Control Strategies of Plant Parasitic Nematodes in Black Pepper Plantation

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Abstract. Plant parasitic nematode is one of important pests causing big loss in crop production. Currently, black pepper is considered as one of major agricultural products in Indonesia, hence its cultivation including plant protection is important in black pepper development. Important parasitic nematodes such as Radopholus similis and Meloidogyne spp., have been known as causal agents of yellow disease in black pepper plantation. These parasitic nematodes could be controlled integrally through several approaches such as using biological control agents, botanical pesticide, and enhancing cultural practice strategies. Biological control using nematode-parasitic bacteria has been proven able to suppress the development of the parasitic nematode in the soil. The use of synthetic pesticide was usually applied as the last effort because of their serious implications for human health not only for the farmers, but also for the consumers and the environment. Therefore, recently, people begin to fascinate the use botanical pesticides known as pesticide of plant origin. Clove, tobacco, and betel vine extract have been proven effectively reduced the penetration rate of the nematode, and suppressed the nematode population development in roots and the percentage of rhizome-knot symptom. These organic materials may be developed for the active ingredient of botanical nematicide. Cultural practices such as amendment of organic materials as mulch becomes important strategy to reduce percentage of total nematode population and its reproduction rates, while at the same time it improves soil quality hence the plant grows well.

1. Introduction
Pepper plant (Piper nigrum L.), which came from India, considered as one of cultivated crops in Indonesia. This King of Spices is one traded spice of the world, which brought many seafarers to the shores of India from the ancient times [1]. In the country pepper usually uses as a spicy and flavouring ingredient for the food industry, and as a constituent of several medicines. This crop is mainly cultivated in some provinces in Indonesia such as Bangka Belitung, Lampung, South Sulawesi, and Southeast Sulawesi Provinces.

However, currently, the production of black pepper in Indonesia facing some problems due to attack of plant parasitic nematode, Meloidogyne incognita (Figure 1) and Radopholus similis (Figure 2) symbiotic with pathogenic fungus, Fusarium sp., causing wilt disease [2, 3]. The disease is reported to be one of the most serious problems in pepper producing countries [4, 5], including in Indonesia [6], causing about 41% of crop lost annually [7]. F. solani f. sp. piperis caused root rots and stem blight in
pepper plant [8], and *F. oxysporum* Schl. f. sp. *piperis* also reported as an important pathogen but less common in pepper plant.

![Image](image1.png)

Figure 1. Root galls caused by *M. incognita* on a primary root (A) and secondary root (B). Adult females of *M. incognita* inside a primary root (C & D) [9]

![Image](image2.png)

Figure 2. Burrowing nematode *R. similis* penetrate and feeds in the cortex of pepper root, and extensive blackish necrosis of the larger laterals gradually develops; causing severe root damage [10]

*M. incognita* is a sedentary endoparasite that feed on vascular system causing hypertrophy and hyperplasia resulting in series of galls on roots, which finally decay due to secondary infestation by microorganisms such as *Fusarium* spp., resulting in total loss of root system [11]. The attacked roots are not fully able to carry the normal function of absorbing moisture and nutrient from soil. The plant then express symptoms on the aerial part initially slight to general yellowing of foliage, stunted growth, and wilting (Figure 3). Wilting occurred two to three months after heavy infection is followed by sunny, warm and dry weather [5].
Figure 3. Pepper plant attack by nematodes showing yellowing leaf disease (a) and wilt disease symptoms (b)

There were thirty-five plant-parasitic nematodes taxa, which were belonging to 19 genera and 11 families, were identified associated with pepper plant in Vietnam in other study [12]. *Meloidogyne incognita* was the most abundant taxa from these thirty-five and was found in all surveyed areas. There were four more taxa that were present in all provinces surveyed. They were *Aphelenchus avenae*, *Tylenchus* sp., *Ditylenchus ausefi*, and *Rotylenchulus reniformis*.

Nematode generally do not directly kills the attacked plants but causing a gradual decline in the health then reduces the overall pepper production [13]. It presence should be controlled because its infestation will only be seen after several months or years when plant has expressed foliar symptoms. It attacking the root system and is quite difficult to realize because of their soil borne nature [14]. Currently, using synthetic nematicide is one of the most important components for its managed. However, environmental pollution causing health hazards due to continuous application pose a challenging problem to scientists to search alternatives control strategies. In term of cultural practices, the grower is encouraged to implement a good cultural practice though the utilization of healthy seed and transplants, growing seedlings in pathogen free peat, disinfection of nutrient solutions, and crop rotation [15].

One of the cheapest and most efficient approaches is stimulation on plant defence through the production of plant defence inducers as well as the use of nematode resistant varieties [16]. However, of 10 pepper varieties released by the Ministry of Agriculture of Indonesia, none are resistant to plant parasitic nematode. Therefore, other strategies for managing nematodes such as using botanical pesticides, natural enemies, and organic materials are much needed.

2. Control strategies

2.1. Natural Enemy

Natural enemies can be utilized as biological control, which is able to manage plant pathogens and stimulate plant growth through several mechanisms, such as antibiosis ability, hydrolysis of chitin, protein, and lipids, production of cyanide (hydrogen cyanide/HCN), phosphate solubilization (P), and fixation of nitrogen (N2). The ability of biological agents in producing antagonistic compounds is influenced by genetic and environmental factors [17]. Although the biological agents have genes that regulate the production of a particular enzyme, such enzyme is not produced due to the absence of environmental supports. Conversely, though the environmental condition is supportive for the enzyme production, the enzyme is not produced because of the absence of gene.
Using fungi, actinomycetes, viruses and bacteria as antagonistic agents in biological control has higher potential to be used for controlling its infestation. They may reduce the disease producing activities of the pathogens [18] by direct action of antagonists. Antibiosis, parasitism, predation, induced resistance of the host plant, and direct competition for space and limited resources are the result of these direct actions and led eventually to reducing the infection level [19].

In the last few years, biological control of plant parasitic nematode has been developed successfully. Antagonistic Pseudomonas sp. strains against nematode has been widely studied by scientist [20] and showed that this bacterial isolates could reduced M. incognita ability to reproduce in soil. The use of Pseudomonas sp. strain A175 and strain F23, as well as Micrococcus sp. strain S54 for controlling nematode shows satisfactory results. Their application in greenhouse trial significantly increase pepper growth performance. Plant height, number of branches number of leaves, number of segments, shoot fresh weight, shoot dry weight, root fresh weight, and dry weight were increased. These three endophytic bacteria significantly reduce the gall formation and nematode population for >23% and >41%. These antagonistic efficacy are at par with chemical treatment with the efficacy percentage of 24% and 40.9%, respectively for gall formation and nematode population reduction [21].

A total 9 of 16 bacteria were safe for plants and mammals which were able to inhibit activity of F. oxysporum (18.6 to 43.7%). Furthermore, the secondary metabolites of the endophytic bacteria that were isolated from pepper plant roots, also promoted lethal effects on M. incognita (16.6 to 65.8%). Seven isolates were able to produce chitinase, and four isolates were able to produce protease. Additionally, 55.6% of isolates were also able to dissolve phosphorus and fix nitrogen. The selected isolates are valuable candidates for the development of broad spectrum biopesticides for controlling phytopathogenic fungi, F. oxysporum, and nematodes, M. incognita. More work is required on product development of all isolates in order to improve their bio-control efficiency and thus provide farmers with a better and reliable product towards phytopathogenic fungi and nematodes management [22].

Some endophytic microbe isolates (ANIC and Trichoderma) in greenhouse experiment could suppress M. incognita population as much as 78,21 and 94,41% on pepper vines; and yellows incidence as much as 60,06 and 90,09% compared with control; and enhanced plant growth [23,24]. Other study showed that bacteria might have beneficial plant growth promoting and insecticidal properties. Both mycelia growth and spore germination of F. oxysporum could be significant controlled by five endophytic bacteria [8]. Among of 23 endophytic bacteria that have been isolated from tropical Piper sp., two Pseudomonas bacteria were found having the potential to control Fusarium sp. This was the caused of root rot disease of black pepper in the Amazon region [25].

The potential of microbial pathogens, endophyte, and antagonists for biological control of Meloidogyne spp. is great when one considers the microbial-based efficacy within a suppressive soil – a soil that totally suppresses nematode multiplication [26]. Endophytic bacteria is ideal candidate for controlling endoparasitic nematodes because it also colonize the internal plant tissue. Chemical control would not be effective if do not know the location of pathogens. Otherwise, endophyte would an effective way although do not know the location of pathogen because they move to internal plant tissue find pathogen itself [27]. Hence, information related to endophytic bacteria as a bio-control agent of wilt disease caused by M. incognita and F. oxysporum in pepper plant is very important.

2.2. Botanical Nematicide

Until now, controlling of nematodes and pathogenic fungi in pepper plant using synthetic both nematicide and fungicide has instead of not effective but also expensive. Registration of new chemicals is an immense hurdle for a prospective control of pests. Using nematicide is frequently cost prohibitive, especially in subsistence agriculture. Furthermore, environmental and human health concerns have resulted in increased restrictions on the use of these toxic materials [28]. Meanwhile, using synthetic nematicide caused no long-term suppression of nematode population densities [29].

Equal with synthetic nematicide, synthetic fungicides are used for managing crop quality and production in pepper plant. However, their environmental effects are so adverse. Its residues could persist in soil, or off-site to surface and ground waters [30]. In other study, it adversely affects the
biochemical parameters such as protein, carbohydrate, and chlorophyll although it also caused the increasing yield of crops. Therefore, the used of synthetic fungicide should be designed as well as possible so the adverse effects could be minimized [31]. The other alternatives to control plant parasitic nematode and pathogenic fungi is botanical pesticides utilizing [32]. These kind of pesticides are commonly considered to be non-persistent because they are easier to transformed by light, oxygen and microorganisms into less toxic products. Therefore no adverse environmental effects are expected [33].

The use of plant-based pesticides in pest control has long been carried out, especially in countries that have known and used herbs in their daily lives [34]. Until now the mode of actions of one plant material with another are known to be very different and are still poorly understood. However, plant-based pesticides are increasingly being considered for use in pest management strategies because of their large potential [35].

Botanical pesticides basically utilize plant secondary compounds as their active ingredients. This compound functions as a repellent, attractant, and killer of pests and as a barrier to appetite for pests. The use of plant materials that have been known to have the above characteristics, as active ingredients of pesticides, are expected to be able to substitute the use of synthetic pesticides, so that the residues of synthetic chemicals in various agricultural products, which are known to bring a variety of negative effects to nature and the surrounding life, can be reduced as low as possible.

The assay of nematicidal activity of plant extracts against M. incognita has been done. The extracts of tobacco (Nicotiana tabacum L.), clove (Syzygium aromaticum L.), and betelvine (Piper betle L.) were the most effective in killing the nematode, with an EC50 that was 5-10 times lower than the synthetic pesticides chlorpyrifos, carbosulfan and deltamethrin. Tobacco, clove and betelvine were highly toxic to the nematodes at 5 mg extract/ ml water, killing more than 80% of the nematodes [36].

Table 1. Toxicity of plant extracts and carbofuran nematicide against M. incognita.

| Common names         | Local names | Concentration (mg/ml) |
|----------------------|-------------|-----------------------|
|                      |             | LC20 | LC50 | LC90 |
| Nicotiana tabacum    | Tobacco     | 1.3  | 1.9  | 3.6  |
| Syzygium aromaticum  | Clove       | 2.8  | 3.9  | 4.9  |
| Piper betle          | Beetle vine | 1.2  | 3.0  | 5.2  |
| Acorus calamus       | Sweet flag  | 4.9  | 11.3 | 18.7 |
| Karbosulfan          | Carbosulfan | 12.7 | 25.3 | 36.1 |

Source: [36]

Forms of nematodes that die after the application of tobacco leaf extracts will be curly in shape resembling nematodes which are killed by organophosphate insecticides such as carbosulfan and chlorpyrifos, which are acetyl cholinesterase inhibitors. Whereas the nematode form that is naturally dies resembles a stick (straight) or like a banana (slightly curved) (Figure 4) [36].

Figure 4. Forms of dead nematodes which were exposed by tobacco leaf extracts (A and B) and nematode die naturally (C)
In line of those finding, tobacco, betelvine and clove oil may be used as the active ingredient of botanical nematicide [37]. Clove based botanical pesticide may also act as a fungicide [38], bactericide, and insecticide [39]. Clove oil has been proven effective to control *F. oxysporum* f. sp. *vanillae* [38] also some diseases caused by bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* and also effective to control *Stegobium paniceum* [40].

From laboratory assay of BioProtektor (clove oil based pesticides) in 2019, revealed that the formula was able to kill about 72.2% of the tested nematodes at a concentration of 1.5%. Mortality increased to 100% at 2.0% test concentration (Table 2); and at 2.0% concentration, the formula did not cause phytotoxic effect to experimental pepper plant in greenhouse condition (data were not published).

Table 2. The percentage of *Meloidogyne* sp. at 1 hour after BioProtektor application

| Repetition | Concentration (%) |
|------------|-------------------|
|            | 0 (control) 0,5 1,0 1,5 2,0 |
| 1          | 0,0 33,3 50,0 100,0 |
| 2          | 0,0 66,7 66,7 100,0 |
| 3          | 0,0 90,0 100,0 100,0 |
| Average    | 0,0 28,6 66,7 72,2 100,0 |

2.3. Cultural practice

There are several kinds of cultural practices such as using nematode resistant or tolerant varieties and mulch. Even none of our varieties are known as resistant varieties to plant parasitic nematode, there are some varieties that known to be tolerant. Lampung Daun Lebar cultivar (LDL; Wide Leaf Lampung) shown tolerant toward *M. incognita* [41]; while Petaling-1 and Bangka cultivar, were tolerant toward both *R. similis* dan *M. incognita* [42, 10].

Application of organic materials as mulch has many advantages. It can improve structure, groundwater reserves and nutrient status of the soil. The nutrient content of the waste varies depending on the source of the raw material (Table 3). Mulch also may inhibit the growth of weeds, and buffer the soil temperature, so that temperature difference is not too extreme. In addition, crop residues may attract soil organisms hence affected the abundance of arthropods due to high soil moisture and the availability of organic material as food source [43] as indicated by more predators and fewer herbivores than controls in apple plantations [44]. Mulching also affects the dynamics of the population density of soil microarthropods such as predators and ants in the corn plot [45]. The presence of organic matter and soil organisms will help improve the structure and carrying capacity of the soil, so that plant growth will be better [43].

Table 3. Nutrient status from patchouli and household wastes as well as from cow dung

| Nutrients | Patchouli waste* | Household waste** | Cow dung** |
|-----------|------------------|-------------------|------------|
| N (%)     | 3.59             | 1.71              | 1.64       |
| P2O5 (%)  | 0.28             | 0.25              | 0.36       |
| K2O (%)   | 1.26             | 0.87              | 0.77       |
| CaO (%)   | 1.7              | 0.61              | 0.21       |
| MgO (%)   | 0.95             | 0.49              | 0.21       |
| C-organic | 35.7             | 18.9              | 31.0       |
| C/N       | 9.94             | 11.7              | 19.35      |

Source: *[46],**[47]*
Mulch application also affects pest populations in the plant canopy. Evaluation of organic mulch in suppressing *Heteroligus* sp. in Nigeria showed that *Cymbopogon citratus* L. and *Ocimum viridum* L. might showed antifeedant activities. The experimental plots significantly produced more tubers and reduced beetle damage but nothing causes beetle mortality [48].

The effectiveness of mulch in suppressing populations of plant-disturbing organisms in the soil will increase if the organic constituent material contains certain chemicals [49] or mixed with substances containing certain chemicals that are poisonous to plant-disturbing organisms [50]. For example, the mulching of vanilla plants mixed with clove plant material was proven to suppress the development of vanilla stem rot disease which caused by the *F. oxysporum* attack [50] and cashew root rot disease in Bali [51] allegedly caused by *R. microporus* (*R. lignosus*) [52], or *F. solani* and *F. oxysporum* [53]. Cloves antifungal function makes a significant role in suppressing the disease development [54].

Patchouli waste mulch can also reduce the level of pest infestation in plantations. The utilization of patchouli waste mulch in pepper cultivation reduced the attack rate of pepper stem borer, *Lophobaris piperis*, thus reducing 5% yield loss [55]. It is suspected that the reduced level of pest infestation is caused by the odour produced by the waste being able to repel existing and future pests to the crop. The nature of patchouli is caused by sesquiterpenes contained in patchouli plants [56].

The process of decomposition of organic material in the soil sometimes contains certain chemicals which are apparently able to suppress the population of plant-disturbing organisms that live in the soil [57, 58] such as plant parasitic nematodes [59]. The suppression of parasitic nematode populations is thought to be closely related to the availability of food sources for various microorganisms in the soil resulting in an increase in population to the point of natural balance. A growing medium for various types of soil bacteria and fungi is the result of organic matter decomposition. The population of bacterial-eating nematodes from the family Rhabditidae, Cephalobidae and fungal-eating nematodes will also increase as the bacterial and fungal population increases [60], the population of the nematode predator, the Mononchidae family, will also increases [61]. Plant parasitic nematodes through competition, antagonism, or due to the creation of unfavourable conditions for the life of the parasitic nematodes. In addition, the process of decomposition of organic matter will produce nitrate and ammoniac nitrogen compounds which are toxic to various plant-disturbing organisms including plant parasitic nematodes [59].

Clove bud and betelvine as mulch in pepper plants have been tested in a greenhouse bioassay. The number of live nematodes treated with clove bud mulch did not differ significantly from that of plants treated with carbofuran (recommended synthetic pesticide). Therefore, clove bud application as a botanical pesticide for future use is highly promising. It also being supported by the fact that clove is the 6th major plant grown on Bangka Island, and the sharply decreased market value of clove over the last years [36].

In short, the sustainable cultivation paradigm invites farmers to re-use mulch from organic material waste. Utilization of organic mulch in agricultural ecosystems produces various benefits such as improving physical and chemical properties of the soil and also balancing the population of soil microorganisms. It makes soil returns its fertility and sustain the maximum plant growth. Utilization of organic mulch is expected to support the success of the development program of organic agriculture which is currently being promoted by the Indonesian government and has become a major demand in the world market, especially in the context of facing free trade.

3. Conclusions and Recommendations

Parasitic nematodes such as *Radopholus similis* and *Meloidogyne* spp. have been known as causal agents of yellow disease in black pepper plantation. These parasitic nematodes could be controlled integrally through several approaches such as natural enemies as their biocontrol agents, botanical nematicides and enhancing cultural practices through the use of mulch and resistant varieties.
References

[1] Cheriyan H 2015 *Good Agricultural Practices Black Pepper (Piper nigrum L.)* (India: Directorate of Arecanute and Spices Development)

[2] Suryanti, Hadisutrisno B, Mulyadi, Widada J 2017 *Jurnal Perlindungan Tanaman Indonesia* 21 127-34

[3] Nelson S and Cannon-Eger K 2009 *Farm and forestry production and marketing profile for black pepper (Piper nigrum)* http://www.agroforestry.net/scps/Black_pepper_specialty_crop.Pdf

[4] Mohandas C and Ramana K 1991 *Journal of Plantation Crops.* 19 41-53

[5] Shahnazi S, Meon S, Vadamalai G, Ahmad K, Nejat N 2012 *Journal of General Plant Pathology* 78 160-69

[6] Daras U and Pranowo D 2017 *Jurnal Penelitian dan Pengembangan Pertanian.* 28 1-6

[7] Ehwaeti M, Elliott M, McNicol J, Phillips M, Trudgill D 2000 *Crop Protection.* 19 739-45

[8] Edkona J E, King W S, Teck S L C, Jiwan M, Ab Aziz ZF 2013 *International Journal of Agriculture and Biology* 15 291-96

[9] Thuy T 2010 *Incidence and Effect of Meloidogyne incognita (Nematoda: Meloidogyninae) on Black Pepper Plants in Vietnam* Dissertation (Katholieke Universiteit Leuven. Heverlee, Belgie) 156 pp

[10] Mustika I 1990 *Studies in the interaction of Meloidogyne incognita, Radopholus similis, and Fusarium solani on black pepper (Piper nigrum L.)* Thesis (Wageningen Agric. Univ. The Netherlands) 127 pp

[11] Ravindra H, Sehgal M, Manu T, Murali R, Latha M 2014 *Journal of Entomology and Nematology* 6 51-55

[12] Thuy T, Yen N, Tuyet N, Te L, De Waele D 2012 *Archives of Phytopathology and Plant Protection* 45 1183-200

[13] Onkendi E M, Kariuki G M, Marais M, Moleleki L N 2014 *Plant Pathology* 63 727-37

[14] Moens M, Perry R N, Starr J L 2009 *Meloidogyne species—a diverse group of novel and important plant parasites - Root-Knot Nematodes (CABI Publishing)*

[15] Abawi G and Widmer T 2000 *Applied Soil Ecology* 15 37-47

[16] Ralmi N H A A, Khandaker M M, Mat N 2016 *Australian Journal of Crop Science* 11 1649

[17] Hardeim P R, van Overbeek L S, van Elsas J D 2008 *Trends in Microbiology* 16 463-71

[18] Symondson W, Sunderland K, Greenstone M 2002 *Annual Review of Entomology* 47 561-94

[19] Beneduzi A, Ambrosini A, Passaglia L M 2012 *Genetics and Molecular Biology* 35 1044-51

[20] Yousif AI, Munif A, Mutaqin K H 2017 *International Journal of Science and Research* 6 2195-99

[21] Wiratno, Syakir M, Pradana A P, Yousif A I A 2018 *Journal of the Pepper Industry* IX 11-22

[22] Wiratno, Syakir M, Sucipto I, Pradana A P 2018 *Biodiversitas* XX 682-87

[23] Munif A and Harni R 2011 *Buletin RISTRI* 2 377-82

[24] Harni R and Munif A 2012 *Buletin RISTRI* 3 201-6

[25] Nascimento S, Lima A, Borges B, de Souza C 2015 *Genetics and Molecular Research.* 14 7567-77

[26] Hallmann J, Davies K G, Sikora R 2009 *Biological control using microbial pathogens, endophytes and antagonists. In: Perry RN, Moens M, Starr JL (eds) Root-Knot Nematodes.* (CABI, United Kingdom)

[27] Ryan R P, Germaine K, Franks A, Ryan D J, Dowling D N 2008 *FEMS Microbiology Letters* 278 1-9

[28] Hussain M, Zouhar M, Rysanek P 2017 *Pakistan Journal of Zoology* 49 205

[29] Starr J L, Bridge J, Cook R 2002 *Resistance to Plant-Parasitic Nematodes: History, Current Use and Future Potential* (CABI Publishing, United Kingdom)

[30] Wightwick A, Walters R, Allinson G, Reichman S, Menzies N 2010 *Environmental risks of
fungicides used in horticultural production systems. In: Carisse O (eds) Fungicides (InTech: Croatia)

[31] Tasneem R, Baftia A, Maheshwari R 2013 International Research Journal of Environmental Sciences 2 1-5

[32] Javed N, Gowen SR, Inam-ul-Haq M, Abdullah K, Shahina F 2006 Crop Protect. 26, 911-6

[33] Ujvary I 2001 Pest Control Agents from Natural Products - Handbook of Pesticide Toxicology. 2nd ed. (Academic Press: San Diego)

[34] Yang R Z and Tang C S 1988 Economic Botany 42 376-406

[35] Regnault R C 1997 Integrated Pest Management Reviews 2 25-34

[36] Wiratno, Tanuwiriyono D, Van den Berg H, Riksen J A G, Rietjens I M C M, Djiwanti S R, Kammenga J E, Murk A J 2009 The Open Natural Products Journal 2 77-85

[37] Asman A, Mesak T, Dyah M 1997 Jurnal Monograf Cengkeh. (Balai Penelitian Tanaman Rempah dan Obat) p90

[38] Tombe M, Sukamto, Zhuhisnain, Efi T 1999 Prosiding Forum Komunikasi Ilmiah Pemanfaatan Pesticida Nobati Bogor, 9-10 Nopember 1999 (Balai Penelitian Tanaman Obat dan Rempah: Bogor) p452-3

[39] Darwis M and Bariyah B 2006 Prosiding Seminar Nasional Pesticida Nobati III-Bogor, 21 Juli 2005 (Balai Penelitian Tanaman Rempah dan Obat: Bogor) p275-276

[40] Wiratno, Siswanto, Ellyda A W 1993 Prosiding Seminar Hasil Penelitian Dalam Rangka Pemanfaatan Pesticida Nabati, Bogor, 1-2 Desember 1993 (Balittro: Bogor) p293-7

[41] Nuryani Y 1984 Pember. Littir X 30-3

[42] Hamid A, Nuryani Y, Kasim R, Sitepu D, Laksmanahardja P, Wahid P 1988 Natar-1, Natar-2, Petaling-1 dan Petaling-2 adalah variasi-variasiet lada yang cocok untuk daerah Lampung dan Bangka (Media Komunikasi Penelitian dan Pengembangan Tanaman Industri

[43] Sugito Y, Nurani Y, Nihayati E 1995 Sistem Pertanian Organik (Fakultas Ilmu Pertanian Universitas Brawijaya) p84

[44] Brown M W and Tworkoski T 2004 Agriculture, Ecosystem, and Environment 103 465-72

[45] Badejo, Tian M A G, Brussaard L 1995 Biol Fertl Soil 20 294-8

[46] Djazuli, M 2002 Prosiding Seminar Nasional dan Pameran Pertanian Organik. Jakarta, 2-3 Juli 2002 p323-32

[47] Tombe M, Mulya K, Zaubin R, Pribadi E R, Indrawanto C, Trisilawati O, Ruhmayat A 2001 Uji coba pemanfaatan dan peningkatan mutu kompos produk pilot plant klder, berikut pemasarannya (Final Report. PT Gas Negara and Balittro (unpublished))

[48] Okiemute T F 2011 Agricultural Journal 6 166-71

[49] Rodriguez-Kabana, Morgan-Jones R, Chet I 1987 Plant Soil 10 237-47

[50] Tombe M, Nurawan A, Sukamto 1993 Prosiding Seminar Hasil Penelitian dalam Rangka Pemanfaatan Pesticida Nabati, Bogor 1-2 Des 1993 p28-35

[51] Tombe M, Wahyuno D, Purnayasa I G N R 2003 Pengendalian penyakit akar putih pada tanaman jambu mente (Laporan Hasil Penelitian PHTBUN)

[52] Arya N and Temaja G R M 1996 Prosiding Pengendalian Penyakit Utama Tanaman Industri Secara Terpadu. Bogor, 13-14 Maret. p224-35

[53] Tombe M, Taufiq E, Supriadi, Sitepu D 1997 Prosiding Forum Konsultasi Ilmiah Tanaman Rempah dan Obat. Bogor, 13 - 14 Maret 1997 p183-90

[54] Serrano M, Martinez-Romero D, Castillo S, Guillen F, Valero D 2005 Innovative Food Science & Emerging Technologies 6 115-23

[55] Wiratno, Wikardi E A, Iskandar M 1991 Seminar Ilmiah dan Kongres Nasional Biologi X, tanggal 24-26 September 1991 p10

[56] Ketaren S 1985 Pengantar Teknologi Minyak Atsiri (Balai Pustaka Jakarta) 427p

[57] Akhtar M and Malik A 2000 Bioresource Technology 74 35-47
[58] Nahar M S, Grewal P S, Miller S A, Stinner D, Stinner B R, Kleinhenz M D, Wszelaki D, Doohan D 2006 *Applied Soil Ecology* **34** 140-51
[59] Mian I H and Rodriguez-Kabana R 1982 *Nematropica* **12** 205-20
[60] Forge T A, Hogue E, Neilsen G, Neilsen D 2003 *Applied Soil Ecology* **22** 39-54
[61] Yeates G W, Wardle D A, Watson R N 1999 *Soil Biology and Biochemistry* **31** 1721-33