**Streptomyces** sp. has different effectify to control two different pathogens

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**Abstract:** Soil microbes can act as biological agents to control pathogens, for example *Streptomyces* sp. which has known to produce secondary metabolites in the form of antibiotics that can inhibit the growth of several pathogenic fungi. If *Streptomyces* sp. sprayed to peanut leaves, it reduced the severity of disease of Gajah, Garuda, Kancil and Hypoma 1 varieties, which indicated by a high disease suppression with efficacy value more than 50%. *Streptomyces* sp. also inhibited 0.42% *Fusarium oxysporum* growth (moler pathogen) in vitro. However, in vivo assay, it did not suppress the development of moler disease which indicated by the high value of severity and infection rate, also low efficacy of disease suppression. So, this was an example that *Streptomyces* sp. was effective for controlling Cercospora leaf spot disease in peanut, but not effective to control moler disease in shallots.

1. **Introduction**

The genus Streptomyces is the most common group because it grows fast in nature and is easy to isolate. This bacterium belongs to the genus that dominates the Actinomycetes group which is the largest antibiotic-producing bacteria. According to [11] *Streptomyces* sp. produce extracellular metabolites which are suspected as antibiotics, so they can inhibit other microorganisms. Therefore, *Streptomyces* sp. is a group of antagonistic microorganisms that have the potential as biological agents to control plant disease.

It stated that *Streptomyces* spp. is one of the biological agents that is effective in controlling Fusarium sprouts and wilt diseases in cotton plants. According to [9] *Streptomyces* sp. which tested in vitro was able to inhibit the growth of *F. oxysporum* on red chili peppers up to 82%, and was effective inhibiting the growth of *F. oxysporum* in tomato plants by 75% [11].

Symptoms of Cercospora leaf spot disease in peanuts can be influenced by the genotype of the host plant and it is environmental factors. The initial symptom of leaf spots small chlorotic spots on the leaves after 10 days infection, these spots will develop larger and brown to black in color. The difference symptoms of early leaf spot (*Cercospora arachidicola*) are generally characterized by a dark brown round spot surrounded by a yellow halo on the upper surface of the leaf, while symptoms of advanced leaf spot (*Cercospora personata*) are rounder spots, smaller spot size, black on the bottom leaf. The symptoms of both are almost the same, namely in the form of leaf spots [15]. It also effectively inhibited the growth of *F. oxysporum* in tomato plants by 75% [11].

Moler disease is one of the most important diseases and is still the main disease in shallot plants until now [4], i.e. in Probolinggo this disease cause a big problem. Farmers complained that the plants are often wilt and they control with pesticides. However, this pesticide still does not work properly
because the disease is soilborne. A wilting plants affected to the yield of shallot bulbs. The weight of the infected bulbs decreased and the bulb became small, so the selling price was sharply decrease. The fungus *Fusarium oxysporum* f.sp.cepae (Foc) decreased the quality and quantity of local shallot bulb production [8].

In this study, it was observed whether the use of *Streptomyces* sp. as a biological agent was equally effective in controlling Moler disease of shallot (soilborne pathogen) and Cercospora disease of peanuts (airborne) that were conducted at the same times.

2. Materials and methods

2.1 Hypersensitive reaction assay

*Streptomyces* sp. was originated from the Research Center for Seed and Crops Protection (BBPPTP) Surabaya, and this was tested by the Gram test and hypersensitive reaction to determine whether this bacteria was not pathogenic to the plant. This test was performed by infiltrate the suspension of *Streptomyces* sp. with a density of 10⁸ cfu per ml to tobacco leaves and then was observed when the first symptoms appeared.

2.2 Inhibition assay *Streptomyces* against to both *F. oxysporum* subsp cepae (Foc) and Cercospora in vitro

Both the fungi caused moler disease in shallot and Cercospora diseases in peanut were carried out in vitro in petri dishes. The antagonist test was done by lay down the fungal mycelia and placing it in the middle of the PDA medium. Then dropped with 1 ose *Streptomyces* sp. at 4 points with 2 cm distance from the fungi tested [9]. The fungal or bacterial isolates were placed at the same time and incubated for 7 days to calculate their inhibition zone by measuring the length of the inhibition zone formed around each fungal colony. The percentage of inhibition growth was calculated with this formula [10].

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\text{Inhibition growth} = \frac{\text{colony control area} - \text{colony treatment area}}{\text{colony control area}} \times 100\%.
\]

The plant reactivity test of Cercospora disease was done by spraying 10 ml *Streptomyces* sp. on 14 days old of peanut plant with density of 10⁸ cfu per ml [6]. Then 10 ml *Cercospora* sp with density of 10⁸ spora/ml was inoculated on peanut at 21 days old by spraying leaves. Application of *Streptomyces* sp.on shallot seeds was conducted at 7 days before planting in order to *Streptomyces* sp. grown optimally in the soil, so it could prevent the growth of Fusarium [16]. This application of *Streptomyces* sp with each density of 10⁷, 10⁸, 10⁹ and 10¹⁰ cfu per ml was carried out by pouring 10 ml per planting hole.

2.3 *In vivo* test

On peanut plant, 10 ml per plant of *Streptomyces* sp. suspension was sprayed on peanut leaves at the age of 14 days after planting with density of 10⁸ cfu per ml [6]. Then *Cercospora* sp was inoculated on plants. Inoculation was carried out by spraying the entire leaf surface by 10 ml per plant with density of 10⁶ spores per ml on 21 days old plant [7]. For shallot, 10 ml per the planting hole of *Streptomyces* suspension was applied to the soil on 7 days before shallot planted. This was to prevent Fusarium infested in the soil. *Fusarium oxysporum* was diluted to 10⁶ and each 10 ml were sprayed to 7 days old shallot plant.

From these two experiments, the disease severity and incidence were calculated using the formula:

Disease Incidence (%) = \( \frac{p_1}{p_2} \times 100\% \), where \( p_1 \) is number of infected plants, \( p_2 \) is number of all plants.

Disease Severity = \( \frac{\sum (n \times v)}{Z \times N} \times 100\% \), where \( n \) is number of infected leaves in each category, \( Z \) is scale value of the highest attack category, \( N \) is number of leaves observed, \( v \) is scale value of each infection category. For Cercospora disease 0 = no symptoms, 1 = leaves
infected 1-20%, 2= leaves infected 21-40%, 3= leaves infected 41-60%, 4= leaves infected 61-80%, 5= leaves infected 81-100% [8].

From these two experiments, the disease severity and incidence were calculated using the formula:
Disease Incidence (%) = p1 / p2 x100%, p1 = number of infected plants, p2 = number of all plants.
Disease Severity = (∑ (nxv)) / ZxN x100%, DS = disease severity (%), n = Number of infected leaves in each category, Z = scale value of the highest attack category, N = number of leaves observed, v = scale value of each infection category. For Cercospora disease Score 0= no symptoms, 1= leaves infected 1-20%, 2= leaves infected 21-40%, 3= leaves infected 41-60%, 4= leaves infected 61-80%, 5= leaves infected 81-100% [8]

For Moler disease, score 0= no symptoms, 1= leaf infected 0 < x ≤ 20%, 2= leaves infected 20 < x ≤ 40%, 3= leaves infected 40 < x ≤ 60%, 4= leaves infected 60 < x ≤ 80%, 5= leaves infected 80 < x ≤ 100% [2].

3. Results and Discussions
The characteristic of Streptomyces sp. was gram positive in 3% KOH and did not show a necrotic lesion on tobacco leaf (HR-). This means that Streptomyces was not a pathogen. Table 1 showed the growth of Cercospora sp. on agar was affected with the kind of growth media.

### Table 1. Interaction between Streptomyces on 5 peanut varieties infected with Cercospora sp.

| Treatment       | Disease severity (%) at 42 dai | Infection rate (Unit/day) | AUDPC   | Efficiency of disease supression (%) |
|-----------------|-------------------------------|---------------------------|---------|--------------------------------------|
| +C-S+Gajah      | 65.8 a                        | 0.1107                    | 908.29  | -                                    |
| +C-S+Kancil     | 56.7 c                        | 0.1015                    | 829.22  | -                                    |
| +C-S+Garuda     | 61.9 b                        | 0.1067                    | 874.34  | -                                    |
| +C-S+Hypomai    | 58.3 c                        | 0.1031                    | 840.39  | -                                    |
| +C+S+Gajah      | 23.3 f                        | 0.0668                    | 373.29  | 50.93                                |
| +C+S+Kancil     | 20.7 h                        | 0.0632                    | 310.36  | 53.27                                |
| +C+S+Garuda     | 23.1 fg                       | 0.0665                    | 349.01  | 51.95                                |
| +C+S+Hypomai    | 22. gh                        | 0.0650                    | 336.90  | 52.92                                |
| -C+S+Gajah      | 43.9 d                        | 0.0893                    | 445.73  | -                                    |
| -C+S+Kancil     | 40.8 e                        | 0.0863                    | 362.60  | -                                    |
| -C+S+Garuda     | 43.4 de                       | 0.0888                    | 420.12  | -                                    |
| -C+S+Hypomai    | 41.9 e                        | 0.0873                    | 395.66  | -                                    |
| -C+S+Gajah      | 15.8 i                        | 0.0553                    | 639.40  | 58.90                                |
| -C+S+Kancil     | 12.5 k                        | 0.0488                    | 579.11  | 62.57                                |
| -C+S+Garuda     | 14.7 ij                       | 0.0533                    | 616.12  | 60.08                                |
| -C+S+Hypomai    | 14.2 i                        | 0.0523                    | 593.83  | 59.91                                |

C= Cercospora sp., S= Streptomyces sp., (+)= treated, (-)= untreated.

The growth of Cercospora on oat meal agar (OMA) was taken after two weeks, while on PDA was taken in 3 weeks. [12] also found that on oat meal agar (OMA), Cercospora sp. sporulate quickly than growing on PDA. [3] found that Cercospora sp. on PDA growing more longer and difficult to sporulate.
Inhibition assay of fungus Streptomyces against A. *Cercospora* sp., B. *F. oxysporum* sp.

*In vitro* assay showed that *Streptomyces* sp. inhibited the growth of *Cercospora* sp. by 0.37 mm (Fig. 1A). This was suggested that *Streptomyces* sp. produced antibiotic that inhibited the growth of fungus pathogen [11]. *In vitro* assay, in the inhibition assay of *Streptomyces* against *F. oxysporum* was 0.42 mm and appeared that *Streptomyces* grown quickly on medium than *Fusarium* at the same ages (Fig. 1B).

**Figure 1.** Inhibition assay of fungus *Streptomyces* against A. *Cercospora* sp., B. *F. oxysporum* sp.

**Figure 2.** Symptoms development for disease scoring (a) fist symptoms: 1, (b) small necrosis with halo: 3, (c) necrosis more appeared with halo: 4

During the research, the RH was high so the symptoms was appeared quickly. Based on disease severity of plant infected with *Cercospora* (Fig. 2). [5] categorized that if the plant has disease severity of 0% (most resistant), 1-20% (resistant), 21-50% (rather resistant), 51-70% (susceptible), 71-100 (more susceptible). Therefore on Tabel 1 showed that disease severity of *Streptomyces* applied on 5 peanut varieties in both –*Cercospora* or + Cercospora was significantly different and this was shown by efficiency disease supression. Based on the disease severity, +C+S was categorized as rather resistance, and +C-S was categorized as susceptible than –C-S (Table 1).
On the other hand with *F. oxysporum*, Streptomyces did not affected to plant infected with *F. oxysporum* in vivo, and this was shown by a high disease incidence (100%) and disease severity (> 90%) (Table 2, Fig. 3). Symptoms of moler disease in all tested plants occurred on 7 days after inoculation (dai) and then increased sharply on 21 d.ai until 42 d.ai. On the control plants, the symptoms appeared more quickly than plant applied with *Streptomyces* sp. when plants were inoculated with spore density of $10^7$, $10^8$, $10^9$, $10^{10}$ cfu per ml. If disease severity more than 50-90%, [14] categorized that this plants has severe infection.

### Table 2. Disease incidence, disease severity and rate infection of moler disease after applied with *Streptomyces* sp.

| Treatment   | Disease incidence 42dai (%) | Disease Severity 42 dai (%) | Infection rate (Unit Per day) | Effication of disease supression (%) |
|-------------|----------------------------|----------------------------|-------------------------------|-----------------------------------|
| *Foc* - *S* | 100a                       | 98a                        | 0,093                         | -                                 |
| *Foc* + *S*. sp 10^7 | 100a                  | 78d                       | 0,036                         | 22,44                             |
| *Foc* + *S*. sp 10^8 | 100a                  | 90bc                      | 0,054                         | 11,51                             |
| *Foc* + *S*. sp 10^9 | 100a                  | 87c                       | 0,048                         | 14,44                             |
| *Foc* + *S*. sp 10^{10} | 100a                  | 94ab                      | 0,066                         | 4,12                               |

The numbers followed by different letters in one column indicate a significant difference in the 5% DMRT test. *Foc = Fusarium oxysporum* f.sp. *cesae*, *S*. 10^7, *S*. 10^8, *S*. 10^9, *S*. 10^{10} = *Streptomyces* sp. concentration

The used of cultivar shallot (biru lancor, Probolingo) which susceptible to moler disease was one of the factors causing the high severity and rate of infection in shallot plants (Table 2). According to [14], cultivar biru planted in Nganjuk during the rainy season showed a high disease severity with a high infection rates, resulted in low yields. Biru cultivar have a small bulbs and did not have many bulb layers so the fungus was easily to penetrate the bulbs [8]. Therefore, this was caused the biru cultivar has a severe infection (80-90%) (Table 2).

### 4. Conclusion

*In vitro* assay, *Streptomyces* sp. was greatly inhibited *Cercospora* sp. growth and *in vivo* tests were able to suppress *Cercospora* sp in all varieties peanut (Gajah, Kancil, Garuda dan Hypoma 1). However, Streptomyces inhibited the growth of *Fusarium oxysporum in vitro*, although *in vivo* it was not able to suppress the development of moler disease and this was shown by slightly efficacion of disease supression. Based on these results we stated that Streptomyces as biological agent may have different affect in controlling two different pathogens or diseases, airbone and soilborne pathogens.
5. References

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