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The efficacy of compost, vermicompost and their combination with antagonistic microbes to reduce potato cyst nematode (*Globodera rostochiensis*)

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Abstract

Potato cyst nematode, (*Globodera rostochiensis*) is one of limiting factors in potato production. Concerning the negative impacts of nematicide application, the environmentally-friendly methods for controlling the nematodes such as organic amendments have been developed. However, the efficacy of organic amendments is often inconsistent. The addition of antagonistic microbes into the organic materials can improve their control effects. The objective of this study was to examine the efficacy of cow manures compost and/or vermicompost with or without addition of antagonistic microbes to suppress *G. rostochiensis*. The experiment was arranged in Randomized Complete Block Design with 11 treatments and three replications. The treatments tested were compost or vermicompost and their combination with or without addition of the microbes, sterile compost and/or vermicompost mixed with the microbes, the check and nematicide treatment. The microbes used were *Paecilomyces* sp. and *Pseudomonas pseudoalcaligense*. The results showed that application of compost solely reduced the numbers of second-stage juveniles, cysts and the females of *G. rostochiensis* by 96.2%, 81.0% and 70.2% respectively. *Paecilomyces* sp. and *P. pseudoalcaligense* mixed with sterile compost and/or vermicompost also effectively suppressed *G. rostochiensis* by 80.8-99.1%. Vermicompost was effective in reducing the numbers of the nematode second-stage juveniles, but it was ineffective in suppressing the cysts and the females. The addition of antagonistic microbes or mixing vermicompost and compost (1:1, v:v) improved the efficacy of vermicompost in suppressing the numbers of *G. rostochiensis* cysts. Based on the overall results, cow manure compost can be recommended as ecofriendly measures for controlling *G. rostochiensis*.

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Introduction

Potato cyst nematode (*Globodera rostochiensis*) is the most important parasitic nematode in potato. In Indonesia, this nematode was recorded at the first time in 2003 (Indarti *et al.*, 2004). The nematode infected the potato roots and lead to poor root system, reduction of plant growth and tuber size (Brodie, 1998; EPPO, 2013). The nematode has become a great concern as it produces cysts that can contaminate the soil and potato tubers.

Potato cyst nematode, *G. rostochiensis*, is difficult to be controlled, because it produces cysts that can survive for long time in the soil (Brodie, 1998). The use of nematicide in the soil may lead negative impacts such as soil pollution, killing the beneficial soil organisms, and the development of pathogen resistance. Concerning this issue, the development of ecofriendly control measures including biological control and organic amendments has been encouraged.

The roles of organic amendments in the management of plant parasitic nematodes have been reported and reviewed (Thoden *et al.*, 2011; Renčo, 2013). The mechanisms of organic matters in controlling the nematodes include the existence of toxic metabolites, the increase in activities of antagonistic microbes and the induction of plant resistance (Oka, 2010; Thoden *et al.*, 2011). The efficacy of organic amendments to suppress the nematodes is often inconsistent (Kimpinski *et al.*, 2003; McSorley, 2011). The addition of antagonistic microbes to the organic materials can increase their efficacy in controlling plant parasitic nematodes (Khan *et al.*, 2001; Oka, 2010; Zakaria *et al.*, 2013).

To support biological control of potato cyst nematode, Istifadah *et al.* (2017) isolated endophytic fungi from potato roots and tubers and found one isolate, *Paecilomyces* sp., that significantly reduced *G. rostochiensis* inocula. Istifadah *et al.,* (2018) also obtained one isolate of bacterial endophytes, *Pseudomonas pseudoalcaligens,* that effectively suppressed the numbers of *G. rostochiensis* cysts and the second-stage juveniles in the soil.

This paper discusses a study that examined the efficacy of compost, vermicompost and their combination in suppressing potato cyst nematode (*G. rostochiensis*). This study also evaluated the effect of the enrichment of the organic amendments with antagonistic microbes (*P. pseudoalcaligens* and *Paecilomyces* sp.) on their efficacy in suppressing the nematode.

Material and methods

Organic materials and their mixtures with antagonistic microbes

Organic materials used were cow manure compost and vermicompost. The C:N ratio of the compost used in this study was 15, while C:N rasio of vermicompost was 16. The non-sterile or sterile organic materials were mixed evenly with the microbial suspension with proportion of 10% (v:v). Sterile compost and vermicompost were prepared by autoclaving (121°C, 1 atm.) the organic materials for 20 minutes. The microbial suspension was prepared by mixing the bacterial suspension (10^8 cfu ml^{-1}) and *Paecilomyces* sp. conidial suspension (10^8 cfu ml^{-1}) with proportion 1:1 (v:v). The mixture was incubated for one week to allow the establishment of the added microbes.

The pathogen inoculum preparation

The inoculum of *G. rostochiensis* was prepared from the nematode cysts, extracted from the infested soil using floatation method (Van Bezooijen, 2006) with modification. The soil was mixed with sterile water containing 0.05% glucose (1: 2, v:v), stirred and incubated for 5 min. The floated debris was filtered through different size of sieves, and the cysts were collected under a stereo microscope. To obtain the nematode second-stage juvenile (J2) and the eggs, the cysts were ruptured and soaked in the sterile water for 2-3 days to encourage the emergence of second-stage juvenile (J2).

The experimental design and treatments

The polybag experiment was arranged in Randomized Complete Block Design with eight treatments and three replications. The treatments examined were the compost, vermicompost, or mixture compost and vermicompost (1:1, v:v), with and without the microbial addition, the sterile compost and/or vermicompost mixed with the antagonistic micobes (10%), untreated check and nematicide treatment (active ingredient : carbofuran).

The planting medium used was pasteurized soil (Andosol type), 2.5 kg per polybag. The potato variety used was Granola. The organic materials and their mixture with the antagonistic microbes were applied in the planting holes 100 g per plant. The nematode was inoculated 14 days after planting by pipetting the inoculum suspension containing about 4000 inocula (J2 and eggs) of *G. rostochiensis* in the five holes surrounding the plants (about 5 cm form the basal stem).

The variables observed and data analysis

To find out the effects of organic amendments and their mixture with antagonistic microbes on potato growth, the plant height was observed at 14 days after planting (before the nematode inoculation). The destructive observation was carried out at seven weeks after the nematode inoculation. The variables observed were the numbers of cysts and second-stage juvenile of *G. rostochiensis* in 100 g soil, the numbers of the female nematode on the roots, also fresh and dry weight of potato shoots and roots.

The data were analysed statistically using IBM SPSS statistics software version 20.The normality of the data was checked and they were transformed if necessary. The significant differences among the treatments were further analyzed using Tukey's HSD (Honestly Significant Difference).

Results

The effect of the treatments on G. rostochiensis in the soil

The results showed that the application of compost and/or vermicompost solely or their combination with antagonistic microbes reduced the numbers of *G. rostochiensis* second-stage juvenile (J2) in the soil by 96.7-99.1%. This reduction was as effective as that of nematicide treatment, which led to 99.5% reduction of the nematode inoculum. The numbers of the nematode second-stage juvenile in all treatments with compost and/or vermicompost and their mixture with antagonistic microbes were also not significantly different (Table 1).

Table 1. The effects of compost and vermicompost and their combination with microbes on the numbers of *G. rostochiensis* propagules in the soil.

Treatments	Second-stage juvenile (J2)		Cysts	
	Numbers of J2 per 100 g soil	Level of suppression (%)	Numbers of cysts per 100 g soil	Level of suppression (%)
Sterile Compost + microbes	0.7 a	99.1	2.7 a	86.2
Sterile vermicompost + microbes	1.7 a	97.6	3.2 a	83.4
Sterile compost and vermicompost + microbes	2.3 a	96.7	3.0 a	84.5
Non sterile compost + microbes	0.3 a	99.5	4.7 ab	75.9
Non sterile vermicompost + microbes	2.3 a	96.7	9.7 ab	50.0
Non sterile compost and vermicompost + microbes	2.0 a	97.1	2.7 a	86.2
Non sterile compost	2.7 a	96.2	4.2 ab	78.2
Non sterile vermicompost	10.0 a	85.7	12.7 bc	34.5
Non sterile compost and vermicompost	7.0 a	90.0	7.0 ab	63.8
Nematicide (carbofuran)	0.3 a	99.5	4.3 ab	77.6
Check	70.0 b	0.0	19.3 c	0.0

Note: The average value in the columns followed by the same letter is not significantly different according to the Tuckey's HSD Test (p<00.5)

The effects of compost and vermicompost on *G. rostochiensis* cysts were relatively differed. The application of compost solely or in combination with vermicompost, with or without antagonistic microbes, reduced *G. rostochoensis* cysts by 63.8%-86.2%. However, the application of vermicompost solely was not effectively reduced the nematode cyst in the soil. The treatment only resulted in 34.5% reduction of the cysts. The mixture of vermicompost with compost (1:1, v/v) led to higher control effects (63.8%) than that of vermicompost solely. However it was still lower than the reduction due to the application of compost solely, which was 81.0% (Table 1). The addition of antagonistic microbes to the non-sterile or sterile vermicompost also increased its efficacy in suppressing the nematode cysts.

Table 2. The effects of the organic amendments and their mixture with antagonistic microbes on the number of *G. rostochiensis* female on the potato roots.

Treatments	Number of the female nematode on the roots	Level of suppression (%)
Sterile Compost + microbes	3.7 a	80.8
Sterile vermicompost + microbes	3.7 a	80.8
Sterile compost and vermicompost + microbes	3.5 a	81.9
Non sterile compost + microbes	5.0 a	73.7
Non sterile vermicompost + microbes	7.3 ab	61.4
Non sterile compost and vermicompost + microbes	1.7 a	71.2
Non sterile compost	5.7 a	70.2
Non sterile vermicompost	10.7 ab	43.9
Non sterile compost and vermicompost	11.0 ab	42.1
Nematicide (carbofuran)	1.0 a	94.7
Check	19.0 b	-

Note: The average value in the columns followed by the same letter is not significantly different according to the Tuckey's HSD Test (p<00.5).

Compost and/or vermicompost sterile that were added with antagonistic microbes significantly reduced the numbers of second-stage juvenile of *G. rostochiensis* by 96.7-99.1%. These treatments also significantly declined the number of cysts in the soil by 82.8-86.2%.

The effect of the treatments on the numbers of G. rostochiensis female on the potato roots

Application of compost with or without the addition of antagonistic microbes significantly reduced the numbers of *G. rostochiensis* female attached on the potato roots by 70.2 – 80.7% (Table 2). The application of vermicompost solely was less effective in suppressing the numbers of *G. rostochiensis* female than the compost. The combination of vermicompost and compost or the addition of antagonistic microbes into the non-sterile vermicompost did not result in sufficient reduction of the nematode female. The numbers of the nematode female in these treatments were not significantly different to that of the check plants.

The effect of the treatments on potato growth

Application of compost and/or vermicompost with or without antagonistic microbes tended to enhance potato growth 1.6-3.2 times than the check plants. However, the plant height in non-sterile compost with or without the addition of antagonistic microbes was not statistically different to that of the check plants (Table 3). A considerable increase was found in sterile or non-sterile vermicompost mixed with the antagonistic microbes.

Based on the observation at the end of experiment (seven weeks after *G. rostochiensis* inoculation) showed that the application of compost and/or vermicompost with or without the addition of antagonistic microbes did not significantly affect the potato growth. Even though the fresh weight of shoot and roots of the treated potato tended to be higher than the check, however, it was not statistically different. At the last observation, the potato plants had already produced potato tubers. The average weight of potato tubers in the treatments with organic amendments with or without microbial enrichment was significantly higher than the check plant. Among the treatments of organic amendments, the potato growth was not statistically different (Table 4).

Table 3. The effects of the organic amendments and their mixture with antagonistic microbes on potato growth before the nematode inoculation.

Treatments	Plant height (cm) at 2 WAP	The increase compared to the check
Sterile Compost + microbes	6.7 bc	2.5
Sterile vermicompost + microbes	8.7 c	3.2
Sterile compost and vermicompost + microbes	5.9 abc	2.2
Non sterile compost + microbes	6.2 abc	2.3
Non sterile vermicompost + microbes	8.1 c	3.0
Non sterile compost and vermicompost + microbes	7.2 bc	2.7
Non sterile compost	6.3 abc	2.3
Non sterile vermicompost	4.3 ab	1.6
Non sterile compost and vermicompost	4.4 ab	1.6
Nematicide (carbofuran)	3.0 a	1.1
Check	2.7 a	1.0

Note: The average value in the columns followed by the same letter is not significantly different according to the Tuckey's HSD Test (p<00.5).

Discussion

The effect of the organic amendments on G. rostochiensis

In this study, the efficacy of the organic amendments in suppressing the nematodes was depended on the kinds of organic amendments and also the kinds of nematode propagules. In this study, both compost and vermicompost effectively suppressed the second-stage juveniles of *G. rostochensis*. For the nematode cysts and the female, however, the effective suppression was only found in the treatment using non sterile compost. The application of vermicompost solely did not significantly reduce those nematode propagules. Renčo *et al* (2011) also found that vermicompost derived from cattle manure also showed relatively low nematicidal effects on *G. rostochiensis* and *Globodera pallida*. However, in other study, vermicompost and its tea significantly decreased *G. rostochiensis* propagules (Renčo and Kováčik, 2015). The efficacy of the same kind of organic material in suppressing plant parasitic nematode may differ, depending on various factors such as the raw materials used, application rate, soil type, and other environmental factors. The raw materials used and the process involved could influence the chemical properties and microbial community in the organic materials (McSorley , 2011).

The combination of two kinds of organic amendments did not increase their efficacy in controlling *G. rostochiensis*. In this study, the efficacy of compost was higher than the vermicompost and also the mixture of compost-vermicompost 1:1 (v:v). Mixing both organic materials reduced the amount of compost and therefore it also decreased the efficacy of the mixture.

The effects of the microbial enrichment of the organic materials on the nematode suppression

In the treatment of sterile compost enriched with antagonistic microbes, the control effects were basically due to the antagonistic activities of the microbes. The sterile organic amendments fortified with *Paecilomyces* sp. and *P. pseudoalcaligens* effectively suppressed all kinds of *G. rostochiensis* propagules. This result confirmed previous studies that also found these microbes significantly reduced the numbers of second stage juveniles and cysts of *G. rostochiensis* in the soil (Istifadah *et al.*, 2017; Istifadah *et al.*, 2018). In the *in vitro* test, the culture filtrate of *P. pseudoalcaligens* also caused high mortality (97.9%) of second-stage of the nematode. The filtrate of the bacteria caused lysis of the juvenile cell walls, indicating the presence of cell wall degrading enzyme (Istifadah *et al.* 2018).

Table 4. The effects of the organic amendments and their mixture with antagonistic microbes on potato growth (seven weeks after the nematode inoculation).

Treatments	Average of shoot fresh weight (g)	Average of root fresh weight (g)	Average of potato tuber weight (g)
Sterile Compost + microbes	17.5 a	4.5 a	33.1 bc
Sterile vermicompost + microbes	18.7 a	4.7 a	46.5 c
Sterile compost and vermicompost + microbes	11.6 a	4.4 a	35.7 bc
Non sterile compost + microbes	22.0 a	5.1 a	37.0 bc
Non sterile vermicompost + microbes	20.5 a	4.7 a	47.4 c
Non sterile compost and vermicompost + microbes	17.9 a	4.9 a	49.5 c
Non sterile compost	23.0 a	5.8 a	39.2 bc
Non sterile vermicompost	23.2 a	6.1 a	38.7 bc
Non sterile compost and vermicompost	27.2 a	6.8 a	42.8 bc
Nematicide (carbofuran)	9.5 a	3.7 a	18.4 ab
Check	10.6 a	3.9 a	10.5 a

Note: The average value in the columns followed by the same letter is not significantly different according to the Tuckey's HSD Test (p<00.5).

The abilities of non-pathogenic *Pseudomonas* to control *Globodera* spp. have also been reported in other studies (ie. Ummaheswari *et al.,* 2012; Trivonova *et al.*, 2014; Seenivasan, 2017). The antagonistic mechanisms of bacteria against plant parasitic nematodes includes the production of secondary metabolites (enzymes, toxins), inhibiting nematode reproduction, egg hatching and juvenile survival, or killing of the nematodes directly (Tian *et al.*, 2007). The efficacy of *Paecilomyces* in suppressing potato cyst nematodes has also been reported (ie. Seenivasan *et al.*, 2007; Ummaheswari *et al.*, 2012; López-Lima *et al.*, 2013; Seenivasan, 2017). This fungus has been known as fungal parasite of nematode eggs and cysts (Jatala, 1986; Cannayane and Sivakumar, 2001).

The addition of antagonistic microbes to the non-sterile organic amendments did not always improve the efficacy of the organic amendments in suppressing plant pathogens. If the efficacy of the organic amendments themselves is already high, the microbial fortification may not necessary. In this study for example, the application of non-sterile compost solely suppressed *G. rostochiensis* by 96.2%, therefore the addition of antagonistic microbes to the compost did not significantly enhanced the suppressive effect. Istifadah *et al.* (2016) also found that the efficacy of chicken manure and compost was not significantly different to those organic amendments enriched with antagonistic microbes, as the efficacy of those organic amendments was already high (90-96%).

For organic amendments with low or moderate efficacy, the addition of antagonistic microbes can improve the level of suppression. In this study, the vermicompost solely did not significantly reduce the number of *G. rostochiensis* cysts. However, the addition of antagonistic microbes into vermicompost significantly improved its efficacy in reducing the number of the nematode cysts. Khan *et al.* (2011) also found that the application of organic material solely only reduced the numbers of galls and egg masses of *Meloidogyne incognita* in tomato by 38.88 and 42.48% respectively. However, the addition of *Paecilomyces lilacinus* and *Trichoderma harzianum* in the organic substrate improved the suppressive effects up to 71.77-74.92% reduction of the nematode galls and egg masses.

The effects of the organic amendments and their combination with the antagonistic microbes on the potato growth

In this study, even though the growth of potato in the treatments using compost or vermicompost tended to be better than the check plants, the growth of potato in all treatments at seven weeks after planting was not significantly different.

The significant effects of the treatments were occurred in the early growth of potato, before the nematode inoculation, in the treatments of vermicompost with or without microbial enrichment. In this study, the experiment was conducted in the polybag using pasteurized soil. The organic amendment at the rate 4% of the soil was apparently sufficient for supporting the early growth of potato (two weeks after planting), but it was insufficient to support the potato growth up to seven weeks after planting. For supporting suitable plant growth in the container media, the rate of compost or vermicompost application is about 20-30% (Hashemimajd *et al.*, 2004). Even though the organic treatments and their combination with antagonistic microbes could not significantly increase potato growth at seven weeks after planting, however, they supported the production of potato tubers better than the check plant.

Overall results of this study showed that in addition to their role as organic fertilizer, compost can be used for controlling plant parasitic nematodes. Compost can also provide suppressive effects on other soil borne pathogens (Noble and Conventry, 2005; Aviles *et al.*, 2011; St. Martin and Brathwaite, 2012; Mehta *et al.*, 2014). Thus, the use of compost as soil amendments provides various advantageous to the soil health and quality, supporting eco-friendly and sustainable crop production.

Conclusion

The application of compost in planting media effectively reduced the numbers of second stage juveniles, cysts and female of *G. rostochiensis*. Sterile compost and vermicompost added with *Paecilomyces* sp. and *P. alcaligense* also effectively suppressed *G. rostochiensis*. Vermicompost was effective in reducing the number of the nematode second-stage juveniles, but it was ineffective in suppressing the numbers of the nematode cysts and the females. Mixing vermicompost and compost (1:1, v:v) or addition of the antagonistic microbes improved the efficacy of the vermicompost in suppressing the nematode cysts.

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