# Extraction of Bioactive Compounds from Luminescent Mushroom (*Neonothopanus nambi*) and its Effect on Root-Knot Nematode (*Meloidogyne incognita*)

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## Abstract

Dry mycelia and culture filtrates from 3 isolates (PW1, PW2 and KKU) of luminescent mushroom (*Neonothopanus nambi*) were extracted in order to obtain bioactive compounds. Extraction using dry mycelium derived bioactive compound powder more than using culture filtrate with extraction efficiency of 8.73-12.90%. The effect of bioactive compound was investigated on infectious larvae (J2) of root-knot nematode (*Meloidogyne incognita*) in laboratory. The result showed that bioactive compound at concentration 500 mg/l caused 100 % mortality of J2 in 1 min. Concentrations of 100 and 50 mg/l affected J2 causing significantly mortalities of 100 % in 30 min and 48 hr, respectively. Effect of this bioactive compound on J2 was also confirmed in screened house experiment and found that concentrations of 100 and 500 mg/l suppressed evidently J2 without root-knot symptom on tomato plants. Root-knot score 1 was detectable on tomato plants treated with the compound of 10 and 50 mg/l. However, it was significantly ($P<0.05$) from control treatment, which exhibited root-knot score 4. This study suggests the efficiency and potential of the application of bioactive compound from *N. nambi* for control root-knot nematode.

**Keywords:** Bioactive compound, extraction, luminescent mushroom, root-knot nematode, tomato

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## Introduction

It was reported that the luminescence mushroom was found in the Plant genetic Conservation Project under Royal Initiation by Her Royal Highness Princess Maha Chakri Sirindhorn at Kok Putaka, Wiang Kao District, Khon Kaen Province. (Saksirirat et al., 2003). This luminescent mushroom was similar to the oyster mushroom by morphological characters. The mushroom was poisonous and found into group, each of which consisted of 4-5 basidiocarps, which normally grew on logs or dead wood. During daytime, the mushroom had gills, white cap, and short stipe. During nighttime, especially during the dark night or in the dark room, the mushroom was luminous. It emits yellowish green light around the gills and stipe that visible within the far range around 10-20 meters. Initially, the study was classified by using nucleotide sequences from the internal transcribe spacer (ITS) of *rRNA* gene. It was compared to the GenBank database at that time. Later, the mushroom looked similar to the one found in Kok Putaka was found in the campus of Khon Kaen University. During daytime, the mushroom was white color. During nighttime, it was luminescent with green and yellow colors. The nucleotide sequence bases of ITS1-5.8S-ITS2 of *rRNA* gene was studied and compared to sequence data of GenBank database. It was revealed that the DNA sequences were the same as DNA sequences of a mushroom, *Omphalotus* sp., based on available data base at that time. Therefore, it was previously reported that was the luminescent mushroom in *Omphalotus* sp., found in Kok Putaka, Khon Kaen. (Saksirirat et al., 2001; 2004). Afterward, Bua-art (2007) studied on the identification of this luminescent mushroom found in Kok Putaka (isolates PW1 and PW2) and the mushroom found in Khon Kaen University campus (isolate KKU). Using the nucleotide sequences of ITS1-5.8S-ITS2 region of *rRNA* gene, it was found that all 3 isolates had the same base sequences as nucleotides of *Neonothopanus nambi* Speg. The identity was 94%, when comparing the appearance, color, and the morphological data from the experts in luminescence mushroom (Prof. Dr. Roy Watling, Kew Garden, Surley, United Kingdom, Dr. Matin Kirchmair, Institute of Microbiology, University of Innsbruck, Innsbruck, Austria and Dr. J.M. Monclavo, Centre for Biodiversity and Conservation Biology, Toronto, Canada; Personal contact). It was concluded that all 3 isolates of luminescent mushrooms were scientifically named as *Neonothopanus nambi* Speg.

In other countries, they studied the advantage and exploitation of luminescent mushroom. The bioactive compound from luminescent mushroom was used to biologically control plant diseases, especially to control root-knot nematode (*Meloidogyne incognita* Chitwood), which caused damage to the economic crops over 3,000 species in temperate and tropical areas. Engler et al. (1998) reported that secondary metabolite was isolated from culture filtrate of luminescent mushroom, *Omphalotus* olearius, in YMG medium. The mushroom *O. olearius* secreted a bioactive compound, omphalotin affecting the growth and development of the root-knot nematode (*M. incognita*). Later, Buchel et al. (1998), identified the structure of that bioactive compound from the luminescent mushroom *O. olearius* using Spectroscopy technique ($^{1}H$ and $^{13}C$ Nuclear Magnetic Resonance Spectroscopy, $^{1}H$ and $^{13}C$-NMR) and known that the bioactive compound affected the root-knot nematode was omphalotin. There were several derivatives of omphalotins, such as omphalotin A, B, C, and D. These bioactive compounds inhibited the nervous system and could be applied in controlling the root-knot nematode.

In Thailand, there was a study on the effectiveness of bioactive compound from culture filtrates of the 2 isolates, PW1, PW2 of luminescent mushroom, found in Kok Phutaka, Khon Kaen and another isolate found in Khon Kaen University (KKU). Culture filtrate from KKU isolate caused the mortality of root-knot nematode and reduced root-knot symptom in tomato 22.50% significantly different from no use of culture filtrate. This showed evidently that the luminescent mushroom can be used to control the root-knot nematode (Bua-art, 2003). The initial extraction and isolation of bioactive compound was done by using Thin Layer Chromatography (TLC). An interesting bioactive compound was found on several layers on TLC. Examples were the substances in pulvinic acid group, the differential of variegatic, or xerocomic acid. The bioactive compound with *Rf* value of 0.41 affected the highest mortality of J2 larvae of the root-knot nematode. In fact, PW1 isolate had the highest effect on the mortality of J2 at up to 100% (Bua-art, 2007). Nevertheless, the bioactive compound with *Rf* value of 0.41 has not been extracted the large amount, identified and determined the chemical property of this compound, as well as the quality of the poisonous property to the root-knot nematode. Therefore, the purpose of this research was to extract the bioactive compound from the luminescent mushroom *N. nambi* and to examine the effect of bioactive compound against root-knot nematode. This can be used and developed further to control the pest with a biological control. This was the beneficial way from the country's biological resources, to preserve the environment, as well as to reduce the usage of pesticides.

## Materials and Methods

### 1. The origin of the luminescence mushroom (*Neonothopanus nambi*)

The 3 isolates of luminescent mushroom (*N. nimbi*) were used. They were PW1 and PW2 isolates from Kok Phutaka, Wiang Kao District, Khon Kaen Province; and KKU isolate found in the area of Khon Kaen University. The mushroom hyphe were grown in potato dextrose agar (PDA) or in sterile sorghum grains. The mushroom was stored at 27 °C in order to be used conveniently in the study.

### 2. The extraction of the bioactive compounds from the luminescent mushroom (*Neonothopanus nambi*)

Three isolates of *N. nambi* were cultured in PDA at the temperature of 28 ± 2 °C for 7 days. Then, the mushroom was grown in liquid culture malt extract broth (MEB). The bioactive compound was extracted biologically. Two extraction methods were used, the extraction method 1 using culture filtrate, and extraction method 2 using dry mycelia as extraction materials. Cork borer with the radius of 0.8 centimeter was used to pierce agar with mycelium of luminescent mushroom. Three pieces of agar plugs were put into the flask with 50 milliliter of the liquid medium (MEB). For Type 2, 5 pieces of jelly were put into the flask with 50 milliliters of the liquid food (MEB). The mushroom culture was incubated in the dark room without shaking 2 hours of light per day at 25 °C for 30 days. The mycelia were filtered using Whatman filter paper No.1 to collect the culture filtrates. The collected mycelia were dried in the oven at 45 °C for 3 days for further extraction.

The culture filtrate was extracted using ethyl acetate (EtOAc) 3 times. The extracted solution was collected and evaporated under pressure using the rotary evaporator. The crude ethyl acetate (EtOAc) was obtained. The crude EtOAc was weighted and stored in the container. The weight of dry mycelia from culture medium was recorded. The dry mycelia was grounded using the blender. The extraction for bioactive compound was performed in the same manner as described previously. The crude extract from the dry mycelia and the culture filtrate were tested to determine the effect of the bioactive compound on the infectious larvae (J2) of root-knot nematode (*M. incognita*). To crystallize the bioactive compound, the crude ethyl acetate was separated using column chromatography. Silica gel was used as stationary phase and EtOAc:hexane as solvent. The concentration of EtOAc was continuously increased starting from 70% EtOAc:hexane. The solution was collected together with the solvent as fraction: 100 milliliters were collected each time. Pole strength of the solvent was increased up to 100% EtOAc, and then stopped. Each fraction was examined using thin layer chromatography (TLC) in order to combine the same fraction together. The fractions with spots on TLC were isolated for purification using chromatography and crystallization until the extracted solution was crystallized into powder.

**3. Test of bioactive compound from luminescent mushroom (*Neonothopanus nambi*) with root-knot nematode (*Meloidogyne incognita*)**

***Laboratory Experiment***

J2 larvae of the root-knot nematode were prepared by adopting the process from Somasri (2001). Tomato roots with the root-knot symptom were cleaned. Clearly brown egg mass were collected using the forceps. They were stored in PVC pipe with 3 centimeters radius and 1 centimeter height. The pipe was sealed with a fine net nylon cloth. It was immersed in 0.5 % of sodium hypochlorite (NaOCl) for 3 minutes. Afterward, the pipe was rinsed with sterilized distilled water 3 times, for 5 minutes each time. The pipe was placed on the cone with 15-centimeters width. The cone had the rubber tube connected at the end with clamp. The water was poured just to the end of the pipe. After 2 days, J2 would hatch out of the eggs. They congregated at the end of the rubber tube ready to be tested. The poeder of bioactive compound from luminescence mushroom was dissolved in dimethylsulfoxid (DMSO) at 50% concentration with sterilized distilled water. Bioactive compound was prepared at concentration of 0, 10, 50, 100, and 500 milligram per liter (mg/l) for further test.

The effectiveness of bioactive compound from luminescent mushroom to the root-knot nematode (*M. incognita*) was tested in the laboratory. Ten J2 larvae of root-knot nematode were tested in the hole of plastic testing tray. There were total of 24 holes filled with 300 µl of bioactive compound at concentration of 0, 10, 50, 100, and 500 mg/l of each hole. The tray was incubated at 25 °C. Completely randomized design (CRD) was used and consisted of 5 treatments (each concentration level of bioactive compound as treatment) and 4 replicates. The mortality rate of the J2 was observed by using stereo microscope every 3 hours after treated the J2 with bioactive compound from luminescent mushroom.

***Greenhouse Experiment***

J2 nematodes tested with bioactive compound from luminescence mushroom at concentration of 0, 10, 50, 100, and 500 mg/l in the laboratory were verified on the effectiveness of bioactive compound on prevention of root-knot disease in the greenhouse. Ten of J2 were

used per concentration level for 3 replicates. They were immersed in bioactive compound at concentration of 0, 10, 50, 100, and 500 mg/l. The solution was infested in the soil around the root of the 21 day-old tomato plants (Sida variety, PA Seeds Company) grown in the plastic pots with 15 centimeters diameter. The crude extract of bioactive compound of 400 µl was used for each pot and for each concentration. Completely randomized design (CRD) consisted of 6 treatments and 3 replicates was used. After pouring the bioactive compound solution with J2 for 7 days, tomatoes were derived urea and irrigated normally. The plants were inspected for root-knot percentage after pouring the bioactive compound solutions with J2 for 30 days according to the scores from Sontirat (1998); 0 meant no root-knot at all, 1 = 1-25% of root-knot, 2 = 26-50% of root-knot, 3 = 51-75% of root-knot, whereas 4 = 76-100% of root-knot (highest level). The root-knot percentages were analyzed statistically the variance and compared the average values using Duncan's multiple range test (DMRT).

# Results

### 1. The extraction of bioactive compound from luminescence mushroom *Neonothopanus nambi*

Bioactive compound was obtained very little by extraction method 1. Using the dry mycelia provided more amount of bioactive compound (Table 1). Total dry mycelia weight of 69.13 grams of from isolate PW1 extracted using EtOAc, was achieved in 15.77 grams of crude compound (22.81% of extraction efficiency w/w). The bioactive compound was extracted from the 2.5 liters of culture filtrate using EtOAc, resulting in 4.63 grams of crude bioactive compound (0.19% of extraction efficiency v/w). On the other hand, 105 grams of bioactive compound was obtained from the dry mycelia from isolate PW2 in crude compound weight of 20.74 grams (19.75% of extraction efficiency w/w). However, using 10 liters of culture filtrate, it provided 16.75 grams of crude bioactive compound (0.17% of extraction efficiency v/w). For KKU isolate, using 98.9 grams of dry mycelia, the bioactive compound was gained with crude compound weight of 18.2 grams (18.40% of extraction efficiency w/w). When using 3.3 liters of culture filtrate to be extracted by extraction method 2, it provided 8.42 grams of crude bioactive compound (0.26% of extraction efficiency v/w). Among isolates tested, the PW1 and PW2 gave rise to gain high extraction efficiency 12.29-12.63 %, as shown in Table 1.

**Table 1.** Comparison on the bioactive compound extraction from dry mycelia and culture filtrate of luminescent mushroom *Neonothopanus nambi.*

| | | | **Extracted from the dry mycelia** | | |
|---|---|---|---|---|---|
| **Isolate** | **Dry weight (g)** | **Crude compound (g)** | **Efficiency of extraction (%)** | **Bioactive compound powder (g)** | **Efficiency of extraction to powder (%)** |
| PW1 | 69.13 | 15.77 | 22.81 | 8.73 | 12.63 |
| PW2 | 105 | 20.74 | 19.75 | 12.90 | 12.29 |
| KKU | 98.9 | 18.20 | 18.40 | 10.62 | 10.74 |
| | | | **Extracted from the culture filtrate** | | |
| **Isolate** | **Volume (l)** | **Crude compound (g)** | **Efficiency of extraction (%)** | **Bioactive compound powder (g)** | **Efficiency of extraction to powder (%)** |
| PW1 | 2.5 | 4.63 | 0.19 | 4.14 | 0.17 |
| PW2 | 10 | 16.75 | 0.17 | 8.18 | 0.08 |
| KKU | 3.3 | 8.42 | 0.26 | 5.58 | 0.18 |

Column chromatography was used to purify the crude bioactive compound and to crystallize the solution. Crude compound extracted from 3 isolates of luminescent mushroom (*N. nambi*) was poured through the column chromatography with reduced pressure and crystallized using 20% MeOH:EtOAc. The powder of bioactive compound type 1 was light yellow color. When subjected to the TLC, it was found that this compound had *Rf* value of 0.46. There was 8.73 grams of PW1 isolate (from dry mycelia), 4.14 grams (from culture filtrate) from 12.90 grams of PW2 isolate (from dry mycelia), 8.18 grams (from culture filtrate) from 10.62 grams of KKU isolate (from dry mycelia), and 5.85 grams (from culture filtrate). In addition, the bioactive compound type 2 was achieved from only the isolate PW2, which was also yellow powder, had *Rf* = 0.42 when tested on TLC (Figure 1).

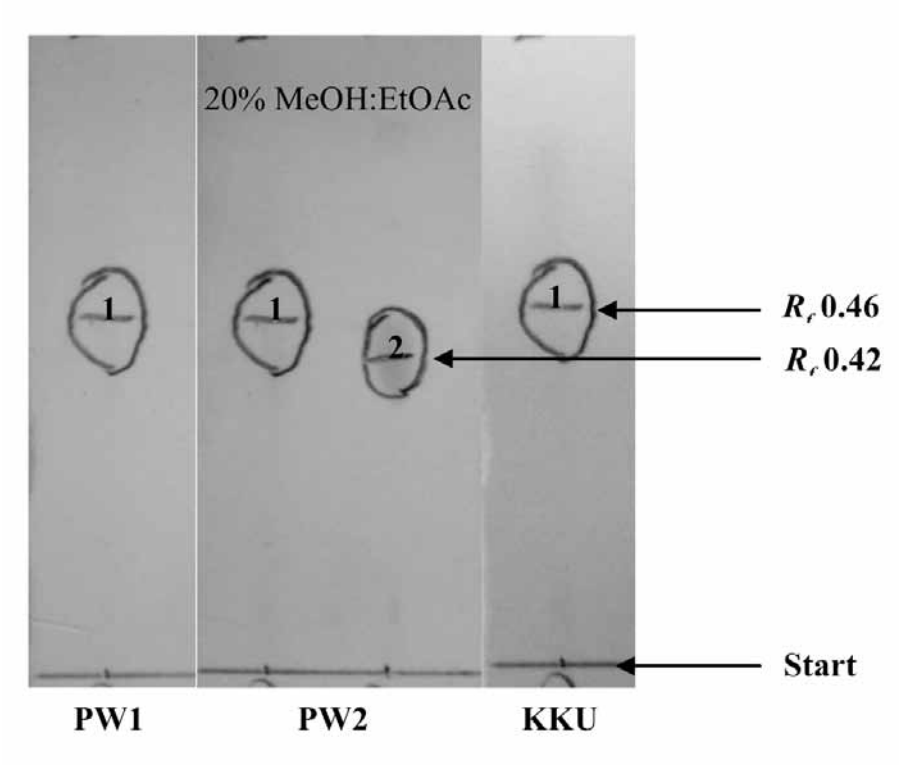


**Figure 1.** Bioactive compound strip from luminescence mushroom (*Neonothopanus nambi*); PW1, PW2, and KKU isolates examined by thin layer chromatography (TLC) using 20% Methanol:Ethyl Acetate (1:1) per volume as the solvent. 1 = bioactive compound type 1; 2 = bioactive compound type 2.

**2. Efficiency of bioactive compound extracted from luminescent mushroom (*Neonothopanus nambi*) against root-knot nematode (*Meloidogyne incognita*)**

***Laboratory Experiment***

Bioactive compound from luminescent mushroom was tested in the laboratory against J2 larvae of the root-knot nematode. It revealed that, at 500 mg/l concentration, the mortality rate of J2 was 100% within 1 minute, followed by 100 mg/l concentration which yielded mortality rate of J2 at 100% within 30 minutes. At the concentrations of 10 and 50 mg/l, the compound caused the mortality of J2 within 48 hours. This was significantly different with control treatment (J2 in DMSO 50%), which indicated that there was no effect on the mortality of J2 (mortality rate at 0 percent).

***Greenhouse Experiment***

The effectiveness of bioactive compound extracted from luminescent mushroom was tested in the greenhouse in order to evaluate the mortality rate of J2 after the test in the laboratory. The result showed that the bioactive compound at 100 and 500 mg/l concentrations were highly effective in controlling the root-knot nematode in the tomatoes. The root-knot roots were not found in the tomatoes of control treatment having root-knot score at level 0 (no root-knot). At the concentration of 10 and 50 mg/l,

1-5 knots were detected in tomato roots (level 1). This was significantly different ($P<0.05$) from control treatment that having J2 alive. The root-knot score level of this control treatment was at level 4 (76-100%), which showing stunt and root-knot symptom. This was clearly different from the tomatoes treated with 10-500 mg/l of bioactive compound and J2 of root-knot nematodes (Table 2 and Figure 2).

This research result indicated that extracting the bioactive compound from *N. nambi* from the dry mycelia gave more bioactive compound than extracting from the culture filtrate. This bioactive compound caused mortality of the root-knot nematode (*M. incognita*) larvae. Besides, it prevented the tomatoes from root-knot disease. The test result in the laboratory and the confirmation from the result in the greenhouse showed the effectiveness and nematicidal property of such bioactive compound to control the root-knot nematode in tomato.

**Table 2.** Percentage of the root-knot in tomato caused by soaking infectious larvae (J2) of the root-knot nematode (*Meloidogyne incognita*) in bioactive compound solutions from luminescent mushroom (*Neonothopanus nambi*) at various concentration levels.

| Bioactive compound | Percentage of root-knot[1] | Root-Knot Score[1] |
|---|---|---|
| 10 mg/l | 6.67 c | 1 |
| 50 mg/l | 11.67 b | 1 |
| 100 mg/l | 0 d | 0 |
| 500 mg/l | 0 d | 0 |
| DMSO (50%) | 82.33 a | 4 |
| Distilled water | 82.67 a | 4 |
| **C.V. (%)** | **9.19** | |

Means followed by the same letter are not significantly different ($P>0.05$, DMRT).

[1] 0 = no root-knot; 1 = 1-25% root-knot; 2 = 26-50% root-knot; 3 = 51-75% root-knot; 4 = 76-100% root-knot. (Sontirat, 1998)

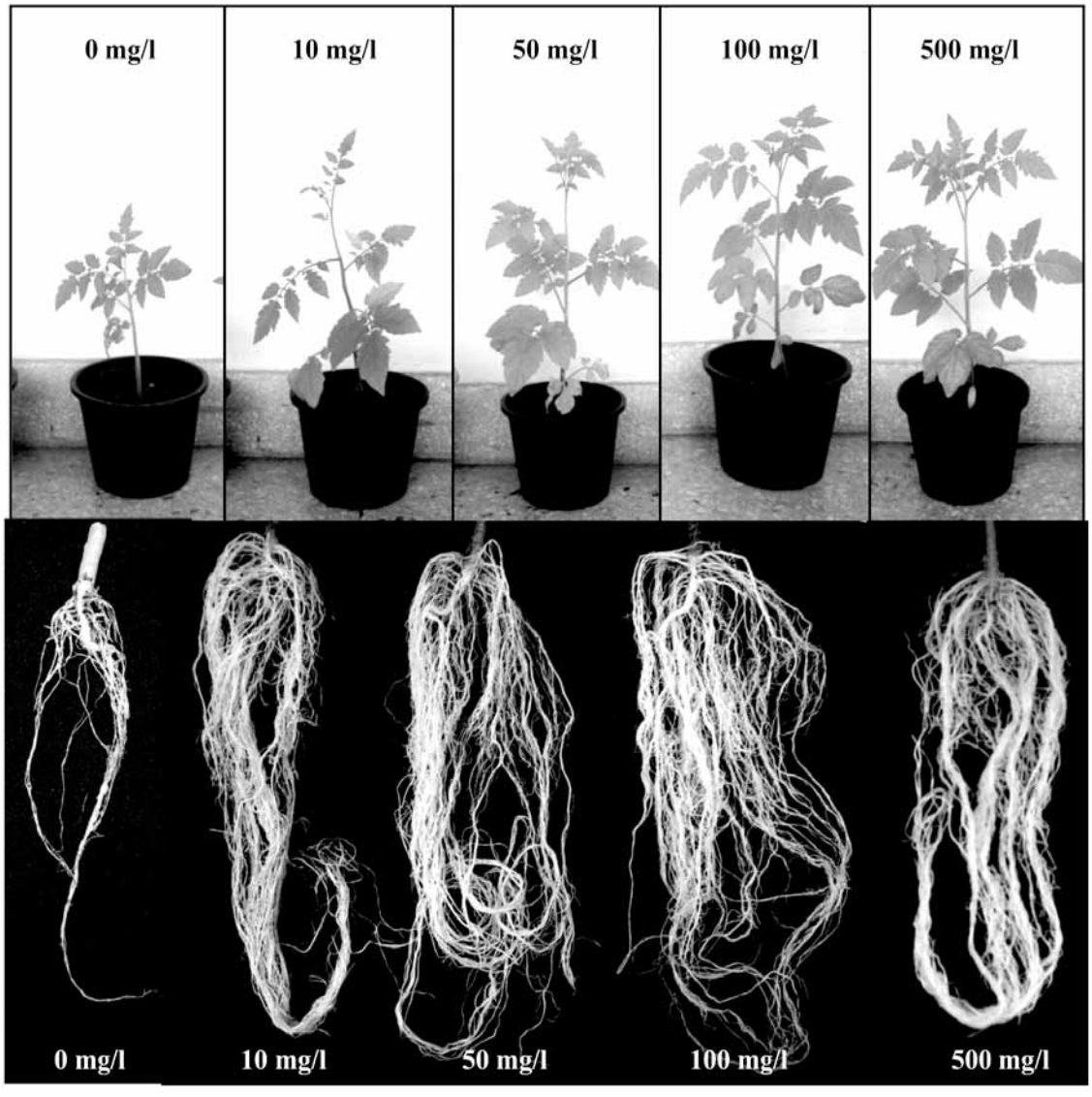


**Figure 2.** Symptom of the tomato plants and roots after treated with the bioactive compound from the luminescent mushroom (*Neonothopanus nambi*) at 0, 10, 50, 100, and 500 mg/l concentration, 400 µl for each concentration level. Ten J2 larvae of root-knot nematode (*Meloidogyne incognita*) were used per concentration level for 30 days.

## Conclusion and Discussion

Luminescent mushrooms (*N. nambi*) were cultivated for the bioactive compound extraction. The dry mycelia gave more extracted compound than using the culture filtrate. The isolate PW1 gave 22.87 percent of crude bioactive compound, which was turned into 12.63 percent of compound powder, followed by those of PW2 and KKU isolates, respectively. It was revealed that there was more bioactive compound from

luminescent mushroom (*N. nambi*) in the mycelia than the secretion outside the mycelia.

Effectiveness of bioactive compound from *N. nambi* was tested with J2 larvae of the root-knot nematode in the laboratory. A concentration of 500 mg/l exhibited the mortality rate of J2 within 1 minute after treated. Using stereo microscopy, J2 were initially moving very quickly, and then slow down afterwards, after about 1 minute, it stopped moving. There was no movement or response from J2 larvae when being pushed using the small bamboo stick. The runner up was the concentration at 100 mg/l. The mortality rate of J2 was within 30 minutes. At the concentration of 10 and 50 mg/l, the mortality rate of J2 was within 48 hours, compared to control treatment (no bioactive compound) that no effect on the mortality rate of J2. In order to verify that this bioactive compound was not only nematostatic substance that caused the nematodes to momentarily stop moving, the bioactive compound was tested in the greenhouse experiment. This was to verify the mortality of J2 in the laboratory and the result recorded. The J2 nematodes were soaked in the bioactive compound solution at the concentration of 100 and 500 mg/l. This can not cause the root-knot symptom in the tomato plants. It meant that this bioactive compound can control the root-knot nematodes in the tomatoes effectively. At the concentrations of 10 and 50 mg/l led the tomato on level 1 of having root-knot. This was different from control treatment that having living J2 of root-knot nematodes (0 mg/l) with root-knot score of level 4. These suggested that the bioactive compound from luminescent mushroom caused the dead of root-knot nematodes and prevent root-knot disease in tomatoes.

The bioactive compounds that affected the nematodes were reported that they can be extracted from various plants and microorganisms. Aminobutyric acid betaine, aminovaleric acid betaine, and glycinebetaine were used in the testing. They were extracted from the brown seaweed (*Ascophyllum nodosum*), which able to reduce the female root-knot nematodes. *M. javanica attacked Arabidopsis thaliana.* (Wu et al., 1998). An example of microorganism is *Pochonia chlamydosporia* YMF 1.00613 that invaded eggs of root-knot nematode, *M. incognita.* The *P. chlamydosporia* can produce aurovertin I (A1) aurovertins E, F and D (A2-A4) bioactive compounds. All 4 types of bioactive compound were one factor that stopped the growth in nematodes (Niu et al., 2010). The bioactive compound in this aurovertin group was not only found in *P. chlamydosporia* bacteria, but also in *Metarhizium anisopliae* that caused the disease in insects and secreted aurovertin D(1), F(2), G(3), and H(4) bioactive compounds (Azumi et al., 2008).

There is previous study from Anke, Sterner (1997), Buchel et al. (1998), on using the bioactive compound from luminescent mushroom to control the root-knot nematodes in plants. The luminescent mushroom (*Omphalotus* sp.) can produce bioactive compound, affecting mortality of J2 of root-knot nematode. Such bioactive compounds were omphalotin A, B, C, and D. The extraction of the bioactive compound and the testing on the effectiveness of the bioactive compound from the luminescent mushroom (*N. nambi*) that were presented in this research were correlated with the research from Bua-art (2003). The test result of secondary metabolite from the culture filtrate of 3 isolates of luminescent mushroom (PW1, PW2, and KKU) against the root-knot nematode (*M. incognita*) was studied. That result indicated after pouring the culture filtrate of isolate KKU at the concentration of 10 milliliter per pot, the number of root-knot of tomato plants had 22.50% reduction. This was not different from using nematicide, carbofuran. This was similar

to the research from Sterner et al. (1997) reported that most luminescent mushroom can control the root-knot nematode (*M. incognita*). This type of mushroom released omphalotin toxic substance. Meyer et al. (2004) demonstrated that mushroom in family Omphalotaceae can mostly create bioactive compound that affected the root-knot nematode, which caused the root-knot symptom. For example, being able to create omphalotin, the *Omphalotus* olearius was poisonous to the nervous system of the root-knot nematode (*M. incognita*).

The bioactive compound showing similar effect from this luminescence mushroom (*N. nambi*) was studied by Boehlendorf et al. (2004). Aurisin A can be extracted from the mushroom (*Panus sp*). It was not luminescent mushroom, but affected several plant pathogens, *Pythium ultimum, Venturia inaequalis, Plasmopara viticola, Puccinia graminis,* and *Phytopthora infestans.* However, there was no report that such compound affected the root-knot nematode. Therefore, this research is the firstly in extracting the bioactive compound from the luminescent mushroom (*N. nambi*) and also including the result of this nematicidal compound against the root-knot nematode (*M. incognita*). This present work showed the way to use the bioactive compound (*N. nambi*) to control the root-knot nematode. Furthermore, the identification of this compound and its effect on antagonistic microorganisms including other beneficial organisms are studied in progress.

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## References

Anke, H., and O. Sterner. 1997. Nematicidal metabolites from higher fungi. **Current Organic Chemistry** 1: 361-374

Azumi, M., Kei-Ichi I., Hiroshi K., Takuya N., Fumio I., Tsuyoshi F., and Yasuhiro I. 2008. Aurovertins F–H from the entomopathogenic fungus *Metarhizium anisopliae.* **J. Nat. Prod.** 7:, 278–280

Boehlendorf, B., S. Neff., T.C. Schuez., L.P. Molleyres, T. Winkler, M. Dobler, and Y. Huang. 2004. **Isolation and characterization of compounds obtained from a fungal microorganism and preparation of some derivatives thereof.** Brit. UK Patent Application

Bua-art, S. 2003. **The effect of secondary metabolite from luminescent mushroom (*Omphalotus* sp.) on the root-knot nematode (*Meloidogyne incognita* Chitwood).** Special problem, Bachelor Degree. Department of Plant Pathology, Faculty of Agriculture, Khon Kaen Universitiy.

Bua-art, S. 2007. **Ribosomal DNA sequences of luminescent mushroom and effect of its bioactive compound against the root-knot nematode (*Meloidogyne incognita* Chitwood).** Master of Science Thesis, Graduate School, Khon Kaen Universitiy.

Buchel, E., U. Martini, A. Mayer, H. Anke, and O. Sterner. 1998. Omphalotins B, C and D, nematicidal cylopeptides from *Omphalotus olearius:* Absolute configuration of omphalotin **A. Tetrahedron** 54: 5345-5352

Engler, M., T. Anke, and O. Sterner. 1998. Production of antibiotics by *Collybia nivalis, Omphalotus olearius,* a *Favolaschia* and a *Pterula* species on Natural Substrates. Z. **Naturforsch.** 53: 318-324

Meyer, L.F., R.N. Huettel, X.Z. Liu, R. A. Humber, J. Jaba, and K. Nitao. 2004. Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile motility. **Nematology** 6(1): 23-32.

Niu, X.-M., Liwang Y., Yan-Sheng C., Hua-X. X., Nan L., Lu-X. W., Ming-H. M., and Ke-Qin Z. 2010. Nematodetoxic aurovertin-type metabolites from a root-knot nematode parasitic fungus *Pochonia chlamydosporia.* **J. Agric. Food Chem.** 58: 828–834

Saksirirat, W., J. Kamkajorn, and N. Sanomuang. 2004. **The comparison of nucleotide sequences of internal transcribes spacer region (ITS) from *rRNA* gene of luminescent mushroom.** Annual Agricultural Conference, 2004, 26-27 January 2004. Faculty of Agriculture, Khon Kaen Universitiy, Khon Kaen.

Saksirirat, W., N. Sanomuang, and T. Winijsanan, 2001. **Species diversity of the macro fungi in plant genetic conservation project, Kok Putaka, Phu Wiang District, Khon Kaen Province.** Annual Agricultural Conference, 2001, 26-27 January 2001. Faculty of Agriculture, Khon Kaen Universitiy, Khon Kaen.

Saksirirat, W., N. Sanoamuang, K. Thomma, J. Kamkajorn, S. Komain, and S. Saepaisan. 2003. **A new record of luminescent mushroom (*Omphalotus* sp.) in Thailand and studies on its cultivation and application.** Pp.251-257 in: Proceeding of Medicinal Mushroom & Biodiversity and Bioactive compound. BIOTEC, PEACH pattaya, Chon Buri, Thailand.

Somasri, K. 2001. **The effectiveness of the soil fungi to control the root-knot nematode (*Meloidogyne incognita Chitwood*) in tomato.** Master of Science Thesis, Graduate School, Khon Kaen Universitiy.

Sontirat, S. 1998. **Nematode.** Ruakeaw Publisher, Bangkok.

Sterner, O., W. Etzel, A. Mayer, and H. Anke. 1997. Omphalotin, a new cyclic peptide with potent nematicidal activity from *Omphalotus olearius* I. Fermentation and biological activity. **Nat. Prod. Lettr.** 10: 33-38

Wu, Y., Jenkins T., Blunden G., Von Mende N., and Hankins S. D.1998. Suppression of fecundity of the root-knot nematode, *Meloidogyne javanica*, in monoxenic cultures of *Arabidopsis thaliana* treated with an alkaline extract of *Ascophyllum nodosum.* **J. App. Phycol.** 10: 91–94