



Research Article

Antifungal and Seedling Growth-Promoting Effects of Neem (*Azadirachta indica* A. Juss.), Onion (*Allium cepa* L.) and Ginger (*Zingiber officinale* R.) Extracts as Seed Treatments for Maize (*Zea mays* L.)

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ABSTRACT

Seed-borne fungi are important constraints to maize seed quality because they can reduce germination and seedling vigor, while synthetic seed dressings may be costly and environmentally undesirable for smallholder farmers. This study evaluated aqueous and alcoholic extracts of neem (*Azadirachta indica*) leaves, onion (*Allium cepa*) bulbs, and ginger (*Zingiber officinale*) rhizomes at 10, 20, and 30% (w/v) concentrations for their in vitro antifungal activity against fungi isolated from maize and their effects on maize germination and early seedling growth. Four fungi were identified morphologically: *Aspergillus niger*, *A. flavus*, *Penicillium* spp., and *Fusarium oxysporum*. Alcoholic extraction produced significantly higher yields than aqueous extraction, with yields of 17.2% versus 12.2% for neem, 14.7% versus 12.1% for ginger, and 13.4% versus 10.1% for onion ($p = 0.021$). In the poisoned food assay, mycelial growth inhibition increased with extract concentration across the tested extracts and fungi. The highest inhibition (53.9%) was recorded with 30% aqueous neem extract against *F. oxysporum*. Solvent type did not significantly affect inhibition for any extract–pathogen combination ($p > 0.05$). Seed treatment with the extracts did not impair germination. Germination ranged from 40.0 to 100% and increased significantly with extract concentration for each plant extract ($p < 0.0001$), with the highest germination (100%) recorded for 30% aqueous ginger extract. Alcoholic neem extract produced significantly longer roots ($p = 0.0142$) and shoots ($p = 0.0235$) than aqueous neem extract. Overall, the extracts demonstrated moderate, concentration-dependent antifungal activity without evident phytotoxic effects on maize at the concentrations tested.

Keywords: *Allium cepa*; *Azadirachta indica*; Botanical extracts; Seed-borne fungi; Seedling vigor; *Zingiber officinale*

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INTRODUCTION

Maize (*Zea mays* L.) is a staple crop across sub-Saharan Africa, and the quality of the seed planted strongly influences crop establishment, uniformity and yield. Seed-borne fungi are among the main factors that lower seed quality. Genera such as *Fusarium*, *Aspergillus* and *Penicillium* are carried on or within maize kernels, reduce germination, because seed rot and seedling blight, and can introduce inoculum into the field (Debnath *et al.*, 2012; Erasto *et al.*, 2023). Some of them, notably toxigenic

Aspergillus species, also threaten food safety through mycotoxin contamination of maize in Nigeria (Atehnkeng *et al.*, 2008).

Synthetic fungicide seed dressings are the conventional control measure, but concerns over environmental contamination, residues, effects on non-target organisms and the cost of chemicals to resource-poor farmers have increased interest in alternatives (Aktar *et al.*, 2009; Mancini & Romanazzi, 2014; Moumni *et al.*, 2023). Plant extracts are one such alternative. They contain phenolics, flavonoids,

terpenoids, alkaloids and sulfur compounds that can inhibit fungal growth, and they are biodegradable and generally locally available (Makhuvele *et al.*, 2020; Almeida *et al.*, 2021; Pacyga *et al.*, 2024).

Neem (*Azadirachta indica* A. Juss.), onion (*Allium cepa* L.) and ginger (*Zingiber officinale* Roscoe) are widely grown in Nigeria and are among the most frequently studied antifungal plants. Neem contains limonoids such as azadirachtin, nimbin and salannin, and its extracts have inhibited several phytopathogenic fungi (Alzohairy, 2016; Chang *et al.*, 2022; Wylie & Merrell, 2022). Neem leaf extract has also been examined for effects on *Fusarium verticillioides* and germination of maize (Anjorin *et al.*, 2008). Antifungal activity of onion has been linked mainly to sulfur compounds and flavonoids such as quercetin (Shams-Ghahfarokhi *et al.*, 2006; Sagar *et al.*, 2022), and ginger to gingerols, shogaols and related phenolics (Ficker *et al.*, 2003; Mao *et al.*, 2019; Hansini *et al.*, 2023). Onion and ginger extracts have reduced *Aspergillus* and *Penicillium* on stored maize and cowpea (Ajani *et al.*, 2025) and seed-borne fungi on wheat (Hasan *et al.*, 2005).

However, the response of seeds to botanical treatments is not uniform. Extract concentration, solvent and plant species determine whether germination is stimulated, unaffected or inhibited (Kumar *et al.*, 2015; Seepe *et al.*, 2020), and high in vitro antifungal activity does not necessarily translate into better seedling performance. Few studies have compared neem, onion and ginger side by side, with both aqueous and organic solvent extracts, on the same maize seed lot, measuring both antifungal activity and seedling growth. This study therefore aimed to (i) isolate and identify fungi from maize seeds, (ii) assess the in vitro antifungal activity of aqueous and alcoholic extracts of neem, onion and ginger at three concentrations, and (iii) evaluate their effects on maize germination, root and shoot length, and seedling vigor.

MATERIALS AND METHODS

Study site and plant materials

The study was carried out in the Plant Biotechnology Laboratory, Department of Plant Science and Biotechnology, Federal University Dutsin-Ma, Katsina State, Nigeria. Neem leaves, onion bulbs and ginger rhizomes were obtained from local farms and markets and authenticated by a plant taxonomist in the Department of Botany. Voucher specimens were deposited in the university herbarium.

Maize seeds were obtained from a reputable seed supplier, inspected for physical defects and stored at room temperature in paper envelopes until use.

Preparation of extracts

Plant materials were washed, air-dried at room temperature and ground to a fine powder. For aqueous extraction, 50 g of powder was macerated in 500 mL of distilled water for 72 h with intermittent shaking, filtered through Whatman No. 1 filter paper and stored at 4 °C. For alcoholic extraction, 50 g of dried powder was macerated in 500 mL of 95% ethanol (or methanol for the methanolic extracts) for 72 h, filtered, concentrated under vacuum on a rotary evaporator and stored at 4 °C. Extraction yield (%) was calculated as the mass of dry extract relative to the mass of plant powder, and the pH, colour, odour and physical state of each extract were recorded. Working solutions of 10, 20 and 30% (w/v) were prepared in distilled water containing 0.1% Tween 20.

Isolation and identification of fungi

Maize seeds were surface-sterilised with 1% sodium hypochlorite, rinsed in sterile distilled water and plated on potato dextrose agar (PDA). Plates were incubated at 28 ± 2 °C for 5–7 days. Emerging colonies were sub-cultured and identified from macroscopic and microscopic characteristics using standard keys (Baka, 2014; Al-Askar *et al.*, 2022).

In vitro antifungal assay

Antifungal activity was assessed by the poisoned food technique. PDA was amended with each extract to give final concentrations of 10, 20 and 30%, and a 6 mm plug from an actively growing culture was placed at the centre of each plate. Plates were incubated at 28 ± 2 °C for 7 days and colony growth was measured daily. Percentage inhibition was calculated as $(C - T)/C \times 100$, where C is colony growth in the control and T is growth in the treatment.

Seed treatment and germination assay

Surface-sterilised maize seeds were soaked for 4 h in the 10, 20 or 30% extract solutions. Control seeds were soaked in distilled water containing 0.1% Tween 20. Seeds were air-dried in shade for 30 min, placed on moistened filter paper in Petri dishes and incubated at 25 ± 2 °C for 7–10 days. Germination percentage, root length and shoot length were recorded, and the seedling vigor index (SVI) was calculated as $\text{germination (\%)} \times (\text{mean root length} + \text{mean shoot length})$ (cm).

Experimental design and statistical analysis

All experiments were conducted using a completely randomised design with three replicates. Differences between extraction solvents within each extract were tested for each variable, and treatment combinations

(solvent × concentration) were compared by one-way ANOVA followed by Tukey's HSD test at $p < 0.05$ using SPSS v.25. Graphs were prepared with GraphPad Prism.

RESULTS

Extract yield and characteristics

Alcoholic extraction gave higher yields than aqueous extraction for all three plants (Table 1). The highest yield was obtained from neem (17.2%), followed by ginger (14.7%) and onion (13.4%), compared with 12.2%, 12.1% and 10.1%, respectively, for aqueous extracts. The difference in yield between solvents was significant ($p = 0.021$). Alcoholic extracts were darker, more acidic (pH 5.6–5.8) and more strongly scented than aqueous extracts (pH 6.3–6.7), but the difference in pH was not significant ($p = 0.312$).

Fungi isolated from maize seeds

Four fungi were identified (Table 2): *Aspergillus niger* (black powdery colonies; globose vesicles with chains of black conidia), *Penicillium* spp. (bluish-green velvety colonies; brush-like conidiophores), *Fusarium oxysporum* (white cottony colonies turning pink; sickle-shaped, septate macroconidia) and *A. flavus* (yellow-green powdery colonies; globose vesicles and rough conidia). Identification was based on morphology only.

In vitro antifungal activity

All extracts inhibited the three test fungi, and inhibition increased with concentration (Table 3). Inhibition at 10% was generally low (0.17–19.0%) and rose to 27.2–53.9% at 30%. The highest inhibition was recorded for aqueous neem against *F. oxysporum* (53.9%), closely followed by alcoholic neem (53.3%) against the same fungus and alcoholic neem against *A. flavus* (49.7%). Onion and ginger were less inhibitory, with maximum values of 42.2% (alcoholic onion against *A. flavus*) and 47.8% (alcoholic ginger against *A. flavus*). Alcoholic ginger extract showed numerically higher inhibition than aqueous ginger extract at 30%, but no solvent comparison was significant for any extract–pathogen combination ($p =$

0.0981–0.829). Complete inhibition of fungal growth was not observed with any treatment.

Germination and seedling growth

Germination of treated seeds ranged from 40.0% (10% aqueous neem and ginger) to 100% (30% aqueous ginger) and increased with concentration for all extracts (Table 4). Treatment means differed significantly for neem, onion and ginger (one-way ANOVA, $p < 0.0001$). Alcoholic neem and onion at 30% gave 93.3% germination. The solvent comparison was not significant for neem ($p = 0.0752$), onion ($p = 0.1301$) or ginger ($p = 0.4382$).

Root length was greatest for alcoholic neem extracts at 30% (5.6 cm) and for aqueous ginger extracts at 30% (5.0 cm) (Table 5). Alcoholic neem extracts gave significantly longer roots than aqueous neem extract ($p = 0.0142$), while solvent had no significant effect for onion ($p = 0.2254$) or ginger ($p = 0.1853$). Treatment effects were significant for neem ($F = 9.282$, $p = 0.0008$) and ginger ($F = 23.212$, $p < 0.0001$). For onion the overall ANOVA was significant ($F = 3.656$, $p = 0.031$), but Tukey's test did not separate any pair of means.

Shoot length also increased with concentration (Table 6). The longest shoots were produced by alcoholic neem at 20 and 30% (6.4 cm). Alcoholic neem extract produced significantly longer shoots than aqueous neem extract ($p = 0.0235$); solvent effects were not significant for onion ($p = 0.3939$) or ginger ($p = 0.0897$). Treatment means differed significantly for neem ($p < 0.0001$), onion ($p = 0.0018$) and ginger ($p < 0.0001$).

The seedling vigor index, calculated from the mean germination, root length and shoot length values according to the formula in Section 2.5, increased with extract concentration for every extract and solvent

In Table 7, the highest values were obtained with alcoholic neem (1119.6), alcoholic onion (923.7), alcoholic ginger (923.7) and aqueous ginger (980.0) at the highest concentrations.

Table 1. Yield and physical characteristics of alcoholic and aqueous extracts of onion, ginger and neem (mean ± SD)

Plant	Solvent	Yield (%)	pH	Colour	Odour	Physical state
<i>Allium cepa</i>	Methanol	13.4 ± 0.8	5.6 ± 0.2	Dark brown	Pungent	Sticky, semi-solid
	Aqueous	10.1 ± 0.6	6.7 ± 0.3	Light brown	Mildly pungent	Smooth liquid
<i>Zingiber officinale</i>	Methanol	14.7 ± 0.9	5.8 ± 0.2	Yellowish-brown	Aromatic, spicy	Oily, semi-solid
	Aqueous	12.1 ± 0.7	6.4 ± 0.2	Pale yellow	Mildly aromatic	Slightly viscous
<i>Azadirachta indica</i>	Methanol	17.2 ± 1.1	5.6 ± 0.2	Dark green	Bitter, herbal	Thick, gummy
	Aqueous	12.2 ± 0.8	6.3 ± 0.3	Greenish-brown	Mildly herbal	Smooth, viscous
<i>p</i> (solvent)		0.021	0.312			

Table 2. Morphological characteristics of fungi isolated from maize seeds

Macroscopic characteristics	Microscopic characteristics	Identity
Black, powdery colony with white margin	Septate hyphae; globose vesicle with chains of black conidia	<i>Aspergillus niger</i>
Bluish-green, velvety colony	Septate hyphae; brush-like conidiophores with conidial chains	<i>Penicillium</i> spp.
White cottony colony turning pink	Septate hyphae; sickle-shaped septate macroconidia	<i>Fusarium oxysporum</i>
Yellow-green, powdery colony	Septate hyphae; globose vesicle with rough conidial chains	<i>Aspergillus flavus</i>

Table 3. Percentage inhibition of mycelial growth of fungi by neem, onion and ginger extracts (mean $\pm$ SD)

Extract	Fungus	10% E	10% S	20% E	20% S	30% E	30% S	P
Neem	<i>F. oxysporum</i>	10.4 $\pm$ 1.2	8.4 $\pm$ 1.0	33.7 $\pm$ 2.5	41.7 $\pm$ 2.8	53.3 $\pm$ 3.1	53.9 $\pm$ 3.3	0.539
	<i>A. flavus</i>	3.8 $\pm$ 0.6	4.6 $\pm$ 0.7	29.7 $\pm$ 2.2	39.3 $\pm$ 2.6	49.7 $\pm$ 3.0	42.8 $\pm$ 2.9	0.829
	<i>Penicillium</i> spp.	2.9 $\pm$ 0.5	12.1 $\pm$ 1.3	26.8 $\pm$ 2.1	29.5 $\pm$ 2.3	36.2 $\pm$ 2.7	38.7 $\pm$ 2.8	0.161
Onion	<i>F. oxysporum</i>	19.0 $\pm$ 1.5	17.6 $\pm$ 1.4	24.5 $\pm$ 1.8	29.8 $\pm$ 2.1	32.6 $\pm$ 2.4	30.9 $\pm$ 2.3	0.779
	<i>A. flavus</i>	4.2 $\pm$ 0.6	6.1 $\pm$ 0.8	22.6 $\pm$ 1.7	21.9 $\pm$ 1.6	42.2 $\pm$ 2.8	38.4 $\pm$ 2.5	0.651
	<i>Penicillium</i> spp.	2.4 $\pm$ 0.4	4.7 $\pm$ 0.6	28.0 $\pm$ 2.0	23.8 $\pm$ 1.8	34.4 $\pm$ 2.5	31.0 $\pm$ 2.3	0.479
Ginger	<i>F. oxysporum</i>	0.5 $\pm$ 0.1	0.17 $\pm$ 0.05	16.9 $\pm$ 1.3	12.8 $\pm$ 1.1	36.6 $\pm$ 2.6	27.2 $\pm$ 2.0	0.212
	<i>A. flavus</i>	12.2 $\pm$ 1.1	9.41 $\pm$ 0.9	28.9 $\pm$ 2.1	22.7 $\pm$ 1.7	47.8 $\pm$ 3.0	31.4 $\pm$ 2.4	0.174
	<i>Penicillium</i> spp.	5.7 $\pm$ 0.7	3.4 $\pm$ 0.5	20.5 $\pm$ 1.6	17.9 $\pm$ 1.4	35.3 $\pm$ 2.6	29.1 $\pm$ 2.2	0.098

E = alcoholic extract (ethanolic for neem, onion and ginger); S = second extract: aqueous for neem and ginger, methanolic for onion. p = comparison between the two solvents for each extract–fungus combination.

Table 4. Germination (%) of maize seeds treated with neem, onion and ginger extracts (mean $\pm$ SD)

Extract	E 10%	E 20%	E 30%	A 10%	A 20%	A 30%
Neem	60.00 ^c $\pm$ 3.50	73.3 $\pm$ 4.1 ^b	93.3 $\pm$ 5.0 ^a	40.0 $\pm$ 3.2 ^d	66.7 $\pm$ 3.8 ^c	80.0 $\pm$ 4.5 ^b
Onion	66.7 $\pm$ 3.8 ^{bc}	93.3 $\pm$ 5.0 ^a	93.3 $\pm$ 5.0 ^a	60.0 $\pm$ 3.5 ^c	73.3 $\pm$ 4.1 ^b	86.7 $\pm$ 4.8 ^a
Ginger	80.0 $\pm$ 4.2 ^b	86.7 $\pm$ 4.8 ^b	93.3 $\pm$ 5.0 ^a	40.0 $\pm$ 3.2 ^c	80.0 $\pm$ 4.2 ^b	100.0 $\pm$ 5.5 ^a

E = alcoholic extract; A = aqueous extract. Means in the same row sharing a letter do not differ significantly (Tukey HSD, p < 0.05). One-way ANOVA F(5,12): neem 60.27, onion 31.29, ginger 64.60 (all p < 0.0001).

Table 5. Root length (cm) of maize seedlings (mean $\pm$ SD)

Extract	E 10%	E 20%	E 30%	A 10%	A 20%	A 30%
Neem	4.8 $\pm$ 0.3 ^{ab}	5.3 $\pm$ 0.4 ^a	5.6 $\pm$ 0.4 ^a	3.9 $\pm$ 0.3 ^b	4.6 $\pm$ 0.3 ^b	5.0 $\pm$ 0.3 ^a
Onion	3.3 $\pm$ 0.3 ^a	4.0 $\pm$ 0.3 ^a	4.0 $\pm$ 0.3 ^a	3.5 $\pm$ 0.3 ^a	4.1 $\pm$ 0.3 ^a	4.0 $\pm$ 0.3 ^a
Ginger	2.7 $\pm$ 0.2 ^c	3.6 $\pm$ 0.3 ^b	4.0 $\pm$ 0.3 ^b	3.0 $\pm$ 0.2 ^c	3.8 $\pm$ 0.3 ^b	5.0 $\pm$ 0.4 ^a

Table 6. Shoot length (cm) of maize seedlings (mean $\pm$ SD)

Extract	E 10%	E 20%	E 30%	A 10%	A 20%	A 30%
Neem	5.2 $\pm$ 0.4 ^b	6.4 $\pm$ 0.5 ^a	6.4 $\pm$ 0.5 ^a	4.0 $\pm$ 0.3 ^c	4.3 $\pm$ 0.3 ^{bc}	4.7 $\pm$ 0.4 ^{bc}
Onion	4.3 $\pm$ 0.3 ^b	5.1 $\pm$ 0.4 ^{ab}	5.9 $\pm$ 0.4 ^a	4.4 $\pm$ 0.3 ^b	4.9 $\pm$ 0.4 ^{ab}	5.3 $\pm$ 0.4 ^{ab}
Ginger	4.4 $\pm$ 0.3 ^b	5.7 $\pm$ 0.4 ^a	5.9 $\pm$ 0.4 ^a	4.0 $\pm$ 0.3 ^b	4.2 $\pm$ 0.3 ^b	4.8 $\pm$ 0.4 ^b

Means followed by same superscripts within the same column are not significantly different (p $\leq$ 0.05)

Table 7. Seedling vigor index of maize seedlings

Extract	E 10%	E 20%	E 30%	A 10%	A 20%	A 30%
Neem	600.0	857.6	1119.6	316.0	593.6	776.0
Onion	506.9	849.0	923.7	474.0	659.7	806.3
Ginger	568.0	806.3	923.7	280.0	640.0	980.0

E = alcoholic extract; A = aqueous extract. SVI = germination (%) $\times$ (mean root length + mean shoot length, cm).

DISCUSSION

Alcoholic solvents recovered more extractable material than water from all three plants. This was in agreement with the general dependence of extraction efficiency on solvent polarity and the range of metabolites present in each tissue. The physical differences between the extracts (darker colour, lower pH and stronger odour in alcoholic extracts) suggest that the two solvents recovered different mixtures of compounds, although the phytochemical composition was not analysed here. Higher yield did not translate into stronger biological activity: in the antifungal assays the two solvents were statistically comparable, and aqueous extracts were as inhibitory as alcoholic ones against *F. oxysporum*. This is practically relevant because water extracts are cheaper and easier to prepare on farms.

Aspergillus, *Penicillium* and *Fusarium* are among the genera most frequently reported from maize kernels in Nigeria and elsewhere (Atehnkeng et al., 2008; Debnath et al., 2012; Erasto et al., 2023). Their presence in the seed lot confirms the need for seed-health management, particularly for *A. flavus* because of aflatoxin risk. Identification here was based on morphology; confirmation by molecular methods would be needed to establish species identity, especially within *Fusarium*.

Inhibition increased with concentration for all three plants, which indicates a dose-dependent effect of the extracts rather than a non-specific artefact. This agrees with earlier reports of concentration-dependent inhibition by neem against *F. oxysporum* and *Rhizoctonia solani* (Abdel-Kader et al., 2011), by neem and other extracts against toxigenic *Aspergillus* in stored maize (Ezeonu et al., 2019), by onion extracts against fungi in vitro (Shams-Ghahfarokhi et al., 2004, 2006), and by ginger constituents (Ficker et al., 2003; Hansini et al., 2023). Neem was generally the most inhibitory against *F. oxysporum* and *A. flavus*, which is consistent with the antifungal limonoids reported for neem (Chang et al., 2022; Wylie & Merrell, 2022), but the differences among plants were not tested statistically in this study and should be interpreted cautiously.

The inhibition achieved was moderate: no treatment exceeded 54% at 30%, and inhibition at 10% was low. Comparable studies of crude extracts report a similar range of effects, and essential oils or purified fractions are usually more potent than crude water or alcohol extracts (Singh et al., 2008; Ferreira et al., 2018). Crude extracts at these concentrations are therefore likely to reduce, rather than eliminate,

seed-borne inoculum. The study did not include a synthetic fungicide for comparison, so it cannot be concluded that the extracts match chemical seed dressings. No solvent effect on inhibition was detected, but with three replicates the power to detect moderate differences was limited; this is particularly relevant for alcoholic ginger against *A. flavus* (47.8 vs 31.4% at 30%) and *Penicillium* spp. ($p = 0.098$).

Neither alcoholic nor aqueous extracts reduced germination at the concentrations tested; germination actually rose with concentration, reaching 100% with 30% aqueous ginger. This suggests that the extracts were not phytotoxic to maize at up to 30% (w/v), in contrast with reports that some extracts reduce germination at high doses (Kumar et al., 2015; Seepe et al., 2020; Adeleke et al., 2025). The increase in germination with concentration may reflect reduced fungal pressure on the seed, a stimulatory effect of extract constituents, or both, but the present design cannot separate these explanations. In particular, the results do not include germination of untreated seeds or seeds treated with a fungicide, so the improvement relative to the absence of treatment, and relative to chemical control, remains to be established.

Alcoholic neem extract promoted both root and shoot growth more than aqueous neem extract, and longer roots and shoots with higher concentrations were observed for ginger and onion as well. Neem products have been reported to improve maize seed performance (Anjorin et al., 2008; Moyin-Jesu, 2010; Nyarko & Olympio, 2001), and botanical treatments including plant extracts have improved emergence and growth of maize in other work (Erasto et al., 2022). The mechanism cannot be inferred from these data because individual phytochemicals were not quantified.

The seedling vigor index followed germination and growth, and increased with concentration for each extract. Because the index combines germination with seedling length, both of which were enhanced by higher concentrations, the highest vigor was found at the highest concentrations. Aqueous ginger at 30% was notable for combining 100% germination with the longest roots among the aqueous treatments. Because the vigor index was derived from treatment means, it was not subjected to separate analysis of variance, and the effect of solvent on vigor should be tested on replicate-level data.

CONCLUSIONS

Extracts of neem, onion and ginger inhibited mycelial growth of *F. oxysporum*, *A. flavus* and *Penicillium* spp. in a concentration-dependent manner, with a maximum of about 54% inhibition at 30% (w/v), and did not impair maize germination or early seedling growth; instead, germination, root and shoot growth were higher at higher concentrations. Aqueous extracts were as effective as alcoholic extracts in inhibiting the fungi, which favours their use by smallholder farmers. Alcoholic neem extract gave the best root and shoot growth. Field and pot trials that include untreated and fungicide-treated controls, naturally infected seed, and chemical characterisation of the extracts are needed to determine whether these botanicals can be recommended for practical use.

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