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FTIR Spectroscopic Analysis of Luminescent Mushroom (*Neonothopanus nambi* Speg.) Induced Disease Resistance against Tomato Root-Knot Nematode, *Meloidogyne incognita* Chitwood

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The objective of this work is to evaluate the efficiency of *Neonothopanus nambi* isolate PW2 on its ability to induce systemic resistance against root-knot nematodes, *Meloidogyne incognita* in tomato plants. Results of split-root experiments showed that tomato roots treated with the combination of culture filtrate and spawn (CF+SP) and the spawn (SP) reduced nematode penetration of 92.3% and 83.5%, respectively. Moreover, both treatments reduced 3.5% of root galls. For evident observation of induced resistant property of *N. nambi*, the defense enzyme activities, phenolic compound, salicylic acid, phenylalanine ammonia-Lyase (PAL), polyphenol oxidase (PPO), chitinase and protease were determined after 10 days of *N. nambi* treatment. Result revealed that most of defense enzyme activities were detected with maximum activities in mycelial spawn of *N. nambi* PW2 which presented highest phenols (0.205 mg^{-1} g fresh weight), PPO (14.15 μmol (quinone) mg^{-1} protein/hr), chitinase (5.23 μmol (GlcNAc) mg^{-1} protein/hr) and protease (26.05 μmol (tyrosine) mg^{-1} protein/hr). The high accumulation of salicylic acid were obtained from tomato root treated with spawn (SP), CF+SP and CF of 0.268 mg g^{-1}, 0.265 mg g^{-1} and 0.250 mg g^{-1} of tomato root fresh weight, respectively. The highest PAL activities were detected from tomato roots treated with combined CF+SP and spawn of 31.51 and 29.17 μmol (trans-cinnamic acid) mg^{-1} protein/hr, compared to control treatment (untrated). Fourier transformed infrared (FTIR) was applied for monitoring the biochemical changes of tomato roots after treated with different treatments of *N. nambi*. Spawn treatment presented higher absorbance peak of protein, lignin and hemicelluloses than that of other treatments and untreated root. This study suggested that the luminescent mushroom, *N. nambi* has inducing resistant property against root-knot nematode, *M. incognita* in tomato plants depended on applying strategies.

Keywords: Induced resistant, luminescent mushroom, *Neonothopanus nambi*, root-knot nematode, tomato.

Root-knot nematode, *Meloidogyne incognita* is widespread and important pest of economic crops around the world, hence it causes severe damage to field, horticultural and vegetable crops particularly to tomato production[1]. This nematode can attack tomato in many part of the world[2] and caused yield loss of tomato estimated 28-47% annually[3]. In order to control or manage the root-knot nematode, chemical control was effective, however it may cause toxic contamination to environment. Therefore, biological control of nematodes is considered as a potential

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management and effective alternative of nematicides[4].

Nematophagous fungi are soil-living fungi potentially as biocontrol agents of various plant parasitic nematodes[5]. Many fungi can produce nematicidal compounds [6]. In particularly, the fungus *Omphalotus olearius*, a luminescent mushroom that produced omphalotin A, showing nematicidal property against the root-knot nematode, *M. incognita* [7]. Engler et al. [8] reported that the secondary metabolite isolated from culture filtrate of *O. olearius* affecting the growth and development of *M. incognita*. In Thailand, there were studies on the effectiveness of luminescent mushroom royal given name in Thai by Princes Maha Chakri Sirindhorn as "Hed Sirin Ratsamee", *Neonothopanus nambi* [9]. The application of culture filtrate, spawn and bioactive compound of *N. nambi* were reported preliminary as having nematicidal effect and suppressed the root-knot disease incidence in tomato plants [9] . Bioactive compounds of this mushroom was extracted and reported on their properties in various evaluations [10,11]. However, previous application of these fungi was reported only beneficial to disease management involved toxic compound production or antagonism directly to the pathogen. It is interesting, therefore, that the luminescent mushroom, *N. nambi* may play a role in inducing systemic resistance in tomato plants against root-knot nematode.

Infrared spectroscopy or Fourier transform infrared (FTIR) is a simple and rapid technique that recently used for monitoring of chemical modification of cell wall components [12]. FTIR spectroscopy provides more detailed chemical structures and composition of plant tissues including roots, stems and leaves, based on specific vibration characteristics of chemical functional group of each sample [13]. Recently, the use of FTIR spectroscopy to identify the chemical structure of plant secondary metabolites has increasingly important within plant pathology research [14]. However, the study on induced resistance by using *N. nambi* against root-knot nematode with FTIR has not been reported. Therefore, the objective of this study is to apply the FTIR spectroscopic analysis for assisted evaluation on luminescent mushroom, *N. nambi* against *M. incognita* in tomato.

MATERIALS AND METHODS

Source of the luminescent mushroom (*Neonothopanus nambi*)

Luminescent mushroom (*N. nambi*) PW2 isolated from the Plant Genetic Conservation Project under Royal Initiation by Her Royal Highness Princess Maha Chakri Sirindhorn at Kok Phutaka, Wiang Kao District, Khon Kaen Province, Saksirirat et al.[15] was used for all experiment. The mushroom hyphae were maintained on potato dextrose agar (PDA) at 28°C. In this study, *N. nambi* fungus was prepared in three formulae, culture filtrate (CF), mycelia spawn (SP) and bioactive compound, aurisin A extracted according to [10]. Culture filtrate of *N. nambi* were prepared on PDA at 28°C for 7 days before transfered of mycelium agar, 5 disks to 500 ml flasks containing 200 ml of Yeast Malt Broth (YMB). After 15 days of incubation at room temperature (25-30°C), tested fungus was filtered through Whatman No.1 filter paper to obtain culture filtrates. Spawn of *N. nambi* was prepared using sorghum grains boiled and autoclaved for 30 minutes. Hyphae of *N. nambi* were inoculated on steriled sorghum grains and incubated at 28°C for 20 days. Ten to twenty sorghum grains with fully colonized by *N. nambi* were inoculated on rubber tree sawdust supplemented with 10% of wheat flour and incubated at 28°C for 26 days before used. Bioactive compound of *N. nambi* were extracted from dry mycelia using method described by Bua-art et al.[10]. The powder of bioactive compound from *N. nambi* was dissolved in dimethyl-sulfoxid (DMSO) at 0.2% concentration with sterile distilled water. Bioactive compound was prepared of a concentrations 500 milligram per liter (mg/l) for further test.

Preparation of root-knot nematode (*M. incognita*) egg inoculums

Egg inoculum was prepared from tomato roots infected with *M. incognita,* which maintained in greenhouse of Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Thailand. The root-knot nematode eggs were extracted from roots with 0.5% NaOCl and collected using the modified technique of [16]. The nematodes inoculums (3,000 eggs per pot) were used for each experiment.

Bioassay in greenhouse experiment

The effect of induced systemic resistant property of *N. nambi* was investigated by evaluation of root gall percentage and number of infective larvae (J2) (*M. incognita*) using the split-root experimental design, which allows inoculation of the fungus and nematode at separated locations on the root system. Three-week-old tomato (Sida cultivar, PA Seed Company) seedlings were uprooted from sterilized soil, washed with tap water, and divided the roots into two halves with a sterilized dissecting scalpel. Each half of the root system was transplanted separately into plastic pots (Ø 12 cm) containing a sterilized mixture of clay soil and sand (1:1 v/v) 300 g soil/pot. One part of the root system was treated with different conditions of *N. nambi* (inducer roots). Soil treated with sterile water served as controls. Ten days after treatments, the other half of the root system was inoculated with 3,000 eggs of *M. incognita* (responder roots) by soil infestation. Complete randomized design (CRD) consisted of 7 treatments and 4 replicates were used (Table 1). Treatments were 1) non treated plants (control) either half-root system; 2) one half-root system added with 3,000 eggs of *M. incognita* (N); 3) nematode added into one half-root system and 0.2% DMSO added into another half-root system (0.2% DMSO); and 4) nematode added into one half-root system and treated with aurisin A (500 mg/L) added into another half-root system (aurisin A); 5) nematode added into one half-root system and the culture filtrate of *N. nambi* PW2 (30 ml) was added into another half-root system (CF PW2); 6) nematode added into one half-root system and the spawn of *N. nambi* PW2 (30 g) added into another half-root system (SP PW2) and 7) nematode added into one half-root system and the combination of culture filtrate (15 ml) and spawn of *N. nambi* isolate PW2 (15 g) were added into another half-root system (CF+SP PW2). Nematode *M. incognita* was applied at 3,000 eggs/half-root system. After 30 days, root galling percentage and numbers of J2 penetrating in roots were recorded. Root galling were evaluated according to; 0 = no root-knot, 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. Each half-root system (responder root) was stained in 0.01% acid fuchsin in lactophenol [17]. Numbers of J2 infecting in tomato roots were quantified per 1 g. roots. Data were analysis of variance (ANOVA) and the differences between means were compared using Duncan's multiple range test (DMRT).

Biochemical analysis

Crude enzyme samples were prepared using 0.5 g of tomato root harvested at 10 days of interval after treated of *N. nambi* into soil without challenge inoculation with nematode. The root were homogenized with 1.2 ml of 0.1 M acetate buffer pH 5.0, centrifuge at 14,000 rpm then transferred supernatant to a new Eppendorf tube 1.5 ml and kept at -20°C until used. Protein content in enzyme solutions were determined by method of Bradford [(18)] using bovine serum albumin as a standard protein.

Assay of phenolic compound

Tomato root samples (0.5 g) were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70°C [(19)]. One ml of the methanolic extract was added to 5 ml of distilled water and 250 µl of Folin-Ciocalteau reagent then the solution was incubated at 25°C. The absorbance of the developed blue color was measured using a spectrophotometer with wave length 725 nm. Catechol was used as the standard. The amount of phenolic compound was expressed as mg^{-1} g fresh weight.

Assay of salicylic acid (SA)

Tomato root samples (0.5 g) were homogenized in 0.5 ml cold-ice buffer (90 ml methanol, 9 ml acetic acid and 1 ml of water) [20]. Then 0.5 ml of 0.05 M ferric ammonium sulfate were added in reaction mixtures and incubated at 30°C 15 min. The absorbance of salicylic acid was measured with wave length 527 nm and compared with a standard curve of SA dissolved in ethyl acetate. The amount of SA was expressed as mg g^{-1} FW.

Assay of polyphenol oxidase (PPO)

PPO activity was determined according to the method of [21]. Tomato root samples (0.5 g) were ground in 2 ml 0.1 M sodium phosphate buffer (pH 6.5) and centrifuged at 16000 g for 15 min at 4°C. The supernatant was used as the enzyme source. The reaction mixture consisted of 0.2 ml of the enzyme solution and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 0.2 ml of 0.01 M catechol were added. The reaction mixture was measured the absorbance at 495 nm.

The PPO activity was expressed as µmol (quinone) mg^{-1}protein/hr.

Assay of Phenylalanine ammonia-lyase (PAL)

PAL activity was determined according to the method of [22]. The reaction mixture consisted of 0.2 ml of the enzyme solution and 0.5 ml of 12 mM L-phenylalanine in 0.1 M sodium borate buffer, pH 8.8 were incubated for 30 min at 30°C. PAL was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid. The reaction mixture was analysed by colorimetric method using spectrophotometer at 290 nm. PAL activity was expressed as µmol (trans-cinnamic acid) mg^{-1}protein/hr.

Assay of chitinase activity

Chitinase activity was evaluated by incubation of 1 ml 1% colloidal chitin and 1 ml enzyme solution in 0.1 M acetate buffer pH 5.0 at 37°C for 1 hour. Reaction mixture was stopped by boiling aliquot for 15 min. The amount of reducing sugar released in reaction mixture was calculated by analysis method of [23] using N-acetylglucosammine (GlcNac) as standard. Chitinase activity was expressed as µmol (GlcNac) mg^{-1}protein/hr.

Assay of protease activity

Protease activity was evaluated by incubation 0.1 ml enzyme solution and 1.0 ml 1% casein dissolved in 0.1 M phosphate buffer, pH 7.0 at 37°C for 1 hour. Reaction mixture was stopped by 3.0 ml of 10% trichloroacetic acid. The enzyme activity was measured the absorbance at 280 nm using tyrosine as standard according to the method of [24]. Protease activity was expressed as µmol (tyrosine) mg^{-1}protein/hr.

FTIR analysis of tomato root

Tomato roots (10 mg) were oven-dried at 60°C for 3 days. The dried tomato roots were ground with a crystal mortar and pestle. FTIR spectra were acquired at the Synchrotron Light Research Institute (SLRI), Nakhon Ratchasima, Thailand, using a Bruker Vertex 70 FTIR spectrometer (Bruker Optics Inc., Ettlingen, Germany) equipped with a nitrogen cooled MCT (HgCdTe) detectors. Absorbance spectra were acquired in the spectral range of 4000-700 cm^{-1}. FTIR samples were recorded in the reflection mode, 4 cm^{-1} spectral resolution, 64 scans per spectrum using Pike MIRacle ATR germanium ATR (PIKE Technologies, Madison, USA).

Data processing and data analysis

Principle Component Analysis (PCA) was performed to distinguish different chemical components between treatment groups. The spectra were preprocessed by taking the second derivative using the Savitzky-Golay algorithm with nine points of smoothing and normalized with Extended Multiplicative Signal Correction (EMSC). PCA was performed using The Unscrambler software (version 9.7, CAMO Software AS, Oslo, Norway) employing the combined spectral ranges of 3000-2800 cm^{-1} and 1750-850 cm^{-1}. Six PCs were selected for data analysis. Score plots (2D) were examined for any clustering of spectra and loading plots identified the spectral features explaining any observed clustering in the scores plots in each data set. The spectra of various treatment of tomato roots were subsequent clustered by Unsupervised Hierarchical Cluster Analysis (UHCA) using the Ward's algorithm method (OPUS 6.5 software; Bruker Optics Ltd., Ettlingen, Germany). UHCA was performed on a regions of 3000-2800 cm^{-1} and 1750-850 cm^{-1} with a total of 60 spectra.

RESULTS

Effect of *N. nambi* on inducing resistance root-knot disease of tomato

Split-root test revealed that luminescent mushroom, *N. nambi*, in all conditions could stimulate tomato resistant to root-knot nematode, *M. incognita* (Table 1) when treated the fungus 10 days before nematode egg infestation. The tomato treated with spawn (SP) 30g/pot or soil drenching by culture filtrate 15 ml/plant combined with spawn 15 g/pot (CF+SP) were the most effective for inducing resistance of tomato against root-knot nematode, they could reduce significantly ($P<0.05$) root gall in tomatoes at 3.5% (83.3% reduction) while the control root exhibited highest root galls (20.58%). Whereas, other treatment such as culture filtrate (CF) of *N. nambi* isolate PW2, 0.2% DMSO and aurisin A (500 mg/L) showed the lower percentage of root galls 4.5, 4.8 and 5.3%, respectively. These results indicated that *N. nambi* could reduce root galls 74-83%. On J2 penetration, treatment of culture filtrate combined with spawn of *N. nambi* isolate PW2 showed the best efficiency in reducing J2 penetration of 92.3% more than other treatments, culture filtrate, spawn, aurisin

A and 0.2% DMSO reduced J2 penetration in root of tomato ranking 17.1-86.6%, while plants inoculated with only nematode (N-only) showed the maximum number of nematode juvenile in tomato roots of 1,036 J2/g root (Table 1).

Determination of defense enzymes by biochemical analysis

Phenol assay

The results of induction of phenolic compound in tomato treated with *N. nambi* with different condition were determined in Table 2. The highest accumulation of tomato root (0.205 mg g^{-1} fresh weight) were obtained from spawn (SP) followed by tomato root treated by aurisin A and culture filtrate (CF) with 0.128 and 0.093 mg g^{-1} fresh weight, respectively compared to control treatment (Table 2).

Salicylic aicd assay

The result showed that SA level was significantly ($P<0.05$) higher in tomato roots

Table 1. Root galling and number of *Meloidogyne incognita* J2 in root 30 days after inoculation on split-root systems of tomato seedlings with different treatments of *Neonothopanus nambi* application on the opposite half-root system at 10 days before nematode inoculation.

Treatment	Root galling (%)[b]	Reduction (%)[c]	No. of J2/g root	Reduction (%)[c]
N+ CF PW2/30 ml[a]	4.50 ± 2.65bc	78.13	138.7 ± 39.11 e	86.61
N+ SP PW2/ 30 g	3.50 ± 3.98c	82.99	170.7 ± 15.01 d	83.52
N+ CF/15 ml+SP/15 g (PW2)	3.50 ± 3.32c	83.87	80.0 ± 15.10 f	92.28
N+ Aurisin A /10 ml	5.30 ± 3.59b	74.25	522.7 ± 75.72 c	49.55
N+ 0.2% DMSO /10 ml	4.80 ± 2.50bc	76.68	858.7 ± 60.58 b	17.11
N-only 3,000 eggs/pot	20.58 ± 3.86a	-	1036.0 ± 55.11 a	-
N-only	20.6e	-	0.0e	-
Control	0.00 ±d	-	0.00 ± 0.00 g	-
C.V. (%)	13.91		16.92	

Means followed by the same letter (s) in a column are not significantly different at P≥0.05 by DMRT.

[a] Control = no organisms, N = 3,000 *M. incognita* J2/half-root system, DMSO=dimethyl sulfoxide, CF = culture filtrate, SP = mycelial spawn, CF+SP = culture filtrate combined with mycelial spawn

[b] Evaluation in 30 days after inoculation with *M. incognita* (3,000 eggs/pot), Treated *N. nambi* 10 days before inoculation with *M. incognita*. Galling index scored based on [25] 0 = no root-knot; 1 = 1-25% root-knot; 2 = 26-50% root-knot; 3 = 51-75% root-knot; 4 = 76-100% root-knot.

[c] Reduction (%) = (Root galling N-only – Root galling Treatment)/Root galling N-only x 100

Table 2. Contents of phenolic compound, salicylic acid and defense enzyme activities in the tomato treated root with different conditions of *Neonothopanus nambi* at 10 days

Treatment	Phenolic[a] compound	Salicylic[b] acid	PAL[c]	PPO[d]	Chitinase[e] activity	Protease[f] activity
CF PW2/30 ml	0.093c	0.250ab	17.85b	8.51b	3.06c	15.89b
SP PW2/ 30 g	0.205a	0.268a	29.17a	14.15a	5.23a	26.05a
CF/15 ml + SP/15 g (PW2) PW2)	0.034d	0.265a	31.51a	6.48b	2.52d	10.42c
Aurisin a /10 ml	0.128b	0.198c	18.52b	6.84b	1.23e	10.72c
0.2% DMSO/10 ml	0.060d	0.220bc	9.69c	8.15b	3.93b	10.65c
Control	0.050d	0.195c	16.24b	2.18c	2.97c	18.46b
C.V. (%)	16.91	18.57	25.99	21.16	15.69	10.40

Means followed by the same letter (s) in a column are not significantly different at P≥0.05 by DMRT.

[a]Phenolic compound evaluated in subunit [mg g^{-1} fresh weight];

[b]Salicylic acid [mg g^{-1} fresh weight];

[c]PAL (Phenylalannine ammonia-lyase) [µmol (trans-cinnamic acid) mg^{-1} protein/hr];

[d]PPO (Polyphenoloxidase) [µmol (quinone) mg^{-1} protein/hr];

[e]Chitinase activity [µmol (GlcNAc)/mg^{-1} protein)/hr]

[f]Protease activity [µmol (tyrosine)/mg^{-1} protein)/hr]

treated with spawn (SP), culture filtrate combined with spawn (CF+SP) and culture filtrate (CF), with 0.268, 0.265 and 0.250 mg g^{-1} fresh weight, respectively compared to control root (Table 2).

PAL assay

The highest activity of PAL enzyme was observed in tomato roots treated with culture filtrate combined with mycelia spawn (CF+SP) and mycelia spawn (SP) with 31.51 and 29.17 µmol (trans-cinnamic acid) mg^{-1} protein/hr compared with control root (Table 2).

PPO assay

All treatment of *N. nambi* increased level of PPO activity (Table 2). The highest activity of PPO was recorded in tomato roots treated with mycelia spawn (SP) with 14.15 µmol (quinone) mg^{-1} protein/hr followed by tomato root treated with mycelia spawn (SP), culture filtrate (CF), 0.2% DMSO, aurisin A, and culture filtrate combined with mycelia spawn (CF+SP) were 8.51, 8.15, 6.84 and 6.48 µmol (quinone) mg^{-1} protein/hr, respectively compared to control root**.**

Chitinase activity

The highest activity of chitinase enzyme were obtained in tomato treated with mycelia spawn (SP) with 5.23 µmol (GlcNAc)/mg^{-1} protein)/hr followed by tomato root treated with 0.2% DMSO were 3.93 µmol (GlcNAc)/mg^{-1} protein)/hr compared to control root (Table 2).

Protease activity

The highest activity of protease enzyme were measured in tomato treated with mycelia spawn (SP) with 26.05 µmol (tyrosine)/mg^{-1} protein)/hr compared to control root (Table 2).

FTIR microspectroscopy

Tomato root samples obtained from six different treatments of *N. nambi* were examined by FTIR spectroscopy in order to monitor biochemical changes during induced resistance process. The average of all quality spectra after normalization and baseline correction over the range of 3000-2800 cm^{-1} are showed in Fig 1(a). Significant spectral changes were observed in a peak position of 1400-1000 cm^{-1} (Fig. 1a). The second derivative spectrum of such regions gives a negative peak, allowing easier identification of individual peaks in complex spectra (Fig. 1b). These spectral changes were associated with alterations in polysaccharide, cellulose and hemicelluloses. Tomato roots treated with *N. nambi* showed higher polysaccharide content region at spectral bands 1515 cm^{-1} (aromatic skeleton of lignin, 1377 cm^{-1} (C-H bending) which were assigned to cellulose, 1107 cm^{-1} (C-C glycosidic link from pectin), and also clear spectral differences in carbohydrate-associated band 1060 cm^{-1} and 1030 cm^{-1} (C-O stretching) assigned to hemicelluloses when compared with untreated roots. The peak area integration of each absorption spectrum was further performed in order to presenting significantly differences between the untreated and treated roots (Table 3).

Our results indicated that the integral area of the tomato roots treated with mycelia spawn shows highest intensity of the cell wall protein

Table 3. The integral area percentage of cell wall regions assignments of tomato root untreated and after treated with *Neonothopanus nambi* in different conditions

Treatment	Cell wall tomato root assignments (integral area %)				
	Protein amide[a] (1716-1560 cm^{-1})	Lignin[a] (1566-1495 cm^{-1})	Cellulose[a] (1487-1337 cm^{-1})	Pectin[b] (1178-1084 cm^{-1})	Hemicellulose[c] (1087-975 cm^{-1})
CF PW2[1/]	30.8 ± 0.3ab	0.23 ± 0.059c	30.6 ± 0.9b	18.2 ± 0.1e	20.1 ± 0.8d
SP PW2	32.8 ± 1.2a	0.57 ± 0.058a	18.6 ± 1.2e	20.2 ± 3.2d	27.8 ± 1.1b
CF+SP PW2	25.6 ± 1.1cd	0.43 ± 0.000b	15.2 ± 0.7f	25.1 ± 0.7b	32.8 ± 2.5a
Aurisin	22.2 ± 0.3d	0.30 ± 0.000bc	33.2 ± 1.9a	22.2 ± 0.2c	22.1 ± 1.8c
DMSO	23.7 ± 0.5d	0.20 ± 0.000c	23.2 ± 0.5c	31.5 ± 0.8a	21.4 ± 1.6cd
Control	28.2 ± 0.4bc	0.40 ± 0.000bc	21.0 ± 0.1d	23.1 ± 0.5c	27.3 ± 0.6b
C.V. (%)	8.14	5.41	4.23	4.28	3.96

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

Control = no organisms, DMSO=dimethyl sulfoxide, CF = culture filtrate, SP = spawn, CF+SP = the combination of culture filtrate and spawn PW2

[a]from [26], [b] from [27]; [28], [c] from [27]

region (1716-1560 cm^{-1}) and also highest absorbance of lignin regions (1566-1495 cm^{-1}) with 32.8 and 0.57%, respectively when compared with the untreated roots (28.2 and 0.40%), respectively. The integral area of tomato roots treated with aurisin A shows highest intensity of cellulose regions (1377 cm^{-1}) with 33.2%, followed by culture filtrate (30.6%), and 0.2% DMSO (23.2%) than the untreated root (21.0%). The integral area of tomato root treated with 0.2% DMSO exhibited highest intensity of pectin regions (1178-1084 cm^{-1}) with 31.5%, followed by culture filtrate combined with spawn (CF+SP) (25.1%) than the untreated roots (23.1%). The tomato roots treated with culture filtrate combined with spawn (CF+SP) also expressed highest intensity of hemicellulose regions (1087-975 cm^{-1}) with 32.8 % than the untreated roots (27.3%) (Fig. 2). These different infrared absorbant spectra of cell wall protein and polysaccharide regions in the tomato roots treated with different condition of *N. nambi* might cause by differences in plant root responses related to induced resistance mechanisms.

To elucidate differences among these infrared spectra of tomato roots, absorption spectrum of each treatment was further analyzed with multivariate statistical techniques based on PCA to assess the spectral differences between average entire spectra as shown in Fig 3a. PCA was performed on second derivative spectra from all 160 spectra. The results showed clearly as separated with six clusters of tomato roots were classified with PC 1 versus PC2 score plot. The first two PCs explained 80% of the total variance in the data set (62% and 18% of the total variance explained by PC1 and PC2, respectively). Spectra from tomato roots treated with mycelia spawn (SP) and culture filtrate combined with mycelia spawn treated root (CF+SP) can be distinguished from other treatments by having positive PC1 scores (Fig. 3a). This spectra were highest negative value for PC1 loading (Fig. 3b) with variables between 1650 cm^{-1}, 1510 cm^{-1}, 1107 cm^{-1}, 1060 cm^{-1} and 1030 cm^{-1}, possibly indicating that protein cell wall, lignin, and cell wall polysaccharide such as pectin and hemicelluloses are most responsible for discrimination. Moreover, spectra from other treatments such as tomato roots treated with culture filtrate (CF), 0.2% DMSO-treated root, aurisin A-treated root and untreated root are clearly separated by the negative correlation of PC1 (62%) (Fig. 3a), which have positive PC1 loadings (Fig. 3b) at 1378 cm-1, suggesting a dominance of cellulose region. Whereas the spectra of mycelia spawn (SP)- and culture filtrate combined with mycelia spawn (CF+SP)-treated tomato root were clustered separately along PC2 (18%). The negative of PC2 loading of the glycosidic linkage (C-O-C)

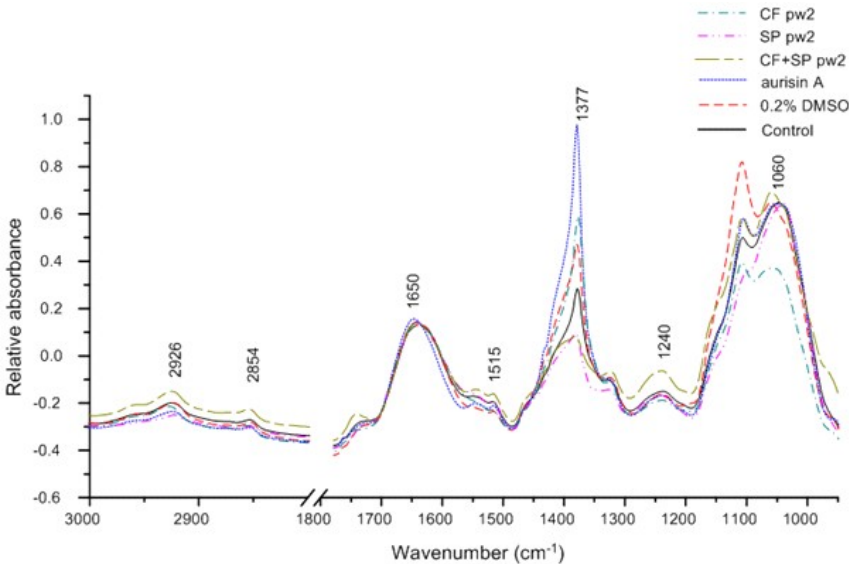

Fig. 1. Average raw FTIR spectra from untreated tomato root (control) and treated tomato root with *Neonothopanus nambi* in different conditions. Spectra were normalized over the range of 3000-1000 cm^{-1}

stretching at band 1030 cm^{-1} attributed to hemicelluloses were used to separate the spectra of the tomato roots treated with spawn (SP) from tomato root treated with culture filtrate combined with spawn (CF+SP). In order to classify and determine the similarity of infrared spectra among the tomato roots both treated and untreated group,the Hierarchical cluster analysis (HCA) with Ward's algorithm were performed using spectral in the regions 3000-2800 cm^{-1} and 1800-950 cm^{-1} (Fig 4). There were two classes which could be distinguished between tomato roots treated with

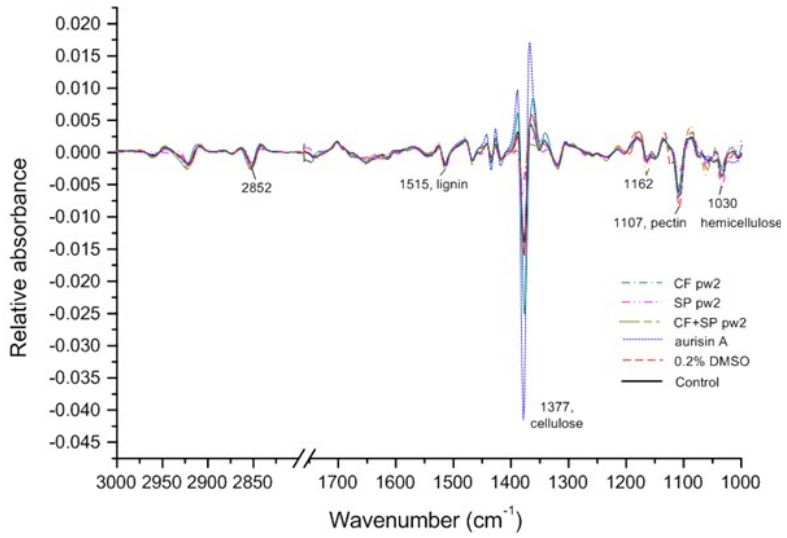

Fig. 2. Representative average 2nd derivative FTIR spectra from untreated tomato root (control) and treated tomato root with *Neonothopanus nambi* in different conditions. The average spectra were taken to the second derivative to enhance the difference between treated and untreated root which were mainly between 1400-1000 cm^{-1}; these include the amide I proteins (1650 cm^{-1}) and 1510 cm^{-1} bands of lignin regions and between 1400-950 cm^{-1} include the poly-saccharide cell wall absorbance regions; the cellulose (1377 cm^{-1}), pectin (1107 cm^{-1}) and hemicelluloses (1060 cm^{-1})

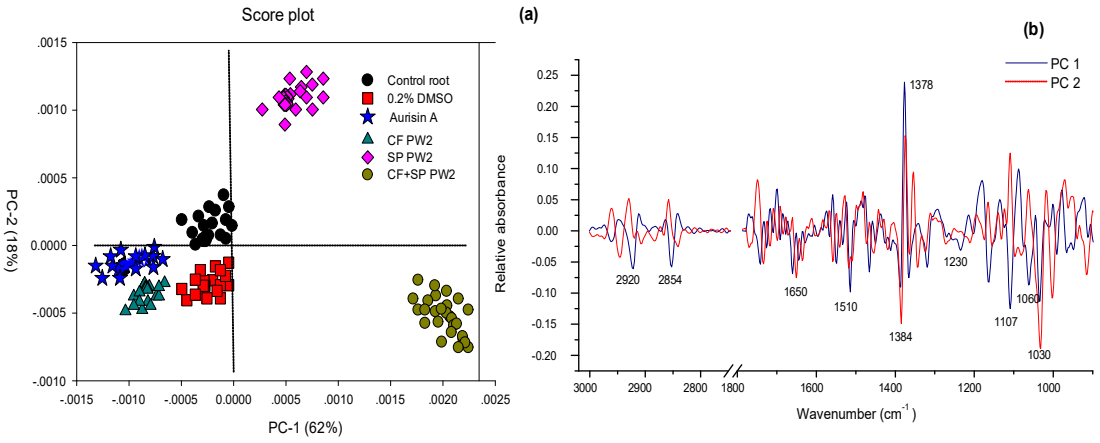

Fig. 3. Principle component analysis (PCA) of FTIR spectral range 3000-900 cm^{-1} giving PCA score plots (a) and PCA loading plots (b). PCA score plots showed distinct clustering between untreated (control) root and treated root after 10 days inoculation. PCA loading plots identify biomarker difference (i.e., discriminating wave numbers) over spectral range of samples

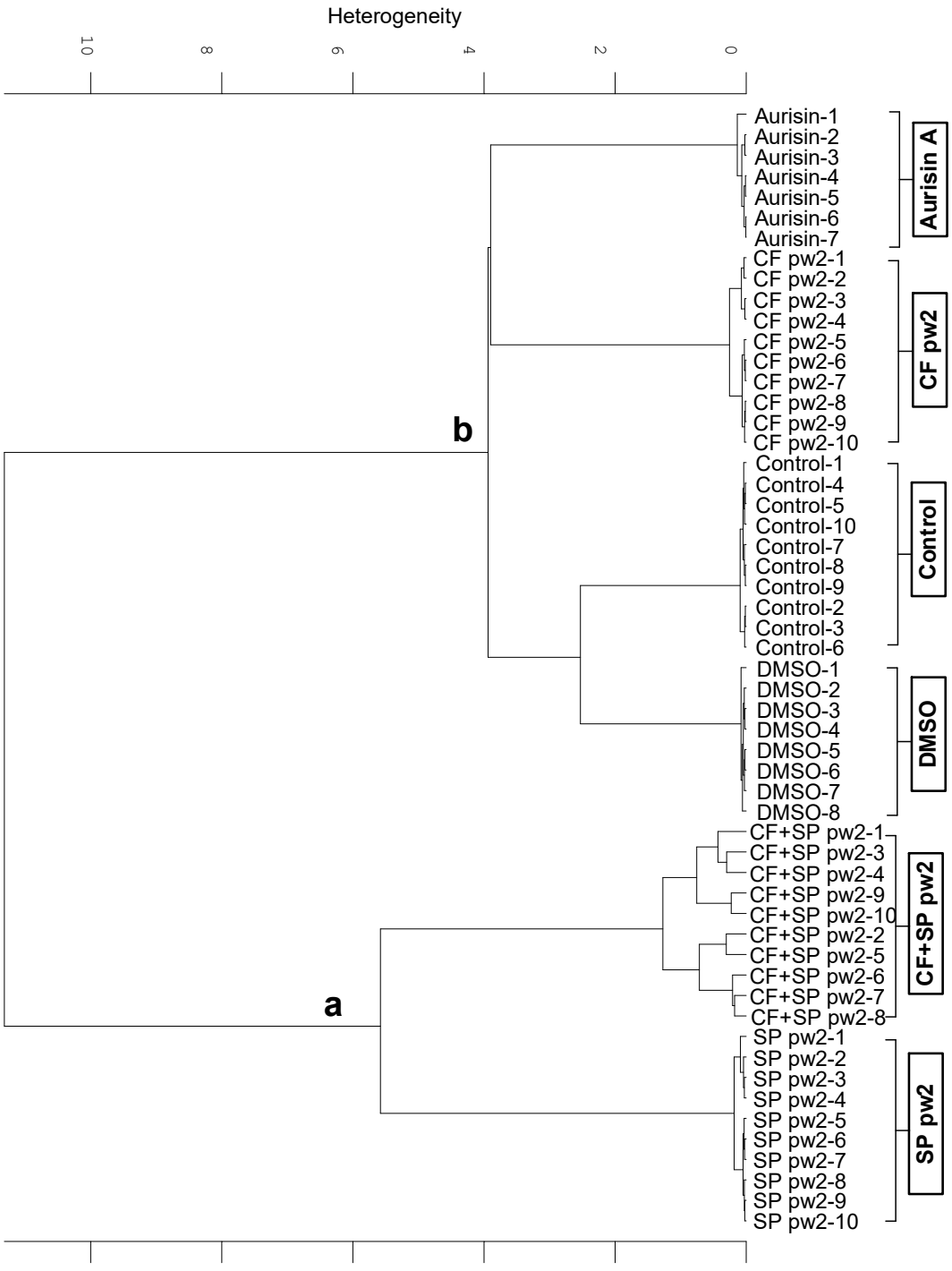

Fig. 4. Cluster analysis of FTIR spectra of untreated tomato root group and root treated group based on spectral range 3000-2800 and 1800-950 cm^{-1}. Classification based on Ward's algorithmn

spawn (SP), culture filtrate combined with spawn (CF+SP) and root treated with culture filtrate (CF), 0.2% DMSO, aurisin A and untreated root below a linkage distance less than 6. Both principle component and cluster analysis indicated that the cell wall protein and polysaccharide regions were clearly discriminated among tomato roots treated with different conditions.

DISCUSSION AND CONCLUSION

The split root experiment presented here was used to elucidate this possible involved mechanism of nematode suppression by luminescent mushroom, *N. nambi*. The results of the experiment revealed that soil treatment with spawn (SP) and culture filtrate combined with spawn (CF+SP) of *N. nambi* was found more effective in enhancing suppress of root-knot nematode, *M. incognita* in tomato. The application of spawn (SP) of *N. nambi* was most effective and significantly reduced root galling percentage and nematode penetration in tomato root when compared to other condition. These findings are in agreement with those of several workers [25, 29]. The work of [30] reported that the activity of nematophagous fungi may increase in response to sawdust to obtain nitrogen balancing in C/N ratio. However, mode of action of sawdust affected on nematophagous fungi is still not fully understood. Defense compounds and enzymes in host plants play an important role in the mechanism of resistance to nematodes. Involvement of biochemical compounds during induction of resistance in tomato was studied in enzyme extracts of tomato roots ten days after treatment of *N. nambi*. It could be concluded in this present study that tomato plants treated with the spawn of *N. nambi* enhances the induction of defense compounds and enzymes such as phenolic compound, salicylic acid, PAL, PPO, chitinase and protease than other treatments and untreated roots. These results are in agreement with previous studies of several researchers. Salicylic acid (SA) is a signal molecule involved in hypersensitive reactions in plants which important to activate defense reactions to root disease caused by nematode. Salicylic acid may activate plant enzymes to limit the nematode development and reproduction[31]. The infestation of lesion nematode, *Pratylenchus zeae* was effected on PAL activity increasing in resistant cultivar of sugarcane when compared with susceptible cultivar [32]. Brueske [33] also reported that the decreasing of PAL activity in susceptible tomato and carrot roots that infected by *Meloidogyne* spp. The increased activity of PAL may associate with the production of trans-cinnamic acid which acts as a substratum for lignin synthesis pathway towards protection against nematode penetration. Moreover, PAL is the most important enzyme playing a role for synthesis of phenols, phytoalexin and lignin. Enhanced activity of highest PPO activity has been reported in tomato that inoculated with *M. incognita* especially, in resistance cultivars [34]. Other defense enzymes include pathogenesis-related proteins such as chitinase and protease were also reported the involvement of induced resistance against plant parasitic nematode. Chitinase may have some effect on the resistance mechanism of plants leading to a reduction of nematode by degrading of the cell wall and cause lysis of the cuticle cell of nematode. The highest accumulation of chitinase was observed in a study of [35] that the tomato cells treated with *Pseudomonas flourescens* strain Pf 128 which reduced nematode penetration in tomato root tissues. FTIR spectra analysis of tomato roots treated with different condition of *N. nambi* exhibited the absorption bands of cell wall proteins and cell wall polysaccharides in spectral range 1700-1000 cm^{-1}. From the quantitative analysis of these components by integral area, it is found that higher cellulose content were observed from tomato root treated with bioactive compound extracted from *N. nambi* (aurisin A) than other treatments. Whereas, highest of cell wall protein and lignin accumulation were obtained from root treated with spwan (SP) of *N. nambi*. The results also confirmed by multivariate data analysis based on PCA and cluster analysis which exhibited that tomato root treated with spawn (SP) alone or root treated with culture filtrate combined with spawn (CF+SP) have more activation of cell wall components such as lignin, pectin and hemicelluloses than other treatments and untreated roots. Lignin is a cell wall component that covalently linked to hemicellulose and crosslinks different plant polysaccharides conferring mechanical strength to the cell wall [36], which plays on important role against many lytic enzymes

produced by pathogens during host tissue colonization. In addition, the lignification in plants cell wall could be a crucial process in resistance to root-knot nematodes [37]. The intensity of PAL, PPO, other defense enzymes and arise of cell wall protein and cell wall polysaccharides may be associated with the resistant mechanism present in the tomato against root-knot nematode. In conclusion, the present study implies that earlier and higher accumulation of phenols, salicylic acid and defense enzymes such as PAL, PPO, chitinase and protease and synthesized of cell wall polysaccharide in tomato roots treated with *N. nambi* involved in induced systemic resistance and resulting in significant reduction of nematode infection. Our study reported previously that the culture filtrate, spawn and the bioactive compound, aurisin A produced by *N. nambi* were effective biocontrol agents against root-knot nematode in tomato[10]. This consequent result of the study reports for the first time on the induced resistance mechanism of luminescent mushroom, *N. nambi* to control the root-knot nematode, *M. incognita* in tomato. This indicated that tomato root applied with *N. nambi* in difference condition may exhibit different modes of induce resistance processes based on their infrared spectral changes. This response may leads to lignifications of cell walls of tomato root which is a mechanical barrier for the pathogens [38].

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