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Development of a Biocontrol Agent Using *Monacrosporium thaumasium* to Control a Root Knot Nematode, *Meloidogyne incognita*

Ye Hoon Choi, Keun Ki Kim[1], Hong Joo Son[1], Hae Soo Shin[2] and Hyean Cheal Park[1]*

Il Shin Chemical Co Ltd., Yangsan, Kyungnam, 626-110, Korea
[1]*School of Applied Life Science, Pusan National University, Miryang, Kyungnam 627-702, Korea*
[2]*Soil Science and Plant Nutrition, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Hwy, Crawley WA 6009*

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The created microbial pesticide was used to examine its effects using the inside-pot test method. The selected microbial pesticide KBC3017 particle pesticide manufactured by using *Monacrosporium thaumasium* was used in the farm-house outdoor test to find the optimum consistency and its effects. The more amount used, the better effect it showed. However, the optimum consistency was 2% and the KBC3017 particle pesticide for which the diatomite and raw jade powder were used as an increaser, when used 2% level of the total amount of soil, showed 71% effect on nematode prevention. The root and the stem of crops were better compared to those without any pesticide used.

Key words : Nematodes, fungi, biocontrol, *Monacrosporium thaumasium*, *Meloidogyne incognita*

Introduction

Plant parasitic nematodes (PPNs) cause serious damage to plant growth and yield in a wide range of crops. These parasitic nematodes attack every part of plants including roots, stems, leaves, fruits and seeds [5,7,10,12]. The most widespread and economically important PPN pathogen in the world is root knot nematodes (*Meloidogyne* sp.). *Meloidogyne* sp. produces 300-1000 eggs per female during its life. This genus spends most of their life time in the roots, except for the second stage of juveniles that hatch from eggs and move through soil to host roots. The PPNs in this genus invade into areas near the growing point of a crop's root and grow by absorbing nutrients of inside the plant's root, and during this process, because of the hormones that these nematodes secretes, the shape of the crop's root turns into lump shape. In Korea, four types of *Meloidogyne* spp. (root-knot nematodes), *M. arenaria, M. hapla, M. incognita,* and *M. javanica* are harming crops. *M. javanica* is more widespread than any other *Meloidogyne* species with high populations in warmer regions [3]. The symptoms of disease induced by these nematodes are the production of galls in the root and resulted leaf wilt with water stress. Therefore, it is difficult to find out how much portion of the yield loss is caused by PPNs. According to nematologists [1,2,6,8,9,11,12], there is 10% yield loss of horticultural crops by PPNs but it would be more than that. Soil effects to PPN life cycle, especially for reproduction, and, therefore modification of the soil environments could be a very easy and affective way to control PPNs. Different plant growth stage cause different level of PPN damage because the level of resistance to PPNs varies depending on different causes the growth stages. Plants under the condition of water stress and lack of nutrients can be more seriously damaged by PPN. Above of all, it is necessary to examine plants, PPNs and soil environments before applying chemicals to control PPNs.

In this study, *M. incognita* (Sweet potato root nematode) was extracted from Sungju, Kyungbuk, Korea on August 2005, and examined its contagion. For biological prevention of the breeding and extermination of the nematode, 32 types of nematode predatory fungi were extracted and tested the predation abilities. Most fungi showed high extermination qualities in the *in vitro*. For further experiments, *Monacrosporium thaumasium* showing the highest extermination quality among the fungi was selected.

Materials and Methods

Isolation of *Meloidogyne incognita*

The nematodes were isolated from soil samples collected from watermelon fields at Sungju, Kyungbuk, Korea on August 2005. *Melodogyne incognita* was extracted by

***Corresponding author**
Tel : +82-55-350-5547, Fax : +82-55-350-5549
E-mail : hcpark@pusan.ac.kr

Whitehead tray method [13]. Fifty gram of soil was spread over the tray (157 mm× 208 mm) for the large scale soil extractions of the roots and soil samples, and maintained at room temperature for 2-3 days. Second stage of juveniles and other species of parasitic nematodes were collected using 38 μm and 26 μm sieves. The collected PPNs were stored in a cold room (4°C) before counting.

M. incognita was also collected from roots using sodium hypochlorite (NaCl) solution and whitehead tray method. Sodium hypochlorite solution was used to dissolve the gelatinous matrices of egg mass [5]. However, sodium hypochlorite method did not completely dissolve the gelatinous matrices of egg mass and it needed to collect all the root fragments on the Whitehead tray to extract the rest of *M. incognita* following hatch. After counting, collect female of *M. incognita* for identification. Female suspension were heated to a temperature of 55-60°C to kill, and then, cooled down at room temperature. Once suspension was cooled down, the females were fixed by formaldehyde solution (4%), and transferred to glycerol and to water for wash. It was transferred again and then anterior position of the body was dissected for examination.

Isolation of nematode predatory fungi

The fungi were collected, isolated and cultivated from soil samples from various cultivating fields. These samples were mixed well and spread soil over the media. After 15 days, single spores were transferred to the water agar medium. All the petri-dishes were sealed and stored in the growth chamber (25°C) for 14 days [12]. After each fungus was isolated, nematodes were inoculated in the centre of media for develop trapping organ to identified fungi species. Each isolated fungus was identified based on morphological characteristics under a stereoscope microscope.

Optimum cultivation conditions for the selected fungi

There are some media optimized for cultivation of select fungi nitrogen source, carbon source and trace element media. Nitrogen source media (yeast extract 0.1%), which was added with 2% of dextrose, sucrose, maltose, xylose, starch (soluble) and cellulose, were stored at 25°C for five days. Dry precipitates of culture fluid were measured after centrifuge at 10000 rpm for 20 minutes. Carbone source media (sucrose 2%), which were inoculated with 0.1% of corn steep liquor, malt extract, peptone, soybean meal, yeast extract and 0.03% of yeast nitrogen base (YNB), casamino acid, YBN + Casamino YBN + inorganic nitrogen were adjusted for pH 7, and stored at 25°C for 5 days. Trace element media were prepared by adding $MnSO_4$, $FeSO_4$, $ZnSO_4$, $CuSO_4$ and $COCl_2$ into sucrose 1%, yeast extract 0.1% and $NaNO_3$ 0.03% media. In addition, optimum temperature for growing fungi on PDB (potato dextrose broth) media is between 25°C and pH 5-10. After centrifuge at 10000 rpm for 20 minutes, dry precipitates of culture fluid were collected from the each media.

Formulation of biological nematocide using *Monacrosporium thaumasium*

For manufacturing biological nematocide using *M. thaumasium*, diatomite was appropriated as an inorganic increaser and raw jade powder for organic increaser, which were shown as the best increaser for the selected mold in the earlier experiment. After analysing the physicochemical disposition, the appropriate pH ranged between 5.9 and 6.4, and 97% of the particles was sized by 1mm. The created microbial nematocide was used to examine its control effects using the inside-pot test method, and to find out the consistency effect using the farm-house outdoor test.

Results and Discussion

M. incognita (Fig. 1) was isolated from soil samples and used in these experiments. The species had pear shape (female) and mean length of 652±25 μm and width of 442± 54 μm. The male head is sharp around stylet and mean length was 1090±104 μm. It was multiplied on tomato and the hatch rate was more than 97%. Soil samples were collected from an watermelon farm and two nematophagous fungi species were isolated (Fig. 2) from 32 soil samples. After 14 days, 10^4/g of these two fungi were produced on the media. These experiments were conducted in a glasshouse at room temperature with water agar media for each treatment. In this room temperature experiment, most of nematophagous fungi were reacted (> 91%) by *M. incognita* and killed by *M. thaumasium* (Fig. 3) in 10 days. *M. thaumasium* produced constructing rings to kill plant parasitic nematodes. Thus, among the isolates, *M. thaumasium* (KBC3017 isolate) was selected by the control rate showed as more than 90% to free living nematodes and root knot nematodes (RKN). Nematophagous fungi were known to be reacted to nematodes hormone, serum, earthworm & yeast extract, rainwater before storm, and etc. to produce

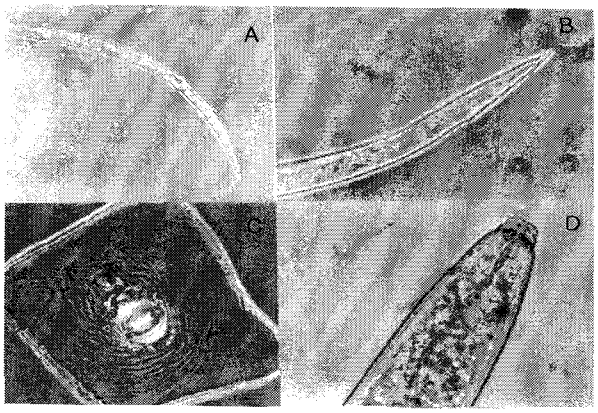

Fig. 1. Isolation and identification of nematode. A: *Meloidogyne* spp. J2, B: Tail of *Meloidogyne* spp. J2, C: Perinea pattern of *Meloidogyne incognita*, D: Head of *Meloidogyne incognita*

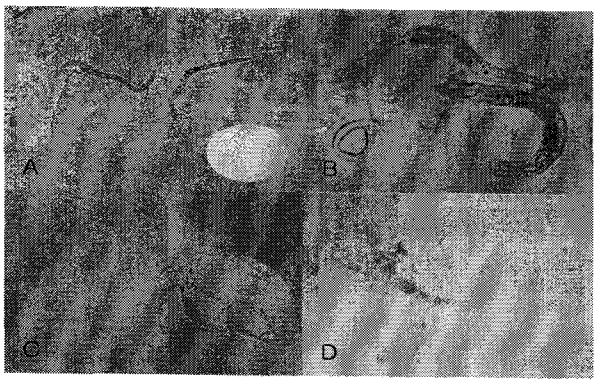

Fig. 2. Isolates of nematode predatory fungi. A and B: Predatory fungi, C: *Monacrosporium* sp., D: Nematode endoparasitic fungi

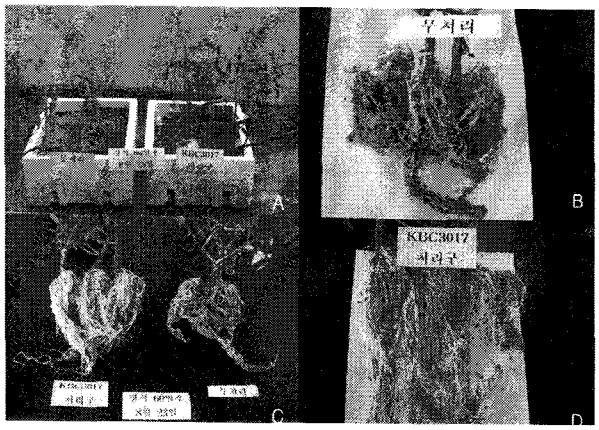

Fig. 3. Effects of the formulation of *Monacrosporium thaumasium* to root knot nematode, *Meloidogyne* sp. in *in vitro*.

trapping organ [1,2]. According to the results, the activity of KBC3017 was 100% in solid culture(+) and 89% in solid culture(-) (Table 1). Both cultures resulted in a smaller number of nematodes per 50g and egg mass per root than the control, especially in the solid culture(+). The number of eggs per root, root biomass and crop biomass of KBC3017 treatments was higher than for the control (Table 2). However, conidia and hypha treatment of *M. thaumasium* inoculum resulted in 47% less egg mass, although root and crop biomass were similar to the control. According to the comparison between KBC3017 and conidia & hypha treatment, KBC3017 treatment was better in relation to the growth of crop, and root and the number of egg per root (68%).

Table 1. Effect of the formulation type for the control of root knot nematode *in vitro*

Formulation Type	Solid culture	Activity (%)	No. of Nematodes per 50g of soil[1]	Egg mass per root (ea)[2]
M. thaumasium	+	100	215	25
	-	89	335	78
Control			603	87

[1]Estimated the number of nematodes per 50g of soil after the treatment of 10 days *in vitro* with 6 applications.
[2]Estimated the egg mass per root after the treatment of 60 days *in vitro* with 6 applications.

Table 2. Effect of treatment type for controlling root knot nematode in the green house experiment

Formulation Type	Treatment	No. of egg per root (% of reduction)	Root biomass (g)	Crop biomass (g)
M. thaumasium	KBC3017	40 (68)	10.0	18.4
	Conidia + hypha	65 (47)	5.7	7.4
Control		124	4.7	7.0

Fig. 4 and 5 show that the number of nematodes after spread a chemical (fosthiazate) and KBC3017 spray. The result showed that KBC3017 was better to control (81%) *M. incognita* than fosthiazate chemical control method (71%). This diagram also shows that the number of nematodes increased dramatically after 30 days.

The result of the field experiments was similar to the results from pot experiments. The biological control with KBC3017 was very effective to control *M. incognita* (Fig. 6).

The selected microbial pesticide, KBC3017 particle pesticide manufactured by using *M. thaumasium*, was tested *in vitro* and in an watermelon field to find the optimum consistency and its effects. The more amount used, the better effect it showed, but the optimum consistency was 2%.

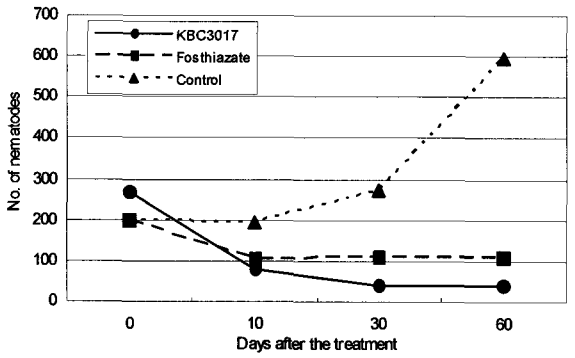

Fig. 4. Comparison of the control effects between the formulation and fosthiazate on the root knot nematodes, naturally occurred in an watermelon field.

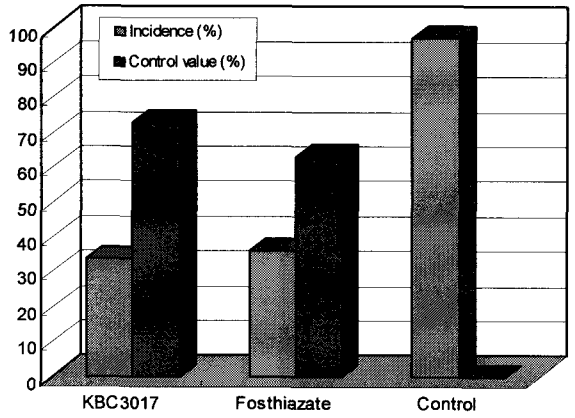

Fig. 5. Comparison of the effects on the incidence of nematodes(%) and the control value(%) between the formulation and fosthiazate in an watermelon field.

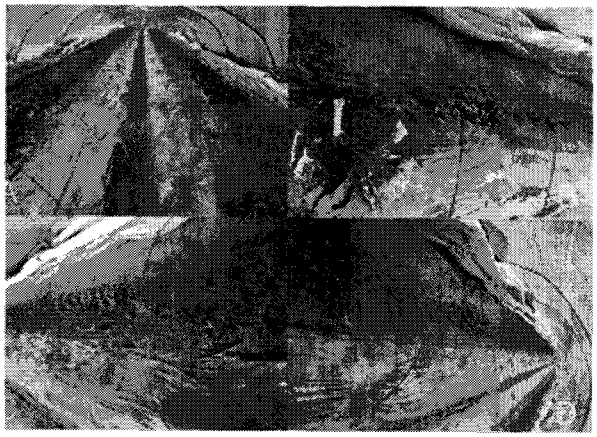

Fig. 6. Control effects of the formulation and a chemical nematocide in an watermelon field infected naturally by root knot nematodes. A and B: Control, C: Treatment of the formulation, D: Treatment of a chemical nematocide.

KBC3017 particle pesticide, for which the diatomite and raw jade powder were used as its increaser showed 71% of the control effect when used 2% level of the total amount of soil. Therefore, farmers should use a mixed or simultaneous combination of the microbial pesticides and chemical pesticides rather than chemical pesticides alone in order to optimize the resisting effects, and this results was very important economically and environmentally. Our present research on manufacturing microbial pesticides for *Meloidogyne* spp. prevention is intended to provide basic data for commercializing purpose.

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초록 : *Monacrosporium thaumasium*을 이용한 뿌리혹선충 (*Meloidogyne incognita*) 방제용 미생물제제의 개발

최예훈 · 김근기[1] · 손홍주[1] · 신해수[2] · 박현철[1]*

((주) 일신케미칼, [1]부산대학교 생명자원과학대학 생명응용과학부, [2]국립서부호주대학교 농업생명과학대학)

미생물 제제의 실내 pot 실험을 통한 효능 실험에서 선발된 *Monacrosporium thaumasium* 을 이용한 KBC3017 제제의 실외 포장 실험을 통해 최적의 구성과 효능 조건을 찾았다. 선발된 균주의 미생물제제 제조를 위해 첨가한 첨가제 중 무기증량제로 Diatomite와 유기증량제로 생옥분이 효과적이었으며, 처리량별 선충 밀도 억제효과는 처리량이 많을수록 효과가 높았으나, 적정 처리량은 2%의 diatomite와 옥가루가 포함된 KBC3017 제제를 이용하여 방제하였을 때 71%의 선충 방제 효과가 나타났다. 이 방제가는 대조구와 비교하였을 때 뿌리와 줄기에서 보다 좋은 효과가 있었다.