

Seed-Borne Mycoflora of Stored Groundnut and Its Control Using Botanical Seed Treatments

Femke Hoekstra¹ ●, Cian Gallagher² ●, Femke Kuipers³ ●

¹ Associate Professor, Department of Plant Pathology, Drenthe Institute of Dairy Science, Assen, Netherlands. Email: f.hoekstra@dids.nl | ORCID: 0000-0001-3754-9126

² Senior Research Fellow, Department of Seed Science and Technology, Tipperary Institute of Animal Science, Clonmel, Ireland. Email: c.gallagher@tias.ie | ORCID: 0000-0001-4388-8431

³ Scientist, Department of Genetics and Plant Breeding, Drenthe Institute of Dairy Science, Assen, Netherlands. Email: f.kuipers@dids.nl | ORCID: 0000-0001-2414-5499

ABSTRACT

*Groundnut kernels are rich in oil and protein, and that richness makes them an attractive substrate for fungi from the field to the store. Seed-borne moulds reduce germination and vigour, spoil the crop, and in the case of *Aspergillus flavus* and its relatives can leave aflatoxins in food and feed. Plant extracts and essential oils are often proposed as inexpensive, locally available seed treatments that could replace or reduce synthetic fungicides. This narrative review asks what the published literature supports about the seed-borne and storage mycoflora of groundnut, the conditions that drive its growth, and the botanical treatments proposed to control it, and it reports no new data. Laboratory studies show that many extracts and oils inhibit fungal growth on agar or in sealed vessels. Far fewer tests have been made on kernels held under realistic storage conditions, and moisture, damage and temperature appear to dominate outcomes whatever is applied. Further concerns include the lack of standardised extracts, uncertain effects on toxin formation at sub-lethal doses, and the safety of residues on food crops. The review concludes that botanical treatments are plausible adjuncts to good drying and storage and not substitutes for them, and it sets out what a convincing storage trial would need to report.*

Keywords: Groundnut; Seed-borne fungi; *Aspergillus flavus*; Aflatoxin; Botanical seed treatment; Essential oils; Narrative review

Citation: Hoekstra, Gallagher and Kuipers [2017]. Seed-Borne Mycoflora of Stored Groundnut and Its Control Using Botanical Seed Treatments. DOI: 10.62649/v5.i04.2017.pp37-46

Copyright: © 2017 by the authors. Open access under CC BY 4.0 license.

Article Information: Received: January 9, 2017 Accepted: June 8, 2017 Published: October 28, 2017

Article Class: Review Article

1. Introduction

Groundnut, or peanut, is grown across the semi-arid tropics and many temperate regions for its oil, protein and use as a food and feed. The same kernel that makes it valuable also makes it vulnerable. Rich in oil and protein, and often harvested, dried and held in conditions that are far from ideal, groundnut offers fungi an excellent substrate at almost every step between the field and the table [1, 2]. Some of these fungi simply spoil the crop; a few leave toxins behind that harm people and livestock [3, 4].

Seed is a particular case. A farmer who keeps groundnut for sowing wants kernels that germinate evenly and grow into healthy seedlings, and seed-borne fungi can undermine both aims. They may kill the embryo, weaken the seedling, or travel with the seed into a new field. Conventional seed dressings with synthetic fungicides are effective but cost money, require careful handling and are not always available to smallholders. It is no surprise that plant extracts and essential oils, which are cheap, local and widely believed to be gentler, are proposed as alternatives [5, 6].

What is less clear is how far the published evidence supports that hope. A paper showing that an oil stops a mould growing on agar tells us something real about the oil. It does not yet tell us what will happen to a bag of kernels in a warm, humid store over several months, and it says little about whether the treatment changes toxin formation or leaves anything unwelcome on the seed. This review sets out what is known about the mycoflora of groundnut, the conditions that govern it, and the botanical treatments proposed against it, and it asks how much weight each line of evidence can carry. The aim is a fair account of what is known and what is merely repeated.

The article is a narrative review. It draws on published work on groundnut and storage fungi, on aflatoxin and its risks, on plant-derived antifungals and on the design of treatment trials, and it reports no new data. Where the literature on stored groundnut is thin, we say so, and we describe the evidence a convincing storage trial would need to supply instead of quoting figures we have not been able to check against their sources.

2. Literature Review

2.1 Fungi Associated with Groundnut Seed and Stored Kernels

The mycoflora of groundnut changes as the crop moves from soil to store. Soil carries a rich community of fungi, and aflatoxin-producing species are common residents of warm agricultural soils, from which they colonise developing pods, especially when the plant is stressed [7, 8]. After harvest, pods and kernels pick up further spores from dust, equipment, containers and the air. Standard accounts of food spoilage describe a succession in which fast-growing moulds appear when material is wet, and more drought-tolerant species take over as it dries [1].

Species of *Aspergillus* are the most discussed. *Aspergillus flavus* is the principal aflatoxin producer in most surveys and is a common colonist of groundnut [4], though the section *Flavi* contains several related species with different toxin profiles, and identification from morphology alone can be unreliable [9]. Black aspergilli, the section *Nigri*, are also frequent on seed and kernels and are better known for causing seedling disease and spoilage, although some members produce other toxins [10]. *Penicillium*, *Rhizopus* and *Fusarium* species are regularly reported as well, and their relative abundance depends on region, season and handling. Table 1 summarises the main groups.

Table 1. Fungal groups commonly associated with groundnut and why they matter.

Group	Typical setting	Main concern
<i>Aspergillus</i> section <i>Flavi</i>	Soil, pods, stored kernels [4, 9]	Aflatoxin formation, kernel decay
<i>Aspergillus</i> section <i>Nigri</i>	Seed surfaces, damaged kernels [10]	Seedling and collar rot, spoilage, some toxin producers
<i>Penicillium</i> species	Cool, damp stores [1]	Decay, off-flavours, other toxins
<i>Rhizopus</i> and allied moulds	Freshly harvested, wet pods	Rapid early spoilage when drying is delayed
<i>Fusarium</i> species	Field-borne, carried on seed	Seedling disease; reduced vigour
Soil-borne pathogens	Pod and seed coat	Poor stand establishment

Note: Qualitative summary; the importance of each group varies with region, season and storage conditions.

An important feature of seed-borne fungi is where they sit. Some are carried on the seed coat as surface contaminants, which treatments can reach. Others are inside the testa or even the cotyledons, where a surface dressing is much less effective. A treatment that reduces surface counts may therefore leave the more damaging infection untouched, and a seed health test that does not distinguish the two can overstate protection. The literature on seed testing and food mycology stresses this distinction and the importance of surface disinfection steps in assays [1, 11].

2.2 Aflatoxin, Health and the Case for Control

Aflatoxins are the reason that stored groundnut attracts so much attention. They are potent liver toxins and carcinogens, produced by certain *Aspergillus* species growing on crops such as groundnut and maize [3, 4]. Reviews of human exposure describe a heavy burden in regions where staple foods are frequently contaminated, and link chronic exposure to liver cancer, especially where hepatitis B is common [12, 13]. Acute poisoning, though rarer, has caused outbreaks in developing countries [14, 15].

Public health authorities and researchers have concluded that reducing exposure requires action along the whole chain, from the farm to the household, and that no single measure is enough [16, 17]. For the seed lot, the consequence is plain: an infected kernel that is sold for food or fed to animals is a risk, and an infected kernel kept for sowing is a risk of another kind. That is part of why treatments that suppress *A. flavus*, and not merely seed rots, attract interest. It is also why any treatment must be judged on toxin outcomes as well as on fungal counts, a point we return to below.

2.3 Moisture, Temperature and Damage in Storage

The fungal ecology of stored grain has been studied for decades, and one conclusion is robust: the water available to fungi decides what grows. Reviews of post-harvest fungal ecology describe how growth and toxin formation respond jointly to water availability and temperature, and how these interact with the condition of the grain [18, 19]. A kernel dried promptly to a safe level and kept dry gives most storage fungi little opportunity; a kernel that is left damp, or that regains moisture in a leaking store, does not.

Damage matters too. Insect holes, cracks from rough handling and broken testa open routes for fungi to the nutrient-rich interior, and field studies of storage practice in West Africa found that how grain was handled and held was strongly tied to contamination [20, 21]. Reviews of peanut management stress drying, sorting out damaged and shrivelled kernels, and clean, dry, well-ventilated storage as the foundation of control [2, 17]. A seed treatment sits on top of that foundation and cannot replace it. A kernel that dries quickly after harvest has a very different future from one left in a damp heap.

2.4 Chemical and Biological Approaches to Control

Several strategies exist beyond good storage. Synthetic fungicides remain the standard seed dressing in many systems. Biological approaches, such as applying non-toxigenic strains of *A. flavus* to compete with toxigenic ones, have been developed, and pre-harvest research programmes have pursued resistant cultivars and crop management [22, 23]. General reviews of mycotoxin prevention organise the options into pre-harvest, harvest and post-harvest measures and caution that each has limits [24, 25].

These approaches have costs that smallholders often find hard to bear, whether in money, equipment or access to inputs. That is the gap that plant-derived materials hope to fill. They are not new: farmers have used neem leaves, wood ash, spices and other local materials in stores for generations, and modern research has tried to test those practices with the methods of mycology [6, 26].

2.5 Botanical Antifungals: What Has Been Shown

Plants make a large array of compounds that inhibit microbial growth. Essential oils, the volatile fractions of aromatic plants, have been reviewed extensively for their antimicrobial properties and

for the many ways in which they act on cells, with an emphasis on their lipophilic character and on the variety of components within a single oil [27, 28]. Reviews aimed at agriculture and food preservation describe their potential against moulds and mycotoxin formation, and emphasise the gap between potential and practice [29, 30].

Laboratory studies from several countries report that oils from spices, herbs and medicinal plants inhibit the growth of storage fungi, in some cases including toxigenic strains of *Aspergillus*, and some also reduced toxin production in culture [31, 32, 33]. Work on thyme oils against *A. parasiticus* found inhibition of growth and aflatoxin formation [34], and a study of a single phenolic compound from clove suggested one mechanism for reduced toxin formation, namely interference with lipid peroxidation [35]. An oil from *Chenopodium ambrosioides* was reported to have antifungal and antiaflatoxigenic activity in the laboratory [36]. These are interesting leads. They are results obtained in specific conditions, usually with a laboratory medium and a defined fungal isolate.

Neem, the best-known botanical in this field, has a long literature covering insecticidal, antifungal and medicinal uses [26], and reviews of plant extracts for disease management list many species with activity against plant pathogens [6]. The breadth of that literature is both a strength and a weakness. Because many extracts show some activity in many tests, a positive result is easy to obtain and hard to interpret. This is why the same plant can look excellent in one report and unremarkable in the next.

2.6 From Assay to Store: The Translation Problem

Critical reviews of plant-derived antimicrobials have argued that laboratory screening needs to be more rigorous before claims are made, with attention to extract preparation, solvents, controls and the definition of activity [37, 38]. Methods such as microplate assays for minimal inhibitory concentration made screening faster and more reproducible [39], though they were developed for bacteria and cannot by themselves speak to growth of a mould on a kernel. A concentration that inhibits a fungus in liquid or on agar is not the concentration that will be present on the seed surface, in a sack, over months.

Volatile oils raise a particular issue. Their activity in a sealed vessel depends on the headspace volume, the temperature and the loss of volatiles over time, none of which resembles an open or semi-open bag. Work applying essential oils directly to maize grain found that effects on fungal

growth and toxin accumulation depended on conditions, notably the water availability of the grain [40]. That result is a useful reminder that a treatment does not act alone: the same oil can look effective in one moisture regime and weak in another.

3. Materials and Methods

3.1 Scope and Question

The review asks a question in two parts: which fungi are consistently reported on groundnut seed and stored kernels, and what does the published literature support about botanical treatments as a means of controlling them? The question was divided into the sub-questions a storage trial would have to answer: what the starting infection was, how the kernels were dried and held, what material was applied and at what dose, how fungal growth and toxin were measured, and how seed quality was judged afterwards. None of these steps is difficult, but each one is easy to skip.

3.2 Literature Assembly

The review draws on peer-reviewed work in four areas: the taxonomy and ecology of *Aspergillus* and related fungi on food crops; aflatoxin toxicology and public health; post-harvest management of groundnut and similar commodities; and the antifungal activity of plant extracts and essential oils. Sources were chosen for relevance, citation in the field and the clarity of their methods. Studies of other crops, notably maize, are used for what they teach about conditions and limits and not as evidence of effects on groundnut.

We did not run a formal systematic search and we do not claim one. This is a narrative synthesis, and the choice of sources reflects the authors' judgement. The practical consequence is that the review is a better guide to what a good storage trial should contain than to the exact size of any reported effect, and it avoids quoting figures from individual studies that could not be checked against the primary papers for this article.

3.3 Appraisal Framework

To judge how far a report can be trusted, we used a short framework of four questions. Was the treatment tested on kernels under storage conditions, or only in a laboratory assay [37, 38]? Were the extract and dose described well enough for another laboratory to repeat them [39, 41]? Were toxin and seed quality measured as well as fungal growth [25, 40]? And were the results replicated across seed lots and seasons, with uncertainty reported instead of only significance

tests [42, 43]? Table 3 lists the items we looked for.

4. Results

4.1 The Mycoflora of Groundnut: A Consistent Picture

Across the literature a broadly consistent picture emerges. Soil-borne and field fungi, including aflatoxigenic *aspergilli*, colonise pods before harvest [7, 8]. After harvest, wet-tolerant moulds appear first if drying is delayed, and species that tolerate drier conditions persist in the store [1, 18]. Black *aspergilli* and *Penicillium* are frequent on the seed surface, and the relative importance of each depends on place and season [9, 10].

This picture means that a single survey of one seed lot at one time describes a moment in a process, not a fixed property of groundnut. A paper that reports the mycoflora of seed from a given market in a given month is useful as a record but should not be generalised to other places, seasons or handling. Where reports differ, the differences often reflect how the seed was sampled, whether the surface was disinfected before plating, and which medium was used, and the literature on isolation media for low-moisture foods illustrates how much the medium shapes the species recovered [1, 11]. Small differences at the start of storage can grow into large ones by the end.

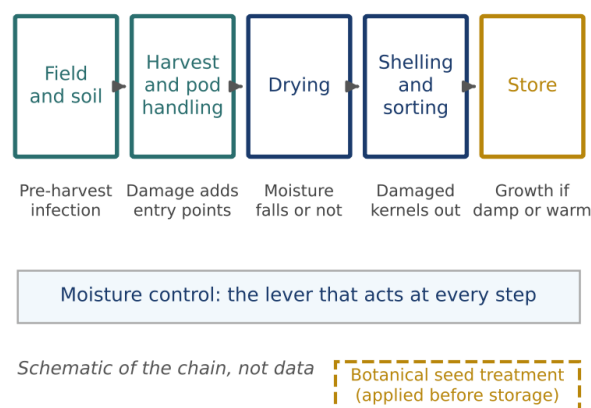


Figure 1. Where fungi enter the groundnut chain and where moisture control acts (schematic, not data).

4.2 Where Control Can Act

Figure 1 sketches the chain from field to store and shows that moisture is relevant at every step. Reducing infection before harvest depends on crop management and, in some programmes, on competitive or resistant approaches [22, 23]. Preventing damage at harvest and shelling reduces the entry points for fungi [2, 20]. Drying and sorting then remove most of the opportunity

for growth [19, 21]. Only at the end of this chain does a seed treatment come into play, and it acts on whatever the earlier steps have left behind.

The logic is important. A treatment applied to seed that is already wet or heavily damaged is asked to do the work of drying and sorting, which it is poorly placed to do. A treatment applied to seed that has been dried and cleaned may add a margin of safety, and its effect may be hard to detect because the baseline risk is already low. In both cases the contribution of the botanical is easy to misjudge, which is one reason why well-designed comparisons with untreated controls matter so much. Read this way, a lack of effect in a single small test is not proof that nothing works.

4.3 What the Botanical Evidence Shows

The evidence for botanical antifungal activity is wide but shallow. Many plants and oils inhibit growth of storage fungi in laboratory assays [31, 32, 33]; some reduce aflatoxin formation in culture [34, 35, 36]; and reviews emphasise their promise as natural preservatives for agri-food commodities [29, 30]. Table 2 lists the main materials and the open questions attached to them.

Table 2. Botanical materials proposed for groundnut seed treatment and the main questions they raise.

Material	Typical evidence	Main open question
Neem seed or leaf extracts	Reviews of activity and use [6, 26]	Batch-to-batch variation
Essential oils (thyme, clove, others)	Growth and toxin inhibition in vitro [33, 34, 35]	Effect on stored kernels
Oils from medicinal plants	Agar and vapour assays [31, 32]	Dose and volatility on a real seed lot
Chenopodium oil	Antifungal activity in laboratory work [36]	Residue and food safety
Crude aqueous extracts	Screening assays [6, 38]	Standardisation; microbial load of the extract
Oils applied to grain	Maize grain experiments [40]	Role of water activity

Note: The last column states the authors' reading of the cited literature and is not a measure of efficacy.

What is missing is depth. The literature contains far fewer experiments in which an extract is applied to kernels that are then held for months under defined conditions, with the starting infection, moisture, toxin and germination all recorded. The studies on maize grain [40] are a welcome example of the kind of work that is needed, and they show that outcomes vary with conditions. For groundnut specifically, we found no body of work of comparable depth that we could cite with confidence, and we have not tried

to fill the gap by extrapolating.

A second weakness is identity. Plants vary with species, variety, part, harvest time, soil and extraction method, and the same name on a bottle can cover materials of very different composition [28, 41]. A result obtained with one oil does not transfer to another sold under the same name, and without chemical characterisation or at least a clear description of the source, the finding cannot be repeated. Regulators of botanical products face the same problem, and the literature on botanical pesticides discusses how standardisation and registration remain unresolved for many materials [41]. A treatment that works only in a sealed dish is not a treatment a farmer can use in a sack.

4.4 The Complication: Safety, Toxin and Standardisation

Three complications deserve emphasis. The first is that reducing fungal growth is not the same as reducing toxin. Stress on a fungus can change its secondary metabolism, and sub-inhibitory doses of an inhibitor may in some conditions leave toxin formation unchanged or even altered; the maize work shows that effects on growth and accumulation do not always move together [40], and compounds such as eugenol appear to act on the biochemical pathways of toxin formation rather than only on growth [35]. A treatment must therefore be evaluated for toxin directly [25].

The second is food and feed safety. An essential oil that is safe as a flavouring at low levels is not automatically safe as a residue on a seed lot that is later eaten, and the toxicological literature on essential oils describes a wide range of biological effects that depend on dose and component [27, 28]. Neem, similarly, has a long record of use but also a literature on its constituents that should not be ignored when it is applied to food crops [26]. Safety questions are rarely addressed in the laboratory papers that report antifungal activity, and a treatment for seed intended for sowing may reasonably be judged differently from one for kernels intended for consumption.

The third is standardisation. Without a defined preparation, dose and application method, results cannot be compared, and advice cannot be given with confidence [37, 41]. Preparations made by farmers from local material will differ again from laboratory preparations. Any recommendation for smallholders must account for that variation, for example by describing a simple, repeatable recipe and testing it in the conditions in which it will be used.

Because these complications are independent of one another, they compound. A promising oil may be hard to standardise; a standardised oil may leave unwanted residue; and a safe, standardised oil may still fail in a damp store. None of this makes botanical treatments unpromising. It does mean that the leap from a successful laboratory assay to a farm recommendation is larger than it appears.

Table 3. What a storage trial of a botanical seed treatment should report.

Item	Why it matters	Common omission
Seed lot description	Initial infection and damage differ between lots [1, 11]	Starting mycoflora not recorded
Moisture or water activity	Largest driver of fungal growth [18, 19]	Moisture not tracked over time
Extract identity and dose	Needed to reproduce the treatment [37, 41]	Plant part, solvent, concentration unstated
Fungal and toxin outcomes	Growth can fall while toxin does not [25, 40]	Toxin not measured
Germination and vigour	A treatment must not harm the seed	Not tested after storage
Controls	Untreated and standard fungicide controls	Positive control missing
Replication	Lots, stores and seasons [42, 43]	Single lot, single season

Note: Items are drawn from the methodological literature cited and from the logic of seed health testing.

4.5 Quality of the Evidence

Reports on botanical control of storage fungi commonly share a set of design limitations. Many use a single isolate, a single concentration series and a single laboratory medium. Positive controls with a standard fungicide are often missing, which makes it hard to judge whether an effect is large or small. Replication is often limited to repeated plates from one preparation, which measures the plate-to-plate variation but not the variation between batches of extract or between seed lots [37, 38].

Statistical practice compounds the problem. Results reported only as significant or not significant discard the size of the effect, which is what a farmer needs, and a p-value is not the probability that an effect is real [43]. In any field where many extracts are screened and positive findings are more likely to be published, a substantial share of the positive findings may not hold up [42]. These problems are shared with much of applied biology and botanicals are not special in having them; they matter here because the case for the treatments rests almost entirely on this kind of study. The point is not that an assay

is poor, only that its result travels less far than its abstract suggests.

Put plainly, the literature supports only a cautious statement. Several botanical materials inhibit storage fungi in the laboratory, and some reduce toxin in culture; whether they protect stored groundnut under realistic conditions has not been established by evidence we can cite with confidence. Figure 2 summarises, qualitatively, the factors that appear to dominate outcomes and the questions that each raises for a test of a treatment, and Figure 3 sketches how a reader might weigh a claim. That is a modest claim, and it is the one the evidence will currently bear.

	Fungal growth	Toxin formation	Test of a treatment
Kernel moisture	Dominant	Strong	Confounds
Temperature	Strong	Strong	Confounds
Kernel damage	Strong	Strong	Confounds
Seed lot history	Moderate	Moderate	Confounds
Extract used	Variable	Unclear	Needs report

Qualitative reading of the literature; cells are labels, not measurements

Figure 2. Qualitative matrix of storage factors and the questions they raise for botanical treatments (not data).

5. Discussion

5.1 Interpretation and Practical Reading

The main finding of this review is a conditional one. Botanical treatments are most likely to help where the kernels have been dried and cleaned, the store is dry and sound, and the residual risk comes from surface contamination, and they are least likely to help where moisture and damage are the real problem. That fits what is known about the ecology of stored grain in general [18, 19], and it implies that the practical question for a grower is not whether a botanical works but what the store is lacking that a botanical would be asked to make up.

The second conditional concerns evidence. A reader meeting a claim that an extract protects stored seed can ask a short sequence of questions (Figure 3). Was it tested on kernels or only on agar? Were moisture and the starting infection described? Was toxin measured alongside growth? Was germination checked afterwards? A report that answers all four is rare, and one that answers none should be read as an interesting observation

and not as a recommendation. Pressing these questions is not hostile to botanical research; it is how a promising lead becomes a usable practice.

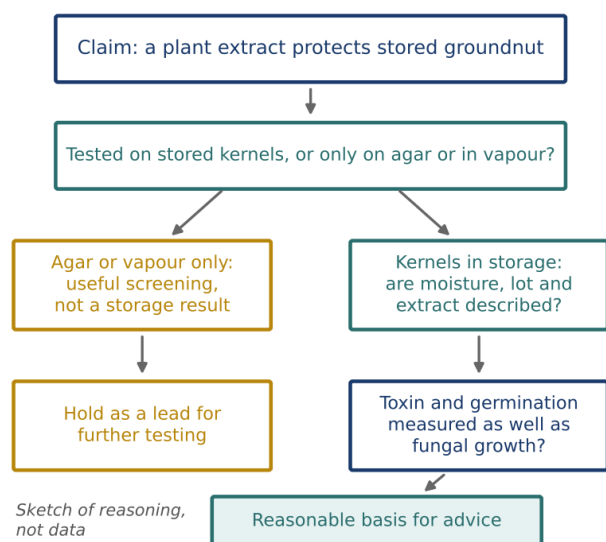


Figure 3. Decision sketch for reading a claim that a botanical treatment protects stored groundnut.

A third point concerns safety and regulation. The use of plant materials on food crops is not automatically benign, and the wide range of biological activities of essential oils [28] argues for attention to dose, residue and the food chain. That is not an argument against use. It is an argument for the same routine checks that apply to any new input, and these are cheap compared with the cost of a contamination problem in a seed lot that is later eaten. These points apply with equal force to any new seed treatment, not only to botanical ones.

Three findings would change this reading. A well-designed storage trial on groundnut showing a clear and repeatable reduction of fungal growth and toxin under ordinary moisture conditions would move botanical treatments from promising to supported. Evidence that a common botanical increases toxin formation at sub-lethal doses would call for caution and for a change in advice. And a standardised, farmer-feasible preparation that keeps its activity over a season would remove the single biggest obstacle to adoption [29, 41].

5.2 Limitations

This is a narrative review and shares the limitations of that form. The selection of sources reflects judgement, no formal search or quality scoring was done, and the review does not quote numerical results from individual studies because these could not be checked against the primary reports for this article. A reader who needs an effect size should consult the original studies directly and apply the appraisal questions above,

or wait for a formal systematic review of storage trials.

The review also draws on evidence from other crops and from in vitro work, which are imperfect proxies for stored groundnut. Studies on maize and on culture media describe conditions that overlap with groundnut but are not the same, and the oil-rich kernel of groundnut may behave differently from cereal grain. We have used those studies to frame hypotheses, and the conclusions should be read in that light. Reviews of this kind are more useful when they say where the evidence stops than when they smooth it over.

5.3 Implications for Trial Design

A trial that could settle the question would begin with characterised seed lots and record the starting infection by a method that distinguishes surface from internal fungi [1, 11]. It would dry the kernels to defined moisture levels, hold them under defined conditions, describe the botanical fully (species, part, preparation, dose and application), and include untreated and standard-fungicide controls. It would measure fungal counts, toxin and germination at intervals, and it would replicate across lots and seasons [37, 42].

Such a trial should also test more than one moisture condition, since the literature suggests that the effect of a treatment changes with water availability [18, 40]. It should report effect sizes with intervals instead of only significance [43]. These requirements are demanding, but they are the ones that distinguish a result a grower can use from one that supports only a hunch.

5.4 Directions for Future Work

Three needs stand out. First, storage trials on groundnut itself, with the design features above, since much of the best-conducted work is on other crops [2, 40]. Second, work on standardised preparations that farmers can reproduce, together with chemical characterisation that allows comparison between laboratories [37, 41]. Third, direct tests of toxin, residue and seed quality, so that a treatment is judged by what matters to the food chain and not only by what is easy to measure [17, 25].

5.5 Practical Reading for Advisers and Smallholders

For an adviser, the sensible position is neither to dismiss botanicals nor to promise results. The foundation remains the same: harvest on time, dry promptly and fully, remove damaged and shrivelled kernels, and store in clean, dry,

well-ventilated containers [2, 17]. A household that wishes to add a botanical can do so as a small, recorded trial, treating part of a seed lot and leaving a comparable part untreated, held side by side, with the same records of moisture, appearance and germination at planting [20, 21].

Such a trial is not a substitute for experimental work and should not be reported as one. It can nonetheless tell a household whether the practice is workable on its own farm, whether the material is available and consistent through the season, and whether any difference is large enough to see against ordinary variation between sacks. Its value lies in local decision-making, and the broader question of efficacy will be settled by designed trials and by synthesis of their results [24, 29].

6. Conclusion

The seed-borne and storage mycoflora of groundnut is well described in outline, and the conditions that govern it are well understood: water, temperature and damage decide most outcomes. Botanical materials show antifungal activity in the laboratory, and some reduce toxin formation in culture, so they are plausible adjuncts to good drying and storage. The evidence, as we can responsibly summarise it, does not yet establish that they protect stored groundnut under realistic conditions, and it leaves open how they affect toxin, residues and seed quality.

For the practitioner the message is practical rather than discouraging. Botanicals can be tried, provided the seed is dry and clean, the preparation is described and repeatable, and the results are recorded. For the researcher, the opportunity is a set of storage trials on groundnut that are replicated, report moisture and starting infection, measure toxin and germination, and use defined materials, to turn a plausible idea into a measured result that growers can rely on.

7. Acknowledgements

The authors thank colleagues who commented on earlier drafts of this review.

8. Funding

This work received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

F.H. conceived the review and wrote the sections on plant pathology and storage fungi. C.G. wrote the sections on seed health testing and botanical treatments. F.K. wrote the sections on mycotoxin risk and appraisal of evidence. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human participants or animals were used and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

13. References

- [1] Pitt, J. I., & Hocking, A. D. (2009). *Fungi and food spoilage* (3rd ed.). Springer. DOI: [10.1007/978-0-387-92207-2](https://doi.org/10.1007/978-0-387-92207-2)
- [2] Torres, A. M., Barros, G. G., Palacios, S. A., Chulze, S. N., & Battilani, P. (2014). Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. *Food Research International*, 62, 11–19. DOI: [10.1016/j.foodres.2014.02.023](https://doi.org/10.1016/j.foodres.2014.02.023)
- [3] Bennett, J. W., & Klich, M. (2003). Mycotoxins. *Clinical Microbiology Reviews*, 16(3), 497–516. DOI: [10.1128/CMR.16.3.497-516.2003](https://doi.org/10.1128/CMR.16.3.497-516.2003)
- [4] Klich, M. A. (2007). *Aspergillus flavus*: The major producer of aflatoxin. *Molecular Plant Pathology*, 8(5), 713–722. DOI: [10.1111/j.1364-3703.2007.00436.x](https://doi.org/10.1111/j.1364-3703.2007.00436.x)
- [5] Isman, M. B. (2000). Plant essential oils for pest and disease management. *Crop Protection*, 19(8–10), 603–608. DOI: [10.1016/S0261-2194\(00\)00079-X](https://doi.org/10.1016/S0261-2194(00)00079-X)
- [6] Gurjar, M. S., Ali, S., Akhtar, M., & Singh, K. S. (2012). Efficacy of plant extracts in plant disease management. *Agricultural Sciences*, 3(3), 425–433. DOI: [10.4236/as.2012.33050](https://doi.org/10.4236/as.2012.33050)
- [7] Horn, B. W. (2003). Ecology and population biology of aflatoxigenic fungi in soil. *Journal of Toxicology: Toxin Reviews*, 22(2–3), 351–379. DOI: [10.1081/TXR-120024098](https://doi.org/10.1081/TXR-120024098)
- [8] Diener, U. L., Cole, R. J., Sanders, T. H., Payne, G. A., Lee, L. S., & Klich, M. A. (1987). Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Annual Review of Phytopathology*, 25, 249–270. DOI: [10.1146/annurev.py.25.090187.001341](https://doi.org/10.1146/annurev.py.25.090187.001341)
- [9] Varga, J., Frisvad, J. C., & Samson, R. A. (2011). Two new aflatoxin producing species, and an overview of *Aspergillus* section *Flavi*. *Studies in Mycology*, 69, 57–80. DOI: [10.3114/sim.2011.69.05](https://doi.org/10.3114/sim.2011.69.05)
- [10] Perrone, G., Susca, A., Cozzi, G., Ehrlich, K., Varga, J., Frisvad, J. C., ... Samson, R. A. (2007). Biodiversity of *Aspergillus* species in some

- important agricultural products. *Studies in Mycology*, 59, 53–66. DOI: [10.3114/sim.2007.59.07](https://doi.org/10.3114/sim.2007.59.07)
- [11] Hocking, A. D., & Pitt, J. I. (1980). Dichloran-glycerol medium for enumeration of xerophilic fungi from low-moisture foods. *Applied and Environmental Microbiology*, 39(3), 488–492. DOI: [10.1128/aem.39.3.488-492.1980](https://doi.org/10.1128/aem.39.3.488-492.1980)
- [12] Wild, C. P., & Gong, Y. Y. (2010). Mycotoxins and human disease: A largely ignored global health issue. *Carcinogenesis*, 31(1), 71–82. DOI: [10.1093/carcin/bgp264](https://doi.org/10.1093/carcin/bgp264)
- [13] Liu, Y., & Wu, F. (2010). Global burden of aflatoxin-induced hepatocellular carcinoma: A risk assessment. *Environmental Health Perspectives*, 118(6), 818–824. DOI: [10.1289/ehp.0901388](https://doi.org/10.1289/ehp.0901388)
- [14] Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M., & Aggarwal, D. (2004). Human aflatoxicosis in developing countries: A review of toxicology, exposure, potential health consequences, and interventions. *The American Journal of Clinical Nutrition*, 80(5), 1106–1122. DOI: [10.1093/ajcn/80.5.1106](https://doi.org/10.1093/ajcn/80.5.1106)
- [15] Kensler, T. W., Roebuck, B. D., Wogan, G. N., & Groopman, J. D. (2011). Aflatoxin: A 50-year odyssey of mechanistic and translational toxicology. *Toxicological Sciences*, 120(Suppl. 1), S28–S48. DOI: [10.1093/toxsci/kfq283](https://doi.org/10.1093/toxsci/kfq283)
- [16] Strosnider, H., Azziz-Baumgartner, E., Banziger, M., Bhat, R. V., Breiman, R., Brune, M.-N., ... Henry, S. (2006). Workgroup report: Public health strategies for reducing aflatoxin exposure in developing countries. *Environmental Health Perspectives*, 114(12), 1898–1903. DOI: [10.1289/ehp.9302](https://doi.org/10.1289/ehp.9302)
- [17] Waliyar, F., Osiru, M., Ntare, B. R., Kumar, K. V. K., Sudini, H., Traore, A., & Diarra, B. (2015). Post-harvest management of aflatoxin contamination in groundnut. *World Mycotoxin Journal*, 8(2), 245–252. DOI: [10.3920/WMJ2014.1766](https://doi.org/10.3920/WMJ2014.1766)
- [18] Magan, N., Hope, R., Cairns, V., & Aldred, D. (2003). Post-harvest fungal ecology: Impact of fungal growth and mycotoxin accumulation on food quality and safety. *European Journal of Plant Pathology*, 109(7), 723–730. DOI: [10.1023/A:1026082425177](https://doi.org/10.1023/A:1026082425177)
- [19] Magan, N., & Aldred, D. (2007). Post-harvest control strategies: Minimizing mycotoxins in the food chain. *International Journal of Food Microbiology*, 119(1–2), 131–139. DOI: [10.1016/j.ijfoodmicro.2007.07.034](https://doi.org/10.1016/j.ijfoodmicro.2007.07.034)
- [20] Hell, K., Cardwell, K. F., Setamou, M., & Poehling, H.-M. (2000). The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, West Africa. *Journal of Stored Products Research*, 36(4), 365–382. DOI: [10.1016/S0022-474X\(99\)00056-9](https://doi.org/10.1016/S0022-474X(99)00056-9)
- [21] Chulze, S. N. (2010). Strategies to reduce mycotoxin levels in maize during storage: A review. *Food Additives & Contaminants: Part A*, 27(5), 651–657. DOI: [10.1080/19440040903573032](https://doi.org/10.1080/19440040903573032)
- [22] Cleveland, T. E., Dowd, P. F., Desjardins, A. E., Bhatnagar, D., & Cotty, P. J. (2003). United States Department of Agriculture—Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Management Science*, 59(6–7), 629–642. DOI: [10.1002/ps.724](https://doi.org/10.1002/ps.724)
- [23] Bhatnagar-Mathur, P., Sunkara, S., Bhatnagar-Panwar, M., Waliyar, F., & Sharma, K. K. (2015). Biotechnological advances for combating *Aspergillus flavus* and aflatoxin contamination in crops. *Plant Science*, 234, 119–132. DOI: [10.1016/j.plantsci.2015.02.009](https://doi.org/10.1016/j.plantsci.2015.02.009)
- [24] Kabak, B., Dobson, A. D. W., & Var, I. (2006). Strategies to prevent mycotoxin contamination of food and animal feed: A review. *Critical Reviews in Food Science and Nutrition*, 46(8), 593–619. DOI: [10.1080/10408390500436185](https://doi.org/10.1080/10408390500436185)
- [25] Marín, S., Ramos, A. J., Cano-Sancho, G., & Sanchis, V. (2013). Mycotoxins: Occurrence, toxicology, and exposure assessment. *Food and Chemical Toxicology*, 60, 218–237. DOI: [10.1016/j.fct.2013.07.047](https://doi.org/10.1016/j.fct.2013.07.047)
- [26] Brahmachari, G. (2004). Neem—an omnipotent plant: A retrospection. *ChemBioChem*, 5(4), 408–421. DOI: [10.1002/cbic.200300749](https://doi.org/10.1002/cbic.200300749)
- [27] Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology*, 94(3), 223–253. DOI: [10.1016/j.ijfoodmicro.2004.03.022](https://doi.org/10.1016/j.ijfoodmicro.2004.03.022)
- [28] Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils—a review. *Food and Chemical Toxicology*, 46(2), 446–475. DOI: [10.1016/j.fct.2007.09.106](https://doi.org/10.1016/j.fct.2007.09.106)
- [29] Prakash, B., Kedia, A., Mishra, P. K., & Dubey, N. K. (2015). Plant essential oils as food preservatives to control moulds, mycotoxin contamination and oxidative deterioration of agri-food commodities—potentials and challenges. *Food Control*, 47, 381–391. DOI: [10.1016/j.foodcont.2014.07.023](https://doi.org/10.1016/j.foodcont.2014.07.023)
- [30] Tripathi, P., & Dubey, N. K. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology*, 32(3), 235–245. DOI: [10.1016/j.postharvbio.2003.11.005](https://doi.org/10.1016/j.postharvbio.2003.11.005)
- [31] Mishra, A. K., & Dubey, N. K. (1994). Evaluation of some essential oils for their toxicity against fungi causing deterioration of stored food commodities. *Applied and Environmental Microbiology*, 60(4), 1101–1105. DOI: [10.1128/aem.60.4.1101-1105.1994](https://doi.org/10.1128/aem.60.4.1101-1105.1994)
- [32] Soliman, K. M., & Badeaa, R. I. (2002). Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food and Chemical Toxicology*, 40(11), 1669–1675. DOI: [10.1016/S0278-6915\(02\)00120-5](https://doi.org/10.1016/S0278-6915(02)00120-5)

- [33] Nguefack, J., Leth, V., Amvam Zollo, P. H., & Mathur, S. B. (2004). Evaluation of five essential oils from aromatic plants of Cameroon for controlling food spoilage and mycotoxin producing fungi. *International Journal of Food Microbiology*, 94(3), 329–334. DOI: [10.1016/j.ijfoodmicro.2004.02.003](https://doi.org/10.1016/j.ijfoodmicro.2004.02.003)
- [34] Rasooli, I., & Abyaneh, M. R. (2004). Inhibitory effects of thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. *Food Control*, 15(6), 479–483. DOI: [10.1016/j.foodcont.2003.07.002](https://doi.org/10.1016/j.foodcont.2003.07.002)
- [35] Jayashree, T., & Subramanyam, C. (1999). Antiaflatoxicogenic activity of eugenol is due to inhibition of lipid peroxidation. *Letters in Applied Microbiology*, 28(3), 179–183. DOI: [10.1046/j.1365-2672.1999.00512.x](https://doi.org/10.1046/j.1365-2672.1999.00512.x)
- [36] Kumar, R., Mishra, A. K., Dubey, N. K., & Tripathi, Y. B. (2007). Evaluation of *Chenopodium ambrosioides* oil as a potential source of antifungal, antiaflatoxicogenic and antioxidant activity. *International Journal of Food Microbiology*, 115(2), 159–164. DOI: [10.1016/j.ijfoodmicro.2006.10.017](https://doi.org/10.1016/j.ijfoodmicro.2006.10.017)
- [37] Cos, P., Vlietinck, A. J., Berghe, D. V., & Maes, L. (2006). Anti-infective potential of natural products: How to develop a stronger in vitro “proof-of-concept”. *Journal of Ethnopharmacology*, 106(3), 290–302. DOI: [10.1016/j.jep.2006.04.003](https://doi.org/10.1016/j.jep.2006.04.003)
- [38] Rios, J. L., & Recio, M. C. (2005). Medicinal plants and antimicrobial activity. *Journal of Ethnopharmacology*, 100(1–2), 80–84. DOI: [10.1016/j.jep.2005.04.025](https://doi.org/10.1016/j.jep.2005.04.025)
- [39] Eloff, J. N. (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, 64(8), 711–713. DOI: [10.1055/s-2006-957563](https://doi.org/10.1055/s-2006-957563)
- [40] Bluma, R. V., & Etcheverry, M. G. (2008). Application of essential oils in maize grain: Impact on *Aspergillus* section *Flavi* growth parameters and aflatoxin accumulation. *Food Microbiology*, 25(2), 324–334. DOI: [10.1016/j.fm.2007.10.004](https://doi.org/10.1016/j.fm.2007.10.004)
- [41] Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*, 51, 45–66. DOI: [10.1146/annurev.ento.51.110104.151146](https://doi.org/10.1146/annurev.ento.51.110104.151146)
- [42] Ioannidis, J. P. A. (2005). Why most published research findings are false. *PLoS Medicine*, 2(8), e124. DOI: [10.1371/journal.pmed.0020124](https://doi.org/10.1371/journal.pmed.0020124)
- [43] Wasserstein, R. L., & Lazar, N. A. (2016). The ASA statement on p-values: Context, process, and purpose. *The American Statistician*, 70(2), 129–133. DOI: [10.1080/00031305.2016.1154108](https://doi.org/10.1080/00031305.2016.1154108)