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Effect of Bioactive Compound from Luminescent Mushroom (*Neonothopanus nambi* Speg.) on Root-Knot Nematode (*Meloidogyne incognita* Chitwood) and Non-Target Organisms

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Abstract

The objective of this study was to investigate the effect of bioactive compounds from a luminescent mushroom (*Neonothopanus nambi*) on root-knot nematode (*Meloidogyne incognita*) and non-target organisms, entomopathogenic nematode (EPN), *Steinernema carpocapsae*; plant pathogenic fungi, *Pythium* sp. and *Phytophthora palmivora*; composting fungus, *Aspergillus* sp.; biocontrol agents, *Trichoderma harzianum.; Bacillus subtilis* and nitrogen fixing bacteria, *Rhizobium* sp. under laboratory conditions. Five concentrations of the bioactive compounds, 0, 10, 50, 100 and 500 mg/l, were exposed to the tested organisms. Mortality percentage of nematodes (*M. incognita* and *S. carpocapsae*) and inhibition zone around paper disk soaked with bioactive compound suspensions were recorded. The results revealed that concentrations of 10, 50, 100 and 500 mg/l were highly toxic to *M. incognita* causing 100 % mortality, but had no adverse effect on *S. carpocapsae*, *Aspergillus* sp., *Trichoderma harzianum; Bacillus subtilis* and *Rhizobium* sp. Inhibition zone was detected on colonies of *Pythium* sp. and *P. palmivora*, but only on the medium with bioactive compound concentration of 500 mg/l. The results suggest the potential use of bioactive compounds from *N. nambi* for control of plant parasitic nematode, *M. incognita* without adverse effects on beneficial organisms.

Keywords: beneficial organism, bioactive compound, luminescent mushroom, nematicidal property, root-knot nematode

1. Introduction

A luminescent mushroom, *Neonothopanus nimbi* Speg. has been newly reported in the area of the Plant Genetic Conservation Project under Royal Initiation by Her Royal Highness Princess Maha Chakri Sirindthorn at Kok Phutaka, Wiang Kao District, Khon Kaen Province (Saksirirat et al., 2003) and was later identified using morphological characters and nucleotide sequences from the internal transcribe spacer (ITS) of *rRNA* gene (Bua-art et al., 2007). There are various reports concerning the advantages and exploitation of luminescent mushrooms. Engler et al. (1998) isolated the secondary metabolite from a culture filtrate of luminescent mushroom, *Omphalotus olearius* cultured in yeast malt glucose medium. This mushroom secreted a bioactive compound, omphalotin, affecting the growth and development of the root-knot nematode, *Meloidogyne incognita* Chitwood (Anke and Sterner, 1997). The chemical structure of the bioactive compound was identified as omphalotin by using spectroscopic techniques (^{1}H and ^{13}C Nuclear Magnetic Resonance Spectroscopy, ^{1}H and ^{13}C-NMR). This compound has several derivatives such as omphalotin A, B, C and D which inhibited the nervous system of the root-knot nematode (Buchel et al., 1998).

Based on that information, our research work has been focused on a similar control of plant pathogens by using *N. nambi*. Saepaisan (2002) reported on the antagonistic property of *N. nambi* against plant pathogenic fungi under laboratory conditions, and found that *N. nambi* inhibited hyphal growth of *Pythium* sp., *Rhizoctonia solani,* and *Phytopthora palmivora.* Thereafter, the nematicidal effect of this mushroom was evaluated against *M. incognita* under laboratory conditions using culture filtrate 80%. The infective larvae (J2) of root-knot nematode were affected by soaking the J2 in a culture filtrate of *N. nambi* with high mortality. The tomato treated with culture filtrate showed lower root galling percentage than non-treated (Bua-art, 2003). The application of culture filtrate and spawn of *N. nambi* was tested in a greenhouse experiment and it was found that both culture filtrate and spawn suppressed the root-knot disease incidence in tomato plants (Bua-art, 2007). In addition, some bioactive compounds were reported preliminary as having nematicidal effect on root-knot nematode (Bua-art, 2007). More recently, our report indicates that the bioactive compound *N. nambi* was extracted by using ethyl acetate and crystallized into powder form, causing 100% mortality of J2. The bioactive compound has potential to be applied as a biological nematicide for control of *M. incognita*. Therefore, the objectives of this study were to examine nematicidal properties and to evaluate its effect on other beneficial organisms including fungal plant pathogens.

2. Materials and Methods

2.1 Source of the luminescent mushroom (*Neonothopanus nambi*) and other tested organisms

Three isolates of luminescent mushroom (*N. nambi*) 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 (KKU). 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. Antagonistic fungus, *Trichoderma harzianum* and *M. incognita* were supplied by KKU. Other organisms, *S. carpocapsae*, *Bacillus subtilis*, *Rhizobium* sp., *Aspergillus* sp., *Pythium* sp. and *Phytophthora palmivora* were obtained from the Department of Agriculture, Ministry of Agriculture and Cooperatives.

2.2 The extraction of the bioactive compounds from the luminescent mushroom (*Neonothopanus nambi*)

Three isolates of *N. nambi* were cultured on PDA at a temperature of 28±2 °C for 7 days. Then, the mushroom was grown in malt extract broth (MEB). The bioactive compound was extracted biologically from *N. nambi* by using the method of Bua-art et al. (2010).

2.3 Test of bioactive compound from luminescent mushroom (*Neonothopanus nambi*) on test organisms

2.3.1 Test on root-knot nematode (*Meloidogyne incognita*)

Infective juveniles (J2) of the root-knot nematode were prepared by adopting the process from Somasri (2001). Tomato roots with the root-knot symptom were washed with sterile water. Brown egg mass was collected from washed gall root of tomato using forceps. The egg mass was stored on a fine net nylon cloth mounted in a PVC pipe with 3 centimeters diameter and 1 centimeter height. The pipe was sealed with a fine net nylon cloth. The pipe was immersed in 0.5 % of sodium hypochlorite (NaOCl) for 3 minutes, and rinsed with sterilized distilled water 3 times, for 5 minutes each time. The pipe was placed on a cone with 15-centimeters width. The cone had a rubber tube connected at the end with a clamp. 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 powder of bioactive compound from luminescent mushroom was dissolved in dimethylsulfoxid (DMSO) at 50% concentration with sterilized distilled water. Bioactive compound was prepared at concentrations of 0, 10, 50, 100, and 500 milligram per liter (mg/l) for further test. The effectiveness of bioactive compound from luminescent mushroom on the root-knot nematode (*M. incognita*) was tested in the laboratory. Ten J2 larvae of root-knot nematode were tested in the holes of a plastic testing tray. There were a total of 24 holes filled with 300 µl of bioactive compounds at concentrations of 0, 10, 50, 100, and 500 mg/l in 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 a stereo microscope every 3 hours after treating the J2 with bioactive compounds from luminescent mushroom.

2.3.2 Test on entopathogenic nematode (*Steinernema carpocapsae*)

The effect of the bioactive compound from *N. nambi* on infective juvenile (IJ) of entomopathogenic nematode (EPN), *S. carpocapsae* was evaluated in the same manner as the test on *M. incognita.* The mortality and movement response were observed for 72 hours after treating with bioactive compound.

2.3.3 Test on beneficial microorganisms

The biological control agents of plant diseases, *Trichoderma harzianum*, *Bacillus subtilis* and nitrogen fixing bacterium, *Rhizobium* sp. including composting fungus, *Aspergillus sp.* were selected to determine the effect of bioactive compound from *N. nambi* in order to evaluate the compatibility for using in combination. The tested microorganisms were spreaded on relevant media: Potato dextrose Agar for *Trichoderma harzianum*. and *Aspergillus* sp., Potato Synthesis Agar (PSA) for *B. subtilis* and Yeast Mannitol Agar (YMA) for *Rhizobium* sp. Thereafter filter paper disks (5 mm diameter) were soaked in bioactive compound suspension at concentrations of 0, 10, 50, 100, and 500 mg/l and placed on the media described above. The diameters of the inhibition zones around the paper disks were measured 3 days after treatment with the bioactive compound.

2.3.4 Test on fungal plant pathogens

The fungal plant pathogens, *Pythium* sp. and *Phytophthora palmivora* were investigated for the fungicidal effect of bioactive compounds from *N. nambi*. Test procedures were similar to those for the test with *Trichoderma* spp. and *Aspergillus* sp.

2.3.5 Experimental design and statistical analysis

Completely randomized design and 4 replications were used in all tests. Observed values were transformed with $\sqrt{(X + 0.5)}$ before analysis of variance. Means were compared statistically by Duncan's Multiple Range Test (DMRT), when significant difference was found.

3. Results

3.1 Nematicidal effect of bioactive compound on root-knot and entomopathogenic nematode

Bioactive compounds from a luminescent mushroom were 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. However, at lower concentrations, 10 and 50 mg/l caused J2 mortality in 48 hours after treatment (Table 1). The dead J2 was observed showing strait larva and not moving as shown in Figure 1A. This was significantly different with the control treatment

Table 1. Mortality percentage of infective juvenile of the root-knot nematode (*Meloidogyne incognita*) and infective juvenile of entomopathogenic nematode (*Steinernema carpocapsae*) after soaking in bioactive compound solutions from luminescent mushroom (*Neonothopanus nambi*) at various concentration levels.

Bioactive compound	**Mortality of *M. incognita* (%)**	**Mortality time (hour)**	**Mortality of *S. carpocapsae* (%)**
0 mg/l	0 b	0	0 a
10 mg/l	100 a	48	0 a
50 mg/l	100 a	48	0 a
100 mg/l	100 a	1/2	0 a
500 mg/l	100 a	1/60	0 a
Distilled water	0 b	0	0 a
C.V. (%)	0.04		0

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

(J2 in DMSO 50%), which indicated that there was no effect on the mortality of J2 (mortality rate at 0 percent). On the other hand, the bioactive compound from *N. nambi* of all concentrations was not effective against *S. carpocapsae*. The entopathogenic nematode moved showing the property of an infectious juvenile until the end of the experimental period (72 hours) with 0% mortality (Table 1). The juvenile still moved (Figure 1B).

3.2 Effect of bioactive compound from *Neonothopanus nambi* on beneficial micro-organisms

The biological control agents of plant diseases, *Trichoderma harzianum*, *Bacillus subtilis* and composting fungus, *Aspergillus* sp. were tested on growth inhibition under laboratory conditions. The results showed that all concentrations of bioactive compound were not effective on growth for all isolates of biological control agents. Inhibition zones around the paper disks containing bioactive compounds on colonies

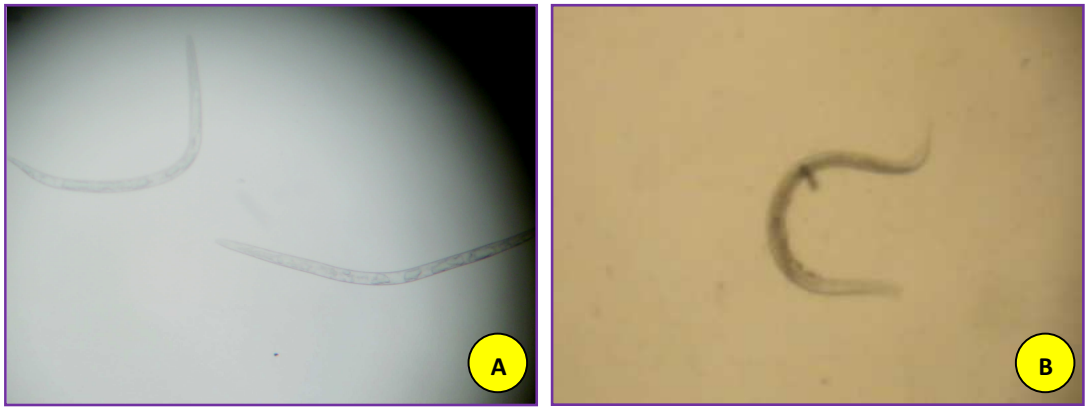

Figure 1. Dead infective juvenile of *Meloidogyne incognita* (A) and living juvenile of *Steinernema carpocapsae* (B)

of tested organisms were not observed (Figures 2A, 2B and 2C). Similar results were achieved in an experiment with nitrogen fixing bacterium (*Rhizobium* sp.). The evidence of inhibitory effect from bioactive compound of *N. nambi* against *Rhizobium* sp. was not detectable (Figure 2D).

3.3 Effect of bioactive compound from *Neonothopanus nambi* on fungal plant pathogens

Two genera of fungal plant pathogens, *Pythium aphanidermatum*. and *Phytophthora palmivora* were tested for growth inhibition under laboratory conditions. The bioactive compound concentration of 500 mg/l from

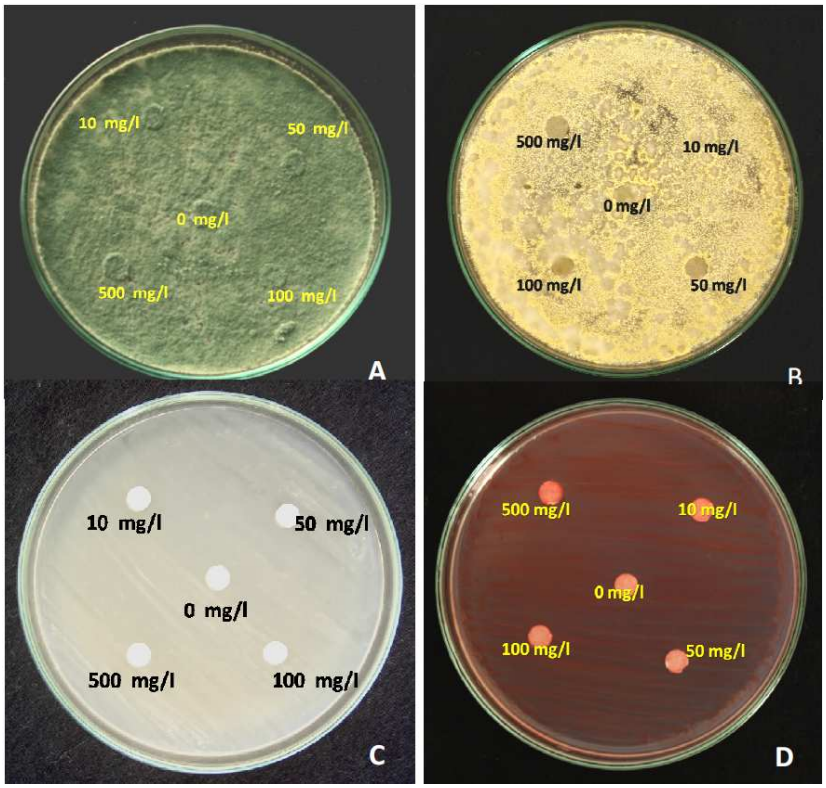

Figure 2. Effect of different concentrations of bioactive compound from *Neonothopanus nambi* on growth of *Trichoderma harzianum*. (A), *Aspergillus* sp. (B), *Bacillus subtilis* (C), and *Rhizobium* sp. (D).

Table 2. Inhibition zone diameter of bioactive compound from *Neonothopanus nambi* on hyphal growth of *Pythium* sp. and *Phytophthora palmivora*.

Bioactive compound concentration	Inhibition diameter (mm) on *Pythium aphanidermatum.*	Inhibition diameter (mm) on *P. palmivora*
0 mg/l	0 b	0 b
10 mg/l	0 b	0 b
50 mg/l	0 b	0 b
100 mg/l	0 b	0 b
500 mg/l	12 a	16 a
C.V. (%)	6.91	7.31

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

N. nambi was effective against hyphal growth of both *Pythium aphanidermatum*. and *P. palmivora*. Inhibition zone diameter of bioactive compound on *P. palmivora* was 16 mm greater than that on *Pythium aphanidermatum*. (12 mm) as shown in Table 2 and Figure 3.

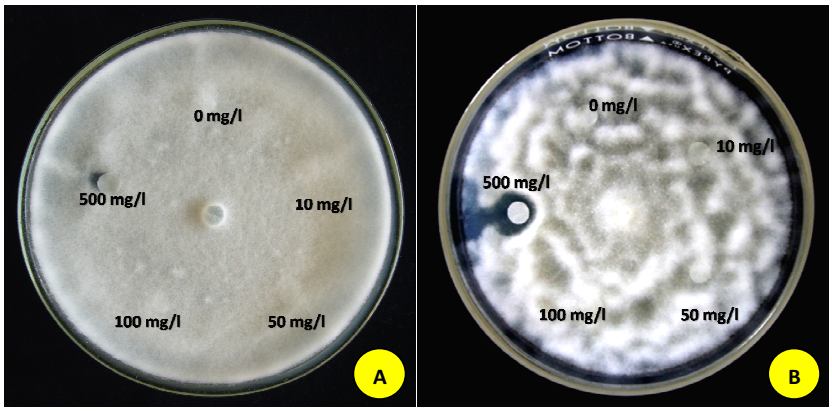

Figure 3. Effect of bioactive compound from *Neonothopanus nambi* on hyphal growth of *Pythium aphanidermatum*. (A) and *Phytophthora palmivora* (B) on PDA.

3.4 Inhibition of bioactive compound from *Neonothopanus nambi* on tested organisms

The effectiveness of the bioactive compound from *N. nambi* against all tested organisms is summarized in Table 3. The bioactive compounds showed nematicidal effect only on *M. incognita* but not on EPN, *S. carpocapsae*. For fungicidal effect, it was found on *Pythium aphanidermatum*. and *Phytophthora palmivora* but not on *T. harzianum*. In addition, no bactericidal property was observed on *Bacillus subtilis*, *Rhizobium* sp. and *Aspergillus* sp.

4. Discussion and Conclusion

The bioactive compound of luminescent mushroom, *N. nambi* has been extracted and reported on the previously (Bua–art et al., 2010). Several scientists reported identifycation and exploitation of bioactive compounds derived from other luminous mushrooms. Sterner et al. (1997) studied on the nematicidal activity of a toxic substance, omphalotin from luminescent mushroom, *Omphalotus olearius* against root-knot nematode *(M. incognita)*. This substance was thereafter characterized and at least 4 derivatives of cyclopeptides such as omphalotin A, B, C and D found (Buchel et al., 1998; Engler et al., 1998). Most luminescent mushrooms in the Family Omphalotaceae released toxin omphalotin affecting the nervous system of *M. incognita* (Meyer et al., 2004). Besides, luminescent mushrooms

Table 3. Summarized effect of bioactive compound from *Neonothopanus nambi* on tested organisms.

Bioactive compound (mg/l)	*M. incognita*	*S. carpocapsae*	*T. harzianum*	*Aspergillus* sp.	*B. subtilis*	*Rhizobium* sp.	*Pythium* sp.	*P. palmivora*
0	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-
50	+	-	-	-	-	-	-	-
100	+	-	-	-	-	-	-	-
500	+	-	-	-	-	-	+	+

+ = Inhibited, – = Uninhibited

O. illulens and *O. olearius* produced not only omphalotin but also illudin S and illudin M, toxic to rat cancer cells. These substances were applied in medical research. The results of our previous studies revealed the extraction of bioactive compounds from *N. nambi*, which were obtained from dry mycelia more than from culture filtrates (Bua–art et al., 2010). The nematicidal effect of culture filtrate and mushroom spawn of *N. nambi* was evaluated on infective larva (J2) of *M. incognita* and it was reported that both culture filtrate and spawn of *N. nambi* caused high percentage mortality of J2 (Bua–art, 2007). Likewise, the report from Heydari et al. (2006) indicated that culture filtrates from oyster mushroom *(Pleurotus ostreatus)* had nematicidal activity and caused mortality of root-knot nematode *M. javanica.* This mushroom is related to the old generic name of *N. nambi;* which has a synonym of *P. eugramus* or *P. lampas* (Moncalvo et al., 2002). In contrast to the EPN our result showed that the bioactive compound from *N. nambi* was ineffective against EPN, *S. carpocapsae*. This result showed the advantage of bioactive compounds from *N. nambi* when used in combination for a pest management program. Normally, the EPN has a stage so called "infective juvenile" (IJ) or "dauer juvenile" (dauers), which is tolerant to desiccation, UV, heat and envi-ronmental fluctuation. This is associated with symbiotic bacteria i.e. *Photorhabdus luminescens, Xenorhabdvs bovienii* and *X. poinarii*. These bacteria play an important role in the release of toxic substance causing mortality of insect host and as food of the EPN. The mass production of the EPN needs the co-culture of symbiotic bacteria and nematode (Torre, 2003). However, the substance has no effect to the EPN larvae. This toxic tolerant property of EPN is different from the root-knot nematode. In addition, there are many research works which show the tolerant property of EPN toward toxin or nematicides as follows. Hartelt et al. (2008) reported that *S. carpo-capsae* could be used in combination with *Metarhizium anisopliae* for control of Lyme disease vector tick *(Ixodes ricinus).* The *M. anisopliae* produced a well-known toxin, destruxin causing mortality of insects. Recently, this fungus has been shown to release a toxin in the aurovertin group (Azomi et al., 2008). The aurovertin was found also in nematopathogenic fungus *(Pochonia chlamydosporia)* (Niu et al., 2010). Similarly to the fungus, the *S. carpocapsae* is able to be used in combination with *Bacillus thuringiensis* (Bt) to control diamond back moth *(Plutella xylostella)* by showing additive effect for managing resistance develop-ment of *P. xylostella* against Bt (Ehlers and Yi, 2006). This emphasizes that the EPN is tolerant to microbial toxin. Moreover, after treatment of some nematode used to control plant parasitic nematodes, Fenamiphos, Oxamyl and Avermectin observed for 11 generations of the EPS reproduction cycle, it increased resistance to Fenaniphos and Avermectin including heat tolerance and reproduction

potential without deterioration in biological control efficacy (Glazer et al., 1997). In another case, *S. feltiae* could be combined with imidaclopid leading to higher mortality rate of *Bemecia tabaci* larvae (Cuthbertson et al., 2003) these suggest the tolerant property of EPN toward toxin, nematicides, insecticides and unfavorable environment. Based on the scientific evidence described previously, the results of our study indicate that S. *caspocapsae* is possibly tolerant or resistant to the bioactive compound of *N. nambi*. It would be an advantage to apply in combination for management of root-knot nematode and insects. Test results for other non-target organisms, *Trichoderma harzianum*., *Aspergillus* sp., *Bacillus subtilis*, *Rhizobium* sp., *Pythium aphanidermatum*. and *P. palmivora*, exhibit the advantages of bioactive compounds from *N. nambi*, because at concentrations of 500 mg/l, they inhibit hyphal growth of plant pathogenic fungi *Pythium aphanidermatum*. and *P. palmivora* but have no adverse effect on other tested beneficial organisms. This result is similar to a study of Boehlendorf et al. (2004) that the Aurisin A extracted from the non luminous mushroom (*Panus* sp.) affected several plant pathogens, *Pythium ultimum*, *Venturia inaequalis*, *Plasmopara viticola*, *Puccinia graminis*, and *Phytopthora infestans*. However, there was no result on *M. incognita*, S. *carpocapsae* and beneficial micro-organisms.

The results indicate the potential use of bioactive compounds from *N. nambi* to control root knot nematodes *Pythium* sp. and *P. palmivora* without adverse effect on *Aspergillus* sp., *Trichoderma* spp., *B. subtilis* and *Rhizobium* sp. However, this result is derived from laboratory experiments. It should be investigated consequently in green-house or field conditions to verify laboratory results. This suggests the nematicidal effect of bioactive compounds from *N. nambi* and potential use on *M. incognita,* fungicidal effect on *Pythium* sp. and *P. palmivora* and non-effect on beneficial micro-organisms such as biological control agents, *Trichoderma harzianum* and *B. subtilis*, nitrogen fixing bacteria, *Rhizobium* sp. and composting fungus, *Aspergillus* sp.

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