

Resistance of *Streptomyces* spp. Isolates towards Active Ingredients of the Nematicide Carbofuran and Dazomet, and its Effect on the Mortality of Nematodes *Meloidogyne* spp.

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ABSTRACT

The decrease in tomato plant production is caused by pest and disease attacks, one of which is the nematode Meloidogyne spp. Control measures still rely on synthetic nematicides, which can have negative effects on health, a broad spectrum of impact, microbial diversity, and resistance to attacks by Meloidogyne spp. Therefore, alternative control using biological agents that are resistant to the active ingredients of nematicides (carbofuran and dazomet) and have the ability to inhibit attacks by Meloidogyne spp. is needed. This study aims to test the resistance of Streptomyces spp. isolates to the active ingredients of synthetic nematicides, carbofuran and dazomet, and evaluate their effect on the mortality of Meloidogyne spp. nematodes. The method used is in vitro with the poisoned food technique and nematicidal activity testing against juvenile nematodes. The results show that the TMP isolate has better resistance than SP against carbofuran, with the second highest colony count of 11.47×10^6 CFU/ml. Meanwhile, dazomet significantly inhibited the growth of Streptomyces spp. colonies. The mortality test showed that the TMP isolate was able to achieve a mortality rate of 59.01% in Meloidogyne spp. This proves the potential of Streptomyces spp. as a biological agent in nematode control and its resistance to nematicide residue active ingredients, making it an environmentally friendly alternative in agricultural practices.

1. INTRODUCTION

The decline in growth and development of tomato plants can be caused by the presence of pests and diseases in the field. One of the important plant pests is the root knot nematode (*Meloidogyne* spp.). *Meloidogyne* spp. nematode attacks cause swelling in the roots (root knots) so that damage occurs to the root tissue which results in hampered translocation of water and nutrients (Raihana *et al.*, 2019). According to Irmawatie *et al.*, (2019) *Meloidogyne* spp. nematodes can reduce the productivity of tomato crop yields by 30% -45%. The control carried out still uses chemical nematicides because they are considered effective and cost-effective (Rana *et al.*, 2019). Most nematode control applications use synthetic nematicides with active ingredients carbofuran and dazomet which have a broad attack spectrum so that continuous use has a negative impact on the environment such as pesticide residues, causing health problems, reducing biodiversity and the population of biological agent microorganisms (Kaur & Gang, 2014). This encourages the development of environmentally friendly nematode control by using biological agents to reduce nematode attacks and have resistance to the presence of active nematicide residues in the field.

Streptomyces spp. isolates are widely found in the soil so they can be used as biological agents against *Meloidogyne* spp. (Suryaminarsih *et al.*, 2020). In addition, *Streptomyces* spp. has high nematicidal ability and is resistant to the presence of chemical pesticide residues in the field. Based on the research results of Bacmaga *et al.*,

(2022) the use of the pesticide tebuconazole field dose did not affect the number and diversity of the genus *Streptomyces* spp. so that it can potentially be a microorganism that can become bioaugmentation in soil contaminated with pesticides. This statement is supported by Fuentes *et al.* (2013) that pure culture isolates of *Streptomyces* spp. are able to reduce pentachlorophenol compounds by ~10% and are able to remove chlorpyrifos substances up to 99.2%. Tondon *et al.* (2018) reported that *Streptomyces* spp. isolates have the ability to degrade the active ingredient carbofuran by 44% in soil samples with a dose of carbofuran of 100 mg/kg and have the potential for biodegradation. Isolates of *Streptomyces* spp. are able to survive in soil media that has been treated with dazomet and then left for 18 months with a residual concentration of dazomet in the soil of less than 0.021 mg/kg (Wang *et al.*, 2021) so that they have the potential to be resistant to soil with dazomet residues.

It can be concluded that *Streptomyces* spp. has the potential to be resistant to chemical pesticide residues. The use of *Streptomyces* spp. is considered effective in controlling the nematode *Meloidogyne* spp. Various studies on the antibiosis mechanism of *Streptomyces* spp. have been carried out. According to the research results of Syahrok *et al.*, (2021) that *Streptomyces* spp. are able to parasitize and damage (lyse) the eggs of the nematode *Meloidogyne* spp. with a parasitization rate of 47.33%. *Streptomyces* spp. isolates have the ability to produce volatile compounds, enzymes, and non-volatile compounds that can damage (lyse) and parasitize the nematode *Meloidogyne* spp., so they are considered effective as biological controls of the nematode *Meloidogyne* spp. Based on the description above, this study aims to determine the resistance ability of the isolate of *Streptomyces* spp. to the active ingredients carbofuran and dazomet and to determine the ability of *Streptomyces* spp. against the mortality rate of J2 *Meloidogyne* spp.

2. RESEARCH METHODS

This research was conducted at the Plant Health Laboratory of the Faculty of Agriculture, National Development University "Veteran" East Java in 2023 – 2024. The research materials were *Streptomyces* spp. specifically SP and TMP isolates from the collection of the Plant Health Laboratory of the Faculty of Agriculture, UPN "Veteran" East Java. The results of root knot nematode exploration on tomato plants, GNA (Glucose Nitrate Agar) media, nematicides with active ingredients carbofuran and dazomet with three concentrations (0.05; 0.125; and 0.2 g/L GNA media), cotton, plastic, tissue, label paper, 70% alcohol, 1% NaOCl, spirits.

The method used in testing the resistance of *Streptomyces* spp. SP and TMP isolates to carbofuran and dazomet nematicides used the in vitro Poisoned Food Technique test with active ingredients carbofuran and dazomet with three concentrations (0.05; 0.125; and 0.2 g/L) GNA media. The control treatment was *Streptomyces* spp. isolates (SP and TMP isolates) grown on media without being given active ingredients and concentrations. The observation parameters were *Streptomyces* spp. isolates (SP and TMP isolates) that were able to grow and count the number of colonies (Soesetyaningsih & Azizah, 2020). The formula is as follows:

$$\text{Number of colonies (CFU/ml)} = \text{Number of colonies per plate} \times \frac{1}{\text{Dilution Factor}} \quad (1)$$

Testing the ability of nematicides of *Streptomyces* spp. isolates on the mortality of juvenile stage 2 (J2) of *Meloidogyne* spp. nematodes. The test was conducted by exploring soil sampling and root results in tomato plantations in the tomato production center in Sumberkembar Village, Pacet District, Mojokerto Regency. Then, identification was carried out by observing the characteristics of nematodes including the presence or absence of stylets, stylet length, stylet shape, nematode length, then comparing the characteristics of the nematodes found with existing literature. The test used as many as ± 30 juveniles inserted into a sterile Eppendorf tube, added with a treatment suspension at a spore density level of 106 as much as 300 μL . Incubation was carried out at room temperature for 24 hours. Nematicide activity was observed based on J2 mortality using a microscope. Juveniles were considered non-motile if they did not move when touched with a needle. As a control, juveniles inoculated with sterile distilled water with the same volume as the isolate being tested were used. The ability of *Streptomyces* spp. isolates to mortality was calculated based on the percentage of juvenile mortality that died among the total number of juveniles tested according to the following:

$$M = \frac{\text{Number of juveniles that died}}{\text{Number of juveniles tested}} \times 100\% \quad (2)$$

where M is the percentage of juvenile mortality

3. RESULTS AND DISCUSSION

3.1. Identification of *Meloidogyne* spp. Nematodes

The results of soil and root exploration with symptoms of *Meloidogyne* spp. nematodes were extracted and isolated using a modified Baerman funnel. Then, identification was carried out by observing the characteristics of nematodes including the presence or absence of stylets, stylet length, stylet shape, and nematode length.

The results of the Koch Postulate test on plants that had been inoculated with exploration nematodes showed symptoms of yellowing leaves, and plants did not grow optimally (Figure 1a), then in (Figure 1b) there was root swelling (gall). This is in accordance with the symptoms occurring in plants infected by *Meloidogyne* spp. nematodes.

The results of observations of juvenile nematodes have elongated bodies like worms, on the head there is a slightly protruding part, relatively short stylets with small-medium basal knob sizes with stomato stylet type, cone-shaped tails. Then, in adult female nematodes, they are round like pears, with a shortened neck and a rounded posterior (back) (Figure 2). The observation results are in accordance with the identification of *Meloidogyne* spp. nematodes (Durahman *et al.*, 2014).



Figure 1. (a) Symptoms of *Meloidogyne* spp. nematodes on tomato plants, and (b) Gall on tomato plant roots

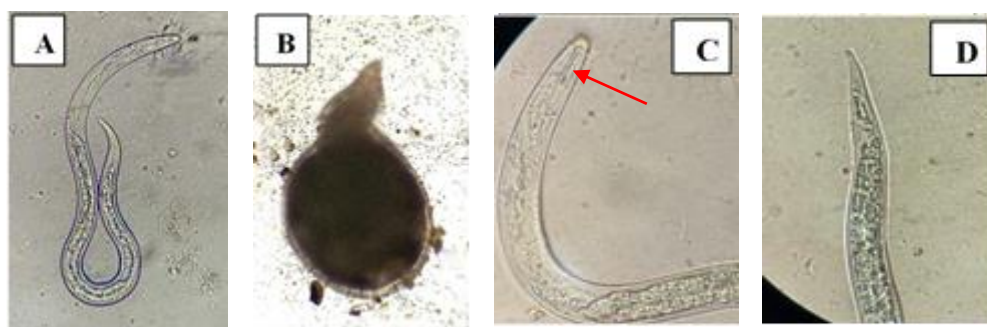


Figure 2. *Meloidogyne* spp. Nematodes Juvenile 2 (A), Adult Female (B), Anterior (C), Posterior (D) (Magnification 100X)

3.2. Resistance of *Streptomyces* spp. to Synthetic Nematicides

The results of the resistance test of *Streptomyces* spp., namely isolates SP & TMP were carried out in vitro with the observation parameters of the number of colonies of *Streptomyces* spp. that grew on the Poisoned Food Technique treatment media with active ingredients carbofuran and dazomet with three concentrations (0.05; 0.125; and 0.2 g/L) of GNA media. The results of the BNJ test analysis on the treatment of active ingredients (carbofuran and dazomet)

had a very significant effect on the number of colonies of *Streptomyces* spp. isolates SP and TMP. The following analysis results are described in Table 1.

Table 1. Effect of active ingredients (carbofuran and dazomet) of synthetic nematicides on the number of colonies of *Streptomyces* spp. SP and TMP isolates

Treatment	Number of Colonies (x10 ⁶ cfu/ml)
Control	13.50 a
TMP isolate, carbofuran	11.47 ab
TMP isolate, dazomet	5.52 c
SP isolate, carbofuran	4.87 c
SP isolate, dazomet	1.54 d

Note: Numbers in the same column followed by the same letter indicate no significant difference in the BNJ test at a 95% confidence level.

Based on Table 1. the highest number of *Streptomyces* spp. colonies in the control was 13.5(10⁶) cfu/ml. The number of colonies in the control was very significantly different from the number of colonies in other treatments. Therefore, it shows that the administration of active nematicide ingredients significantly affects the decrease in the number of colonies. According to [Liu et al., \(2024\)](#) the application of nematicides can reduce the population of beneficial soil microbes such as *Pseudomonas* spp. bacteria, and *Flavobacterium* spp. Furthermore, in Table 1. shows that in the treatment of *Streptomyces* spp. isolate TMP grown on media with the addition of the active ingredient carbofuran has the second highest number of colonies of 11.47(10⁶) CFU/ml and is not significantly different from the control treatment. This shows that the resistance ability of *Streptomyces* spp. isolate TMP is better than *Streptomyces* spp. isolate SP to the presence of the active ingredient carbofuran. Based on the results of the study by [Chen et al., \(2024\)](#) the nematicide active ingredient carbofuran did not show an inhibitory effect on the growth of *Streptomyces* saraceticus strain 31 (SS31) isolate, even the combination treatment of *Streptomyces* saraceticus strain 31 (SS31) isolate and the active ingredient carbofuran was effectively able to control *Meloidogyne incognita*. The treatment with the lowest number of colonies in the SP isolate with the active ingredient dazomet. This is because the active ingredient dazomet is able to reduce (inhibit) the growth of *Streptomyces* sp. This is in line with the research results of [Zhu et al., \(2020\)](#) that after the application of dazomet there was a decrease in the percentage of abundance of *Streptomyces* sp. compared to land without dazomet application during the first harvest of watermelon plants.

Table 2. Effect of active ingredients concentration of nematicides on the number of colonies of *Streptomyces* spp. SP and TMP isolates

Treatment	Number of Colonies (x10 ⁶ CFU/ml)
Control	13.50 a
TMP Isolate, 0.05 gram/L	6.61 b
TMP Isolate, 0.12 gram/L	4.06 bc
TMP Isolate, 0.2 gram/L	3.03 bc
SP Isolate, 0.05 gram/L	2.33 c
SP Isolate, 0.12 gram/L	2.20 c
SP Isolate, 0.2 gram/L	2.06 c

Note: Numbers in the same column followed by the same letter indicate no significant difference in the BNJ test at a 95% confidence level.

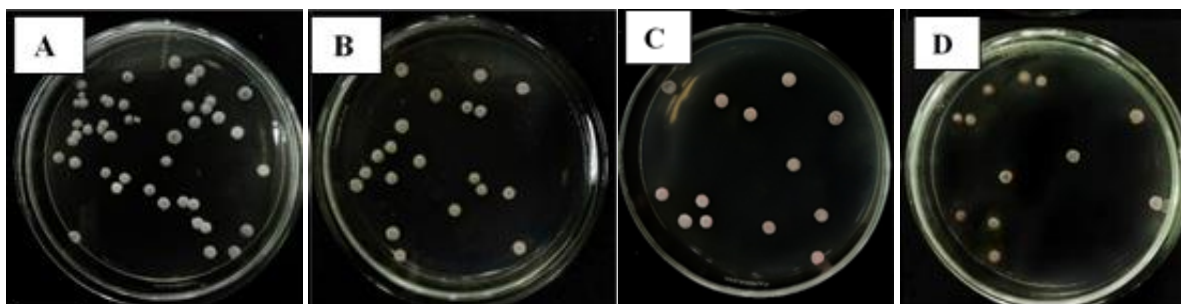
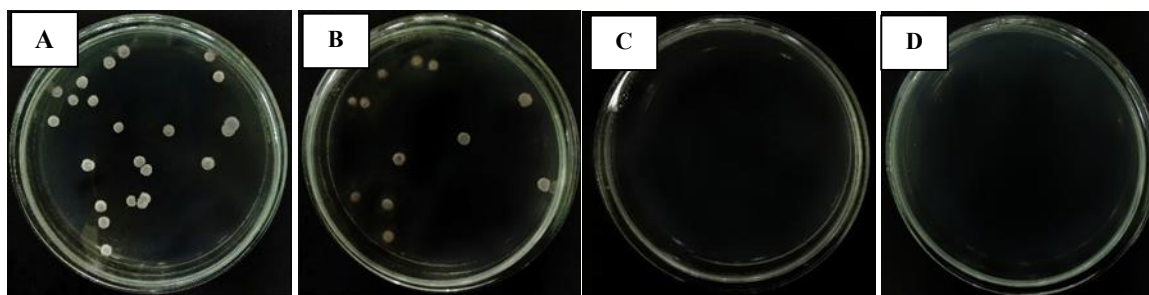
The results of the analysis in Table 2 show that the interaction of the active ingredient concentration treatment components (0.05, 0.125, 0.2 grams/L GNA media) has a very significant effect on the number of colonies of *Streptomyces* spp. isolate SP and TMP. The highest number of colonies was in the control while the lowest was in the treatment of *Streptomyces* spp. isolate SP and the active ingredient concentration of 0.2 grams/L GNA media. This is influenced by the large concentration of the active ingredient of the nematicide. Based on research by [Shahid et al., \(2021\)](#), there was a significant decrease in the number of colonies of *Sinorhizobium saheli* bacteria at concentrations (12.5, 25, 50, 75 and 100 mg/L) of the active ingredient carbofuran, so that the concentration greatly affects the number of bacterial populations.

Table 3. Effect of active ingredients (carbofuran and dazomet) and their concentration on the number of *Streptomyces* spp. colony

Treatment	Number of Colonies ($\times 10^6$ CFU/ml)
Control	13.50 a
Carbofuran, 0.05 gram/L	7.80 b
Carbofuran, 0.12 gram/L	6.27 b
Carbofuran, 0.2 gram/L	5.10 b
Dazomet, 0.05 gram/L	0.65 c
Dazomet, 0.12 gram/L	0 c
Dazomet, 0.2 gram/L	0 c

Note: Numbers in the same column followed by the same letter indicate no significant difference in the BNJ test at a 95% confidence level.

Based on the analysis results in Table 3, the control treatment was significantly different from the other treatments. Then, the active ingredient treatment significantly affected the number of *Streptomyces* colonies. The number of *Streptomyces* colonies was greater in the media containing the active ingredient carbofuran compared to the active ingredient dazomet. This is because the *Streptomyces* spp. isolate has the ability to degrade the active ingredient carbofuran by 44% of the soil sample with a carbofuran dose of 100 mg/kg and has the potential for biodegradation. *Streptomyces* spp. isolate with hydrolysis enzymes has the ability to hydrolyze (break down) carbofuran compounds into methylamine compounds which are used as a source of carbon and nitrogen for the growth of *Streptomyces* spp. colonies (Mishra *et al.*, 2021). The treatment of the active ingredient dazomet greatly affects the existence and reduces the population of *Streptomyces* sp. The study of Wu *et al.* (2025) support this statement, where after the treatment of dazomet fumigation on strawberry land in a greenhouse at a dose of 25 kg in 667 m², the population of beneficial soil microorganisms such as *Streptomyces* sp. decreased compared to the population before the dazomet treatment. Figure 3 to 6 documented the results of the resistance test on TMP and SP isolates that grew in the treatment media of the active ingredients carbofuran and dazomet at concentrations of 0; 0.05; 0.125; and 0.2 g/L.

Figure 3. *Streptomyces* spp. TMP isolates on active ingredient carbofuran: (A) Control, (B) 0.05 g/L, (C) 0.125 g/L, (D) 0.2 g/LFigure 4. *Streptomyces* spp. TMP isolates on active ingredient dazomet: (A) Control, (B) 0.05 g/L, (C) 0.125 g/L, (D) 0.2 g/L

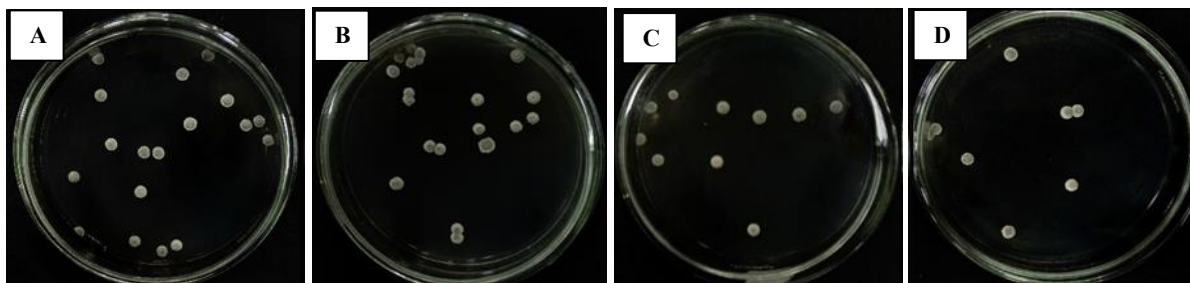


Figure 5. *Streptomyces* spp. SP isolates on active ingredient carbofuran: (A) Control, (B) 0.05 g/L, (C) 0.125 g/L, (D) 0.2 g/L

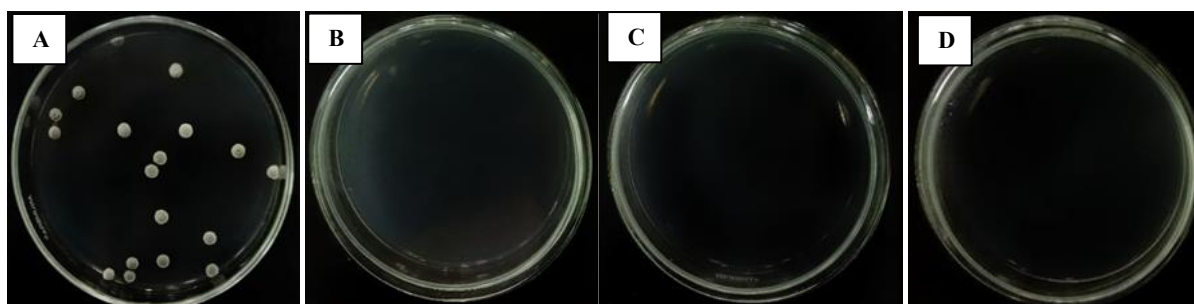


Figure 6. *Streptomyces* spp. SP isolates on active ingredient dazomet: (A) Control, (B) 0.05 g/L, (C) 0.125 g/L, (D) 0.2 g/L

3.3. *Streptomyces* spp. Isolates on the Mortality of J2 *Meloidogyne* spp.

The test results on two isolates of *Streptomyces* spp. namely isolates SP & TMP on the mortality of J2 nematodes *Meloidogyne* spp. were carried out in vitro with observation parameter the percentage of J2 mortality. The results of the LSD test on the treatment of *Streptomyces* spp. isolates SP and TMP had a very significant effect on the percentage of mortality of J2 nematodes *Meloidogyne* spp. The following analysis results are described in Table 4. Treatment with the highest mortality percentage was the TMP treatment with 59.01%, this was very different from the control treatment which had the lowest mortality of 1.58%. This is in line with the results of the study by Efendi *et al.*, (2021) that the *Streptomyces* sp. isolate had a J2 *Meloidogyne incognita* mortality rate of 83.57% compared to the control treatment. Research by Atif *et al.* (2013) found that *Streptomyces* spp. isolates with high chitinase activity can increase the mortality of J2 *Meloidogyne* spp. by up to 67%. The increase in mortality is because *Streptomyces* spp. isolates can produce chitinase enzymes to degrade cell walls and cause damage (lysis) to the chitin layer of J2 *Meloidogyne* spp. and adult nematodes (Rajendran *et al.*, 2023). In the TMP and SP isolate treatments, the values were not significantly different, so both isolates had the ability to kill *Meloidogyne* spp. nematodes in the J2 phase.

Table 4. Effect of *Streptomyces* spp. Isolate SP and TMP Treatment on the Mortality of J2 *Meloidogyne* spp.

Treatment	Mortality
Control	1.58% a
SP Isolate	47.67% b
TMP Isolate	59.01% b

Note: Numbers in the same column followed by the same letter indicate no significant difference in the BNJ test at a 95% confidence level.

4. CONCLUSION

This study shows that the *Streptomyces* sp. isolate. have resistance to synthetic nematicide active ingredients such as carbofuran and dazomet. From the results of the study, *Streptomyces* isolate TMP has better resistance than SP isolate to these active ingredients, especially to carbofuran. In addition, this isolate is also effective in increasing the mortality of juvenile nematodes *Meloidogyne* spp., with the highest mortality rate of 59.01% in the TMP isolate. This indicates

that *Streptomyces* spp. not only has the potential as a biological agent to control nematodes, but is also able to survive in environments exposed to residues of nematicide active ingredients, making it an environmentally friendly solution for controlling plant pests.

DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest concerning the study, authorship, and/or publication of this article.

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