

## Use of starch and biochar-based essential oil formulations from three aromatic plants as antifungal components to improve peanut (*Arachis hypogaea* L.) seed storage

### Abstract

Poor storage conditions of foodstuffs are responsible for their deterioration on many microorganism species with substantial quantitative and qualitative losses. During storage, formulating essential oil of some antifungal plants' based on cassava starch and corn cob biochar efficacy was evaluated on fungi associated with peanut seeds (*Arachis hypogaea* L.). Two hundred peanut seeds were dispersed in plastic bottles; 5 g of essential oil formulation starch-based (3 and 7  $\mu\text{l/g}$ ) were sprinkled on the seeds. Biochar pieces were added to the seeds in the bottles for biochar-based essential oils. Laboratory conditions were used to store both treatment and non-treatment seeds for 70 days. Infection rates of fungal infections were estimated at 10, 20, 40, 60, and 70 days of storage. From the results obtained, five fungal genera were isolated from peanut seeds, including four species of *Aspergillus*, *Cercospora* sp, *Fusarium* sp, *Phomopsis* sp and *Rhizopus stolonifer*, with seed infection percentages ranging from 4 to 62%. The antifungal activity of starch-based formulations revealed that at a concentration of 3  $\mu\text{l/g}$ , *Cymbopogon citratus* essential oil is the most effective antifungal agent, 13% infection for 70 days resulted storage of peanut seeds. After 70 days of storage, at a dose of 7  $\mu\text{l/g}$ , *C. sempervirens* essential oil had the best antifungal efficacy with 23.50% of infected seeds for the biochar-based formulation. As a result of these findings, starch and biochar can be used to formulate essential oils for the conservation of peanut seeds. Moreover, *Cymbopogon citratus* essential oil formulated with starch is the most effective.

**Keywords:** *Cymbopogon citratus*, *Cupressus sempervirens*, *Thymus vulgaris*, essential oil, Starch, Biochar, Antifungal activity, Peanut seeds, Conservation.

### Introduction

Peanut (*Arachis hypogaea* L.) is one of the most essential cultivated legumes in sub-Saharan Africa [1-2] (Snapp *et al.*, 2018; Kpatinvoh *et al.*, 2017). Global annual groundnut production is estimated at 47 million tonnes of dry pods, of which nearly 29% is produced in Africa and 594,000 tonnes from Cameroon [3] (FAOSTAT, 2018). Pulse in general and groundnuts in particular are essential contributors to global food security, health and poverty eradication [1] (Snapp *et al.*, 2018), especially in developing countries. These legumes are a significant resource of essential protein, micronutrients, and amino acids [4] (Asif *et al.*, 2013). They can thus contribute to the fight against iron deficiency anemia, one of the most critical micronutrient deficiencies observed in Africa and against protein-energy deficiency [1] (Snapp *et al.*, 2018).

Poor storage conditions are sometimes observed in rural areas. High seed moisture content (insufficient drying), inappropriate relative humidity and temperature of storage structures, thus promoting mould growth[5-6](Nadjet *et al.*, 2016 ; Taruvinga *et al.*, 2014).The damage caused by these stock moulds is manifested not only by a significant alteration of the aesthetic quality and organoleptic and chemical characteristics of the foodstuffs[5](Nadjet *et al.*, 2016),but also by the production of mycotoxins such as aflatoxins. Molds of the genus *Aspergillus* mainly produce the latter, recognized as a major contaminant of peanuts[7](Torres *et al.*, 2014). Due to their varied toxic effects, mycotoxins have the potential to cause both acute and chronic poisoning in both humans and animals, sometimes fatal such as carcinogenicity, immune toxicity, neurotoxicity, and hepatotoxicity[8](Agriopoulou *et al.*, 2020). It has been noticed that the many hazards caused by molds on agricultural products generally lead to their removal during sorting for the market, resulting in a worldwide loss of food production estimated at between 5 and 10 % [9](Yiannikouris and Jouany, 2002).

Nowadays, to reduce the incidence of mold in stored commodities, chemical control through the use of synthetic fungicides seems to be the most effective and widespread control method in the world, with a substantial increase in agricultural productivity[10-2] (Aoudou *et al.*, 2010; Kpatinvoh *et al.*, 2017).Nevertheless, synthetic pesticides are currently a global concern since their adverse effects on human health and the environment have been demonstrated. Indeed, the application of these synthetic chemicals at high concentrations for post-harvest control increases the risk of toxic residues in foodstuffs[11-12](Adjou *et al.*, 2013; Rosenbaum *et al.*, 2015) and the risk of developing resistant fungal strains[13](Rapp *et al.*, 2004). The toxic effects of chemical pesticides on human beings are carcinogenic, immunosuppressive, mutagenic, and neurotoxic[14](Mostafalou and Abdollahi, 2016). [5](Nadjet *et al.*, 2016)showed that mycotoxin toxicity could be increased by human exposure to synthetic pesticides, since the latter inhibit many enzymes, particularly those capable of detoxifying certain mycotoxins.

Because problems associated with chemical pesticides, using of natural products of plant origin to preserve foodstuffs is experiencing significant growth and constitutes a major challenge to be met. Essential oils are a new category of biodegradable plant protection products that have demonstrated their antifungal properties, with the particularity of presenting no toxicity to humans[15-16](Abd-Alla and Haggag, 2013; Zhu *et al.*, 2016). Many previous studies on essential oils antifungal activity of *Thymus vulgaris*[17-18,

19][Kritzinger *et al.*, 2002; De Vincenzi *et al.*, 2004; Vitoratoset *al.*, 2013),*Cymbopogon citratus*[20-21, 22](Yousef, 2013; Aoudou *et al.*, 2017; Premathilake *et al.*, 2018), and *Cupressus sempervirens*[23-24](Mazari *et al.*, 2010; Ismail *et al.*, 2013)have been demonstrated against some stock fungi such as *Aspergillus niger*, *A. flavus*, *Penicillium sp.**Alternaria alternata*, *Fusarium tricinctum*, *Phomopsis* and *Rhizopus stolonifer*. However, although essential oils do not harm human health (without overdoses) and the environment, some factors limit their full adoption.In actual storage conditions, essential oils have a short shelf life because of their high volatility and unstable constituent molecules.[25-26](Hsieh *et al.*, 2006 ; Baptiste Hzounda Fokou *et al.*, 2020).Additional doses are necessary to ensure their antifungal activity,but essential oils typically yield low extraction yields. Hence, the importance and necessity of developing formulations of essential oils capable of optimizing the effectiveness of low doses of these essential oils as well as improving the persistence of their antifungal properties during food storage is maintained.With this in mind, the objective of this study is to contribute to improve the conservation of peanuts, by fighting against the development of mold using essential oil formulations.

## Materials and methods

### Plant material and procedure for extracting Essential Oils

In January 2020, fresh leaves of *Thymus vulgaris*, *Cymbopogon citratus* and *Cupressus sempervirens* were collected in the locality of Dschang and then air-dried for five days at room temperature ( $25^{\circ}\text{C} \pm 2$ ). The leaves were hydrodistilled with the help of a Clevenger-type apparatus for about 5 hours at the Research Center for Microbiology and Ontibral Substances of the University of Dschang. Following the recommendations of [27-28]Sessouet *al.* (2012) and Rguez *et al.*(2018), the extracted oils were dried with anhydrous sodium sulfate and stored at  $4^{\circ}\text{C}$  away from light until used to prevent spoilage.

### Starch and biochar aremade from corn cob

Cassava starch (*Manihot esculenta*) was obtained using the method described by [29](Maptue *et al.*, 2021). Biochar based on maize cobs was obtained using the methods described by[30] Ioannidouet *al.* (2009), [31] Liu *et al.* (2014) and taken up by[29](Maptue *et al.*, 2021). Biochar, specially corn-cobbiochar, has the particularity of having a high relative porosity of nearly 80%[32](Djousse *et al.*, 2018).This feature has aroused particular interest in its use, as it can retain essential oils in its microporosity and allow increasing volatility over time.

## Essential Oil Formulations

Starch-based essential oil formulations (*Cymbopogon citratus*, *Cupressus sempervirens*, and *Thymus vulgaris*) were made regarding the previous work of [33]Camara (2009) and the technique described by [29](Maptue *et al.*, 2021). For biochar-based essential oil formulations (*Cymbopogon citratus*, *Cupressus sempervirens*), the technique described by Maptue and collaborators in 2021 was applied. After preparing the formulations, they were allowed to rest for 10 hours to ensure that the volatile elements of the essential oil diffused effectively.

## Treatment of peanut seeds

After removing rotten, cracked, or damaged seeds and waste, two hundred peanut seeds were dispersed in plastic bottles. Regarding the starch-based essential oil formulation, 5 g was sprinkled on the seeds for each concentration. The powder and seeds were mixed in each container to ensure that they had excellent contact. Each piece of biochar soaked in essential oil was placed in the middle of the seeds in the bottles. Deltamethrin, a synthetic pesticide for the protection of stored seeds, used at the manufacturer's recommended dose (2g/kg), was a positive control. Untreated groundnut seeds were used as a negative control. The experiment was performed three (03) times.

## Effects of Essential Oil Formulations

For 70 days, treated and untreated seeds were stored at a laboratory room temperature of  $25 \pm 2^\circ\text{C}$ . Fungal infection rates were evaluated at 10, 20, 30, 40, 50, 60 and 70 days of storage, using Potato Dextrose Agar and the blotter technique, following the protocol described by [34] ISTA (2023) with some modifications.

## Seed health

Disinfected peanut seeds on the surface (200 seeds) were placed on PDA (Potato Dextrose Agar) medium to isolate the associated storage fungus. After 7-10 days of incubation at  $25 \pm 2^\circ\text{C}$  and daily observation, fungal colonies produced around the seeds were collected and purified for identification using conventional fungal identification keys as reported in other studies [35-36, 37] (Champion, 1997; Warham *et al.*, 1997; Mathur and Kongsdal, 2003).

## Statistical analysis

Analysis of variance was performed on the collected data before using R software version 4.2.2 to conduct the Duncan test with a 5% probability threshold.

## Results and discussion

### Fungi associated with peanut seeds and percentage of infection before treatment

The fungal species that were isolated from groundnut seeds and the percentage of infection for each are depicted in Table 1. According to this result, groundnut seeds collected in Dschang are infected mainly by eight (08) fungal species of interest. These are *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus parasiticus*, *Cercospora* sp, *Fusarium* sp, *Phomopsis* sp, and *Rhizopus stolonifer*. The infection of seeds caused by these different species ranges from 4 to 62%.

**Table 1:** Percentage of peanut seed infection prior to treatment with essential oils by isolated parasites

Identified fungi	Fungal infection rate (%)
	Peanut
<i>A. flavus</i>	39,00
<i>A. fumigatus</i>	11,50
<i>A. Niger</i>	18,00
<i>A. parasiticus</i>	3,50
<i>Cercospora</i> sp	15,50
<i>Fusarium</i> sp	41,50
<i>Phomopsis</i> sp	11,00
<i>Rhizopus stolonifer</i>	22,00

### Antifungal activity of different formulations of essential oils

The *in vivo* antifungal efficacy of essential oil formulations of *C.citratus*, *C. sempervirens*, and *T. vulgaris* based on starch and biochar is presented in Table 2. It is apparent from these findings that all formulations have an inhibitory effect on fungal infection of peanut seeds. Depending on the type of essential oil, dose, storage period, and formulation support, this inhibition can vary.

**Table 2:** Fungal infection rate of peanuts treated with starch- and biochar-based essential oil formulations as a function of storage period

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**Infection rate (%)**

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Formulations	Doses ( $\mu\text{l/g}$ )	10 days	20 days	40 days	60 days	70 days
Starch +	3,00	38.50 <sup>b</sup>	14.50 <sup>a</sup>	22.00 <sup>b</sup>	16.00 <sup>BC</sup>	13.00 <sup>a</sup>
<i>C. citratus</i>	7,00	32.50 <sup>b</sup>	24.50 <sup>b</sup>	19.00 <sup>b</sup>	4.00 <sup>a</sup>	24.16 <sup>bc</sup>
Starch +	3,00	40.50 <sup>b</sup>	30.00 <sup>bc</sup>	18.66 <sup>b</sup>	15.00 <sup>b</sup>	22.50 <sup>bc</sup>
<i>C. sempervirens</i>	7,00	39.00 <sup>b</sup>	41.00 <sup>d</sup>	13.00 <sup>a</sup>	29.00 <sup>d</sup>	28.50 <sup>cd</sup>
Starch +	3,00	66.50 <sup>e</sup>	27.00 <sup>b</sup>	11.50 <sup>a</sup>	14.50 <sup>b</sup>	18.50 <sup>ab</sup>
<i>T. vulgaris</i>	7,00	18.50 <sup>a</sup>	24.50 <sup>b</sup>	9.00 <sup>a</sup>	14.50 <sup>b</sup>	25.00 <sup>bc</sup>
Biochar +	3,00	65.66 <sup>e</sup>	55.66 <sup>e</sup>	44.66 <sup>c</sup>	21.50 <sup>bcd</sup>	34.00 <sup>e</sup>
<i>C. citratus</i>	7,00	54.00 <sup>cd</sup>	54.66 <sup>e</sup>	52.00 <sup>d</sup>	14.66 <sup>b</sup>	57.50 <sup>fg</sup>
Biochar +	3,00	40.16 <sup>b</sup>	34.00 <sup>c</sup>	22.50 <sup>b</sup>	24.66 <sup>d</sup>	38.50 <sup>e</sup>
<i>C. sempervirens</i>	7,00	52.66 <sup>c</sup>	41.16 <sup>d</sup>	20.16 <sup>b</sup>	23.16 <sup>cd</sup>	23.50 <sup>bc</sup>
Control	Control -	62.00 <sup>de</sup>	62.00 <sup>F</sup>	62.00 <sup>th</sup>	62.00 <sup>f</sup>	62.00 <sup>g</sup>
	Control +	50.83 <sup>c</sup>	50.83 <sup>e</sup>	50.83 <sup>d</sup>	50.83 <sup>e</sup>	50.83 <sup>fg</sup>

Means assigned with the same upper letter do not differ significantly according to Duncan's test at the probability threshold  $P = .05$

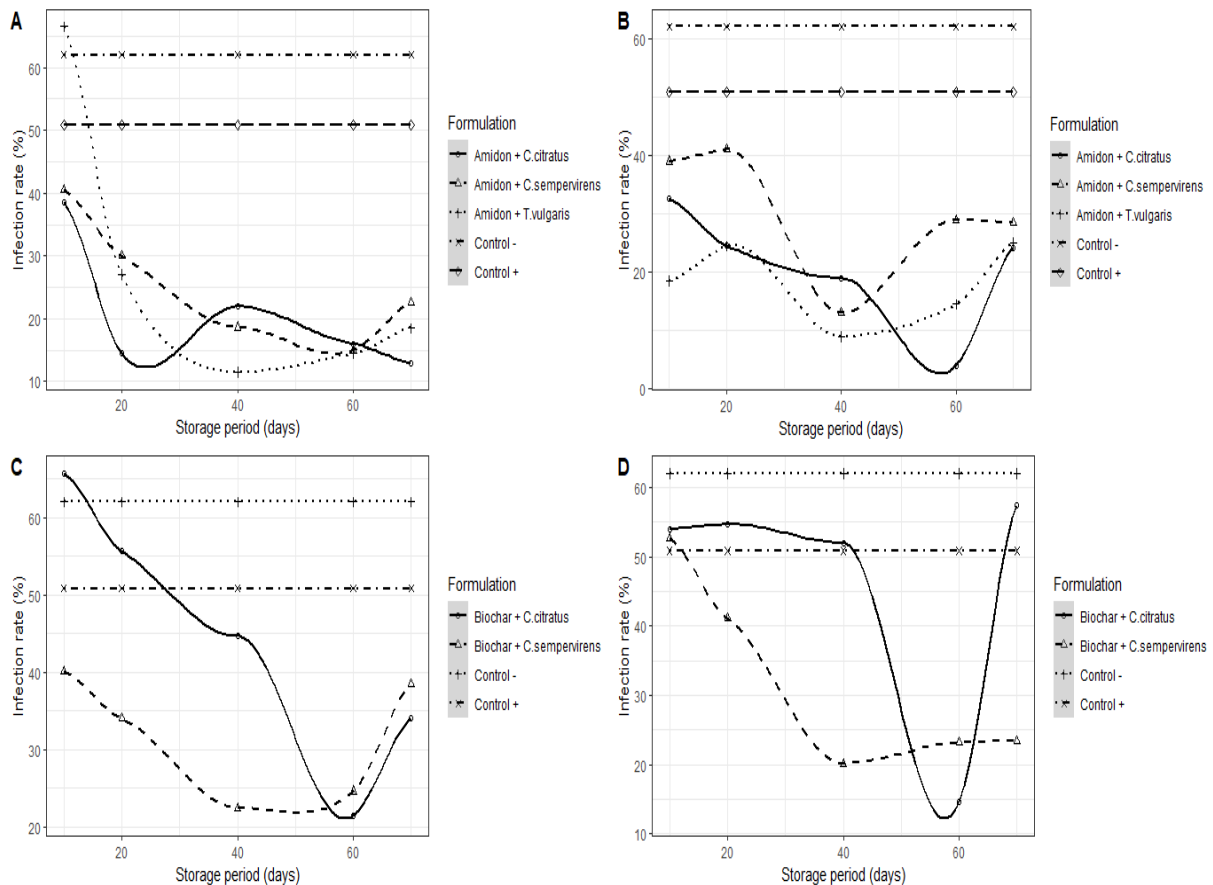
### Antifungal activity of starch-based essential oil formulations

Figures 1 (A-B) and 2 (A-B) show, respectively, the percentage of infection as a function of time and the percentage of infection after 70 days of storage of peanut seeds treated with the essential oil formulations of *Cymbopogon citratus*, *Cupressus sempervirens*, and *Thymus vulgaris* starch-based. Based on these results, the inhibitory effects of the formulations were significantly influenced by the storage period, the dose applied and the type of essential oil. Seeds treated with essential oils had a decrease in fungal infection percentage throughout the storage period. Upon ten days of storage, the infection rate of treated seeds was high and ranged from 18.50% to 66.50%. After 40 days of storage, the infection percentages of treated seeds were lower than those of positive (58%) and negative (62%) controls and ranged from 9% to 2.2%. These values are significantly lower ( $P = .05$ ) than those of the control and those obtained after then days of storage. After 60 days of storage, the infection percentages of treated seeds, which range from 14.50% to 29%, are significantly lower ( $P = .05$ ) than those of the controls but still higher than those obtained after 40 days of storage. According to these findings, the antifungal activity of different formulations is determined by both the type of essential oil and its dose. For this purpose, the

starch formulation + *Cymbopogon citratus* is the one with the highest antifungal activity with a fungal infection rate of 13.00% at the concentration of 3 µl/g after 70 days of storage, followed respectively by starch + *Thymus vulgaris* (18.50%) and starch + *Cupressus sempervirens* (22%) at a concentration of 3 µl/g after 60 days of storage.

### **Antifungal activity of biochar-based essential oil formulations**

Figures 1 (C-D) and 3 (A-B) show the percentages of fungal infection of peanut seeds treated with essential oil formulations of *Cymbopogon citratus* and *Cupressus sempervirens* based on vegetable charcoal. After ten days of treatment, the percentages of fungal infection in seeds range from 97 to 100%; these mean values are statistically greater than those of negative and positive controls 62.50% and 85.50%, respectively. After 60 days of storage, the percentages of infection of the treated seeds, which vary from 10% to 12%, are significantly lower ( $P = .05$ ) than those of the control and those obtained after 40 days of storage. Notably, these percentages of fungal infection observed after 60 days were also lower than those recorded after 70 days of storage (9% to 27%). The seeds' lowest percentages of fungal infection were obtained after 60 days of storage, and there was no significant difference between the doses applied. However, following a storage period of 70 days, the biochar + *C. citratus* formulation exhibited superior antifungal activity compared to that which was observed after 60 days at a concentration of 7 µl/g, with a seed infection rate of 9%, this is an improvement over the 7 µl/g (12%) recorded at 60 days.



**Figure 1:** Seed infection rate by substrate type, dose and storage period. A – B: Formulation at a dose of 3 µl/g. C – D: Formulation at a dose of 7 µl/g

### Effect of formulation support and dose on the effectiveness of formulated essential oils

Table 2 presents the effect of substrate type on the antifungal activity of essential oil formulations at doses of 3 and 7 µl/g. The influence of formulation support on seeds' fungal infection percentages is not statistically significant ( $P > .05$ ) at a dose of 3 µl/g. Whilst figure 1 illustrates the lowest percentages of fungal infection were detected at a 60-day storage period, starch-based essential oil formulations exhibited greater activity than biochar-based formulations, with no significant disparity. Looking at the effect of formulation support on the antifungal activity of *Cymbopogon citratus* essential oil formulations at a dose of 7 µl/g, a significant difference ( $P = .05$ ) was observed between starch and biochar formulations at 40 and 70 days of storage. Biochar-based formulations exhibited the most prominent inhibitory impact on fungal infection of peanut seeds following 70 days of storage.

## Discussion

*In vivo*, antifungal tests using the essential oil compositions of *Cymbopogon citratus*, *Cupressus sempervirens*, and *Thymus vulgaris* against fungi associated with peanut seeds revealed a substantial decrease in the rate of fungal infection of treated seeds compared to untreated seeds and positive controls. *Thymus vulgaris* essential oil had the highest antifungal activity in starch-based formulations, followed by formulations of *Cupressus sempervirens* and *Cymbopogon citratus* oils at a concentration of 7 µl/g. Similarly, for biochar-based formulations, the essential oil of *C. sempervirens* at the concentration of 7 µl/g showed the highest antifungal activity. The ability of these formulations to prevent fungal infections can be explained by the active chemicals in essential oils, which can either prevent the development of fungi or stimulate the host plant's defenses, such as pathogenic fungi [38](Liu *et al.*, 2002). Numerous studies have indicated that essential oils possess biologically active compounds, such as terpenes and terpenoids, including monoterpenes and sesquiterpenes with their hydrocarbon and oxygenated derivatives, which contribute to their antimicrobial properties and chemical makeup[39-40](Smigielski *et al.*, 2018; Tchoumboungang *et al.*, 2009). These studies have reported the antifungal effects of oxygenate and monoterpene hydrocarbons against a diverse group of phytopathogenic fungi[38-19, 41](Liu *et al.*, 2002; Vitoratos *et al.*, 2013; Regnier *et al.*, 2014).

Previous studies [42-18](Thompson *et al.*, 2003; De Vincenzi *et al.*, 2004) showed that *Thymus* spp essential oil composition consists primarily of oxygenated monoterpenes, including carvacrol, thymol, linalool, geraniol, and p-cymen. These findings support the obtained results and corroborate the work of [17] Kritzinger *et al.* (2002), which demonstrated that under *in vitro* conditions, *Thymus vulgaris* essential oil significantly inhibited the growth of *Aspergillus flavus*, *A. niger*, *Fusarium oxysporium*, *F. equiseti*, *Penicillium chrysogenum* and *Rhizopus* sp associated with cowpea seeds in storage. Similarly, under *in vivo* conditions, thyme markedly decreased the incidence of these fungi on naturally infected seeds.

On the other hand, it has been shown that lemongrass essential oil has as its majority compounds citral aldehyde, β-Citral, geraniol, cis-Verbenol, acetal diethyl citral and nerol, which are oxygenated monoterpenes, as well as myrcene and α-pinene, which are hydrocarbon monoterpenes[43-22] (Shahzadi and Shahzadi, 2017; Premathilake *et al.*, 2018). Other authors[44-45](Paranagama *et al.*, 2003; Kakarla and Ganjewala, 2015) explained that citral is the chemical constituent responsible for the antifungal properties found within lemongrass essential oil. The work of [10] Aoudou *et al.* (2010) showed complete inhibition of

the growth of *A. parasiticus* mycelia by citral. Much other research has been conducted on the chemical composition of essential oils of evergreen species [46-47, 48, 23, 24] (Chéraif, 2005; Sacchetti *et al.*, 2005; Emami *et al.*, 2006; Mazari *et al.*, 2010; Ismail *et al.*, 2013).

Studies have shown that this oil consists mainly of monoterpene hydrocarbons, such as  $\alpha$ -pinene,  $\delta$ -3-carene, myrcene, and limonene, with only a small amount of oxygenated monoterpenes present. The predominance of monoterpene hydrocarbons is the likely reason for its limited ability to inhibit fungi associated with peanut seeds. According to [23] Mazari *et al.* (2010), found that *C. sempervirens* essential oil had a moderate inhibitory effect on the growth of *A. flavus* and *F. oxysporum*. However, when compared to the positive control (amphotericin B), the oil did not show significant activity against *Rhizopus stolonifer*.

## Conclusion

Essential oil formulations based on starch and biochar have significantly reduced fungal development in peanut grains during storage. Use starch-based formulations revealed that *Thymus vulgaris* essential oil almost completely inhibited fungi associated with peanut seeds for up to 60 days of storage at 7  $\mu$ l/g. The biochar-based formulation of essential oil from *Cymbopogon sempervirens* demonstrated a satisfactory impact and inhibitory effect after a storage period of 60 days. Consequently, these outcomes indicate that... starch, and biochar can be used to formulate essential oils to preserve peanut seeds. *Thymus vulgaris* essential oil has been shown to be the most efficient, particularly when combined with starch. Further research to investigate the endurance of these formulations would be intriguing.

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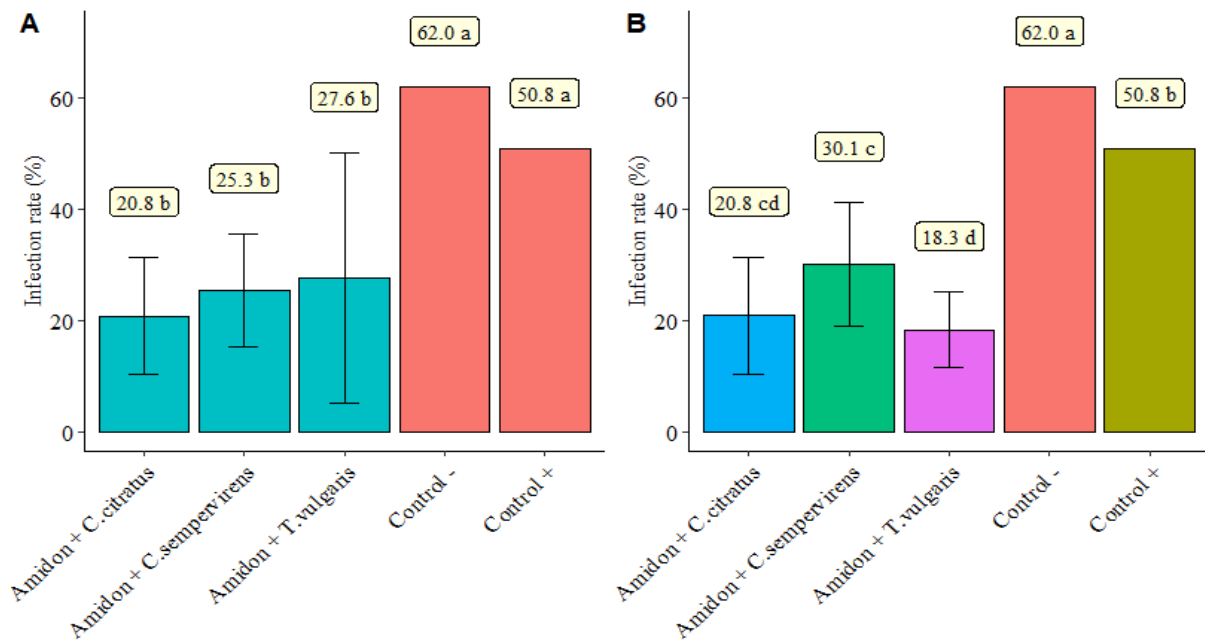
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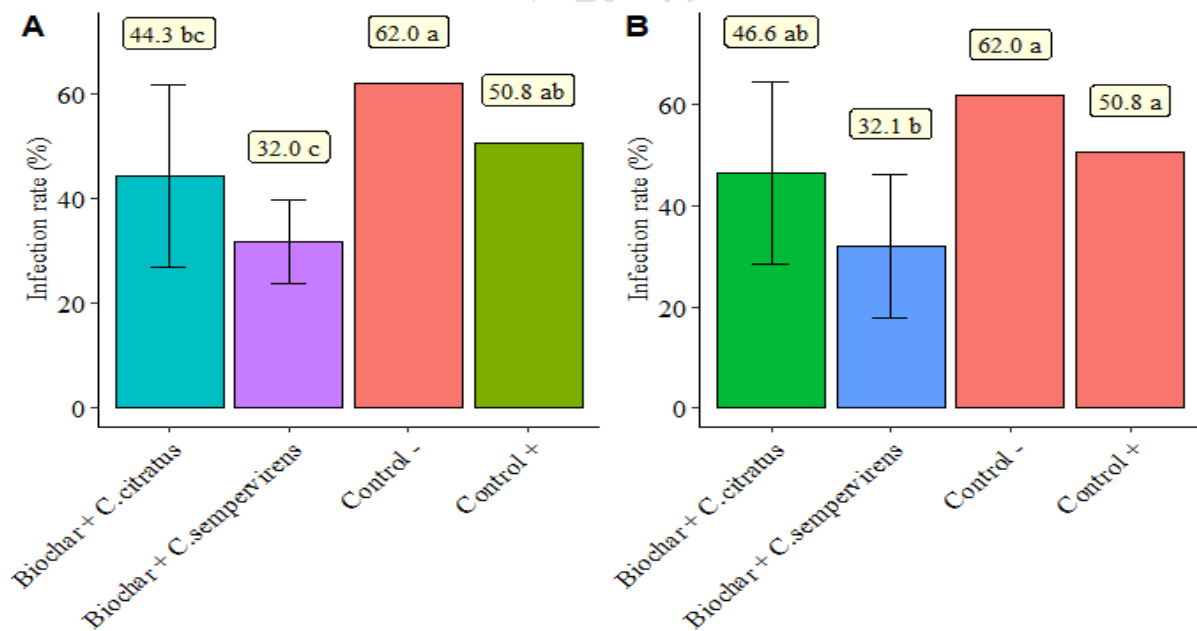
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**Figure 2:** Fungal infection rate of treated peanut seeds based on starch-based essential oil formulations and dose after 70 days of storage. A: Starch formulation at a dose of 3 ug/l. B: Starch formulation at a dose of 7 ug/l.



**Figure 3:** Fungal infection rate of treated peanut seeds based on biochar-based essential oil formulations and dose after 70 days of storage. A: Biochar formulation at a dose of 3 ug/l. B: Biochar formulation at a dose of 7 ug/l.