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Antifungal Effect of Citronella Essential Oil Against *Colletotrichum capsici* Causing Anthracnose Disease in Green Chili Pepper

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ABSTRACT

Green chili pepper is one of the most valuable crops in the Philippines and is extensively cultivated throughout the world due to its versatility. However, its production is affected by pests and diseases, particularly anthracnose diseases caused by *Colletotrichum capsici,* which is one of the most destructive pathogens of this fruit, reducing the crop's yield and sustainability. With this, several studies explored the antimicrobial activities of plant-derived essential oils (EOs), and one of them is the citronella (*Cymbopogon nardus*) oil (CO), and its potential as an eco-friendly alternative to synthetic pesticides. However, limited studies were conducted on the phytotoxicity level of citronella oil and its antifungal effects against anthracnose disease of green chili pepper. Therefore, this study was conducted to determine the non-phytotoxic level of CO and evaluate its effects against the symptom development of anthracnose disease. A phytotoxicity test was conducted, and the *in vivo* antifungal potential of non-phytotoxic CO concentrations was evaluated against the disease caused by *C. capsici*. All tests were replicated four times with five samples per treatment, and arranged in a Completely Randomized Design (CRD). The phytotoxicity test resulted that below 0.7% CO concentrations were non-phytotoxic, while 0.8% to 2% exhibited discoloration on the fruits' epidermis. Subsequently, non-phytotoxic 0.5% to

0.7% CO inhibited the growth and development of *C. capsici*, implying that its effects were in a dose-dependent manner. Hence, further studies may evaluate the long-term and potential synergistic effects of CO with other natural antifungal agents for enhanced efficacy on various crops and under different conditions.

Keywords: *Green chili pepper, colletotrichum, phytotoxicity, antifungal, citronella*

Introduction

In the Philippines, the green chili pepper (*Capsicum spp.*), also referred to as "siling haba," is valued as a spice with economic importance. It is a significant cash crop for smallholder farmers due to its market demand. Moreover, it is a staple in Filipino cooking, adding flavor to various meals (Besa, 2014). However, in the year 2020 to 2023, production of this fruit was 2390.01 MT, 2469.18 MT, 2535.38 MT, and 2548.81 MT, respectively. Slow production in 2023 was noted, producing only 0.53% increase from 3.31% and 2.68% in the year 2021 and 2022, respectively (PSA-Openstat, 2025). This could be related to factors such as the soil health, environment, poor postharvest handling (Juacalla et al., 2021), and plant diseases that can occur at any stage of plant growth (Edusei et al., 2022). Diseases are one of the major constraints that could negatively impact the supply relative to rapid population growth, proportional to increased food demand (Mateo et al., 2024).

One of these diseases is the green chili pepper anthracnose disease, commonly caused by *Colletotrichum capsici*, which infects the host plant tissues via wounds or natural openings (Balendres, 2023). The pathogen can produce appressoria and hyphae, allowing itself to invade, spread, and colonize the host tissue, leading to symptom development characterized as a sunken lesion with a dark, concentric ring (Eaton et al., 2021). It can also produce a range of enzymes and toxins, aiding them to infect and overcome host defenses (Singh et al., 2011). Factors such as the humidity, temperature, and rainfall density and duration play a vital role in the severity of infection (Agrios, 2005).

To manage plant diseases, several researchers explored the antimicrobial efficacy of various essential oils (EOs) against various plant pathogens, such as those from the neem (Schuch et al., 2024), thyme, peppermint, lemongrass (Tančinová et al., 2022), and orange peel (Sheikh et al., 2021). One promising oil is the citronella oil (CO) derived from citronella (*Cymbopogon nardus*) plant, containing flavonoids, saponins, alkaloids, tannins, and phenols, mostly known for their antibacterial, insecticidal, and fungicidal properties. Primarily, it contains geraniol, limonene, citronellal, citronellol, and linalool, all have pesticidal ability by degrading the pathogen cell wall during penetration (Santiwithchaya, 2004). Nakahara et al. (2003) concluded that species of *Aspergillus*, *Penicillium*, and *Eurotium* were suppressed by citronellal compound. Additionally, Maurya et al. (2024) also stated that CO was effective against *F. oxysporum* and *A. alternata.*

With this, citronella oil was used in this study based on several studies discussing its antifungal activity against *C. capsici* (Schulman, 1959, as cited in Dela Cueva & Balendres, 2018; Pavoni et.al., 2019), its availability, cost-effectiveness, and

environmental safety compared to synthetic fungicides since nowadays, conventional farming systems are commonly practiced in the country (Cablinan, 2024). However, citronella oil at higher concentrations was reported to be toxic; thus, several studies focused on its efficacy as weed control (Race, 2023; Singh et al., 2006; Somala et al., 2023) while studies on its toxicity to crops were limited. Hence, this study was conducted to determine the non-phytotoxic concentration of CO on green chili pepper fruits, and subsequently evaluated its antifungal effects against *C. capsici*. This study could aid in establishing an eco-friendly measure and a compatible integrated control against plant pathogens. Findings of the study showed the potential use of citronella oil as a biopesticide alternative to synthetic pesticides.

Methods

Preparation of Culture Media

Potato Dextrose Agar (PDA) was prepared following the procedure of Gupta et al. (2017). The medium was composed of 10g dextrose, 20g agar, and 250g potatoes. Peeled potatoes were sliced and boiled in 500 ml of distilled water until they softened. Then, the supernatant was filtered, and the melted agar was mixed. The volume was restored to 1000 mL and boiled again for 5 minutes, during which dextrose was added. The medium was then poured into flasks with cotton plugs and sterilized in a pressure cooker at 15 psi for 30 minutes, with a slight modification: a pressure cooker was used instead of an autoclave due to availability. Thereafter, the medium was poured into petri plates measuring 15 ml, and were allowed to solidify for 10 minutes to cover, then were placed upside down. All works were done aseptically inside the laminar flow hood in a UV-sterilized isolation room.

Collection and Isolation of *Colletotrichum capsica*

Diseased green chilli peppers with typical symptoms of anthracnose disease, the soft-whitish to dark brown to black necrotic lesions with a sunken appearance on fruit surfaces, were collected. Diseased samples were wrapped with clean paper, stored in UV-sterilized zip bags, labeled, and brought to the laboratory in a box. Specimens were directly examined under the microscope before isolation for confirmation of the causal pathogen based on its morphological structures. Green chili pepper anthracnose disease, causative agent *C. capsici,* was morphologically confirmed; thereby, the tissues underwent surface sterilization using sterile water following the procedure described by Anggrahini et al. (2020). Then, the advancing portion was swabbed with 70% ethanol, and a flame-sterilized scalpel was used to cut sections of infected fruit tissues measuring 5 mm towards the healthy portions, where the pathogens are likely more active. Thereafter, tissues were dipped using flame-sterilized forceps into previously sterilized petri plates containing 1% sodium hypochlorite (NaOCl) with slight modification, where the plant tissues were dipped in NaOCl for 1 minute instead of 30 seconds for higher disinfection activity, then the tissues were rinsed three times in sterile distilled water (SDW), and were blotted dry using UV-sterilized blotting paper. All works were done aseptically.

Pure Culture and Mass Production of the Pathogen

Pure culture was done from a five-day-old isolated culture, which was microscopically identified by transferring a mycelial disc (5 mm) from an advancing portion to a new sterile PDA. Another microscopic fungal identification was done after

five days based on its morphological characteristics, such as the growth patterns, color of mycelia, and the vegetative and reproductive structures. For the mass production of *C. capsici*, culture discs were obtained from the advancing mycelial growth, then were transferred to fresh plated sterile PDA, incubated at room temperature for another seven days until further tests.

Pathogenicity Test

Virulence of the cultured pathogen was confirmed through a pathogenicity test, conducted on 10 green chili pepper fruits sprayed with *C. capsici* spore suspension, and another 10 samples as a negative control. Fungal suspension was prepared following Sepúlveda et al. (2024) with slight modification, where the culture pathogen in 60 mm x 60 mm petri plates was dislodged, added with 10 ml sterile distilled water, and filtered using a three-layer gauze to separate the spores. Thereafter, spores were diluted by adding 1ml spore suspension to 9 ml sterile water. Spores were counted at 1.75 x 106 per ml using a hemocytometer. Each fruit received 1 ml of diluted suspension, then was incubated for three days at room temperature and was observed daily.

Procurement of Citronella Oil (CO)

Citronella oil available in the market was used in the study. It was obtained from 7As' Farm Project Co. under the name of Argus Craig B. Gomez, located at 2030 3rd Avenue, Teacher's Village, Tagum City, Davao del Norte. The product was labeled as an insect repellent and was refined through steam distillation.

Phytotoxicity Test

Essential oils (EOs) were reported to cause deleterious effects at the cellular level, such as photosynthesis and mitochondrial respiration inhibition (Werrie et.al., 2020). Thus, a phytotoxicity test was conducted to determine the non-phytotoxic CO concentration. This method was also used to evaluate the effects of the compound and to provide a rapid, efficient mechanism of pre-screening potential oil concentrations causing damage to fruits. Lutensol A 8 AP, as an emulsifier, was used using a micropipette. And, CO concentrations tested were 0.1% (+0.005 ml E), 0.3% (+0.015 ml E), 0.5% (+0.025 ml E), 0.7% (+0.035 ml E), 1% (+0.060 ml E), 1.3% (+0.075 ml E),1.5% (+0.085 ml E), 1.7% (+0.1 ml E), and 2% (+0.15 ml E) in 100 ml oil-water emulsion. Selection of these concentrations was based on the study of Brum et al. (2014), but with narrower intervals, and the green chili fruits used in the study were obtained from a single source nearby. The experiment was laid out in a Completely Randomized Design (CRD) with 10 treatments replicated four times, with five sample fruits per treatment.

Fruits were observed and evaluated daily after treatment application for any symptoms of phytotoxic injuries such as modifications in color, browning, reddening, and even rotting on stems and fruits, which may be localized, necrotic, or even local death of tissues, or generally appearing first as discoloration but would end up to rotting of the calyx and pedicel. Any changes were monitored and recorded daily using the rating scale used by Nalini and Parthasarathi (2018), where 0-no symptoms, 1-very slight discoloration, 2-slightly severe but not lasting, 3-moderate and more lasting, 4-medium and lasting, 5-moderately heavy, 6-heavy, 7-very heavy, 8-nearly destroyed, 9-destroyed, and 10-completely destroyed. The non-phytotoxic citronella oil concentrations were further optimized in the succeeding *in vivo* test.

***In vivo* Test**

The sample fruits were arranged in a Completely Randomized Design (CRD) with five treatments replicated four times, with five sample fruits per treatment. Fruits were treated with non-phytotoxic concentrations of CO as follows: T1 – Control (distilled water); T2 – 0.3% CO + 0.025 mL E; T3 – 0.5% CO + 0.035 mL E; T4 – 0.7% CO + 0.045 mL E in 100 mL oil–water emulsion; and T5 – Mancozeb (chemical check, RR). The treatments were prepared following the procedure from the previous experiment, and the chemical was applied at its recommended rate (2.8 ml/ 1000 ml DW). Thereafter, fungal spore suspension was prepared following the procedures described by Sepúlveda et al. (2024), as mentioned in the pathogenicity test. Then, the spores were examined based on their colony color and appearance, and the morphology of the reproductive and vegetative structures under the microscope.

Treatment Application and Pathogen Inoculation

Treatments were applied 48 hours before *C. capsici* inoculation to green chili pepper fruits as a preventive measure by foliar spraying using a hand atomizer. Thereafter, the green chili pepper fruits were kept in a humid condition for three days to induce infection.

Gathered Data

Disease incidence percentage was assessed using the formula, disease incidence (%) = number of infected fruits/total number of fruits assessed x 100. The number of days to symptom appearance was also counted. Severity of infection was also evaluated daily, following the scale used by Restrepo-Leal et al. (2022) where 0-no visible symptoms apparent, 1-few minute lesions to about 5% of the total area is affected and usually confined to the bottom of the fruits, 2- fruits in about 10% of the total area was infected, 3- about 25% of the total area was infected, 4- about 50% of the total area was infected, 5-fruits were severely infected >50% and dead. Disease severity index was calculated using the formula, $\%DI = 0n_0+1n_1+ 2n_2+3n_3+4n_4+5n_5 / 5 (N) \times 100$, where, %DI= percent disease index; $0n_0+1n_1+2n_2+3n_3+4n_4+5n_5$ = the samples with a rating of 0, 1, 3, 4, and 5; N= total number of samples; and 5= highest rating.

Statistical Analysis

Data collected were analyzed using Analysis of Variance (ANOVA), set at $p \leq 0.05$ significance level, following the Completely Randomized Design (CRD), and the difference among treatment means was compared using Tukey's Honest Significant Difference (THSD).

Ethical Considerations

The study was conducted in a controlled laboratory setting, and all experiments were designed to minimize waste and prevent environmental harm. The researchers ensured that all chemicals and materials used in the study were handled and disposed of in accordance with local and institutional regulations and guidelines.

Results and Discussion

Morphological Characterization

Colletotrichum capsici displays distinct morphological features with hyaline, single-celled, rod-shaped spores, typically measuring 18-27 µm x 2.1-4.1 µm (Ventura et al., 2004). Its colony morphology varies, with colors ranging from cottony white to gray, and the presence of appressoria was observed, crucial for pathogenicity and identification. The mycelium of *C. capsici* was typically white to gray (Figure 1), and can be darkened with age compared to closely related species such as *C. gloeosporioides*, whose colony is described as white to pale grey with mycelia containing bright orange conidial masses produced in concentric rings on the colonies (Photita et al., 2005). The formation of acervuli—the asexual fruiting body, which were small, dark, and cushion-like structures—was another key characteristic, as they characteristically produce numerous spores. Additionally, the presence of setae, the hair-like or spine-like structures, was observed, which usually aided in distinguishing this species from other fungi within the *Colletotrichum* genus (Joshi et al., 2024).

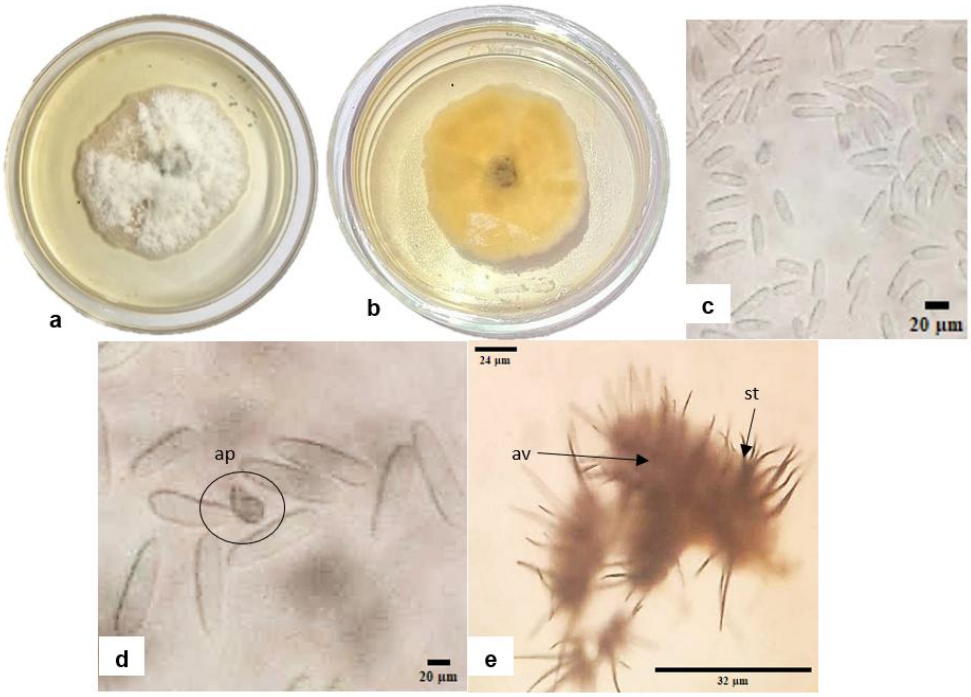

Figure 1. Morphology of *C. capsici* in plates at the (a) front, (b) back, (c) microscopic view of the fungal spores at 40x magnification, (d) appressorium (ap)-the structure which is usually melanized to combat turgor pressure during attachment and penetration, and (e) acervuli (av) – the fruiting bodies of the pathogen where asexual sores like conidia are formed, and setae (st) – the hairlike structure on the fruiting body which aids spore dispersal.

Pathogenicity Test

Colletotrichum species typically produce several distinct symptoms that can severely affect the fruit quality and marketability. The initial symptom of the infection often includes the appearance of small, circular spots that may have a dark brown or

black center, sometimes surrounded by a lighter halo. As the infection progresses, these spots become enlarged and coalesce, leading to larger areas of decay.

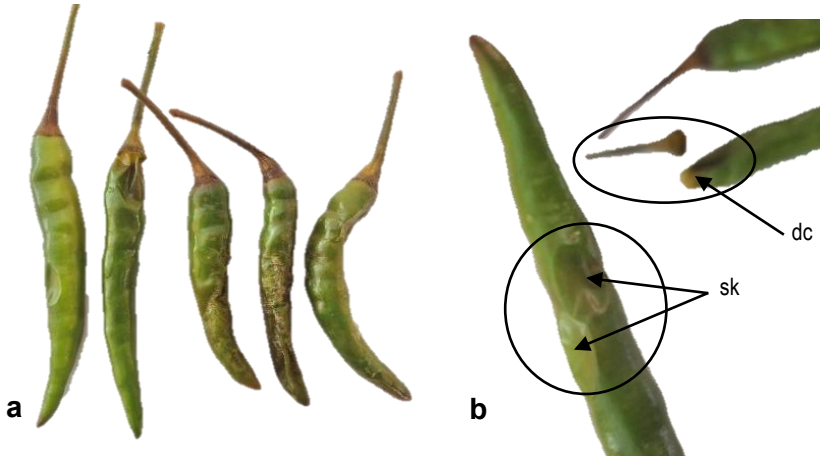

Figure 2. *Pathogenicity test for the virulence of the pathogen Colletotrichum capsici inoculated in green chili pepper, (a) appearance of the fruits with the pathogen infection, (b) closer view of the sunken (sk) and detached calyx (dc) symptom appearance of chili pepper caused by C. capsici.*

Soft rotting was observed, where the fruit tissue becomes mushy and water-soaked. This decay resulted in a foul odor, indicating advanced deterioration. It also exhibited a premature drop of the fruit calyx, thereby reducing yield and affecting harvest quality. Additionally, the presence of white or grayish fungal structures, such as spores and mycelium, was visible on the surface of the decaying fruit (Figure 2). Re-isolation and microscopic examination of the pathogen from the diseased fruits were conducted, revealing that the observed symptoms were caused by the inoculated pathogen; therefore, it is pathogenic and virulent.

Phytotoxic Effect of Citronella Essential Oil

The phytotoxic response of citronella oil at different concentrations on the physical appearance of green chili pepper fruits stored in room conditions was assessed. The results revealed that the 1% to 2% citronella oil concentrations showed phytotoxic effects on the treated sample of this experiment (Table 1).

Table 1. Mean Percentage of the Phytotoxicity of the Different Citronella Oil (CO) Concentration Levels Applied in Green Chili Pepper

TREATMENT	DAYS AFTER TREATMENT APPLICATION						
	1 DAT**	2 DAT**	3 DAT**	4 DAT**	5 DAT**	6 DAT**	7 DAT**
T1-Control	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}
T2-0.1% CO	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}

T3-0.3% CO	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}
T4-0.5% CO	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}
T5-0.7% CO	0.00±0.00^{d}	0.00±0.00^{d}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{d}
T6-1% CO	0.83±1.67^{cd}	1.67±1.92^{cd}	2.50±1.67^{c}	3.33±2.72^{c}	5.00±1.92^{c}	5.00±1.92c	5.00±1.92^{cd}
T7-1.3% CO	0.00±0.00^{d}	2.50±1.67^{cd}	2.50±1.67^{c}	5.00±1.92^{c}	5.00±1.92^{c}	6.67±2.72^{c}	6.67±2.72^{c}
T8-1.5% CO	4.17±3.19^{b}	5.83±4.19bc	10.83±3.19b	18.33±3.33b	20.00±2.72b	20.83±3.19^{b}	24.17±5.69^{b}
T9-1.7% CO	5.83±3.19^{b}	9.17±3.19^{ab}	11.67±3.33^{b}	15.83±5.00^{b}	20.00±2.72^{b}	20.83±1.67^{b}	25.83±1.67^{b}
T10-2% CO	10.00±0.00^{a}	13.33±3.85^{a}	22.50±5.00^{a}	35.00±6.38^{a}	42.50±8.77^{a}	47.50±11.98^{a}	56.67±3.85^{a}
Pr (> F)	0	0	0	0	0	0	0

*** - Significant at 5% level; mean values with (±) standard deviation having the same letter superscripts are (ns) not significantly different at 5% level of significance using Tukey's Honest Significant Difference (THSD); values are means of three replications*

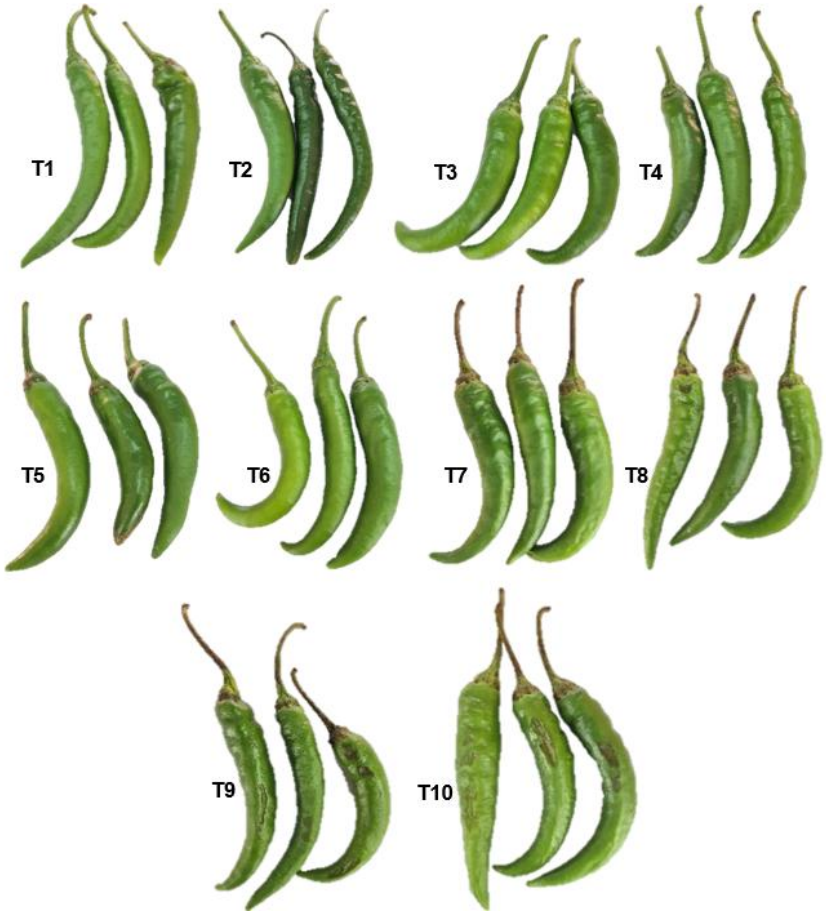

Figure 3. *Appearance of green chili pepper fruits as affected by the different citronella essential oils concentration levels within seven days of observation, where higher CO concentrations (T8-T10) exhibited rotting and discoloration. Citronella oil at higher concentrations (1%-2%) significantly caused phytotoxicity.*

The application resulted in visible injury such as necrosis, chlorosis, yellow to brown patches, rotting, and as well as calyx removal, and even death of the whole fruit (Figure 2b). The statistical analysis showed that the 0.1% to 0.7% CO concentrations have no significant difference from the untreated samples after seven days of application (Table 1). This assessment determined that a 0.1%-0.7% citronella oil concentration can be further optimized for the succeeding *in vivo* experiment against *C. capsici*. Responses to the present experiment corroborate the report of Werrie et al. (2020), which stated that the compound present in citronella essential oil caused phytotoxicity to the plant in higher concentrations but not in lower concentrations, concluding that these low doses simulate mild stress. Singh et al. (2006) also demonstrated that the application of citronellal, which is a compound abundant in CO, caused symptoms in weeds like chlorotic or necrotic spots with varying levels of injury. Furthermore, their study resulted that at lower concentrations (7.5 and 15 mg/ml), injuries were less severe and reversible, whereas, at higher concentrations (60 mg/ml), it causes a very severe and

irreversible injury followed by complete wilting and even shedding of leaves, in agreement to the symptoms observed in this study. Therefore, the compound citronellal could probably be the cause of severe injury to the stem calyx and fruits of green chili pepper at higher concentrations.

Days to Fungal Symptom Appearance

The earliest symptom appearance was significantly observed in untreated sample fruits at an average of 2.05±0.19 days after pathogen inoculation (see Figure 4). Meanwhile, the symptom appearance of fruits treated with 0.3% and 0.5% was delayed to 6.75±6.08 days after inoculation, whereas application of 0.7% further delayed the symptom appearance to 8.12±4.48 days (Table 2), which was longer than the untreated one. The result of this study implied that numerically, the number of days to symptom appearance was delayed when treated with non-phytotoxic 0.3%-0.7% CO, comparable to untreated samples.

Table 2. Mean Percentage of the Number of Days Symptom Appearance After Application of Different Citronella Oil (CO) Concentration Levels on Green Chili Pepper

Treatment	Number of Days of Symptom Appearance
T1-Control	2.05±0.19^{ns}
T2-0.3% CO	6.75±6.08^{ns}
T3-0.5% CO	6.75±6.08^{ns}
T4-0.7% CO	8.12±4.48^{ns}
T5-Mancozeb (chemical check)	-
Pr (> F)	0.0767

*** - Significant at 5% level; mean values with (±) standard deviations having the same letter superscripts are (ns) not significantly different at 5% level of significance using Tukey's Honest Significant Difference (THSD); values are means of three replications*

Figure 4. Symptom appearance in fruits with the application of non-phytotoxic CO at different concentrations within seven days of observation, where the untreated fruit samples exhibited severe infection, while application of non-phytotoxic 0.7% CO was comparable to the chemical (T5) used.

CO Effects on the Disease Incidence (%)

Non-phytotoxic CO concentrations were significantly different from untreated fruits in the mean percentage of disease incidence, where untreated samples exhibit 100% incidence. More so, this study resulted in an incidence was at 40% to 20% when applied with 0.3% to 0.7% CO, respectively, implying that the application of CO delayed the rate of infection of *C. capsici,* which could be due to reduced inoculum (Gouveia et al., 2023).

Table 3. Mean Percentage of the Disease Incidence Caused by *C. capsici* as Affected by the Different Citronella Oil (CO) Concentration Levels Applied in Green Chili Pepper

TREATMENT	DAYS OF OBSERVATION						
	1 DAT**	2 DAT**	3 DAT**	4 DAT**	5 DAT**	6 DAT**	7 DAT**
T1-Control	55.00±1 0.00^{c}	70.00±1 1.55^{c}	85.00±1 0.00^{c}	85.00±1 0.00^{c}	100.00± 0.00^{c}	100.00± 0.00^{c}	100.00± 0.00^{c}
T2-0.3% CO	30.00±2 5.82^{b}	30.00±2 5.82^{b}	30.00±2 5.82^{b}	40.00±2 8.28^{b}	40.00±2 8.28^{b}	40.00±2 8.28^{b}	40.00±2 8.28^{b}
T3-0.5% CO	15.00±1 9.15^{ab}	15.00±1 9.15^{ab}	20.00±2 3.09^{ab}	20.00±2 3.09^{ab}	20.00±2 3.09^{ab}	20.00±2 3.09^{ab}	20.00±2 3.09^{ab}
T4-0.7% CO	0.00±0.0 0^{a}	0.00±0.0 0^{a}	10.00±1 1.55^{ab}	10.00±1 1.55^{a}	15.00±1 9.15^{ab}	20.00±2 3.09^{ab}	20.00±2 3.09^{ab}

T5-Chemical Check	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Pr (> F)	0.0005	0	0	0.0001	0	0	0

*** - Significant at 5% level; mean values with (±) standard deviations having the same letter superscripts are (ns) not significantly different at 5% level of significance using Tukey's Honest Significant Difference (THSD); and values are means of three replications.*

The result of this study was in agreement with the study of Hoyos et al. (2024), where *Cymbopogon citratus* essential oil reduced anthracnose incidence and severity by causing serious ultrastructural damage to conidia, such as vacuolization, cytoplasm leakage, and invagination of the plasma membrane. Moreover, the study of Gouveia et al. (2023) indicated that citronellal was fungicidal on *Candida albicans,* probably because it can cause damage to the cell wall and membrane of the pathogen. However, in the present study, CO has not totally inhibited the pathogen growth because its major components are highly affected by prevailing temperature, precipitation, light, and humidity according to Le et al. (2024). In addition, Chang et al. (2008) also explained that the quality and composition of the oil can be affected as the temperature increases. Since CO components are relevant in inhibiting fungal pathogen growth, a decreased amount of these compounds would result in its reduced ability. On the other hand, the fast development of the pathogen infection due to the time of incubation influenced the disease infection according to Yan et al. (2014).

CO Effects on the Disease Severity (%)

Application of a non-phytotoxic CO concentration significantly reduced the disease severity level, comparable to a synthetic pesticide. In this study, 0.5% to 0.7% exhibited no significant difference with 4% to 3% disease severity, respectively, comparable to the synthetic pesticide within seven days of observation (Table 4). Application of citronella oil at 0.5% and 0.7% concentration resulted to significantly higher disease reduction, showing the efficacy of CO.

Furthermore, the results obtained in this study were in agreement to the study of Lucas, et.al., (2012) where they demonstrated that the essential oil from cinnamon promoted 49%, 21%, 27% protection when applied with different time intervals, within the entire cultivation period. This event can also be explained by prevailing conditions such as the host susceptibility, virulence of the pathogen, and the environment (van der Waals et al., 2013).

Another possible reason for not totally inhibiting the growth and development of the pathogen was the volatility of essential oils as described by Schuck et al. (2001). Moreover, duration of treatment application and inoculation are the major factors to activate the mechanisms of CO, as demonstrated by Itako et al. (2013) by applying lemongrass (*C. citratus*) essential oil 72 hours before inoculation of A. solani. Observations of this study were in partial agreement with Abreu (2006) who observed reductions of 26%, 62%, and 95% in the incidence of tomato black spots treated with the essential oil of cinnamon, concluding that increasing the concentration of the

essential oil of cinnamon and decreasing the application intervals boosts the efficiency of the oil in controlling the disease.

Table 4. Mean Percentage of the Disease Severity Caused by *C. capsici* as Affected by the Different Citronella Oil (CO) Concentration Levels Applied in Green Chili Pepper

TREATMENT	DAYS OF OBSERVATION						
	1 DAT**	2 DAT**	3 DAT**	4 DAT**	5 DAT**	6 DAT**	7 DAT**
T1-Control	11.00±1.73c	16.00±2.83c	24.00±7.48b	32.00±8.64c	54.00±5.16b	70.00±4.00c	85.00±3.83c
T2-0.3% CO	6.00±4.47b	6.00±4.47b	6.00±4.47b	8.00±5.66b	51.00±2.00b	51.00±2.00b	51.00±2.00b
T3-0.5% CO	3.00±3.32ab	3.00±3.32ab	4.00±4.00a	4.00±4.00ab	4.00±4.00a	4.00±4.00a	4.00±4.00a
T4-0.7% CO	0.00±0.00a	0.00±0.00a	2.00±2.00a	2.00±2.00ab	3.00±3.32a	3.00±3.32a	3.00±3.32a
T5-Chemical Check	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a	0.00±0.00a
Pr (> F)	0.0005	0	0	0	0	0	0

*** - Significant at 5% level; mean values with (±) standard deviations having the same letter superscripts are (^{ns}) not significantly different at 5% level of significance using Tukey's Honest Significant Difference (THSD); values are means of three replications.*

Conclusion and Future Works

Phytotoxicity assessment revealed that 0.1 to 0.7% CO concentrations were non-phytotoxic on green chili pepper fruits, while higher concentrations significantly showed phytotoxic symptoms. An *in vivo* study revealed that preventive application of CO significantly lessens the incidence and severity of anthracnose disease. This could probably be due to the concentration of active compounds present in the oil. Therefore, this study concluded that 0.5% and 0.7% citronella (*C. nardus*) oil concentrations were non-phytotoxic and effective against the development of *C. capsici,* causing anthracnose disease in green chili pepper. This antifungal activity can aid as an alternative measure to synthetic fungicides against plant pathogens. Further studies may also be conducted to further increase the efficacy of *C. nardus* essential oil against *Colletotrichum* spp. and other phytopathogens to study the efficacy of citronella oil for a longer duration and further test the efficacy of the non-phytotoxic citronella (*C. nardus*) oil under field conditions.

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Conflict of Interest

The authors declare that they have no conflict of interest in the publication of this research paper. No financial or personal relationships with other people or organizations have influenced the conduct of this research or the preparation of this manuscript.

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