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RESEARCH ARTICLE

BIOCONTROL OF RUST AND LEAF SPOTS DISEASES OF PEANUT USING CULTURE FILTRATES OF *AKANTHOMYCES LECANII* IN NATURAL CONDITIONS IN BURKINA FASO

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Abstract

Leaf spots and rust are the most destructive diseases of peanut in Tropical Africa, but it has shown that *Akanthomyces lecanii* is known as hyperparasite of rust and leaf spots fungi. Previous studies have investigated on the use of conidia of *A. lecanii* in biocontrol of these diseases. However, the conidia are influenced by temperature and relative humidity, which could result in low efficiency of biocontrol agents in natural conditions. The use of secondary metabolites could help to fight against the plant diseases in natural conditions. Thus, we sprayed peanut plants with culture filtrates from 21-day-old cultures of two strains of *A. lecanii* grown in potato dextrose broth, to assess their efficiency against leaf spots and rust diseases in natural conditions. A susceptible variety “SH470P” and a moderately susceptible variety “PC79-79” of peanut to rust and leaf spots diseases were used. Our findings revealed that *A. lecanii* culture filtrates have significantly reduced the severity of rust and leaf spots diseases in "SH470P" only. In "SH470P", the lowest severity of leaf spots in 2020 (59.26%) and 2021 (48.10%) were recorded in plots sprayed with culture filtrates of *A. lecanii*. The lowest severity of rust (29.60%) was recorded in the plots sprayed with the *A. lecanii* culture filtrates in 2021 in "SH470P". From our findings, the use of *A. lecanii* culture filtrates is a promising alternative in the control of peanut rust and leaf spots. But, further studies are required to determine the effective doses and active secondary metabolites against these diseases.

Introduction:-

Peanut (*Arachis hypogaea*) is a multipurpose and highly nutritious crop containing oil, food and its foliage or haulm provides a valuable fodder for livestock (Hasan et al., 2014). However, several factors, including poor soil fertility, high soil salinity, inappropriate crop management practices, pests and diseases affect peanut production. Rust, early leaf spot and late leaf spot caused by *Puccinia arachidis*, *Cercospora arachidicola* and *Phaieosariopsis personata*, respectively, are major diseases affecting the peanut crops worldwide (Kumar et al., 2010 ; Koïta et al., 2018 ; Kumhar et al., 2018 ; Alidu et al., 2019). During severe disease outbreaks, leaf spots, in addition to rust can result in yield losses up to 50% (Ghewande, 2009).

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The use of synthetic fungicides has proven to be efficient for increasing yield, but they can potentially cause harm to environment and human heath through residues. In addition, the continuous use of traditional fungicides may cause bioaccumulation of the toxic residues besides giving rise to resistant strains of pathogens. Increased public concern about pesticide utilization and the health hazards necessitates the exploitation of alternative methods of disease control (Kumhar et al., 2018). In the last decades, several studies have been carried out on the antagonistic nature of several fungal biocontrol agents (Kishore et al., 2005; Gómez-De La Cruz et al., 2017). Among the antagonistic fungi with the capacity to control peanut diseases, *Akanthomyces lecanii* (previously known as *Lecanicillium lecanii* or *Verticillium lecanii*) [Kepler et al. (2017)] is the one with capacity to control rust and leaf spots (Nana et al., 2022, 2023). Nana (2015) assessed the effects of conidial suspensions of *A. lecanii* in the control of rust, early and late leaf spots diseases in peanut. This work revealed that the efficiency of conidial suspensions of *A. lecanii* was lower in field conditions than controlled conditions. This might particularly be due to the effects of environmental factors (temperature and relative humidity) on the conidia of the biological control agent, under field conditions.

Recently, in some studies, the use of culture filtrates of bio-agents to control certain plant pathogens and pests have been reported (Zou et al., 2007; Elkot et al., 2011; Kim et al., 2013). Wang et al. (2007) and Gurulingappa et al. (2011) showed that culture filtrates of the entomopathogenic fungi *A. lecanii* reduce aphid survival rates and deter feeding by whitefly and larva of *Spodoptera littoralis*. According to Saksirirat et al. (1991) cell-free culture filtrates of some *Verticillium psalliotae* isolates caused degradations, when rust spores were treated with them for 24 or 72 h. Nana (2015) reported that suspension of *A. lecanii* cultures partially inhibited the germination of spores of fungi causing rust and leaf spots diseases in peanut. The purpose of this experiment was to assess the effectiveness of culture filtrates of two strains of *Akanthomyces lecanii* to control leaf spots and rust diseases of peanut in natural conditions.

Materials and Methods:-

Culture filtrates preparation

A. lecanii strains 4184 and 4181, obtained from the National Museum of Natural History (MNHN) in Paris were used to prepare culture filtrates. In practice, 15 explants (0.4 cm in diameter) of each isolate cultivated for 10 days on potato dextrose agar was inoculated into 2000 ml potato dextrose broth (PDB) in Erlenmeyer flasks and incubated at 25-28°C and 500 rpm during 21 days. After the culture period, the culture fluid was filtered through a Whatman filter paper and a cellulose acetate syringe filter (0.2 µm) for removal of spores (Kim et al., 2013). Culture filtrates were kept in the refrigerator until biological assay.

Experiment location and ecological conditions

The experiment was carried out at Gampèla district, located at longitude 12°22 W and latitude 12°25 N, in the central part of Burkina Faso, during the 2020 and 2021 cropping seasons. The mean annual rainfall for the two seasons was 855 mm and 974 mm, respectively. The annual temperature is ranged between 21.5°C and 42.8°C (Koïta et al., 2017).This area is mainly devoted to crops farming experiment in Burkina Faso.

Experimental design and treatments

Two peanut varieties "SH470P" and "PC79-79", susceptible and moderately susceptible to both leaf spots and rust diseases were used, respectively. Two treatments including *A. lecanii* 4181 culture filtrate and *A. lecanii* 4184 culture filtrate, as well as, a control treatment without any fungus were laid out in split plot design, in three replicates. In each plot, each variety was sown in two rows (2.4 m length) with holes 0.15 m apart. Row to row distance was 0.5 m; while from plot to plot distance was one meter. The distance between two consecutive blocks was two meters. In each plot, plants were sprayed with one liter of culture filtrates (15%), using a hand-held Solo branded sprayer at 30 days after sowing (DAS), 45 DAS, 60 DAS and 75 DAS. The control plants were sprayed with distilled water. The recommended cultural practices were applied, and a Nitrogen-Phosphorus-Potassium (N-P-K) fertilizer of formula 14−23−14 was applied at 100 kg per hectare at 21 DAS.

Data collection and statistical analysis

The disease severity of rust and leaf spots was assessed on 10 randomly selected plants from each plot at 85 DAS using disease rating scale ranging from one (for no symptom) to nine (for severe symptom) (Subrahmanyam et al., 1995). From the scores, disease severity was calculated by using the following formula (Kumhar et al., 2018):

$$\text{Disease severity } (\%) = \frac{\sum n}{N \times 9} \times 100.$$

where: Σn – sum of individual ratings, N – total number of plants assessed, 9 – highest score on the severity scale.
Pod yield and 100 seed weight were also determined after harvesting per plot and per variety. All data were submitted to analysis of variance based on split plot design using XLSTAT 2016 software and the difference between means compared using Duncan's Multiple Range Test at 5%.

Results:-

Effect of *Akanthomyces lecanii* cultures filtrates on the severity of peanut leaf spots diseases

Figure 1 shows the effectiveness of the cultures filtrates of the both strains of *A. lecanii* on leaf spots severity in 2020 and 2021. The severity of the leaf spots was generally higher in 2020 than in 2021. Statistical analysis revealed that there was a significant difference between the treatments at 85 DAS in both years ($p \leq 0.041$).

In the case of SH470P variety, the cultures filtrates of the both strains of *A. lecanii* significantly reduced the severity of leaf spots compared to the control in the both years. At 85 DAS, leaf spot severity was 70.37% and 63% in the control, and 59.26% and 48.1% in the plants treated with culture filtrates of *A. lecanii* for 2020 and 2021, respectively. However, statistical analysis did not reveal a significant difference between the effects of the both strains of *A. lecanii* on leaf spots severity.

In the case of PC79-79 variety, there was no difference in leaf spots severity in plants treated with the culture filtrates compared to the control plants. The diseases severity ranged from 40.74% to 48.15% and 22.20% to 33.30% in 2020 and 2021, respectively.

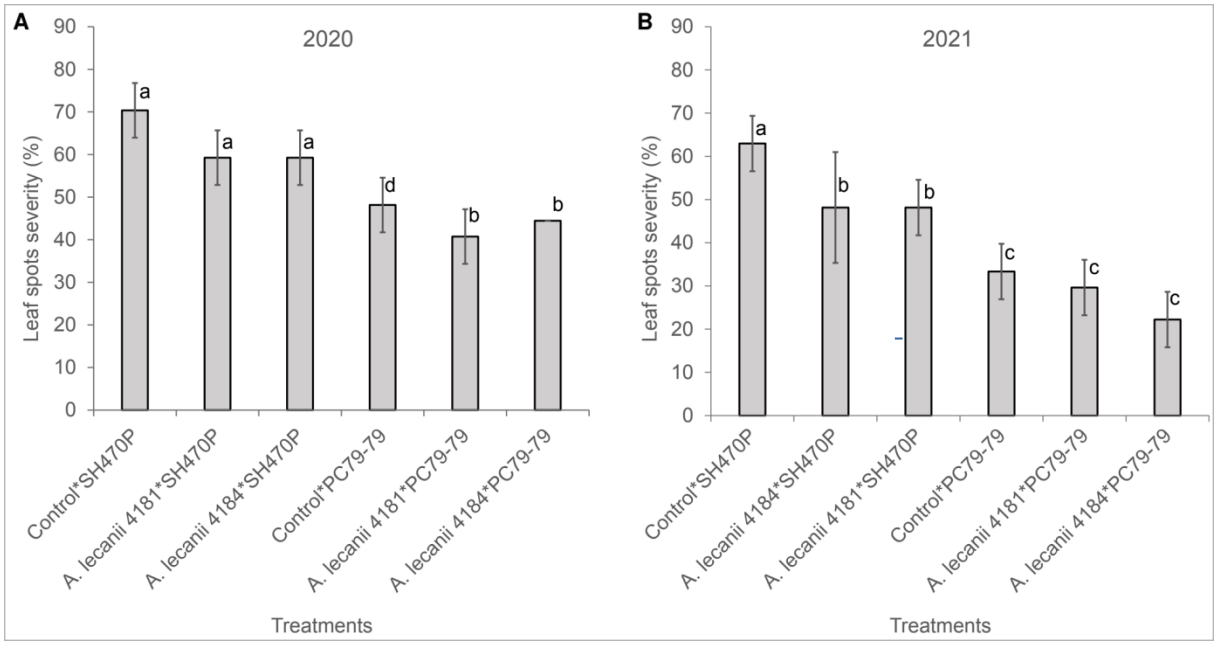

Fig. 1:- Effect of *A. lecanii* cultures filtrates on the severity of peanut leaf spots in (A) 2020 season and (B) 2021 season. Bars are means ±SD. Bars with the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test. DAS – days after sowing.

Effect of *Akanthomyces lecanii* cultures filtrates on the severity of peanut rust

Figure 2 shows that severity of the peanut rust was higher in 2021 compared to 2020. The culture filtrates of the two strains of *A. lecanii* significantly reduced the severity of rust compared to the control in the peanut variety "SH470P" for the season 2021 (p = 0.006). However, there was no significant difference between the effects of culture filtrates of the two strains of *A. lecanii* on rust severity. The severity of rust was 51.90% in control and 29.60% in plants treated with the filtrates of the two strains of *A. lecanii* for the variety "SH470P" in 2021. For both varieties, the statistical analysis did not reveal any significant difference between the treatments in 2020 (p = 0.215) that ranged from 14.80% to 29.60%.

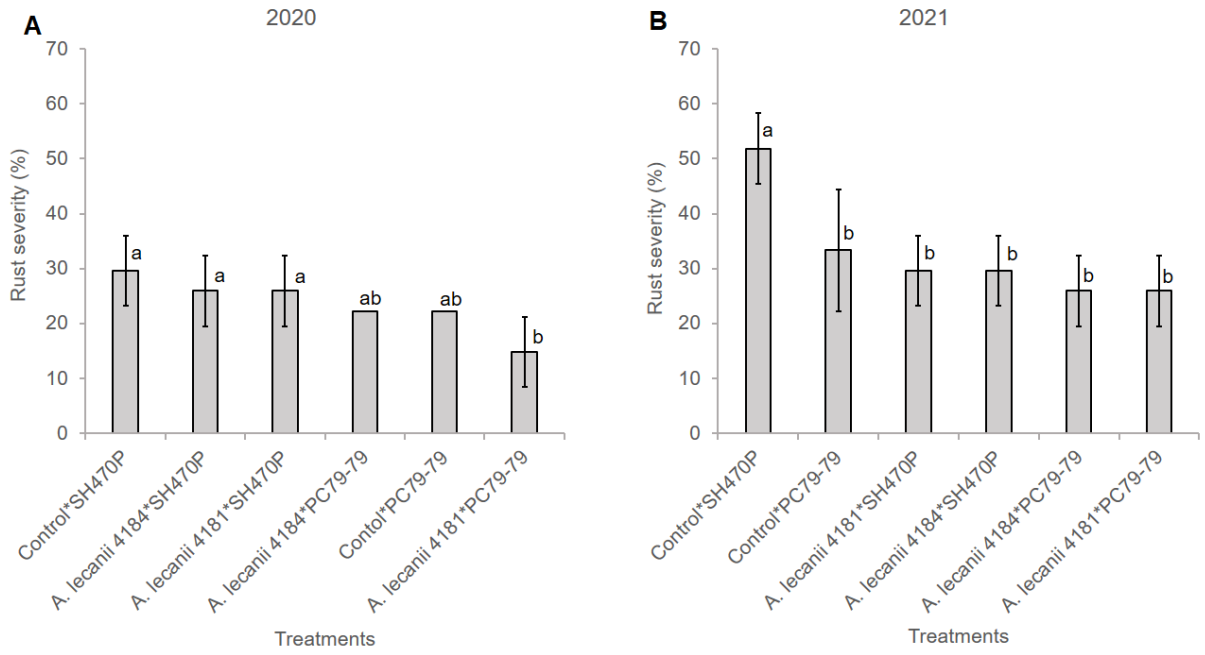

Fig. 2:- Effect of *A. lecanii* cultures filtrates on the severity of peanut rust at 85 days after sowing in (A) 2020 season and (B) 2021 season. Bars are means ±SD. Bars with the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test.

Effect of *Akanthomyces lecanii* cultures filtrates on peanut production

The results revealed that *A. lecanii* 4181 culture filtrates have significantly increased the yield of peanut pods compared to the control in 2020 (Table 1). During this season, the best pod yields, 1597.3 Kg/ha and 1545.1 Kg/ha, were recorded in plots treated with *A. lecanii* 4181 culture filtrates in PC79-79 and SH470P varieties, respectively. In contrast, yields were low (1257.5 Kg/ha and 1206.9 Kg/ha) for control in 2020 with the varieties PC79-79 and SH470P, respectively. In 2021, the analysis did not revealed a statistical difference between the yields of the treated plots and the yields of the control plots for the both varieties (p = 0.300). From hundred seed weight measurement with values ranging from 30.4 to 35.6 g and 35.6 to 37.1 g in 2020 and 2021, respectively, we did not reported a statistical difference between the treatments.

Table 1:- Effect of *A. lecanii* cultures filtrates on pods yield and 100 seed weight in 2020 and 2021 seasons.

Peanut varieties	Treatments	Pods yield (Kg/ha)		100 seed weight (g)	
		2020	2021	2020	2021
PC79-79	Control	1257.5±128.5bc	1716.7±256.6a	35.0±0.9 ab	36.2±1.3 a
	A. lecanii 4181	1597.3±69.8a	1796.7±205.0 a	35.6±2.3 a	36.4±1.4 a
	A. lecanii 4184	1247.6±197.5bc	2033.3±450.9 a	33.1±1.3abc	37.2±2.3 a
SH470P	Control	1206.9 ±155.4c	1866.6±230.9 a	31.9±2.6 abc	36.2±0.7 a
	A. lecanii 4181	1545.1 ±261.3ab	1983.3±104.1 a	31.4±1.5 bc	35.6±0.8 a
	A. lecanii 4184	1131.8 ±157.0c	2050.0±229.1 a	30.4±2.6 c	37.1±0.9 a
p-value traitements (A)		0.004	0.300	0.255	0.327
p-value varieties (B)		0.387	0.368	0.004	0.598
p-value A*B		0.932	0.847	0.806	0.876

Column means (±SD followed by the same letter are not significantly different at p = 0.05 according to Duncan's Multiple Range Test

Discussion:-

The results of the present study indicate that the spraying of peanut plants with *A. lecanii* culture filtrates significantly reduced rust and leaf spots diseases severity. This suggests that *A. lecanii* culture filtrates contain active compounds against these diseases. Some authors have shown that many fungal biological control agents produced secondary metabolites that are active against some pathogenic fungi of plants. For example, Vey et al. (2001) reported that *Trichoderma* spp. produced several secondary metabolites (e.g., Harzianic acid, alamethicins, tricholin, peptaibols, antibiotics, 6-pentyl-α-pyrone, massoilactone) with antifungal modes of action. Also, Lozano-Tovar et al. (2013) demonstrated that secondary metabolites of *Metarhizium brunneum* and *Beauveria bassiana* inhibited mycelial growth and germination of propagules of *Verticillium dahliae* and *Phytophthora megasperma*. In the regard of *Akanthomyces* genus, Arai et al. (2003) and Khan et al. (2014) have reported that several species produce secondary metabolites with antifugal activity (e.g., Radicicol, monorden, Ascochlorin, Bigutol).

Moreover, *A. lecanii* is well known to produce hydrolytic enzymes such as chitinases and proteases active against certain insects and plants infecting fungi (Mayorga-Reyes et al., 2012; Hasan et al., 2013; Nguyen et al., 2015; Nana et al., 2023). Rocha-Pino et al. (2011) reported that *A. lecanii* could produce chitinases in solid-substrate culture or submerged cultures. According to Ma et al. (2012) and Mathivanan et al. (1998), the antifungal activity of chitinases can result from the inhibition of growth of hyphae, spores germination of fungi including *Puccinia arachidis*. Nana et al. (2022, 2023) shown that *A. lecanii* suspension inhibited the germination of the conidia of leaf spots fungi and lysed the wall of urediniospores of peanut rust fungus. As a result, *A. lecanii* is widely used as an effective biocontrol tool against different fungal diseases (Gómez-De La Cruz et al., 2017). However, most researches have focused on the use of conidial suspensions of *A. lecanii* against rust and leaf spots diseases of peanut, as demonstrated in Nana et al., (2022, 2023). From our knowledge, this study reveals for the first time the effect of *A. lecanii* culture filtrates on the severity of rust and leaf spots diseases in natural conditions. We found that the severity of rust was higher in the 2021 season compared to 2020. This could be explained by the heavy rainfall (974 mm) during the season 2021 compared to low rainfall (855 mm) recorded in 2020. The increase peanut pod yield is probably due to effects of treatments with *A. lecanii* culture filtrates, as confirmed by others authors (Koïta et al., 2017; Bdliya and Gwio-Kura, 2007) who have demonstrated that the control of peanut leaf spots can improve peanut production.

Conclusion:-

The results of this study revealed that the use of *A. lecanii* culture filtrates reduced the severity of peanut rust and leaf spots diseases and increased pod yield. Nevertheless, it is necessary to carry out a chemical analysis of the culture filtrates in order to determine the active compounds against peanut rust and leaf spots and to formulate an effective biofungicide against these diseases.

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