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Full Length Research Paper

In vivo effect of cashew apple powder on fungi isolated from stored maize seed (EV8728-SR)

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One of the most significant cereal crops in the world is maize (*Zea mays* L.), which is susceptible to a number of illnesses, particularly those spread through seeds. Plant extract research offers a viable area for replacement research for fungicides used to treat seeds. In order to evaluate its antifungal impact on the sanitary and physiological quality of maize seeds, this study examined the phytochemical profile of the hydroalcoholic extract from cashew apple powder. The EV8728-SR variety of seeds was utilized, and they were subjected to the following treatments: Control (untreated seeds), cashew apple powder at 1, 2, 4, and 8 g, and cashew apple powder at 1, 4 and 8 g. In an entirely random experimental design, the seeds were put through sanitation and germination tests. Alkaloids, tannins, flavonoids, and saponins were found in the hydroalcoholic extracts of cashew apple powder, according to phytochemical prospecting. The cashew apple powder reduced the incidence of *Aspergillus* spp., including *Aspergillus niger*, *Aspergillus flavus* and *Cladosporium sphaerospermum, Fusarium moniliforme and Curvurlaria lunata* at all concentrations compared to the control. It should also be noted that this powder positively affected seed viability with an improvement in seed germination rates (92-97%) after one month of treatment. These results represent sources of information for the implementation of an integrated control method against the fungal agents of maize seeds that cause the decline in its productivity.

Key words: *Zea mays* L., cashew apple, biofungicide, fungi, conservation.

INTRODUCTION

The cornerstone of global food security is maize (Zea mays L.), one of the key cereal crops grown worldwide (Cheikh, 2018). It is a significant staple food in Africa and helps many smallholder farmers significantly with their food and income security (Opoku et al., 2023). In Eastern and South Africa, maize makes up close to half of the calories and protein ingested, and makes up one-fifth of the calories and protein in West Africa. Maize is the primary food and source of income for more than 300 million people in sub-Saharan Africa. Ishengoma et al., 2021). Maize is the main food source for rural communities in Ivory Coast and the majority of West

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African nations. It is a naturally occurring source of vitamins, fiber, lipids, carbs, and other macro- and micronutrients (Seepe et al., 2020). Maize is farmed in Ivory Coast for commercial, dual, and subsistence uses. The maize is grown by small farmers, who rely on old, crude methods and local expertise to prevent crop illnesses both in the field and during storage. Climate change, crop diseases, grain deterioration during storage, and poor seed germination are only a few of the problems that the small farming system must deal with (Seepe et al., 2020). Production of maize has been limited by post-harvest losses. Microbial infections can have a deleterious impact on seed germination, yield, and nutritional value. This is partly related to poor storage practices and attacks by mycotoxigenic fungi such Fusarium moniliforme and Aspergillus flavus (Baka, 2014). Most often, pesticide resistance is a result of the usage of synthetic pesticides to control weevils or fungi diseases. Synthetic compounds used to manage postharvest illnesses have a number of drawbacks, including high and acute toxicity, a slow rate of degradation, environmental pollution, and possible carcinogenic effects. Additionally, small farmers have little access to and less ability to buy these chemicals, and seeds treated with artificial fungicides run the risk of problems or food poisoning. It is imperative to create sustainable, reasonably priced methods that can be utilized to prevent or control grain rotting, especially when it occurs during storage in underdeveloped rural areas. Alternative approaches for the development of newer biopesticide that give almost distinct standards have been on the rise due to these issues connected to synthetic pesticides across the globe (Tia et al., 2021; Seepe et al., 2020). These requirements include being ecologically friendly, created from locally accessible resources, less expensive, less prone to pest resistance, less expensive, and pest-specific (Tia et al., 2021; 2020; 2019). As a result, traditional techniques for employing plant derivatives to prevent microbial contamination of stored goods have been examined. Plants are widely available and biodegradable, which has given the use of botanicals as an alternative source of bio-pesticides in crop protection and post-harvest losses impetus (Tia et al., 2021; Cissé et al., 2020; 2019).

A tropical evergreen tree called the cashew (Anacardium occidentale) is grown in a number of nations, including Ivory Coast. It is cultivated for the cashew nut market. Cashew apples are underutilized since the majority of them are discarded in the field as agricultural waste. In this connection, investigating the cashew apple's biochemical potential is intended, as is the requirement for innovative remedial measures to deal with seed-related fungi. In this work, the hydroalcoholic extract from cashew bagasse powder's phytochemical profile was determined, and its antifungal activity was assessed in relation to the physiological and sanitary quality of maize seeds of the cultivar EV8728-SR in the Ivory Coast.

MATERIAL AND METHODS

Collection of maize samples

The seeds of maize (*Zea mays*), cultivar EV8728-SR, were collected from two locations, Korhogo and Yamoussoukro, and brought to the National Seed Laboratory (LANASEM) located in Yamoussoukro, in the centre of Ivory Coast, for further study. A total of four kilograms of maize seed were sampled in each location. The maize collected in Korhogo had already been treated with the synthetic chemical in storage, while the sample from Yamoussoukro had not received any synthetic chemical before. Following transportation of the samples to the LANASEM, the seeds underwent purity analysis to weed out crop remnants and old seeds.

Collection of plant parts and preparation of the cashew apple powder

The cashew apples used for the production of powder were harvested at Djédriville and Wédala in Yamoussoukro, and transported to the National Institute Polytechnic Félix Houphouët Boigny for the production of powder after extraction of the apple juice. These apples were rinsed several times with tap water and the nuts were removed manually. They were then pressed using a mechanical press. Cashew apple bagasse, obtained after juice extraction were first dried in a solar dryer for 3 days to reduce the water content considerably. This bagasse was then transferred to the laboratory and dried on a bench in the shade at a temperature of 25±2°C for a fortnight. The dried samples of bagasse were ground to a fine powder using a mechanical sieve mill. The powder obtained was stored in clean, tightly closed jars and kept in a cool, ventilated place until further use.

Phytochemical screening of the cashew bagasse powder extract

We chose to macerate the powder in a hydroalcoholic solution in order to prepare the liquid extract. For 24 h at room temperature (25 2 °C), 150 g of cashew bagasse powder was immersed in a beaker containing 0.5 Ll of hydroalcoholic solution. The solution was then filtered through paper to produce the hydroalcoholic extract. The qualitative examination of this hydroalcoholic extract involved reactions that characterize its phytochemical composition. It entails the creation of insoluble compounds through precipitation processes, carried out in accordance with the methodology outlined by Abbad et al. (2023).

Detection of alkaloids

On a steam bath, 5 ml of 1% aqueous HCl were mixed into 1 mL of the hydroalcoholic extract before being filtered while still hot. In order to get a brown precipitate, a positive test for alkaloids, distilled water was added to the residue, and 1 ml of the filtrate was treated with a few drops of either Mayer's reagent (Potassium mercuric iodide-solution).

Detection of flavonoids

To 5 ml of extract, 3 ml of 1% aluminum chloride solution ($AlCl3$) was added. The presence of flavonoids was indicated by the yellow coloration that was seen. The aforementioned mixture received 5 cc of a diluted ammonia solution, then concentrated $H2SO4$ was added. If the test for flavonoids is positive, the coloring will be yellow.

Detection of tannins

One milliliter of the hydroalcoholic extract and one milliliter of the 1% aqueous solution of FeCl33 were combined. The emergence of a greenish or blackish blue colour is a sign that tannins are present.

Detection of saponins

20 ml of distilled water were used to boil 5 ml of the hydroalcoholic extract, which was then filtered. In order to create a stable and persistent foam that would last for at least 15 minutes, 10 ml of the filtrate was combined with 5 ml of distilled water and forcefully agitated.

Health status of maize seeds testing

The blotter paper and agar plate approach was used to isolate and find seed-borne mycoflora (Khan et al., 2023). Using the wet blotting paper method as described by Mathur and Kongsdal (2003), the health state of the seeds was evaluated. Using a 9 cm Petri dish, evenly distribute 25 maize grains on blotting paper that has been dampened with distilled water. Four Petri plates with 25 grains each are required for one test, for a total of 100 grains. For seven days, these plates were incubated in a cycle of 12 h of light at 20°C and 12 h of darkness at 22°C. Seven days after incubation, all of the fungi on the seeds were examined under a binocular microscope as part of the evaluation of the seeds.All these procedures were done under sterile working environments. The existence and features of the fruiting structures were then visually assessed using spore color and colonization after subculturing was carried out using PDA to obtain pure cultures. Taxonomic characteristics like conidia and hyphae were used to identify the seed-borne fungal diseases. For the purpose of identifying the fungi, keys (Malone and Muskette, 1964; Misra and Mew, 1994; Mathur and Kongsdal, 2003), as well as several books, manuals, and periodicals, were reviewed.

Maize seed treatment and health and germination test

The identical procedures used for the seed health test were used for the antifungal essay. A fully randomized design was used to conduct the testing in a lab setting at ambient temperature (26 2°C). 100 maize kernels weighing a total of 32 g were placed in clean, airtight, transparent test boxes, and each received 0, 1, 2, 4 or 8 g of cashew bagasse powder to assess the antifungal effect on seed-colonizing fungi. Each box's contents were combined to ensure that the powder coated all of the seeds. Throughout the testing period of one month, these treated seeds in the test boxes were observed weekly. For each dose per week, each trial was repeated four times (100 seeds per repetition. A batch of trials was gathered for hygienic analysis every seven days using the blotting paper technique described above. The germination rate and rate of infected grains (infestation level) were estimated using the formula given by Djeugap et al. (2018).

$$\boldsymbol{Ir}\ (\%) = \left(\frac{\text{Number of infected grains}}{\text{Total number of incubated grains}}\right) x100$$

Statistical analyses

Using STATISTICA 7.1 statistical software, the Fisher's test was used to analyze the variance in the data, and the Tukey's test (p 0.05) was used to compare the means. The histograms displayed in the paper were created using Excel (Microsoft Office 2013).

RESULTS

Quantitative phytochemical screening

Table 1 presents the results of the preliminary identification of phytochemicals in cashew bagasse powder. Regarding the identified phytochemicals, alkaloids, flavonoids and tannins were found in cashew bagasse powder extract.

Health status of seed testing

Figure 1 display the findings of the seed sanitation investigation. A. flavus, A. niger, C. sphaerospermum, F. moniliforme, and C. lunata were the seed-borne fungus isolated from the grains coming from the two zones. *Immature Aspergillus* flavus had white heads that became yellowish cream to green as they grew older. They were also noted to have bulbous crowns and lengthy, hyaline conidiophores. C. *sphaerospermum* extensively growing, covering the whole seed. They had short whitish conidiophores. Short, dark conidiophores with clusters of black, glossy conidia at the tips were present on *C. lunata. Microconidia on F. moliniforme* were chains of distinctively powdery white color. A. niger possessed long, erect, hyaline, solitary conidiophores with brown to black globose conidial heads. The results obtained indicate that there are regional differences in the frequency of these fungi. More seed-borne fungus are found in Korhogo seeds than Yamoussoukro seeds. However, compared to the other detected fungal species, *A. flavus, A. niger, and F. moniliforme* had higher occurrences.

Antifungal effect of cashew bagasse powder assessment

Figures 2 and 3 depict the impact of cashew bagasse powder on the fungi that live on maize seeds, *including A. flavus, A. niger, C. sphaerospermum, F. moniliforme, and C. lunata*. Results showed that, when compared to the control, all treatments were positively effective in lowering the growth of all tested fungus. Regardless of where the maize seeds were collected, the bagasse powder generally exhibits a dose-dependent efficacy for a certain fungus. Therefore, the rates of fungal infestation of the seeds are reduced with increasing doses. As early as 1 g of powder, the infestation rates of fungi such *A. flavus, A. niger, C. sphaerospermun, F. moniliforme, and C. lunata* were reduced. With higher doses, this reduction significantly changed. In the meantime, the increase in dose, particularly starting at 2 g of powder, had an impact on the infestation rate of *A. flavus* on the seed of two

Table 1. Phytochemical constituent found in cashew bagasse powder.

Phytochemical constituents of interest	Reactions
Alkaloids	+++
Flavonoids	+++
Tannins	+++
Saponins	-

Positive test: ++ + (Presence); *Suspicious test*: - (Absence).
Source: Authors

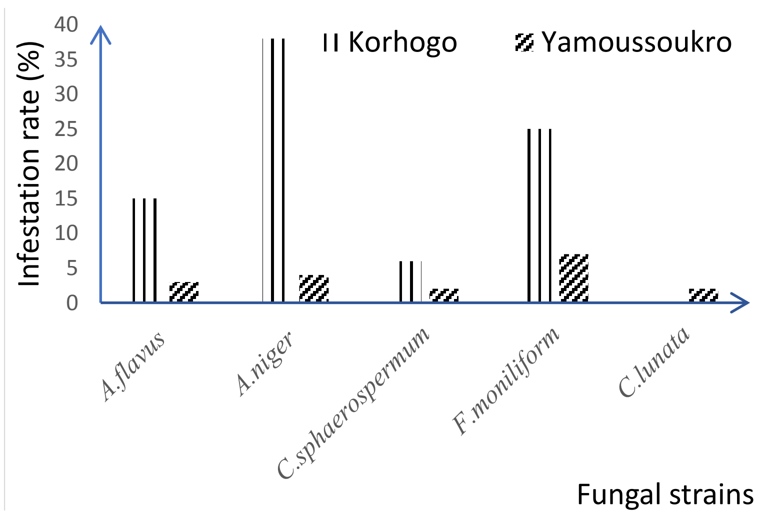

Figure 1. Maize seed health status before treatment.
Source: Authors

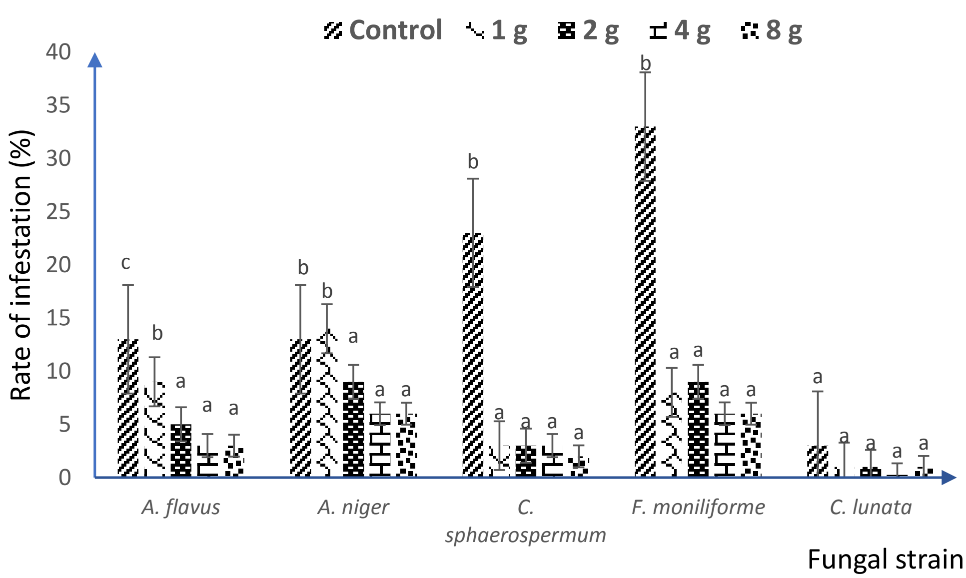

Figure 2. Antifungal effect of cashew bagasse powder on fungi hosted by maize seeds from Yamoussoukro.
a,b,c, Means followed by the same letter are not significantly different from each other according to the LDS Fisher (P<0,05).
Source: Authors

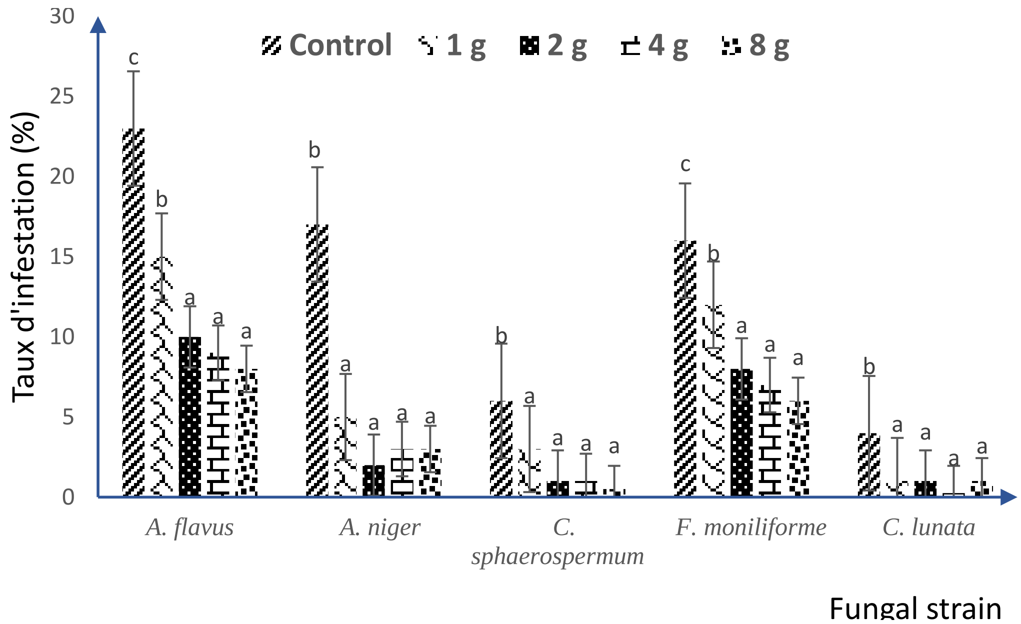

Figure 3. Antifungal effect of cashew bagasse powder on fungi hosted by maize seeds from Korhogo. a,b,c, Means followed by the same letter are not significantly different from each other according to the LDS Fisher (P<0,05).
Source: Authors

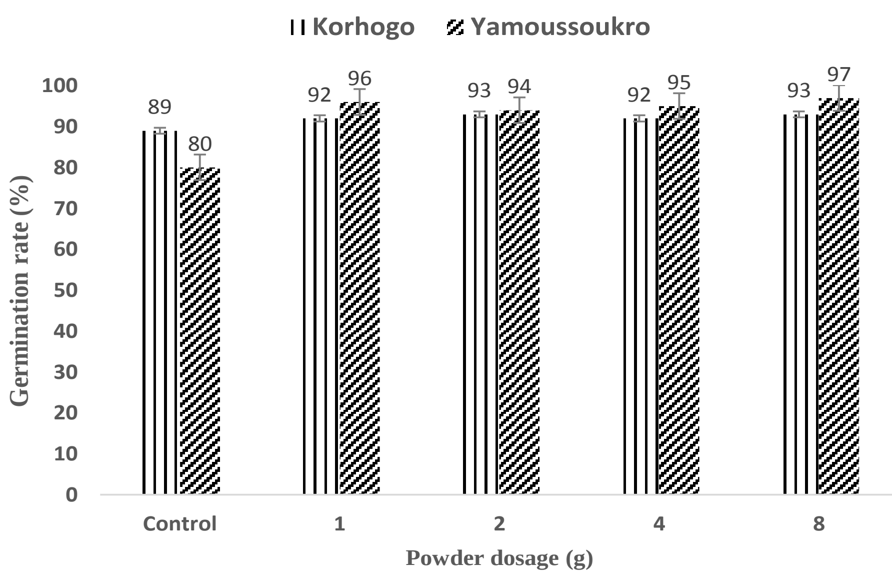

Figure 4. Effect of treatment with cashew bagasse powder on maize seed viability.
Source: Authors

origins.

Phytotoxicity on maize seed germination

Furthermore, an improvement in the germination of treated maize grains (about 98%) was observed after one month of storage. Figure 4 revealed the effectiveness of cashew bagasse powder in seed conservation in the two localities with germination rates (Tg > 90%) compared to the controls. Maize seeds treated with cashew bagasse powder had the highest percentage germination (92-97%) as compared to untreated. There is significant difference in seed germination of untreated seeds (80-

89%) in comparison to all other treatments. It is noted that this plant powder improved the germination of seeds with germination rates Tg varying from 94 to 97% for those of Yamoussoukro and 92 to 93% for those of Korhogo after one month of treatment.

DISCUSSION

The phytochemical prospecting showed the presence of different groups of secondary metabolites, suggesting the availability of alkaloids, tannins and flavonoids. The importance of these metabolites is that they have shown pharmacological and biological activities.

These secondary metabolites act as a plant's defensive system against herbivores, insects, and microbial predators. These elements give cashew apple extracts their antibacterial properties (Okey-Ndeche et al., 2020).

The preliminary identification of phytochemicals test marked positive for alkaloids in the cashew bagasse powder analysed in this work. In plants, alkaloids are considered part of their defense mechanism since they act as poisons and repellents to predators (Luis et al., 2022).

Moreover, flavonoids were identified in the cashew bagasse powder. They are known to be synthesi~~z~~sed by plants in response to microbial infection. Also, they have a positive impact on human health (Medeiros et al., 2021) and have been reported to significantly affect the microorganisms (Okey-Ndeche et al., 2020). Tannins belong a diverse group of polyphenolic compound widely distributed in cashew apples. An essential property of tannins, he has been known as astringency, their relative antimicrobial action can be related to their ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins~~,~~ (Luis et al., 2022).

With the information obtained from the preliminary phytochemical identification, it was observed that cashew bagasse powder has a wide range of valuable natural compounds.

Different species of fungi including *A. flavus, A. niger, C. sphaerospermum, F. moniliforme* and *C. lunata*, were identified and isolated from the grains coming from the two zones. The results for the isolation and identification of seed-borne fungal pathogens associated with stored maize grain were in agreement with the findings of Goko et al. (2021).

The occurrence of *A. flavus, A. niger, C. sphaerospermum* and *F. moniliforme* in Korhogo was comparatively high compare to fungal strains in Yamoussoukro. On the other hand, the incidences of *A. flavus*, *A. niger*, *F. moniliforme* are higher than incidence of the rest of the identified fungal species. Nevertheless, *C. lunata* was detected on the seed from Yamoussoukro only.

The results show that certain fungal species are the most predominant in the corn kernels collected in Korhogo. These results are consistent with the literature that the development of certain mold species can be attributed to factors such as heat and high relative humidity with low temperatures. These conditions can lead to inadequate drying of maize that predisposes maize to mold in the field or in storage (Goko et al., 2021). The literature indicates that variable conditions, such as humidity and temperature in different storage, determine seed quality and fungal growth. However, the different climatic conditions in the two collection areas would facilitate the growth of a particular fungal pathogen, as they have different requirements for development and mycotoxin production.

According to Erasto et al. (2023), higher fungal incidences endanger seed germination, since they contribute to losses in the quality and quantity of seeds. The higher the incidence of fungal species on seeds the lower the germination of those seeds.

In an early study, Khan et al. (2023) indicated that *A. niger* has been reported to reduce the seed germination and seed loss during storage and *Curvularia lunata* causes delay in seed germination due to rot of seeds. In the same vein, the amount of infection caused by *Fusarium moniliform* stay high in seed storage. The species of *Aspergillus* has been reported to cause a significant loss in the seed quality and nutritional value of grains (Medeiro et al., 2021).

Our preliminary results in the current study indicate that each dose of the extracts of cashew bagasse reduced the natural infection frequency of *A. flavus*, *A. niger*, *C. sphaerospermum*, *F. moniliforme* and *C. lunata*.

In general, a dose-response for all four concentrations was observed for the fungi evaluated. In this study, it was observed that bagasse powder caused growth inhibition of all fungi from the lowest concentration of (1 g) except *A. flavus*, probably due to its different sensitivity. The result also reveals that the effectiveness of cashew bagasse powder was dosage dependent, the higher the dosage the greater the reduction in prevalence of the fungi on the seed. To our knowledge, this study would be one of the very first on the biological activity of cashew bagasse powder. These results show that the cashew bagasse powder is well endowed with biologically active compounds that some pathogenic fungi are sensitive to them.

Cashew bagasse powder is a mixture of many compounds such as alkaloids, flavonoids, tannins and many other compounds proved to have pesticidal properties. It is known that plants synthesi~~z~~se a variety of bioactive compounds in plant tissues reported to have *in vitro* antifungal properties (Aftab et al., 2019; Umaru et al., 2018). Very similar and profound results were observed in work of Navoda and Anupama (2022). They noticed higher level exhibiting in controlling fungal pathogens such as *Aspergillus* spp. According to them, this inhibition may be due to the alkaloid present in *Allium sativum*. Also, Okechukwu et al. (2019) mentioned that the high flavonoid content in *A. sativum* plays a significant role in its antibacterial and antifungal effect against fungal pathogens. The results also agree with Behiry et al. (2022) who illustrated that some plant metabolites (phenolics) could suppress aflatoxin production in *A. flavus*.

On the other hand, this study found that cashew bagasse powder showed significant activity in promoting seed germination compared to nontreated maize. The ability of the bagasse powder to increase seed germination could be attributed to the suppression of the incidence of the seed-borne fungi that could have killed the embryo of the seeds.

For the variables percentage of germination rate, there were no significant difference between the treatments evaluated, which indicates that rate of germination was not affected by the cashew bagasse extract concentration used.

This is possibly due to the fact that this treatment does not have an allelopathic effect on maize seeds germination. Several authors have revealed that extracts in powder form have positive effects on protection, seed germination, seedling emergence of various crop species such as maize, bean, wheat, tomato and chilli (Arzoo and Biswas, 2013; Alam et al., 2014).

Authors like, Patrick et al. (2018) were able to show that preservation of maize grains for three months and more without any treatment leads to a loss of germination power. However, the use of plant powders such as *Ocimum canum, Corymbia citriodora, Tephrosia vogelii* and *Capsicum frutescens* in the conservation of the grains allows the latter to keep their germinative power beyond 70% for three months. The results obtained in this current study regarding fungal growth and germination of maize seed after treatment may be explained in light of the findings of Turner (2013). The author found that the activity of the powder extracts on the viability and germination power of maize grains could be explained by the fact that the powders would absorb the moisture contained in the seeds. This absorption would thus reduce the respiration of the seeds and consequently their metabolic activity which creates unfavourable conditions for the development of fungi.

According to Erasto et al. (2023), higher fungal incidences endanger seed germination, since they contribute to losses in the quality and quantity of seeds. The higher the incidence of fungal species on seeds the lower the germination of those seeds. This is proven by treated seeds, which have lower incidences of fungal species, hence, the respective effects on seed germination, in the current study. Even ~~if~~ though seed treatment using this powder might not eradicate all the fungal species coloni~~z~~sing seeds, their efficacy is still remarkable, simply because seed germination from seeds treated with biofungicide are improved.

Conclusion

Alkaloids, tannins, and flavonoids were found in cashew apple powder after a phytochemical prospecting analysis. The cashew apple powder has an antifungal effect on fungus colonizing maize seedlings, according to in vivo investigations. All of the tested fungi were active in response to this powder's antifungal effects. Taking into account the amount of powder used and the kind of fungi, this cashew bagasse powder not only gradually decreased the seed infection rates but also shown a good impact on seed germination. Almost majority of the tested doses had no adverse effects on the germination of maize seeds. Because it is biodegradable and environmentally benign as well as being effective, cashew bagasse powder is a possible candidate to be utilized in seed treatment. In order to manage seed-borne pathogens for improved seed germination, which can help increase crop productivity, this study calls for additional research to investigate better ways to improve the effectiveness of the biofungicide (fine characterisation of bioactive compounds and molecular characterisation of fungi species isolated).

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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