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Evaluation of fungal diseases of strawberries (*Fragaria vesca*) and control methods based on essential oils

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ABSTRACT

The strawberry (Fragaria vesca) is an emerging fruit crop in Burkina Faso with strong economic and nutritional potential, but its sustainability is compromised by numerous fungal diseases that cause considerable losses both in the field and post-harvest due to rot and wilt. The objective of this study is to investigate the symptoms, identify the main pathogenic fungi associated with strawberry cultivation in central Burkina Faso, and evaluate a control method based on essential oils. The methodology relies on field surveys, the collection of symptomatic leaves, followed by macroscopic and microscopic analyses after isolation in the laboratory. In vitro tests were conducted with three essential oils Cymbopogon citratus, Eucalyptus camaldulensis, and Azadirachta indica at concentrations of 5% and 15% to evaluate their antifungal potential against the isolated pathogens. Our work identified three pathogens: Botrytis cinerea, Mycosphaerella fragaria, and Aspergillus niger. These pathogens appear at different stages of plant growth and cause a range of symptoms, from necrosis and chlorosis to leaf death. They reduce photosynthetic activity, cause premature leaf drop, and lead to fruit rot. The three plant extracts demonstrated complete fungicidal efficacy. Specifically, Cymbopogon citratus, Eucalyptus camaldulensis, and Azadirachta indica at concentrations of 5% and 15% completely inhibited the growth of Botrytis cinerea, Mycosphaerella fragaria, and Aspergillus niger 72 hours after treatment. Understanding host-parasite-environment relationships, as well as the effectiveness of certain plant extracts, offers potential solutions for implementing environmentally friendly control methods.

Keywords: *Fragaria vesca, biological control, fungus, isolation, essential oil, symptomatology.*

Introduction

Strawberry (*Fragaria ananassa* Duch.) is an important commercial fruit crop with rich organoleptic qualities such as a pleasant flavor, texture, and taste, and numerous health benefits [1]; [2]. Strawberries are widely consumed as fresh fruit or processed into various food products. Based on the most recent statistics, global strawberry production has increased by 41% in the last decade, and China, the United States of America (USA), the European Union (EU), Mexico, Turkey, Egypt, and South Korea are the leading producers [3]. In 2019, the annual strawberry yields in the USA and China were 1,021,490 t from 18,130 ha (563,425 hg/ha) and 3,221,557 t from 126,126 ha (255,424 hg/ha), respectively [1]. The increase in strawberry productivity over the past decade could be attributed to scientific innovations in the areas of breeding, improving cultural practices, and pest and disease management [4]; [5].

In Burkina Faso, strawberry cultivation has taken hold since the 1980s and is now experiencing remarkable growth. It is sometimes nicknamed "red gold" because of its profitability for local market gardeners.

National production is estimated at around 2,000 tons per year, concentrated mainly around cities like Ouagadougou and Bobo-Dioulasso. Three main varieties are produced: Festivale, Camarosa, and Selva, introduced for their adaptation to Sahelian climatic conditions. An improved local variety called Amine has been developed, but is produced in very small quantities, despite being excellent for preservation and processing.

Phytosanitary constraints explain the constant search for effective and environmentally friendly control methods. Several methods exist, including chemical control, biological control, and cultural control [6]; [7]. However, each of these methods, taken individually, has shown its limitations. Genetic control could be a sustainable solution, but this requires an understanding of host-parasite interactions and, therefore, knowledge of the parasites themselves [6].

Post-harvest losses remain very high due to the presence of pathogenic fungi. There is little specific scientific data on the incidence and epidemiology of fungal diseases in Burkina Faso. Accurate identification of pathogens in the field and post-harvest is essential for developing integrated management methods adapted to the local context.

Materials and Methods

Study Site

The study was conducted in central Burkina Faso, and samples were taken from the Boulmiougou market gardening area, located west of Ouagadougou, on the road to Bobo-Dioulasso. Strawberry cultivation in Burkina Faso is concentrated mainly in the peri-urban areas of Ouagadougou and in certain specific zones of the Kadiogo, Houet, and Boulgou provinces. The peri-urban area of Boulmiougou was chosen because it is the only site where strawberry producers can be found at any time of year. Producers from Loumbila come to Boulmiougou to obtain runners for later transplanting.

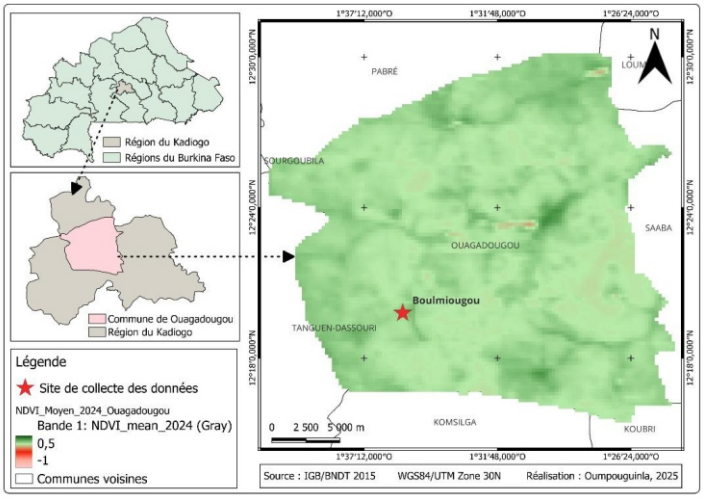


Figure 1 : Map representing the study area

Plant material for identification

The plant material for disease identification consists of strawberry leaves and runners exhibiting symptoms of fungal diseases described in the literature.

Plant material for treatments

Three plant extracts, namely essential oils of *Cymbopogon citratus*, *Eucalyptus camaldulensis*, and *Azadirachta indica*, were used in the study. These species have demonstrated antifungal efficacy against other pathogens and on other crops in previous studies.

Cymbopogon citratus (D.C.) STAFF. (Lemongrass): From the Poaceae family, lemongrass is a perennial herb with aromatic, linear, ribbon-like leaves that form compact clumps and reach 1 m in height. The leaves are light green and measure 1.5 cm to 60 mm wide. It flowers every two years (Kouame et al., 2015).

Eucalyptus camadulensis DEHNH: This is a tree from the Myrtaceae family, with a slender habit, reaching 12 m to 20 m in height, with a generally straight and more or less whitish trunk, a narrow crown with drooping and sparse branches. The essence extracted from its leaves (Eucalyptol) is especially renowned as an expectorant and cough suppressant (cough, bronchitis, asthma, cold, rhinitis, etc.) and is also used in perfumery [9].

Azadirachta indica A. Juss (Neem): The neem tree, or margosa, is native to India and belongs to the Meliaceae family. It is generally cultivated in tropical and semi-tropical regions. Its fruits and seeds are the source of neem oil. The neem tree is a fast-growing monoecious tree that can reach a height of 20 meters, and more rarely, 35 to 40 meters. Its foliage is evergreen but may become deciduous during severe droughts. The branches are broad and spreading. The fairly dense crown is rounded and can reach a diameter of 15-20 meters on older trees. The opposite, pinnate leaves are 20 to 40 centimeters long, with 20-30 dark green leaflets about 3 to 8 centimeters long. The terminal leaflet is often absent. The petioles are short. The white, fragrant, protandrous flowers appear from January to May in tropical zones [10]. They are arranged on more or less drooping axillary panicles measuring up to 25 centimeters long. The inflorescences, which branch to the third degree, bear 150 to 250 flowers.

Sampling and Data Collection

Surveys were conducted in the irrigated areas of Boulmiougou and Loumbila. During these surveys, samples exhibiting symptoms of fungal diseases as defined in the literature were collected. The Loumbila site no longer has any plants between March and November, making it impossible to collect samples during this period. Each sample taken in Boulmiougou consisted of 20 leaves placed in plastic bags and sealed with parafilm. A total of three collections were carried out at different times. A total of sixty (60) leaves were collected during the survey in the visited area. After sorting, leaves and stolons exhibiting symptoms were retained. Incubations were initiated as the samples were brought from the field. Sampling was carried out randomly using the diagonal sampling technique described by [11].

Preparation of the Culture Medium

PDA (Potato-Dextrose-Agar) culture medium was used for the isolation of fungi. In the case of certain obligate parasites, direct observations were made under a microscope. The culture medium was prepared by taking 39 g of synthetic PDA powder, weighing it using an electronic balance, and adding it to 1 liter of distilled water in a jar. The mixture was stirred to obtain a homogeneous solution. The jar was then placed in an autoclave at a pressure of 1 bar and 121 °C for 15 minutes. The jar was removed from the autoclave and cooled to between 45 °C and 50 °C to prevent condensation. Finally, the medium was transferred under a sterile fume hood into sterile 9 cm diameter Petri dishes, adding approximately 15 ml of medium per dish.

Incubation

The samples were first washed thoroughly under running water. They were then cut into small fragments of approximately 25 mm^2 (5 mm on each side), selecting areas close to the spots or lesions, near necrotic zones [12]. These fragments were then disinfected for a few seconds in a 2% sodium hypochlorite solution, followed by 30 seconds of rinsing with sterile distilled water. This rinsing was repeated three times to remove as much sodium hypochlorite as possible. This disinfection process eliminated exogenous microflora. Afterward, the fragments were placed on sterile Watmann filter paper to dry for 30 seconds under a fume hood. Finally, the cut fragments were placed on culture media prepared beforehand in Petri dishes. The incubation was done under alternating light (12h) and darkness (12h) to induce better fungal development.

Isolation

After colony development during incubation, successive subculturing was carried out under the same conditions to obtain pure colonies in Petri dishes. The pure colonies obtained using the [12], which consisted of taking a thin portion of the mycelium with a scalpel blade and then depositing it in a new Petri dish containing a PDA-based medium, were subjected to macroscopic and microscopic identification.

Symptomatology

Symptomatology consists of the study of symptoms. Symptoms are the visible manifestation of disease. They are characterized by changes in different parts of the plant, such as leaves and stems. Several symptoms are characteristic of diseases. Necrosis is the death of cells, chlorosis is the degradation of chlorophyll, wilting is the loss of turgor, and yellowing is a change in color. Galls and many other symptoms can also appear on the plant.

Macroscopic Identification

Macroscopic identification of fungi is performed by observing pure colonies with the naked eye after successive subculturing on culture medium. Two criteria are considered: the appearance and color of the colonies. The first criterion is based on the appearance of the colonies, which may appear downy, woolly, cottony, velvety, powdery, or granular. As for the second criterion, color, the most common colors are white, cream, yellow, orange, brown, and even black. The pigments may be localized within the mycelium or diffused throughout the culture medium [13].

Microscopic Identification

Microscopic examination consisted of using a scalpel to remove a thin portion of mycelium from a cultured colony and placing it on a slide with a drop of sterile water. Observation under 40x and 100x objectives (400x and 1000x magnification) allowed for the distinction of the main morphological elements, including the presence of spores (shape, size, color) and the structure of the hyphae (presence or absence of septa), in accordance with the identification keys proposed by [14].

Treatment Methods

To evaluate the antifungal efficacy of certain essential oils against pathogens isolated from strawberry leaves, three essential oils were used:

Eucalyptus camaldulensis, essential oil in a 10 ml bottle

Azadirachta indica, neem oil in a 500 ml bottle

Cymbopogon citratus, essential oil in a 10 ml bottle

All oils were at 100% concentrations.

The oils were purchased from the Institute of Research in Applied Sciences and Technologies (IRSAT) in Ouagadougou.

Dilution of Essential Oils

The pure essential oils were diluted in sterile distilled water and shaken before use. The solutions were prepared at two concentrations: 5% and 15%. Approximately 1 ml of each solution was used for each treatment per Petri dish.

The dilution was performed according to the following formula:

$Ci \times Vi = Cf \times Vf$ where:

Ci = initial concentration (100%)

Vi = initial volume (ml)

Cf = desired concentration (5% or 15%)

Vf = final volume (ml)

Treatment

Petri dishes with PDA medium were prepared. Pure colony fragments of each previously isolated pathogen were placed in the Petri dishes. Using a 10 ml graduated syringe, each treatment was applied directly to the surface of the mycelial cultures in the Petri dishes at a rate of approximately 1 ml per dish.

The treated dishes were then incubated at room temperature with alternating light and dark cycles to observe the evolution of colony growth compared to untreated controls.

Results

Symptomatology

Observations conducted on strawberry production plots in Boulmiougou revealed and described several types of symptoms. These symptoms appeared primarily on the leaves, with varying degrees of severity. They manifested as necrotic spots (Photo 2) and spherical necrotic spots with a light center (Photo 3). Two types of symptoms were identified during the observation. The first, characterized by progressive necrosis, is a general symptom, indicating that several pathogens could be responsible. The second, marked by spherical necrosis, is a specific symptom, suggesting the probable involvement of a single pathogen.



Photo 1: Symptoms of necrosis



Photo 2 : Symptoms of purple spots with a white center

Identifications

Botrytis cinerea

On PDA medium, the colony grows rapidly, occupying almost the entire surface of the Petri dish by the fourth day of incubation at room temperature. The observed colonies were initially white and cottony (Photo 3), gradually becoming gray to light brown in the center, with dense sporulation at maturity. Under the microscope (100x objective), spherical, cluster-like spores were observed (Photo 4). The branched conidiophores bear hyaline to slightly brownish ovoid conidia arranged in clusters. The abundant sporulation observed after a few days of incubation, as well as physiological characteristics such as the ability to grow at different temperatures and in various pH ranges, confirm the identification of *Botrytis cinerea*.

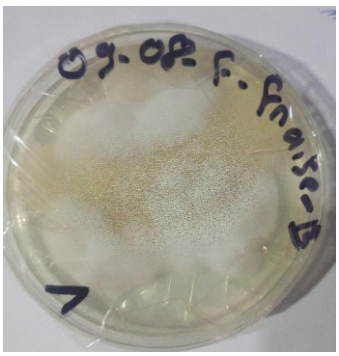


Photo 3 : Botrytis cinerea on PDA medium

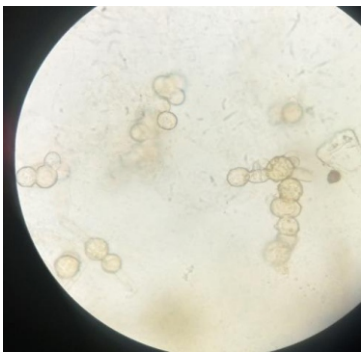


Photo 4 : Spores of Botrytis cinerea (G×100)

Aspergillus niger

Colonies observed on PDA medium are black at maturity (Photo 5), with a cottony texture. Under the microscope (100x objective), a dense, septate mycelium is observed (Photo 7). The conidiophores are long, ending in a globose vesicle bearing round-headed phialides (Aspergillus head), which are responsible for the formation of spherical conidia (Photo 6). Based on this description, the identification keys allowed us to identify the pathogen as *Aspergillus niger*. This opportunistic fungus frequently colonizes weakened tissues, particularly under conditions of high humidity and temperature. Affected leaves turn dark brown to black, often accompanied by a distorted appearance. A black, powdery deposit may be visible on necrotic areas: this corresponds to the abundant sporulation of the fungus, consisting of black conidia characteristic of *A. niger*.

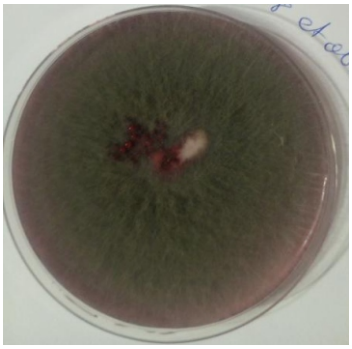


Photo 5 : Pure colony on PDA medium

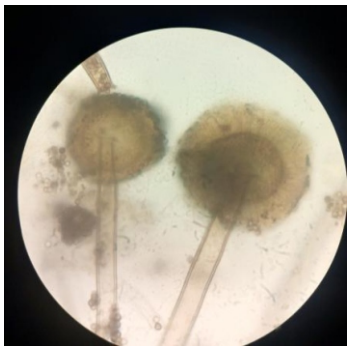


Photo 6 : Spores d'Aspergillus niger (G×100)



Photo 7 : Mycelium d'Aspergillus niger

Treatment with Essential Oils

After treating pure colonies with the essential oils of *Azadirachta indica*, *Cymbopogon citratus*, and *Eucalyptus camaldulenis* at concentrations of 5% and 15%, no visible colony growth was observed on PDA plates after 72 hours of incubation. The negative controls showed normal fungal growth starting at 24 hours. These results demonstrate the antifungal activity of these three essential oils against *Aspergillus niger*, *Mycosphaerella fragaria*, and *Botrytis cinerea*.

Discussion

Observations made in Boulmiougou identified three major pathogens responsible for strawberry leaf diseases: *Botrytis cinerea*, *Aspergillus niger*, and *Mycosphaerella fragariae*.

The morphological characteristics and symptoms observed on strawberry leaves are consistent with descriptions reported in the literature.

The isolation and identification of *Botrytis cinerea* from strawberry leaves collected in Boulmiougou confirm the presence of this pathogen as one of the main causes of strawberry leaf diseases. The observed morphological characteristics, particularly the grayish colonies on PDA medium, the branched conidiophores, and the hyaline to light brown ovoid conidia, correspond to the classic descriptions of the fungus reported by [15]. Similarly, rapid growth accompanied by dense mycelial mating is characteristic of *B. cinerea* as described in the literature [16]. *Botrytis cinerea* thrives particularly well under conditions of high humidity, low aeration, and moderate temperature, generally between 15 and 25°C [17]. In the vegetable farming systems of Ouagadougou, regular irrigation and sometimes high planting density create a humid microclimate conducive to infection. This finding aligns with the observations of [18], who indicate that the combination of humidity and temperature is a key factor in the development of gray mold. According to [19], the fungus preferentially colonizes damaged tissues before invading healthy tissues, which explains its widespread distribution in intensive production systems. Observations made in Boulmiougou, where some leaves showed pre-existing lesions, corroborate this mechanism. These results underscore the importance of regular phytosanitary monitoring and the implementation of integrated pest management methods combining judicious cultural, biological, and chemical measures to limit the impact of this disease on strawberry production in Boulmiougou.

Regarding *Aspergillus niger*, observations confirm its presence as a fungal agent associated with foliar lesions on strawberries. The morphological characteristics and symptoms are consistent with studies conducted in West and North Africa, highlighting the ecological plasticity and variable virulence of the pathogen depending on local conditions. In the present study, colonies isolated on PDA medium are characterized by a black color at maturity, a dense septate mycelium, and conidiophores terminating in globular vesicles bearing phialides, in accordance with classic descriptions of the species [20]. The abundant sporulation observed in the form of black conidia is also a well-documented distinguishing characteristic in the literature [21].

The presence of *A. niger* on strawberry leaves can be explained by its generalist behavior. This fungus is widespread in tropical and subtropical soils and readily colonizes weakened tissues, particularly under high humidity and temperature conditions [22]. In Boulmiougou, the agro-environmental conditions regular irrigation, high temperatures, and high humidity are particularly favorable to its development. Furthermore, undecomposed plant debris often serves as a source of inoculum, promoting secondary infection. The presence of *A. niger* in strawberry plots, therefore necessitates rigorous management, including soil sanitation, improved drainage, and reduced leaf wetness. The presence of *Aspergillus niger* on strawberry leaves can be explained by its widespread distribution in the soil and on plant debris. These results highlight the need for continuous monitoring and an integrated pest management approach combining sanitation, good cultural practices, and the use of biological alternatives.

For *Mycosphaerella fragariae*, the observed leaves revealed the presence of purple spots with whitish centers, characteristic of common leaf spot disease.

These symptoms are consistent with those described by [23], who reports that the first lesions appear as small, circular spots with purple edges, later evolving into a pale or grayish center. Similarly, [24] observed similar symptoms on strawberry crops in Ohio (USA). These authors specify that the disease develops primarily under conditions of high humidity, which promotes spore germination and the spread of the fungus through water splash. These findings align with our observations, as the studied plot also exhibited high relative humidity, a condition favorable to the development of *M. fragariae*. The results of the present study are also consistent with those reported by [25], which emphasize that *Mycosphaerella fragariae* infections are more frequent on older leaves and that the coalescence of lesions can cause premature drying of the foliage.

This loss of leaf area reduces the plant's photosynthetic capacity and leads to a decrease in yield, a phenomenon also observed in our trials, where severely affected plants showed a significant weakening of their vigor. The observations made on the leaves in this study confirm the presence of common leaf spot disease and illustrate the symptomatic similarity with previous work carried out in other regions of the world. These results show that local climatic conditions (high humidity and moderate temperatures) are determining factors for the expression of the disease. Finally, the findings are consistent with those of the [3], which reports that *Mycosphaerella fragariae* can persist on plant debris and spread by wind and water splash, thus promoting its survival from one season to the next.

The neem (*A. indica*) essential oil, known for its high azadirachtin content, likely caused cell membrane alteration leading to fungal cell death. Similarly, the phenolic and aldehyde compounds (citral, geraniol, citronellal) present in *C. citratus* and the terpene oxides of *E. camaldulensis* may have interfered with the cellular metabolism and respiration of the fungi.

The results obtained are consistent with several previous studies demonstrating the effectiveness of essential oils in controlling phytopathogenic fungi. [27] and [28] reported a complete inhibition of the growth of *Aspergillus niger* and *Fusarium oxysporum* by citronella and eucalyptus essential oils, with fungicidal effects comparable to those of synthetic chemical fungicides. These results suggest that the tested essential oils represent promising alternatives to the synthetic chemical fungicides often used to control strawberry foliar diseases. The complete absence of growth observed 72 hours after application suggests comprehensive fungicidal activity rather than simple temporary inhibition (fungistatic). This result is encouraging from an integrated pest management perspective, where essential oils could reduce the use of chemical products while preserving the health of strawberries and the ecological balance of the soil.

Conclusion

This study has led to a better understanding of the pathological diversity affecting strawberries, an emerging crop with strong economic potential. Investigations conducted at the Boulmiougou sites resulted in the identification of three fungal pathogens: *Botrytis cinerea*, *Aspergillus niger*, and *Mycosphaerella fragariae*. These fungi, responsible respectively for leaf destruction, black necrosis, and whitish to light gray spots, pose major constraints to strawberry productivity. Laboratory experiments demonstrated the effectiveness of extracts from three plant species *Azadirachta indica* (neem), *Cymbopogon citratus* (lemongrass), and *Eucalyptus camaldulensis*, on the growth of these pathogens.

The complete absence of colony development after 72 hours of incubation is likely linked to the bioactive compounds present in these extracts. These results confirm the relevance of biocontrol approaches as ecological and sustainable alternatives to synthetic fungicides. This study highlights the need to integrate extracts from local plants into strawberry plant protection strategies in Burkina Faso. Their use could contribute to reducing the use of chemical products and improving the quality of the fruit. Thus, integrating these natural solutions into local agricultural practices represents a promising avenue for healthier, more sustainable, and environmentally friendly strawberry production, contributing to food security and the valorization of Burkina Faso's endogenous resources.

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