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# Assessing the effects of *Lecanicillium lecanii* in the biological control of early and late leaf spot of peanut *in vitro* (Burkina Faso, West Africa)

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**Early and late leaf spots caused by *Cercospora arachidicola* and *Phaeoisariopsis personata* respectively, are the most widespread peanut fungal diseases in West Africa. These diseases lead to notable crop losses in the rural area. In the recent decades, chemical fungicides are used to fight against crop losses, but these chemical substances cause big damages to the environment including the animals and human beings. Thus, the biological control is encouraged by scientific community and all government because it is well known to be safety and cost effective. For this reason, we investigated on the effectiveness of *Lecanicillium lecanii* on leaf spots of peanut in Burkina Faso. To do so, spore suspensions ($10^6$ spores / ml) from four strains of *L. lecanii* was used *in vitro*. The results revealed that *L. lecanii* 4181 inhibited the pathogen conidia germination up to 87%, as well as elongation germ tube with highest rate of 56%. Compared to the distilled water control, the severity scores vary between 5.7 and 8, but our results showed a notable decrease of score from 2.3 and 4.7. From our findings, the treatments with *L. lecanii* spore suspensions on peanut leaves significantly reduced the severity of leaf spots and may be potentially used to promote organic farming in West Africa.**

**Key words:** Foliar diseases, biological control, peanut, agriculture.

## INTRODUCTION

Early and late leaf spots are the most common foliar diseases of the peanut. The most obvious effect of this disease is the loss of photosynthetic tissue, which leads to premature defoliation. Early and late leaf spot contribute to significant loss of crops over the world (Subrahmanyam et al., 1992; Shokes and Culbreath,

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1997). These diseases can cause up to 70% yield loss (Subrahmanyam et al., 1980). Previous studies havedemonstrated a significant impact of these pathogens in peanut farms in West Africa, particularly in Burkina Faso (Neya, 2017). Some authors have focused their activities on detecting resistant varieties, and the use of plant biopesticides (Koïta et al., 2017; Zongo et al., 2019). However, biological control of plant diseases is now considered as a promising tool for sustainable agriculture in rural area and may help to fight against poverty and hunger in developing countries. Some authors have demonstrated that the antagonistic fungi, such as *Dicyma pulvinata* (Berk. & Curt.) v. Ar x (= Hansfor dia pulvinata (Berk. & Curt.) Hughes) and *Verticillium lecanii* (Zimmerm.) Viegas (*Lecanicillium* sp.) can be used to fight against early and late leaf spots of the peanut, as well as *Darluca filum* (Biv.) (Cast.), *Tuberculina costaricana* (Syd.) and *V. lecanii* (*Lecanicillium* sp.) are used against peanut rust (Zambettakis and Sankara, 1985). In India, Subrahmanyam et al. (1990) have proven that *V. lecanii* parasitezed the uredospores of *Puccinia arachidis* (Speg.) and reduced the extent of rust and late leafspot on peanut leaves. Some studies in Burkina Faso were only limited to the biology and reproduction of *L. lecanii* (Nana et al., 2014*).* The main objective of this study was to evaluate the effectiveness of *L. lecanii* in the biological control of peanut leaf spots. To reach our goal, three specifics objectives were carried out: (1) to determine the effect of *L. lecanii* on conidia germination of pathogens, (2) to determine the effect of *L. lecanii* on the germ tube elongation of pathogens, and (3) to establish the severity scores of leaf spots.

## MATERIALS AND METHODS

### Preparation early and late leaf spot pathogens inoculum

The conidia of *Cercospora arachidicola* and *Phaeoisariopsis personata* were collected from infected peanut leaves at the centre part of Burkina Faso, named Gampela. The area is located at longitude 12°22 W and latitude 12°25 N. Once, the peanut leaves collected, they were preserved in blotting paper and closed in Petri dishes, and the samples were kept in laboratory at 25°C for one week. Then, the conidia of *C. arachidicola* and *P. personata* were gathered by using a scalpel after submerging the leaves in distilled water containing 0.02% Triton × -100. Conidia concentrations of pathogens were determined using Neubauer hemocytometer and adjusted with sterile water containing 0.02% Triton × 100.

### Preparation of *L. lecanii* inoculum

Four strains of *L. lecanii* (4184, 2711, 1052 and 4181) were used as antagonist fungi in this study. These strains were gained from the National Museum of Natural History (MNHN) in Paris, France.
For the germination tests, the inoculum required for the experiments were obtained from 14-day-old cultures grown on potato dextrose agar (PDA) plates (Gurulingappa et al., 2010). The conidia were harvested by scraping the surface with a sterile scalpel after flooding the plates with sterile water containing 0.02% Triton X 100.

The conidial suspension was filtered to remove hyphal debris, and the conidial concentration was determined using again Neubauer hemocytometer and adjusted to $1.25 \times 10^6$ conidia. $mL^{-1}$ with sterile water containing 0.02% Triton × 100. To treat the peanut leaves*, L. lecanii* were grown in liquid Czapek medium during 14 days. The inoculum obtained was stored in a sterile bottle at 4°C. For treatments, solution was filtered to remove hyphal debris, and the concentrations were determined, and then adjusted to $10^6$ conidia. $mL^{-1}$ with sterile distilled water. In addition, Triton X-100 was added in the solution, and adjusted to 0.02% before application.

### Testing the effect of *L. lecanii* on conidial germination and germ tube elongation

To do so, one milliliter of the conidial suspension of each pathogen (5000 conidia / ml) was added in 4 ml of *L. lecanii* containing $1.25 \times 10^6$ conidia / ml. The final solution was incubated in tubes at 25°C and stored in laboratory without light during 24 h. The essay was replicated three time. The rate of germination was determined based on a total of 100 conidia of pathogen selected randomly. The germ tube elongation was measured by micrometry method. The percentage inhibition was determined following to the formulae 1 and 2, adapted from (Greche et al., 2000):

$$\mathrm{Ig} = \frac{\mathrm{NE}-\mathrm{NL}}{\mathrm{NE}} \times 100\% \quad (1)$$

Where Ig is the rate germination inhibition, NE = the number of conidia that have germinated in distilled water and NL: the number of conidia that have germinated in the solution of *L. lecanii*

$$\mathrm{Ite} = \frac{\mathrm{LE}-\mathrm{LL}}{\mathrm{LE}} \times 100\% \quad (2)$$

Where (Ite) is the percent inhibition of germ tube elongation, LE = Germ tube length in distilled water, LL: Germ tube length in *L. lecanii* solution.

### Testing the effect of *L. lecanii* on early and late leaf spot

In order to determine the effect of *L. lecanii* on development of early and late leaf spot on peanut leaves, a susceptible variety of peanut “TS32-1” was used. For this experiment, the healthy leaves of TS32-1 30-day-old were collected from glasshouse-grown plants. Here, one peanut leaf was placed on blotting paper and preserved in Petri dish (size 90 mm diameter). Leaf and blotting paper were kept in moist during the experiment period to maintain the leaf in life. The experiment was replicated three time. In this experiment, we treated each lower face of peanut leaf with 100 µl of the solution of *L. lecanii* ($10^6$ conidia.$mL^{-1}$), and then we added 100 µl of the suspension of the pathogen ($10^5$ conidia.$mL^{-1}$). The samples were kept at 25°C in an oven refrigerant (Aqualytic), first in the dark for 12 h, and then alternately 12 h of light-12 h of darkness. The treatments of *L. lecanii* solution were realized each week for one month. From the appearance of the first spot, we have determined the scores of leaf spot severity every fifth days, following Subrahmanyam et al. (1982).

### Data analysis

The means of conidial germination rates and germ tube length were calculated. The data was subjected to an analysis of variance and a comparison of average according to Duncan's test at 5% level. All data were computed using XLSTAT Pro 2007.

**Table 1.** Effect of inoculation with *L. lecanii* on conidia germination of *Cercospora arachicola*.

| Inoculation treatment | Percentage of germination of conidia | | Germ tube elongation | |
|---|---|---|---|---|
| | Percentage of germination of conidia (%) | Percent inhibition (%) | Germ tube length (µm) | Percent inhibition (%) |
| Distilled water | 88.67[a] | 0 | 13.50[a] | 0 |
| *L. lecanii* 4184 | 56.67[b] | 36 | 12.67[ab] | 6 |
| *L. lecanii* 1052 | 52.83[c] | 40 | 9.33[bc] | 31 |
| *L. lecanii* 2711 | 51.33[c] | 42 | 8.83[c] | 35 |
| *L. lecanii* 4181 | 38.33[d] | 57 | 8.33[c] | 38 |
| Standard deviation | 17.36 | | 2.64 | |
| P values | < 0.0001 | | 0.031 | |

**Table 2.** Effect of inoculation with *Lecanicillium lecanii* on conidia germination of *Phaeoisariopsis personata*.

| Inoculation treatment | Percentage of germination of conidia | | Germ tube elongation | |
|---|---|---|---|---|
| | Percentage of germination of conidia (%) | Percent inhibition (%) | Germ tube length (µm) | Percent inhibition (%) |
| Distilled water | 84.00[a] | 0 | 6.97[a] | 0 |
| *L. lecanii* 4184 | 41.00[b] | 51 | 4.59[c] | 34 |
| *L. lecanii* 1052 | 33.67[c] | 60 | 6.03[b] | 13 |
| *L. lecanii* 2711 | 32.67[c] | 61 | 4.38[c] | 37 |
| *L. lecanii* 4181 | 11.00[d] | 87 | 3.09[d] | 56 |
| Standard deviation | 24.9 | | 1.45 | |
| P values | < 0.0001 | | < 0.0001 | |

## RESULTS

### Effects of *L. lecanii* on conidia germination of *C. arachidicola*

The results from Table 1 show that the *L. lecanii* significantly reduce the germination percentage and germ tube elongation of *C. arachidicola*. The lowest germination percentage was observed with *L. lecanii* 4181 (38.33%). We found that, all strains of *L. lecanii* presented the lowest germination percentages than that those recorded in distilled water. The rates of inhibition of conidial germination vary between 36 and 57%. We found the lower values of germ tube length (8.33 and 8.83 µm) in suspensions of *L. lecanii* strains 4181 and 2711 with inhibition rates of 38 and 35%, respectively.

### Effects of *L. lecanii* on conidial germination of *P. personata*

Table 2 shows variation of values of germination rates and germ tube length of *P. personata*. The four strains of *L. lecanii* significantly reduced the germination rate but also slowed the elongation of the germ tube ($P < 0.05$). We recorded the lowest values of germination percentage (11%) and germ tube length (3.09 µm) of the pathogen in *L. lecanii* 4181. The percentages of inhibition range from 51 to 87% for germination rate and 13 to 56% for germ tube elongation.

### Effects of *L. lecanii* on the development of early leaf spot on peanuts leaves

Inoculations with *L. lecanii* significantly reduced the severity of early leaf spot (Figure 1). We found that strains 4181 and 4184 delayed the manifestation of symptoms until twelve days after inoculation (DAI). The lowest severity scores were reported with *L. lecanii* 4184 during the experiment period. We observed evidence of the reduction of spot density and leaf area damage in Figure 2 (Photos A and B). Statistical analysis revealed significant differences between *L. lecanii* strains and distilled water treatments at 17 DAI ($P = 0.001$), 22 DAI ($P < 0.0001$) and 27 DAI ($P < 0.0001$).

### Effects of *L. lecanii* on the development of late leaf spot on peanuts leaves

In Figure 3, we observed that the inoculation treatment

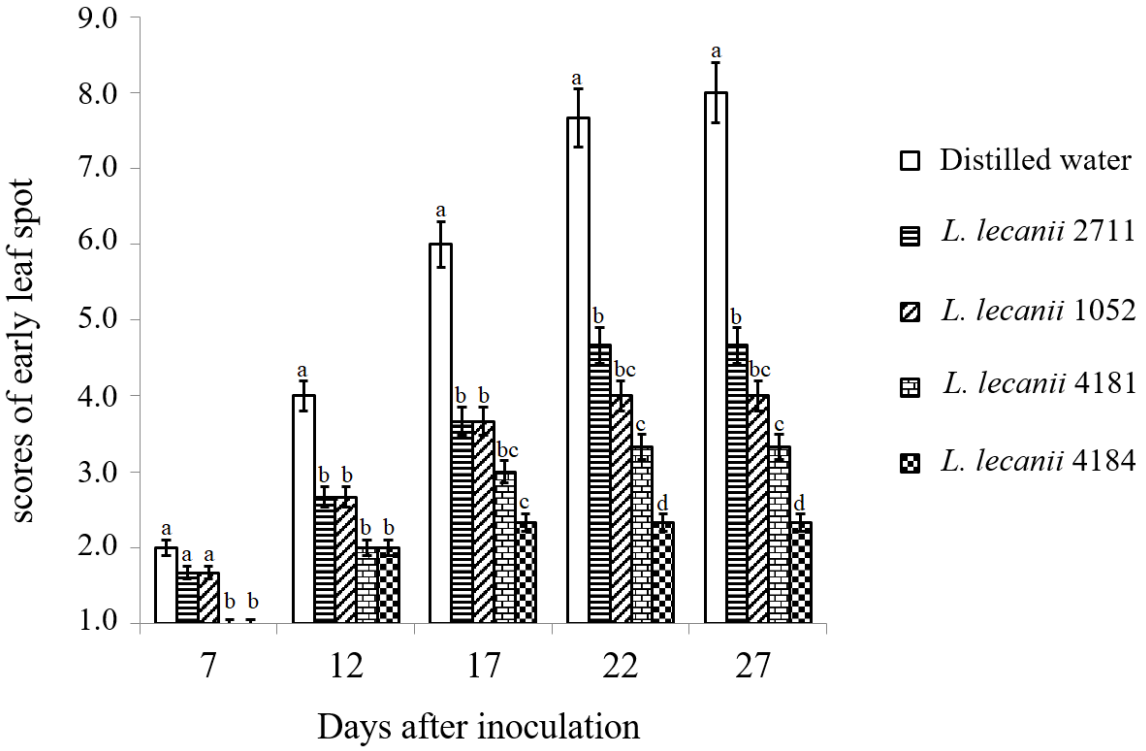


**Figure 1.** Effect of inoculation with *L. lecanii* on early leaf spot development on peanut leaves. Bars are means ±SE, n = 3 repetitions.

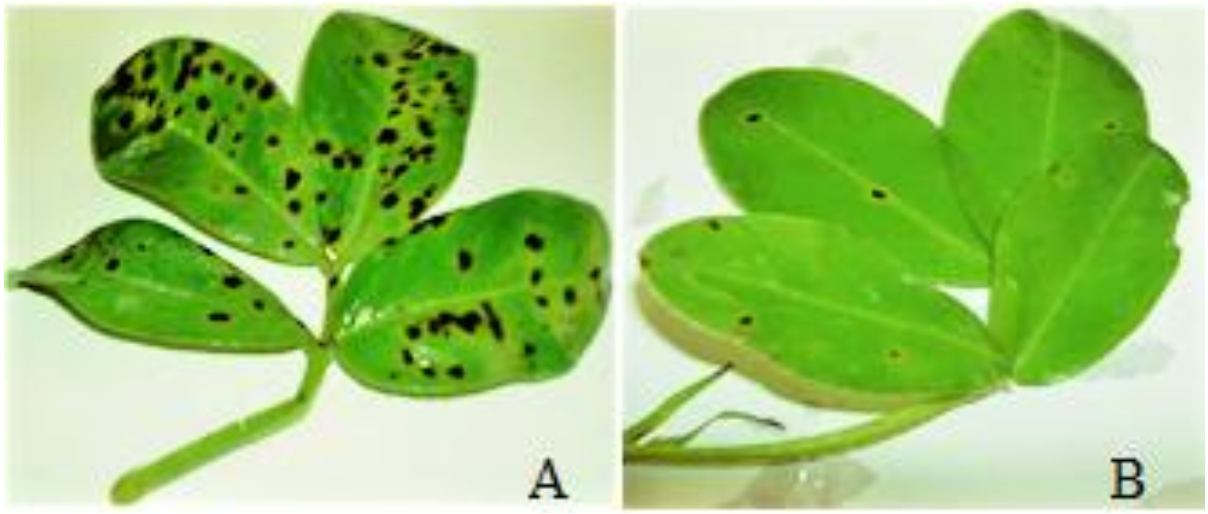


**Figure 2.** Early leaf spot lesions on peanut leaf (A) inoculated with distilled water, (B) inoculated with *Lecanicillium lecanii* 4184.

with *L. lecanii* reduced the severity late leaf spot. Up to 15th day after inoculation, we did not observe late leaf spot lesions on peanut leaves treated with *L. lecanii*. From the 20th to the 30th day after inoculation, we observed lesions on peanuts leaves treated with *L. lecanii* range from 2 to 3.7, and the lowest severity scores compared with the distilled water control (from 2.3 to 5.7). These evidences were observed in the Figure 4 (Photos A and B). Statistical analysis revealed significant differences between treatments on the 25th and 30th

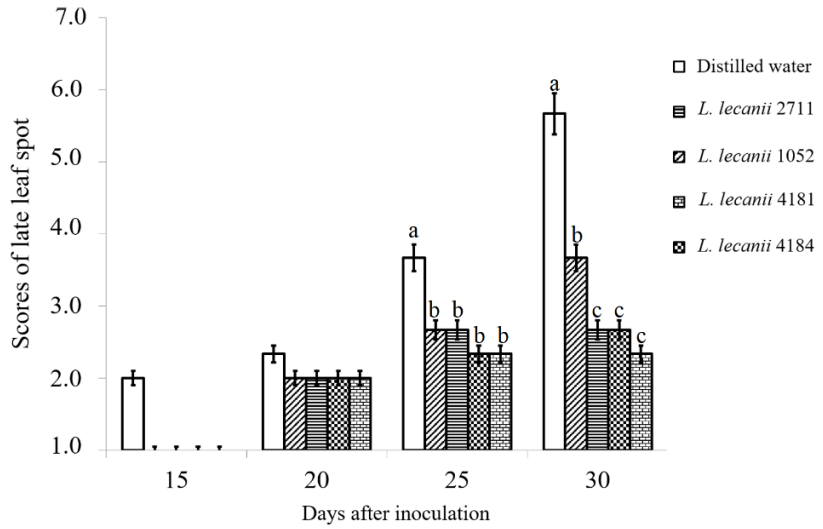


**Figure 3.** Effect of inoculation with *L. lecanii* on late leaf spot development on peanut leaves. Bars are means ±SE, n = 3 repetitions.


**Figure 4.** Late leaf spot lesions on peanut leaf (A) inoculated with distilled water, (B) inoculated with *L. lecanii* 4181.

days after inoculation (P = 0.005 and P = 0.0001). The best performance (lowest severity score 3.7) was observed with *L. lecanii* 4184 on the 30th day after inoculation.

## DISCUSSION

*L. lecanii* had an inhibitory action on conidial germination and germ tube elongation of *C. arachidicola* and *P. personata*. We also observed a weak development of leaf spots when the leaves are inoculated with the solutions of *L. lecanii*. The action of *L. lecanii* on disease development resulted in a delay in the onset of symptoms and a decrease in the leaf area damaged by pathogens. The best results were obtained with strains 4184 and 4181.

The potential use of *Lecanicillium* sp. in biological control against insect pests of crops has been demonstrated by many authors (Kim et al., 2007; Xie et al., 2019; Trinh et al., 2020), as an entomopathogen. Many findings carried out the biological control of plant

diseases by *Lecanicillium* sp. Indeed, it is known as mycoparasites of pathogens responsible of rust found in many species of plant. For example, some authors highlighted ability of *L. lecanii* to colonize lesions of coffee rust (Setiawati et al., 2021; Merle, 2019; Gómez-De La Cruz et al., 2017; Vandermeer et al., 2009; Jackson et al., 2012). Some hyperparsites have been studied in the control of peanut leaf spots. *Dicyma pulvinata* (Berk. & Curt.) V. Arx was found in leaf spot pathogens of peanut because of its effectiveness in controlling late leafspot both under field and greenhouse conditions (Mitchell et al., 1987). Subrahmanyam et al. (1990) has already showed the parasitism of *V. lecanii* (*Lecanicillium* sp.) on rust and late leaf spot of peanut in greenhouses. These studies showed that the pre-inoculation of peanut leaves with a suspension of *V. lecanii* conidia reduced the density of spots and damage leaf area due to rust and late leaf spot pathogens. However, the inhibitory effects of *L. lecanii* on the germination of conidia of *C. arachidicola* and *P. personata* were not yet well documented. This is also true for the inhibitory action of *L. lecanii* on the early leaf spot of peanut. According to (Mahfud et al., 2006; Kushalappa and Eskes, 1989), *Lecanicillium* sp. inhibits the germination of *Hemileia vastatix* spores. This could be explained by the fact that *L. lecanii* produces hydrolytic enzymes (e.g chitinases and proteases) in the culture media (Nguyen et al., 2015; Deshpande, 1999). The inhibitory action of chitinases in the germination of the spores of phytopathogenic fungi has been proven by many authors. Thus, Huang et al. (2005) have purified from the bacterium *Bacillus cereus* strain 28-9 an antifungal chitinase (ChiCW) which reduced the rate of germination and germ tube elongation of *Botrytis elliptica* conidia. Many findings revealed that, the *L. lecanii* can also endophytically colonize various plants (Gurulingappa et al., 2010). In fact, *L. lecanii* has already been isolated from asymptomatic cotton leaf strains (*Gossypium hirsutum* L.) (McGee, 2002). Gurulingappa et al. (2010) have demonstrated that *L. lecanii* colonized the leaves of cultivated plants such as cotton, wheat, tomato when they were inoculated with conidia. Our findings indicate the potentiality of *L. lecanii* use in the control of early and late leaf spot on peanut in semi-arid area.

## Conclusion

In conclusion, this study demonstrated the effectiveness of *L. lecanii* in reducing damage caused by pathogens responsible for peanut leaf spot disease. The results also indicated the inhibitory action of *L. lecanii* on germination and germ tube elongation of *C. arachidicola* and *P. personata* conidia. Our results indicate the potential use of *L. lecanii* as a biological control agent against peanut leaf fungal diseases. In future, field studies are necessary to promote the use of *L. lecanii* to control early and late leaf spot in large scale.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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