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ABUNDANCE OF NEMATODES IN COMBINATION OF SOIL SOLARIZATION AND COW MANURE APPLICATION

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A B S T R A C T

Applying cow dung and solarizing the soil are two methods that may be used to improve soil fertility and inhibit soil-borne diseases. The purpose of this study was to ascertain how cow manure application and soil solarization affected the density of nematodes in shallot. An investigation was conducted using a single-factor Randomized Complete Block Design (RCBD) in Gotakan Village, Panjatan District, Kulon Progo Regency, and Yogyakarta Special Region, Indonesia. The interventions included three types of treatment with five replications each: (1) solarization + cow manure, (2) cow manure, and (3) control. Three phases of nematode abundance observations were made: prior to application, following application, and right after harvest. Principal Component Analysis (PCA) was used for evaluating the data in regard to nematode feeding behavior and their abundance. Principal Component Analysis (PCA), which was developed on nematode feeding behavior, was used to examine the data. The results of the main component analysis at each observation stage based on the feeding behavior have an eigenvalue > 1 and a cumulative diversity value of 100%, which is divided into 2 main components: PC1 and PC2. The abundance of each feeding behaviors nematodes were able to be decreased by combination of soil solarization and cow dung application, especially plant parasitic nematodes and non-plant parasitic nematodes such as, fungal feeder, bacterial feeder, predatory nematodes, and omnivores was not significantly reduced. The application of cow dung enhanced the number of fungal feeder and bacterial feeder. The results of this investigation should give more insight into how cow manure treatment and soil solarization combine to impact the nematode abundance in shallot. This research is important for future agricultural and soil management implications and could be a potential combined treatment for controlling plant parasitic nematodes.

Keywords: Cow manure, feeding behavior, nematode abundance, soil solarization.

INTRODUCTION

Microorganisms found in soil, such as bacteria, fungi, viruses, protozoa, and archaea, form symbiotic relationships with plants and play a crucial role in preserving the quality and balance of soil ecosystems, including decomposition and nutrient cycling (Calderón *et al.,* 2017; Sahu *et al.,* 2017, Muñoz-Rojas *et al.,* 2023). Nematodes are essential as an indication of the health of the soil ecosystem and the balance of the food web (Zhao *et al.,* 2013; Choudhary *et al.,* 2023) and they play a significant role in a number of soil processes, particularly in the nutrient cycles (Zhang *et al.,* 2013; Becquer *et al.,* 2014; Zhao *et al.,* 2014; D. Zhao *et al.,* 2022). Nematode populations are relatively stable and can adapt to distinctions in soil temperature and moisture, so their presence can be used as an indication of the quality of the soil (Nielsen *et al.,* 2014). Nematodes are also crucial to the breakdown of nutrients, regulating soil fertility, and influencing how nutrients are used and altered in the soil (Nisa *et al.,* 2021; Khanum *et al.,* 2022).

The presence of soil microorganisms, one of which is nematodes, is strongly influenced by environmental conditions and cropping patterns (Suyadi and Rosfiansyah, 2017). Soil microorganisms can be manipulated biotically and abiotically to inhibit plant pathogens and maintain soil ecosystem balance (De Corato, 2020). Soil solarization and cow manure applications are two techniques to maintain the soil

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ecosystem's balance, manipulate environmental conditions, and inhibit nematode development. Optimizing soil management techniques and ensuring sustained agricultural production, especially in crops like shallots, need a thorough understanding of the particular effects of soil solarization and cow manure application on nematode populations. Since soil solarization and the addition of organic matter are both affordable and environmentally friendly, they are an excellent combination for controlling soil-borne diseases (Gilardi *et al.,* 2014), including plant parasitic nematodes. According to Gebreegziher *et al.* (2023) and Wang *et al.* (2023), combining soil solarization with various manures might enhance soil solarization's ability to control weeds and pathogens.

A hydrothermal process known as "soil solarization" uses solar radiation to raise soil temperature, which lowers the number of soil pathogens (Balakrishna *et al.,* 2015; Putri *et al.,* 2021; Ramdan *et al.,* 2022). Solarization can be utilized as an alternative to anaerobic disinfestation of soil to manage diseases and parasitic nematodes (Melero-Vara *et al.,* 2012; Castronuovo *et al.,* 2023). Previous research (Kokalis-Burelle *et al.,* 2016; Gill *et al.,* 2017) proved that soil solarization can suppress plant parasitic nematode populations, such as *Meloidogyne* spp., *Heterodera* spp., *Pratylenchus* spp., and *Rotylenchus* spp. Additionally, the use of manure suppresses plant parasitic nematode populations and supports the presence of non-parasitic nematodes (Chauvin *et al.,* 2015; Damaryono *et al.,* 2018; Indarti *et al.,* 2023). The application of cow manure can increase the abundance of non-parasitic nematodes from the group of bacterial feeders and fungal feeders (Utami *et al.,* 2017).

The application of manure affects the soil ecosystem, by enhancing soil fertility as well as the microorganisms and microflora population of nematode egg parasites. The addition of organic fertilizer as microbial inoculum can maintain microbial activity in the soil, increase soil microbial community abundance, increase soil temperature, and effectively reduce solarization time (Di Mola *et al.,* 2021). Melero-Vara *et al.* (2012) and Indarti *et al.* (2023) have reported that the incorporation of organic matter into soil solarization treatments is beneficial in reducing nematodes and slows down their recolonization processes. Therefore, soil solarization and cow manure application can be an alternative cultivation technique for shallots to suppress parasitic nematodes and enhance soil fertility.

Nematode species, populations, and variety in agricultural areas may be affected by the combination of solarization and cow dung treatment. However, the reports concerning the combination of solarization with manure and the plenitude of nematode genus in the soil are only a few. Thus, the goal of this study was to determine how cow dung and soil solarization influenced the variety of nematode species present in shallot.

MATERIALS AND METHODS

Experiment Area: Field research was performed from December 2022 to March 2023 at the shallot planting center in Gotakan Village, Panjatan District (7°53'28" S 110°09'35" E), Kulon Progo Regency, Yogyakarta Special Region, Indonesia. At the Nematology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Gadjah Mada, observations on nematode genus, diversity, and abundance were made.

Research design: This study used a single-factor *Randomized Complete Block Design* (RCBD) with three treatments and five replications. The treatments were: (1) Solarization+cow manure, (2) cow manure, and (3) control, and the control treatment which was the farmer usually do to the shallot land by using chicken manure.

Land preparation and treatment procedures: Through the soil was suitable for planting, the land was prepared by tilling and loosening it. Cow manure application is carried out before the installation of transparent polyethylene plastic. Dose of cow manure and chicken manure used was ten tones/ha that tilled into soil. Covering every soil plot as an experimental unit for solarization treatment included installing transparent plastic with a 0.25 mm thickness. Plot layouts (30 x 15 cm) were implemented at random on the field. After turning over and watering the soil, transparent plastic was placed on top of it. Solarization and cow manure treatments were applied before planting. Solarization time of the soil was 30 days. The soil then was uncovered from the plastic before planting.

Soil sampling: Randomized soil samples were taken from each plot (30 x 15 cm) to measure the starting population of nematodes (Pi). The nematode population was observed from the soil samples in three observation periods: before treatment, after treatment, and the harvest period of shallot. The soil samples were collected in a methodical zigzag pattern, at a depth of 0 to 30 cm from 5 points sampling to be composited as one sample for each replication. Early in the first week, the first soil samples were taken from experimental areas of

December 2022.

Nematode population analysis: In accordance with Kaya and Stock (1997), soil samples from the field were separated and extracted using a modified Whitehead-tray technique. Tissue paper was placed on a whitehead tray that had been prepared for this technique. In addition, 100 milliliters of the field's soil were put on the filter tray and smoothed with water until it reached the filter paper's surface. To isolate resident nematodes, water-soaked soil was allowed to stand at room temperature for 48 hours. Additionally, the soaking water that was left over after the nematode isolation process was suspended and left for fifteen minutes. After the suspension was adjusted to ±55 mL, the suspension result was placed in water containing nematodes at a progressively lower volume. To determine the species and number of nematodes, a 5 mL sample was obtained and examined using a 400x magnification microscope binocular (Olympus CX-22, Japan).

Genera of the nematode were identified descriptively based on the morphological characteristics of nematodes (Bogale *et al.,* 2020) both physically and structurally (such as shape, and size), as well as anatomical characteristics including overall body shape, sections of the oral cavity (stylet type and presence/absence), the esophagus, the esophagus position to the intestine, the type of reproductive organs, and the shape of the tail. Moreover, nematode genera were also grouped based on their feeding behavior, including plant feeders, bacterial feeders, fungal feeders, omnivores, and predatory nematodes (Kanwar *et al.,* 2021).

DATA ANALYSIS

An analysis of the nematode population abundance data was conducted using ANOVA. In the meantime, to ascertain the impact of solarization and cow manure on nematode communities based on feeding behavior, the Duncan Multiple Range Test (DMRT) was carried out at a 5% level whenever the result was significantly different using IBM® SPSS Statistics software and Principal Component Analysis (PCA) by Minitab software.

RESULTS

Nematode population analysis: Genera identified in this study included *Labronema, Dorylaimus, Rhabditis, Tylenchus, Aphelenchus, Cephalobus, Mononchus, Pratylenchus, Helicotylenchus, Aphelenchoides, Hirschmaniella*, and *Meloidogyne.* The plenitude of plant parasitic nematodes decreased in every treatment, including the control, with the highest decrease in plant parasitic nematode populations in the solarization + cow manure (94%). Even though the solarization+cow manure treatment could reduce the plant parasitic nematode population significantly; the non-plant parasitic nematode population wasn't significantly reduced. In the cow dung treatment and control treatment, the number of non-parasitic plant nematodes rose, with the maximum rise of 90% (Figure 1).

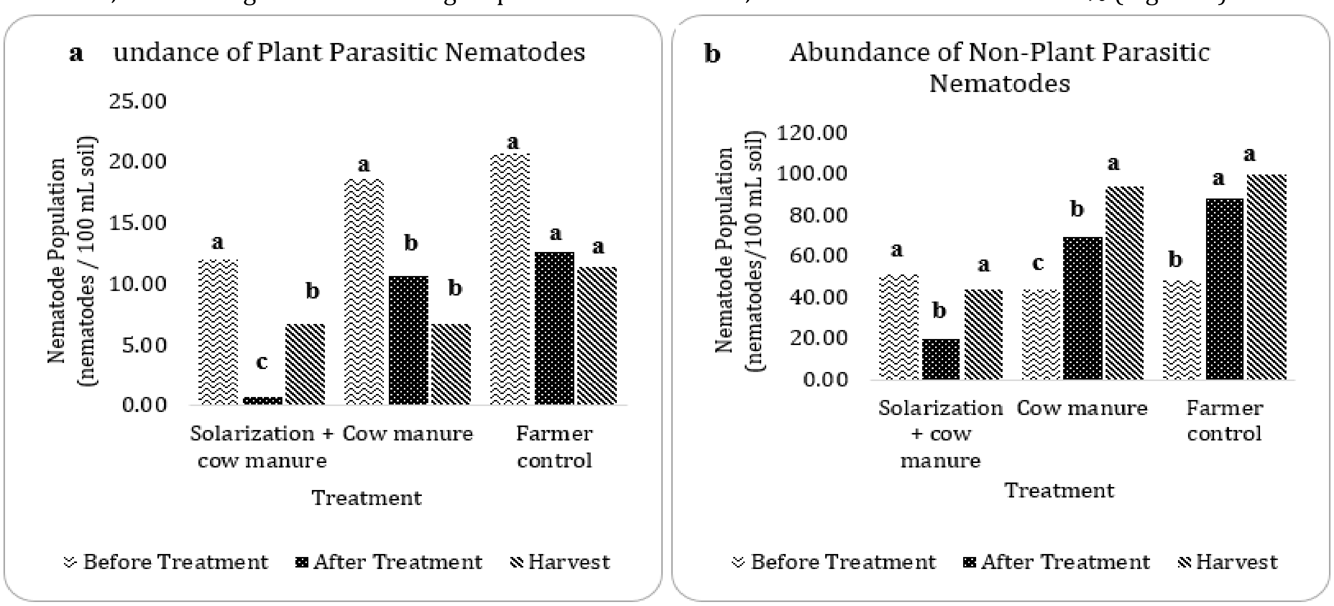

Figure. 1. Effect of solarization + cow manure, cow manure, and farmer control treatments before application, after application, and harvest on the abundance of (a) plant parasitic nematodes and (b) non-plant parasitic nematodes. Note: Values in the same graphs followed by the same letter(s) are not significantly different ($p \leq 0.05$) using Duncan's Multiple Range test.

Impact of soil solarization and cow manure on nematode communities: PCA (Figure 2) showed 100% cumulative variation with a proportion of PC1 of 82.6% and PC2 of 17.4%, indicating that different treatments had a major impact on the distribution of nematodes based on their eating behavior through population decrease. PC1 axis was related to plant feeder, bacterial feeder, and fungal feeder on the treatment of manure. There were no nematodes grouping in the solarization and cow manure treatments. While, PC2 was related to predatory nematodes, omnivores, and plant feeders on the group of control.

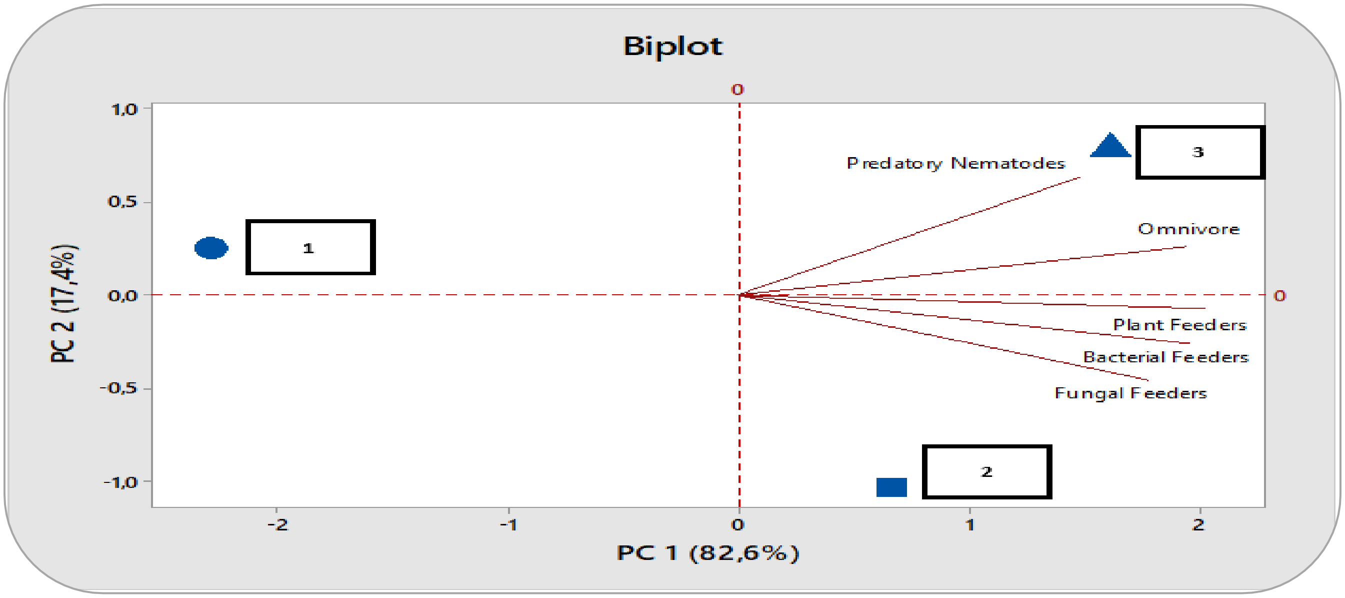

Figure 2. Principal Component Analysis (PCA) biplot of soil nematodes with different feeding behaviors on different applications after treatment (1 = solarization + cow manure; 2 = cow manure; 3 = farmer control). The eigenvalues of PC1 (4.131) and PC2 (0.868). Vectors that are in the same direction have a positive correlation, those that form a right angle are uncorrelated, and those that form an obtuse angle have a negative correlation.

DISCUSSION

Some techniques, including the use of a biosecurity system, a nematode monitoring program, crop rotation and cropping plans, antagonistic or biofumigant crops, planting schedule adjustments, solarization, tillage, organic amendments input, and the application of nematodes, can be used to manage plant parasitic nematodes combined (Stirling, 2023). According to some research, using bionematicides from other sources, such as *Bacillus, Pseudomonas*, and dried castor leaves, might be an alternate method of nematode management (Asyiah *et al.,* 2022; Isnaini *et al.,* 2023; Kouakou *et al.,* 2023). In this study, we examined additional implementations, such as the implementing manure and solarization on the impact of nematode populations. Due to its ability to raise soil temperature, solarization is recognized as one effective method for managing weeds, plant pathogenic fungus, and parasitic nematodes (Elkarmi *et al.,* 2008; Abd Elgawad *et al.,* 2019; Pavlović *et al.,* 2019; Putri *et al.,* 2021). Nematode abundance and distribution are strongly correlated with thickness and temperature of the soil (Ilieva-Makulec *et al.,* 2014). In this study, solarization was able to increase soil temperature to 42°C, causing the mortality of nematodes. This finding supported the previous research that optimal soil nematodes live at temperatures of 20-25°C, whereas temperatures of < 10°C and > 35°Care extreme temperatures for nematodes (Majdi *et al.,* 2019). Some research results showed that solarization can affect soil temperature. Soil temperature increased due to the use of soil solarization to reach 39.5-45.8ºC with a duration of 8 weeks (Shofiyani and Budi, 2014), also revealed that solarization for 14 days could increase soil temperature by up to 41°C (Putri *et al.,* 2021).

When the summer is fallow, transparent plastic which cover the crop in greenhouses is useful for capturing solar radiation that causes soil temperature to increase, increases the soil's temperature and moisture content and modifies the microbial populations activity, and suppresses the presence of pathogens in the soil (Martínez-Escudero *et al.,* 2022). Butler *et al.* (2012) proposed that the use of transparent polyethylene, sometimes referred to as solarization treatment, is an alternative approach to disease management and plant parasitic nematode population reduction. Conversely, a number of variables, including nematode species, climate, and exposure time, affect how well solarization suppresses nematode populations (D'Addabbo *et al.,* 2005). For instance, applying solarization for two to three years in a row increases the technique's effectiveness. For this reason, the nematode population density is progressively lowered down below the profitability threshold due to the ongoing solarization treatment, which inhibits nematode growth and development in the soil (Candido *et al.,* 2008).

Applying manure encouraged the growth of soil microorganisms in addition to improving soil fertility and structure (Wang *et al.,* 2013; Hartmann *et al.,* 2015). Ammonium is released during the microbial breakdown of manure, and plant parasitic nematode populations are poisoned by it. As diverse soil fauna activities break down organic matter, volatile compounds such as ammonium, nitrate, sulfur dioxide, organic acids, and others are produced. These compounds can either directly cause nematodes to hatch or alter the mortality of juvenile nematodes (Khatamidoost *et al.,* 2015; Zhai *et al.,* 2018). Liu *et al.* (2016) conducted research that supported this result, finding that applying manure increased nematode abundance overall by 37%. According to Fard and Doryanizadeh (2022) using a chicken dung treatment resulted in a decrease in the number of plant parasitic nematodes. The use of chicken manure has been shown by El-Deeb *et al.* (2018) to reduce root damage and the number of *M. incognita* egg masses infecting cucumber plants by 60.98%. Consequently, M. incognita, a kind of nematode that causes knotting in roots, may be controlled by chicken manure. Chicken manure has been reported could increase the population of nematodes *Tylenchulus semipenetrans* by 49.75 %, *Pratylenchus* spp. by 56.97%, *Tylenchorhynchus* spp. by 55.47%, *Hoplolaimus* spp. by 50.53%, and *Helicotylenchus* spp. by 52.52 % (El Metwally *et al.,* 2019). According to reports, the population of nematodes *Tylenchulus semipenetrans* might rise by 47.75%, *Pratylenchus* spp. by 56.97%, and *Tylenchorhynchus* spp. by 55.47% when chicken dung was used. by 50.53% for *Hoplolaimus* spp. and 52.52% for *Helicotylenchus* spp. (El Metwally *et al.,* 2019). Chicken manure application able to increase non-parasitic nematodes (Figure1). Bacterial feeder nematodes richness was observed in the addition of chicken manure with high concentrations of N (Shaw *et al.,* 2019). Compost made from chicken and cow dung is a potentially effective way to control phytoparasitic nematodes, boost non-parasitic nematodes, and enhance grapevine output and plant development (El-Ashry, 2021).

The feeding behavior of the nematode group revealed the difference between the two treatments (Solarization+cow manure treatment and cow manure treatment). The omnivore and predatory nematode groups in the chicken manure treatment were higher than the bacterial and fungal feeders in the cow treatment (Figure2). Manure increases soil microbial activity and abundance (Geisseler and Scow, 2014). The diversity and structure of soil microorganisms are significantly influenced by the types of chemicals (natural or artificial) that are given (Liu *et al.,* 2016). According to Liu *et al.* (2016), soils with a high organic matter concentration exhibit great heterogeneity in biodiversity. This discovery matched the previously published Forge *et al.,* (2005), where populations of soil microbial, including nematodes, increased in soil treated with dairy manure. This conclusion is consistent with Shaw *et al.* (2019) findings, which show that the presence of fungal feeder nematodes can be impacted by compost or raw cow dung. Furthermore, adding mature chicken manure has been shown to enhance the number of omnivorous and predatory soil nematodes (Steel *et al.,* 2010; Nahar *et al.,* 2006). An increase in predatory nematodes, such as *Mononchoides fortidens*, was also reported by Khan and Kim, (2005) with the addition of chicken manure compost. Increasing the density of omnivore nematodes is beneficial for soil health because omnivore nematodes stimulate the soil, mineralize nutrients, and prey on other nematodes (Khan and Kim, 2007). Therefore, the addition of organic soil amendment could be an alternative for plant parasitic nematode management, which has been shown to substantially improve soil health (Zafar *et al.,* 2022), and sustainable crop production (Jauregi *et al.,* 2023). Furthermore, our research findings showed that a combination of solarization+cow manure application could reduce the plant parasitic nematode population and keep the environment safe due to the un-significant reduction in the non-plant parasitic nematode population.

CONCLUSIONS

Finally, it has been demonstrated that application of solarization+cow manure could be a potential combined treatment for plant parasitic nematodes control. Those combinations could reduce all types of nematode population, and didn't have any significant impact to non-parasitic nematode. The genera found in this investigation were *Labronema, Dorylaimus, Rhabditis, Tylenchus, Aphelenchus, Cephalobus, Mononchus, Pratylenchus, Helicotylenchus, Aphelenchoides, Hirschmaniella*, and *Meloidogyne.* This research could be an option for practical implication to agricultural land with other vegetations and recommended to conduct further research on effect to other organisms, such as weeds, pathogens, and biological agents.

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