room temperature (30+5 ºC)	100 a	100 a	100 a	100 a	96 a	96 a
C.V. (%)	7.3	5.47	3.86	8.9	12.03	6.5

Means in the same column with the same letter are not significantly different (P > 0.05,DMRT)

Table 4. Percentage of germination rate of *S. rolfsiisclerotia* after soil solarization in micro plot at a depth of 5 cm.from soil surface

Treatment	Percentage of sclerotia germination(%)					
	week 1	week 2	week 3	week 4	week 5	week 6
No mulching	100 a	98.4 a	72 b	58.4 b	44.4 b	35.2 b
Mulching	100 a	80 b	33.6 c	0 c	0 c	0 c
Room temperature (30+5 ºC)	100 a	100 a	96 a	96 a	100 a	96 a
C.V. (%)	2.13	15.8	11.4	9.36	11.2	2.47

Means in the same column with the same letter are not significantly different (P > 0.05,DMRT)

three weeks for no germination of *S. rolfsii*, but mulching reduced germination faster than did no mulching from 98.4 to 16 % (Table 5). The difference between no mulching and room temperature control was significant at six weeks after exposure of the soil to the sun and the germination was still high (96%), whereas the difference between mulching and room temperature was as early as four weeks

Table 5. Percentage of germination rate of *S. rolfsiisclerotia* after soil solarization in micro plot at a depth of 10 cm.from soil surface

Treatment	Percentage of sclerotia germination(%)					
	week 1	week 2	week 3	week 4	week 5	week 6
No mulching	100 a	100 a	100 a	97.6 a	82.4 a	74.4 b
Mulching	98.4 a	95.2 a	82.4 a	71.2 b	40.8 b	16 c
Room temperature	100 a	100 a	100 a	100 a	96 a	96 a
C.V. (%)	7.3	5.47	3.86	8.9	12.03	6.5

Means in the same column with the same letter are not significantly different (P > 0.05, DMRT)

Table 6. Disease score of stem rot of Jerusalem artichoke in green house experiment

Treatment	Disease score[1/]
Uninoculated control	0.0 f
Inoculated *S. rolfsii*control	5.0 a
Carboxin	0.8cd
T. harzianum T9+carboxin	0.2ef
B. firmus BSR032+carboxin	0.6 de
T. harzianum T9+ *B. firmus* BSR032	1.2 c
T. harzianum T9	0.4def
B. firmus BSR032	1.8b
Mulching	0.0 f
Mulching+*T. harzianum* T9	0.0 f
Mulching+*T. harzianum* T9+carboxin	0.0 f
Mulching+*T. harzianum* T9+*B. firmus* BSR032	0.0 f
Mulching+BSR032	0.0 f
mulching+*B. firmus* BSR032+carboxin	0.0 f
mulching+carboxin	0.0 f
mulching+*B. firmus* BSR032+*T. harzianum* T9+carboxin	0.0 f
C.V.(%)	31.99

Means in the same column with the same letter are not significantly different (P > 0.05, DMRT) [1/]Di=disease score 0 = no symptom, 1 = small necrosis, 2 = small brown lesion, 3 = brown lesion 4 = large lesion, plant wilt, 5 = sclerotia abundant, plant dead

Table 7. Average stem rot dead plant and yield of tuber of Jerusalem artichoke in micro plot experiment

Treatment[2/]	yield (kg)/ kg/m^2	Dead plant/plot
S. rolfsii-inoculated control	1.16 b	9.33 a
Mulching+*S. rolfsii*	1.47 a	8.33 a
Mulching+Carboxin+*S. rolfsii*	1.46 a	0.66 b
Mulching+*Trichoderma harzianum* T9+*S. rolfsii*	1.57 a	1.67 b
Mulching+*Trichoderma harzianum* T9+*Glomus clarum*+*S. rolfsii*	1.69 a	0.33 b
C.V.(%)	10.54	27.73

Means in the same column with the same letter are not significantly different (P > 0.05, DMRT) [2/]S S= *S. rolfsii*, T =*Trichoderma harzianum* T9, C = Carboxin, So = soil solarization, M = *Glomus clarum*

after initiation of the treatments and the difference continued until six weeks. However, at six weeks, the sclerotia of *S. rolfsii* still germinated although the germination was very low (16%). Similar to germination at 5 cm below soil surface, room temperature control did not significantly reduce germination of *S. rolfsii*.

Integrated management control in greenhouse

Integrated management control methods were evaluated for disease score under greenhouse condition. Significant differences (P≤0.05) among disease control treatments were observed for disease score, and all disease control treatments significantly (P≤0.05) reduced disease score, ranging from 1.8 to 0.0 compared to *S. rolfsii*-inoculated control (5.0) (Table 6). All integrated management control treatments with mulching did not show disease symptoms and had disease score rating of 0 similar to that of un-inoculated control.

Integrated management control in micro plots

All mulching treatments had significantly (P≤0.05) higher tuber yield (ranging from 1.46 to 1.69 kg/m^2) than did *S. rolfsii*-inoculated control (1.6 kg/m^2), and the highest tuber yield was obtained from mulching+*Trichoderma harzianum* T9+*Glomus clarum*+*S. rolfsi*(1.69 kg/m^2), which was not significantly different from other mulching treatments (Table 7). Most mulching treatments had significantly (P≤0.05) lower dead plants (ranging from 0.33 to 1.67 plants/plot) than did *S. rolfsii*-inoculated control (9.33 plants/plot) except for mulching+*S. rolfsii* (8.33 plants/plot).

CONCLUSIONS

S. rolfsii had 100% germination at 45 °C for 1 hour, and the germination percentage was reduced with the increase in soil temperature from 45 ° C to 50 and 60 °C, respectively. *S. rolfsii* did not germinate at 60 ° C.

Under micro plot experiment, exposure to the sun with soil mulching caused total death of s. rolfsii at 5 cm below the soil surface at four weeks of exposure time, and, at 10 cm below the soil surface, the exposure time took six weeks to achieve total death of *S. rolfsii*. Longer exposure time is required under micro plot condition because of better heat transfer compared to pot experiment. The results indicated that low temperature of 42 ° C will be effective in controlling *S. rolfsii* if exposure time is longer.

Integrated management control of *S. rolfsii* consisting of the use of carboxin, *T. harzianium* T9, *B. firmus* BSR032 and soil mulching in Jerusalem artichoke indicated that soil mulching alone, soil mulching+carboxin, soil mulching+*T. harzianium* T9 and soil mulching+*B. firmus* BSR032 were effective in controlling germination of *S. rolfsii* as all sclertotia did not germinate. Among the treatments without soil mulching, carboxin alone, carboxin+*T. harzianium* T9 and carboxin+*B. firmus* BSR032 were not significantly different for germination of S. rolfsii. For biological control, applications of *T. harzianium* T9 alone and in combination with *B. firmus* BSR032 were not significantly different, whereas application of *T. harzianium* T9 alone was better than application of *B. firmus* BSR032 alone. Soil solarization plus either carboxin or *T. harzianum* T9 alone or in combination with *G. clarum* are effective methods for control stem rot of Jerusalem artichoke.

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