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

Tounwendsida Abel NANA¹*, Adama ZONGO², Bawomon Fidèle NEYA³ and Philippe SANKARA⁴

¹Equipe Phytopathologie et Mycologie Tropicale (PM-Prop), Unité de Formation et de Recherche en Sciences de la Vie et de la Terre (UFR/SVT), Université Joseph Ki-ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso.
²Institut des Sciences de l’Environnement et du Développement Rural (ISEDRA), Université de Dévoussi, BP 176 Dévoussi, Burkina Faso.
³Université Nazi BONI/Centre Universitaire de Gaoua 01, P. O. Box 1091, Bobo-Dioulasso 01, Burkina, Faso.
⁴Université Aube Nouvelle 06 P. O. Box 9283 Ouagadougou 06, Burkina Faso.

Received 28 October, 2021; Accepted 15 December, 2021

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 known well 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 (10⁶ 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, 1996).
1997). These diseases can cause up to 70% yield loss (Subrahmanyan et al., 1980). Previous studies have demonstrated 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 (Kolta 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 × (= 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, Subrahmanyan et al. (1990) have proven that *V. lecanii* parasitized the uredosporas 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 X 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 × 10⁵ 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 10⁶ 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 × 10⁵ 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 times. 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 formula 1 and 2, adapted from (Greche et al., 2000):

\[
Ig = \frac{NE - NL}{NE} \times 100\% \tag{1}
\]

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*

\[
Ite = \frac{LE - LL}{LE} \times 100\% \tag{2}
\]

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 times. In this experiment, we treated each lower face of peanut leaf with 100 μl of the solution of *L. lecanii* (10⁶ conidia mL⁻¹), and then we added 100 μl of the suspension of the pathogen (10⁶ conidia mL⁻¹). The samples were kept at 25°C in an oven refrigerator (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 five days, following Subrahmanyan 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 arachidicola.

| Inoculation treatment | Percentage of germination of conidia (%) | Percent inhibition (%) | Germ tube length (µm) | Percent inhibition (%) |
|-----------------------|-----------------------------------------|------------------------|-----------------------|------------------------|
| Distilled water       | 88.67<sup>a</sup>                       | 0                      | 13.50<sup>a</sup>     | 0                      |
| L. lecanii 4184       | 56.67<sup>b</sup>                       | 36                     | 12.67<sup>ab</sup>    | 6                      |
| L. lecanii 1052       | 52.83<sup>c</sup>                       | 40                     | 9.33<sup>bc</sup>     | 31                     |
| L. lecanii 2711       | 51.33<sup>c</sup>                       | 42                     | 8.83<sup>c</sup>      | 35                     |
| L. lecanii 4181       | 38.33<sup>d</sup>                       | 57                     | 8.33<sup>c</sup>      | 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 (%) | Percent inhibition (%) | Germ tube length (µm) | Percent inhibition (%) |
|-----------------------|-----------------------------------------|------------------------|-----------------------|------------------------|
| Distilled water       | 84.00<sup>a</sup>                       | 0                      | 6.97<sup>a</sup>      | 0                      |
| L. lecanii 4184       | 41.00<sup>b</sup>                       | 51                     | 4.59<sup>c</sup>      | 34                     |
| L. lecanii 1052       | 33.67<sup>c</sup>                       | 60                     | 6.03<sup>b</sup>      | 13                     |
| L. lecanii 2711       | 32.67<sup>c</sup>                       | 61                     | 4.38<sup>c</sup>      | 37                     |
| L. lecanii 4181       | 11.00<sup>d</sup>                       | 87                     | 3.09<sup>d</sup>      | 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
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 hyperparasites 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). Subrahmamyan 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 well documented. This is also true for the inhibitory action of *L. lecanii* on the early leaf spot of peanut. According to (Mahtud 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.

**ACKNOWLEDGEMENTS**

The authors are grateful to Dr Kadidia KOITA and Dr Idrissa KABORE for many insightful comments and suggestions which largely helped to improve the manuscript, and English proof reading.

**REFERENCES**

Deshpande MV (1999). Mycospedicide production by fermentation: Potential and challenges. Critical Reviews in Microbiology 25(3):229-243.

Gómez-De La Cruz I, Pérez-Portilla E, Escamilla-Prado E, Martínez-Bolaños M, Carrón-Villanovo GLL, Hernández-Leaf TL (2017). Selection in vitro of mycoparastites with potential for biological control on Coffee Leaf Rust (*Hemileia vastatrix*). Revista Mexicana de Fitopatología 36(1):172-183.

Greche H, Hajjali N, Jamali-Nejrou M, Mrabet N, Benjilali B (2000). Chemical Composition and Antifungal Properties of the Essential Oil of *Tanacetum anuum*, Journal of Essential Oil Research 12(1):122-124.

Gurulingappa P, Sword GA, Murdoch G, McGee PA (2010). Colonization of crop plants by fungal entomopathogens and their effects on two insect pests when in planta. Biological Control 53(1):34-41.

Huang CJ, Wang TK, Chung SC, Chen CY (2005). Identification of an Antifungal Chitinase from a Potential Biocontrol Agent. *Bacillus cereus* 28-9. Journal of Biochemistry and Molecular Biology 38(1):82-88.

Jackson D, Skillman J, Vandermeer J (2012). Indirect biological control of the coffee leaf rust, *Hemileia vastatrix*, by the entomogenous fungus *Lecanicillium lecanii* in a complex coffee agroecosystem. Biological Control 61(1):89-97.

Kim JJ, Goettel MS, Gillespie DR (2007). Potential of Lecanicillium species for dual microbial control of aphids and the cucumber powdery mildew fungus, *Sphaerotheca fuliginea*. Biological Control 40(3):327-332.

Koita K, Sofogba HK, Nana TA, Neya BF, Campa C, Sankara P (2017). Integrated management of leaf spot of peanut with aqueous leaf extracts of Lippia multiflora Moldenke and Chlorothalonil. International Journal of Applied Microbiology and Biotechnology Research 6:8-14. http://www.bluepenjournals.org/ijamb/pdf/2018/January/Koita_et_al. pdf

Kushalappa AC, Eskes AB (1989). Advances in coffee rust research. Annual Review of Phytopathology 27:503-531. https://doi.org/10.1146/annurev.py.27.090189.002443

Mahtud MC, Mor Ahmad ZA, Meon S, Kadir J (2006). In Vitro and In Vivo Tests for Parasitism of Verticillium *psaliiotae* Treschow on *Hemileia Vastatrix* BERK. and BR. Malaysian Journal of Microbiology 2(1):46-50.

McGee PA (2002). Reduced growth and deterrence from feeding of the insect pest Helicoverpa armigera associated with fungal endophytes of cotton. Australian Journal of Experimental Agriculture 42(7):995-999.

Merle I (2019). Effets du microclimat sur le développement de l’épidémie de rouille orangée du caféier Arabica (*Hemileia vastatrix* – *Coffea arabica*) dans une gamme de situations de production. Thèse de doctorat, Université de Montpellier 186 p.

Mitchell JK, Smith DH, Taber RA (1987). Potential for biological control of *Cercosporidium personatum* leafspots of peanuts by *Lhyma pulvinata*. Canadian Journal of Botany, 65(11):2263-2269. https://doi.org/10.1139/b87-308

Nana TA, Zongo A, Neya BF, Koita K, Sanon E, Ouedraogo A, Zagre
MB, Sankara P (2014). Influence de la température sur la croissance radiale in vitro de Lecanicillium lecanii, candidat de lutte biologique contre les cercosporoses et la rouille de l’arachide (Arachis hypogaea L.). Les Annales de l’Université de Ouagadougou, Série C, 10:1-27.

Neya BF (2017). Héritabilité de la résistance aux cercosporoses de l’arachide, (Arachis hypogaea L.) et de quelques caractères associés au rendement. Thèse de doctorat unique, Université OUAGA I Pr. Joseph Ki-ZERBO, Ouagadougou 220 p.

Nguyen HQ, Quyen DT, Nguyen SLT, VU VH (2015). An extracellular antifungal chitinase from Lecanicillium lecanii: purification, properties, and application in biocontrol against plant pathogenic fungi. Turkish Journal of Biology 39:6-14. https://doi.org/10.3906/biy-1402-28

Setiawati R, Widiastuti A, Wibowo A, Priyatmojo A (2021). Variability of Lecanicillium spp. Mycoparasite of Coffee Leaf Rust Pathogen (Hemileia vastatrix) in Indonesia. Pakistan Journal of Biological Sciences 24(5):588-598.

Shekes FM, Culbreath AK (1997). Early and late leaf spots. In: Kokalis-Burell N., Porter D.M., Rodriguez-Kabana R., Smith D.H., Subrahmanyam P. (eds): Compendium of Peanut Diseases. 2nd Ed. APS Press, St. Paul: 17-20. http://oar.icrisat.org/id/eprint/7612

Subrahmanyam P, Gibbons RW, Nigam, SN, Rao VR (1980). Screening methods and further sources of resistance to peanut rust. Peanut Science 7:10-12.

Subrahmanyam P, Mc Donald D, Gibbons RW, Nigam SN, Nevill DJ (1982). Resistance to rust and late leaf spot disease in some genotypes of Arachis hypogaea L. Peanut Science 9:6-10. https://doi.org/10.3146/0095-3679-9-1-2

Subrahmanyam P, Reddy PM, McDonald D (1990). Parasitism of Rust, Early and Late Leafspot Pathogens of Peanut by Verticillium Lecanii. Peanut Science 17(1):1-3.

Subrahmanyam P, Wongkaew S, Reddy DVR, Demski JL, Mc Donald D, Sharma SB, Smith DH (1992). Diagnostic au champ des maladies de l’arachide. ICRI SAT, Bulletin d’information No. 36, Patancheru, Andhra Pradesh 5020324, Inde, ISBN 92-9066-256-5, 79 p. http://oar.icrisat.org/id/eprint/1227

Trinh DN, Ha TKL, Qiu D (2020). Biocontrol Potential of Some Entomopathogenic Fungal Strains Against Bean Aphid Megoura japonica (Matsumura). Agriculture 10:114. https://doi.org/10.3390/agriculture10040114

Vandermeer J, Perfecto I, Liere H (2009). Evidence for hyperparasitism of coffee rust (Hemileia vastatrix) by the entomogenous fungus, Lecanicillium lecanii, through a complex ecological web. Plant Pathology 58:636-641. https://doi.org/10.1111/j.1365-3059.2009.02067.x

Xie T, Jiang L, Li J, Hong B, Wang X, Jia Y (2019). Effects of Lecanicillium lecanii strain JMC-01 on the physiology, biochemistry, and mortality of Bemisia tabaci Q-biotype nymphs. PeerJ, 7:e7690. https://doi.org/10.7717/peerj.7690

Zambettakis C, Sankara P (1985). Les hyperparasites fongiques de Puccinia arachidis en Afrique. PremiCres JournCes d’Etudes sur les Maladies des Plantes 1:133-349.

Zongo A, Konate AK, Koïta K, Sawadogo M, Sankara P, Ntare BR, Desmae H (2019). Diallel Analysis of Early Leaf Spot (Cercospora arachidicola Hori) Disease Resistance in Groundnut. Agronomy 9(1):15.