Phytopathology Research

RESEARCH

Open Access

Botanical extracts control the fungal pathogen *Colletotrichum boninense* in smallholder production of common bean

Tamia M. Kushaha[1], Angela G. Mkindi[1], Ernest R. Mbega[1], Philip C. Stevenson[2,3] and Steven R. Belmain[2*]

Abstract

Anthracnose caused by *Colletotrichum* spp. remains an intractable problem in the most common bean (*Phaseolus vulgaris*) production areas worldwide and can cause total yield loss. Many smallholder farmers are familiar with using botanical extracts to control insect pests; however, there is less familiarity with their use to control fungal diseases due to a lack of evidence. Here, we demonstrate that anthracnose could be controlled effectively by pesticidal plant species that are used for insect control. In laboratory trials, water extracts from 11 plant species could inhibit fungal growth (100%) and spore germination (75–100%) equally well to two commercially available fungicides, the synthetic Mancolaxyl and biofungicide Bioderma. In screenhouse trials, anthracnose disease was reduced by the extracts of three plant species. Moreover, bean crop growth in these botanical treatments did not differ significantly from that observed in the commercial fungicide treated plants. Field trials in a smallholder community reporting severe problems with anthracnose showed an effect similar to the screenhouse results. Field trials resulted in bean seed yields approximately 350 kg/ha higher in bean plants treated with *Azadirachta indica* and *Lippia javanica* at 10% w/v compared to the negative control untreated plants. In all trials, botanical extracts were as effective as commercially available fungicides, suggesting that these botanical extracts could provide dual-purpose pest and disease management for anthracnose and crop pest insects. The outcomes of this research show that prospects for using locally available resources to control anthracnose on common bean are credible and can be combined with controlling insect pests.

Keywords Botanical, Crop disease management, Fungicide, Legume, Pesticidal plant

Background

The endophytic genus *Colletotrichum* is considered one of the most economically detrimental fungal pathogens in agriculture (Dean et al., 2012). *Colletotrichum* spp. cause anthracnose disease in a wide range of woody and herbaceous crops, and each species within the genus can infect a range of crops (Cannon et al. 2012) including many legumes, particularly common bean (*Phaseolus vulgaris*). *Colletotrichum* spp. are adapted to diverse environmental conditions and remain problematic on crops in most countries at multiple latitudes (Damm et al. 2012). *Colletotrichum* spp. attacking common bean are hemibiotrophic, with asymptomatic biotrophic and

*Correspondence:
Steven R. Belmain
s.r.belmain@gre.ac.uk
[1] Department of Sustainable Agriculture, Biodiversity and Ecosystem Management, The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
[2] Department of Agriculture, Health and Environment, Natural Resources Institute, University of Greenwich, Chatham Maritime ME4 4TB, UK
[3] Department of Trait Diversity and Function, Royal Botanic Gardens Kew, Kew Green, Richmond, Surrey TW9 3AE, UK

destructive necrotrophic phases (González et al., 2015). The pathogen can be transmitted through contaminated seed, but also remains in the soil, crop residues, and through cross-infection of other crop and non-crop plant species (Mohammed, 2013). Improving the availability of clean seeds and practices, such as crop rotation, can help to reduce the problem (Yesuf and Sangchote 2007). Breeding resistant bean varieties continues to be a focus of research to improve bean production (Martiniano-Souza et al., 2021). However, for many smallholder producers where land resources are limited and only local susceptible bean varieties are available, the immediate choices available to them are often between doing nothing in the hopes of achieving some yield or using a commercially available fungicide (Mohammed, 2013). Consequently, persistent problems with anthracnose remain for smallholder producers of common bean where the pathogen can cause total yield loss (Yesuf and Sangchote 2007). Commercially available fungicides are available and can be effective, but these are often expensive for smallholders, and many farmers are concerned about using such products for health and safety reasons (Mkindi et al. 2021).

Botanical pesticides have mostly been developed to control insect pests (Isman 2020; Stevenson et al. 2020). Natural products also exist to control fungal pathogens, such as the biocontrol agent *Trichoderma* spp. (Gutiérrez-Moreno et al., 2021; Khan and Javaid, 2020a) and botanical extracts (Marak et al. 2021; Naqvi et al., 2023; Vasuki et al., 2020). Botanical extracts are environmentally friendly and cost-effective alternatives normally used to control insects but offer potential solutions to mitigate anthracnose and other fungal diseases. Such natural products could help to reduce reliance on synthetic fungicides in agricultural fields (Marak et al. 2021). Some plant species, such as *Allium sativum*, *Azadirachta indica*, and *Cymbopogon citratus*, have been shown to have antifungal activity against anthracnose disease. *Ximenia caffra* has also been identified to have antifungal properties attributed to high levels of phenolics and flavonoids effective against several plant fungal pathogens (Maroyi, 2016). *Lippia javanica* and *Ocimum gratissimum* have been reported to impede conidial germination in *Colletotrichum* spp., possessing inherent antifungal properties suitable for seed treatment (Andrade Pinto et al. 2010; Ganiyu et al. 2018; Masangwa et al. 2017).

Widespread use of botanical extracts to control fungal pathogens is not commonly reported, but if plant species that are known to control insect pests could also control fungal pathogens, this would increase farmer incentives and economic cost-benefits of using a single application to control both insects and fungal diseases. Previous research has documented the feasibility of smallholder farmer use of botanicals in crop protection, where qualitative (Mkindi et al. 2021) and quantitative (Mkenda et al., 2015) cost-benefit analyses have shown that using botanical extracts are more economically and socially acceptable in comparison to using synthetic pesticides. It is expected that adoption of more agro-ecologically sustainable crop protection practices by smallholder bean farmers can be facilitated by promoting multifunctional plant materials that are able to control both pests and pathogens whilst facilitating other ecosystem services such as conservation biocontrol (Belmain et al., 2022). Thus, the objectives of the study were to evaluate pesticidal plants with existing information on their insecticidal properties but where information on their fungicidal crop protection properties was unknown.

Results

Laboratory evaluation of botanical extracts to inhibit fungal growth

Diseased bean plants collected from farmer's fields were confirmed to be infected with several closely related species or strains of the genus *Colletotrichum*, and cultures confirmed as *C. boninense* were used in all trials. Screening of extracts from 11 botanical species to inhibit anthracnose growth on nutrient agar plates showed that they were all able to stop fungal growth in comparison to the untreated control (Fig. 1, Table 1). Ethanol extracts were generally more effective than water extracts, showing complete inhibition of fungal growth on the first assessment period at day 3. Some water extracts did not achieve 100% inhibition on day 3; however, inhibition was still high even for the poorest performing treatments (> 90%). The least effective was *X. caffra*, followed by *Zingiber officinale*, *Carica papaya,* and *O. gratissimum*; however, the difference between all botanical treatments was not significant by day 9 of the trial. Although not statistically significant, the efficacy of the positive controls of synthetic fungicide (Mancolaxyl) and biofungicide (Bioderma) was observed to decline slightly over time, with inhibition at 100% on day 3, declining to 70% and 88% on day 6 and 9, respectively.

Laboratory evaluation of botanical extracts to inhibit spore germination

A suspension of fungal spores derived from *C. boninense* was exposed to each of the 11 botanical extracts and fungicide controls, where the untreated control showed 100% spore germination, whilst spore germination was significantly reduced by all treatments by 75% to 100% (Fig. 2). Four species (*A. indica*, *C. papaya, Dysphania ambrosioides*, and *Tagetes minuta*) were able to inhibit

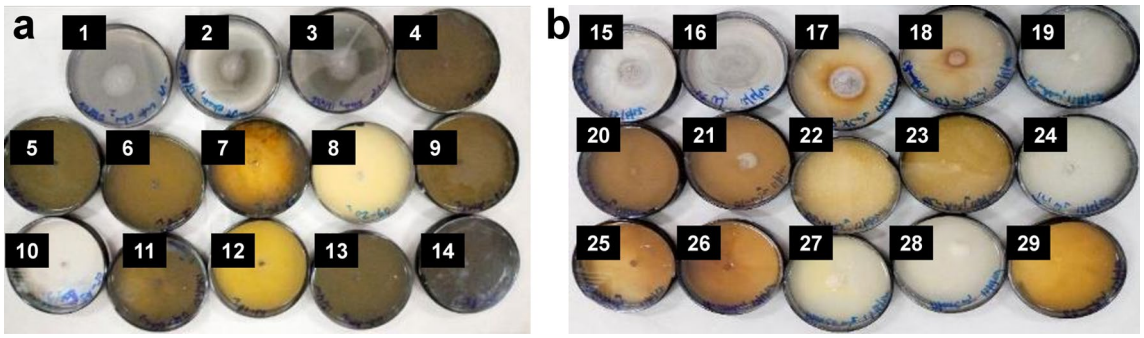

Fig. 1 Agar plates supplemented with botanical extracts were inoculated with *C. boninense*. Images show mycelial inhibition 9 d after inoculation. **a** The ethanolic extracts of botanicals where 1, Untreated control; 2, Untreated control; 3, Control Bioderma; 4, *Dysphania ambrosioides*; 5, *Azadirachta indica*; 6, *Lippia javanica*; 7, *Carica papaya*; 8, *Zingiber officinale*; 9, *Tagetes minuta*; 10, Control Mancolaxyl; 11, *Ocimum gratissimum*; 12, *Ipomoea batatas*; 13, *Annona muricata*; 14, *Ximenia caffra*. **b** The water extracts of botanicals where 15, Untreated control; 16, Untreated control; 17, *Ximenia caffra*, 18, *Annona muricata*; 19, *Allium sativum*; 20, *Lippia javanica*; 21, *Tagetes minuta*; 22, *Zingiber officinale*; 23, *Azadirachta indica*; 24, Control Bioderma; 25, *Ocimum gratissimum*; 26, *Dysphania ambrosioides*; 27, Control Mancolaxyl; 28, Control Mancolaxyl; 29, *Carica papaya*. The colour of the agar is influenced by the plant extracts

Table 1 Fungal growth inhibition rate of *C. boninense* on agar plates by botanical extracts

Treatment	Day 3		Day 6		Day 9	
	Water	Ethanol	Water	Ethanol	Water	Ethanol
Control – untreated	0.0 c	0.0 c	0.0 d	0.0 c	0.0 b	0.0 c
Allium sativum	100.0 a	100.0 a	95.0 a	100.0 a	100.0 a	97.6 b
Annona muricata	100.0 a	100.0 a	95.0 a	100.0 a	89.6 a	100.0 a
Azadirachta indica	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Carica papaya	94.5 b	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Dysphania ambrosioides	100.0 a	100.0 a	95.0 a	100.0 a	95.5 a	100.0 a
Ipomoea batatas	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Lippia javanica	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Ocimum gratissimum	94.5 b	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Tagetes minuta	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a
Ximenia caffra	91.1 b	100.0 a	42.9 c	100.0 a	77.7 a	100.0 a
Zingiber officinale	94.5 b	100.0 a	72.5 b	100.0 a	81.9 a	100.0 a
Control + Mancolaxyl	100.0 a	100.0 a	87.6 ab	100.0 a	70.1 a	100.0 a
Control + Bioderma	100.0 a	93.6 b	95.1 a	90.6 b	88.9 a	100.0 a
R^2 (%)	0.998	1.000	0.964	0.994	0.870	0.999
F	996.355	515420.616	57.533	388.762	14.473	1758.538
Pr (> *F*)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Analysis of Variance (n = 3) followed by Fisher's LSD test where treatments in the same column with different letters are significantly different at the 95% confidence interval

spore germination completely. The worst performing extracts were *O. gratissimum* (75% inhibition) and *Annona muricata* (78% inhibition).

Impact of botanical extracts on potted common bean plants inoculated with anthracnose

In the potted bean plant trial carried out in a screenhouse, all botanical and positive control treatments induced a 70% reduction in the number of infected leaves and stems compared to the untreated control (Fig. 3). Bean plant growth was adversely affected by anthracnose, where the untreated control showed a reduced average plant height of 19 cm, whereas plant height across all treatments was 5–13 cm higher (Fig. 4). Untreated control plants also had fewer total leaves and pods per plant than all the botanical and positive control treatments (Fig. 4). In terms of number of pods per plant, the best performing treatments in this study were *D. ambrosioides*

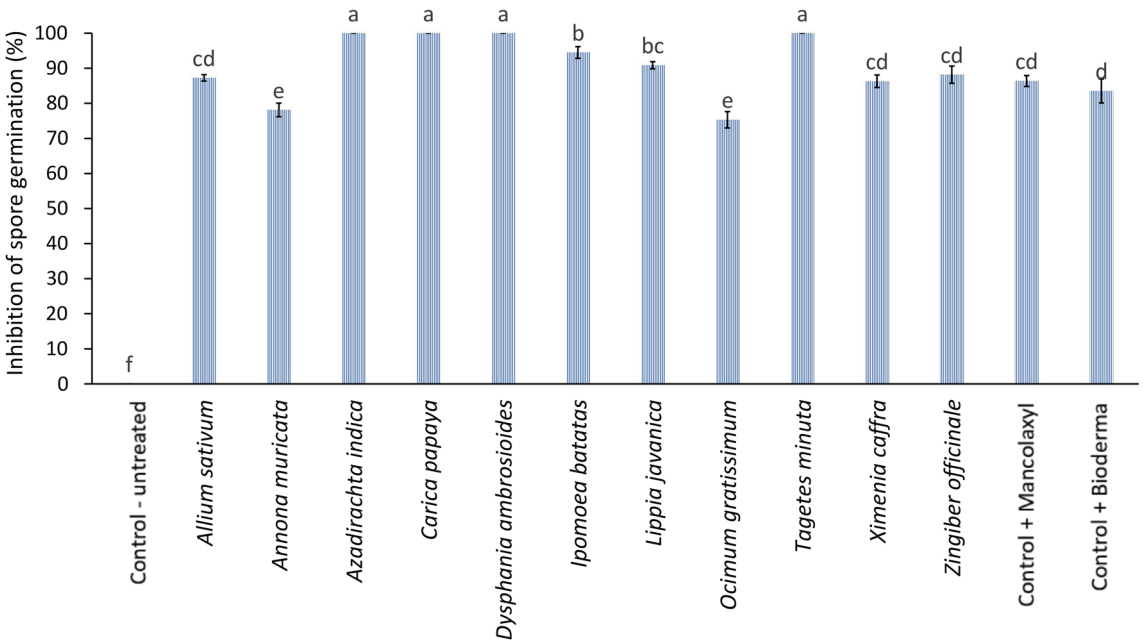

Fig. 2 Inhibition of spore germination of C. *boninense* in presence of different botanical water extracts (10% w/v). Data shown are means ± SE (n = 3). Analysis of Variance followed by Fisher's LSD test where treatments with different letters are significantly different at the 95% confidence interval, $P < 0.0001$

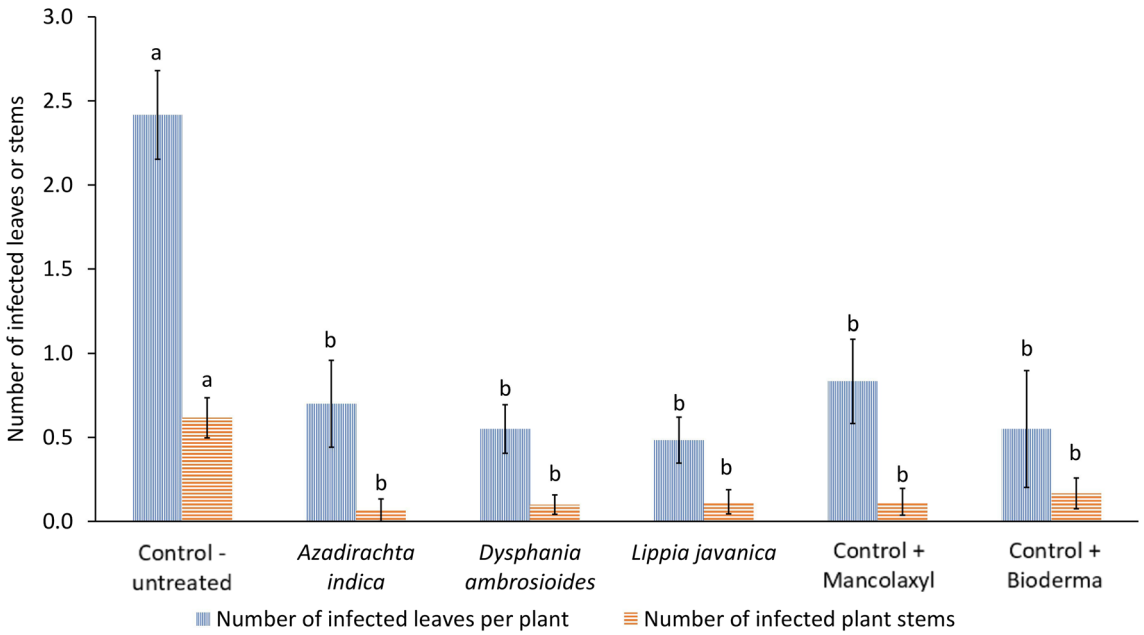

Fig. 3 Impact of botanical extracts on the anthracnose disease of common bean plants. Potted bean plants were initially infected with the fungal pathogen C. *boninense* and subsequently treated with botanical extracts. Disease levels were analysed five weeks after the appearance of anthracnose infection. Data shown are means ± SE. Analysis of Variance followed by Fisher's LSD test where treatments in the same column with different letters are significantly different at the 95% confidence interval, $P < 0.0001$

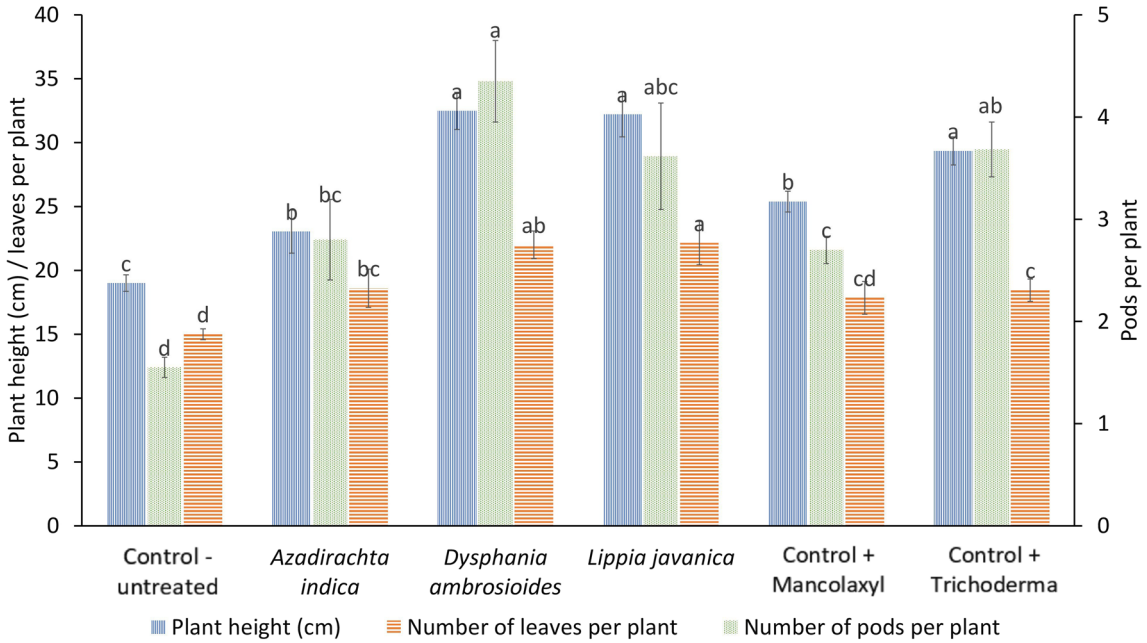

Fig. 4 Impact of botanical extracts on common bean growth parameters. Potted bean plants were initially infected with the anthracnose pathogen *C. boninense,* and data were collected at the time of harvest. Data shown are means ± SE. Analysis of Variance followed by Fisher's LSD test where treatments in the same column with different letters are significantly different at the 95% confidence interval, $P < 0.0001$

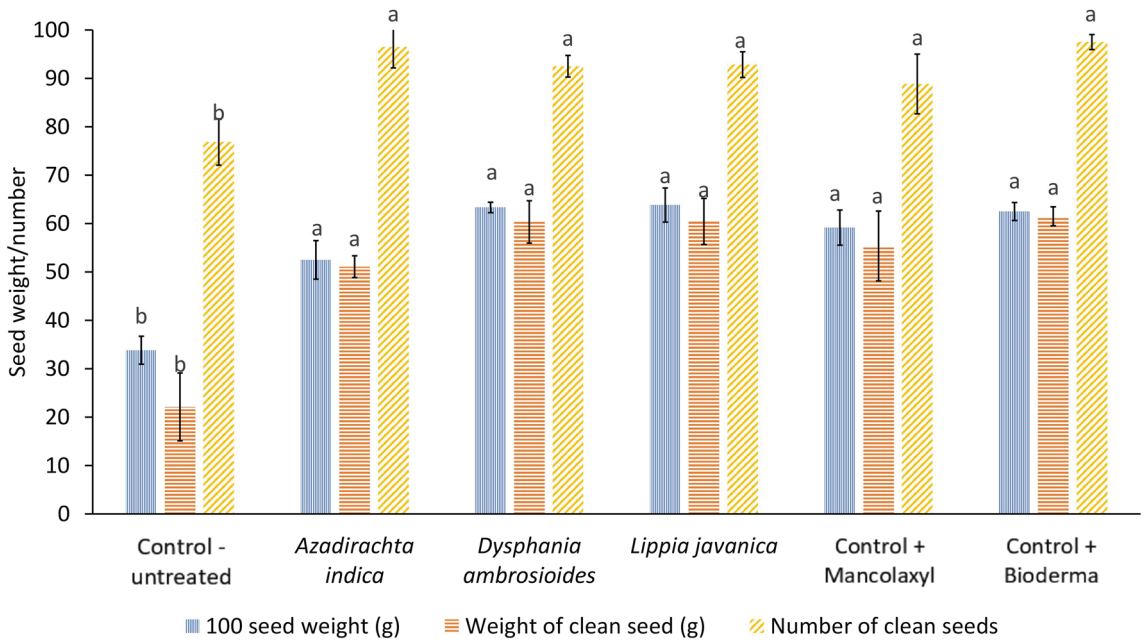

Fig. 5 Impact of botanical extracts on common bean seed yield and quality. Bean seeds were initially infected with the anthracnose pathogen *C. boninense* and subsequently treated with botanical extracts. Treated seeds were planted in pots and grown in a screenhouse. Data shown are means ± SE. Analysis of Variance followed by Fisher's LSD test where treatments in the same column with different letters are significantly different at the 95% confidence interval, $P < 0.0001$

(4.4 pods/plant), Bioderma (3.7), and *L. javanica* (3.6). *A. indica* (2.8) and Mancolaxyl (2.7) were still significantly better than the untreated group, which had 1.5 pods/plant. The impact of anthracnose on plant health ultimately affected bean seed quality where, at harvest, the weight of 100 randomly chosen seeds from the untreated control was almost 50% less than that from any of the treatments (Fig. 5). From these seeds, the proportion of seeds assessed as clean and free of anthracnose symptoms was significantly higher from the treatments than that of the untreated control (Fig. 5).

Impact of botanical extracts on common bean planted in the field

In the field trial, common bean seeds were sown and then spray-inoculated with *C. boninense* after plant emergence and sprayed 7 d after with botanical extracts and the positive control fungicides. Results were similar to those observed in the screenhouse trial. Anthracnose disease severity was significantly higher in the untreated control bean plants, with higher numbers of infected leaves, stems, and pods, than in the botanical and positive control fungicide treatments (Table 2). The overall impact of anthracnose on plant growth was subtler, where statistical differences in mean plant height and total number of leaves were observed between the untreated control and treatments. These biological effects were small, with a difference of about 1 cm in plant height between treatments and control and, on average, about one leaf less per untreated control plant compared to the treatments (Table 2). However, anthracnose infection still reduced the average weight of 100 randomly selected seeds by about 10% in the untreated control compared to the treatments. The overall bean yield was 10–15% higher in the botanical and fungicide treatments than in the untreated control (Fig. 6). With respect to bean yield, the best-performing botanicals were *A. indica* and *L. javanica,* which were as good as the Mancolaxyl and Bioderma fungicide treatments (Fig. 6).

Discussion

Our data show that extracts from all 11 plant species were effective, with some as effective as two commercially available products, the synthetic fungicide Mancolaxyl and the bio-fungicide Bioderma, in inhibiting germination of *C. boninense* spores. Some of the plant species were less effective; for example, *X. caffra* and *Z. officinale* water extracts had slightly less fungal growth inhibitory effects, and *A. muricata* and *O. gratissimum* were slightly less effective in preventing spore germination. These less effective species were omitted from further trials. However, there is considerable evidence that these four species do have antifungal properties for crop protection (Madjouko et al., 2019; Maroyi, 2016; Radice et al., 2022; Tsala et al. 2022). Three plant species (*A. indica, L. javanica,* and *D. ambrosioides*) were chosen that are known to be highly abundant in the study area as well as more cosmopolitan throughout

Table 2 Impact of botanical extracts on anthracnose disease progression and on common bean growth in farmer field trial

Treatment	Number of plants infected	Disease severity score	Disease severity (%)	Plant height (cm)	Number of infected leaves	Total number of leaves per plant	Number of infected stems per plant	Number of infected pods per plant	Total number of pods per plant
Control—untreated	0.55 a	1.23 a	20.55 a	22.09 c	0.57 a	19.20 b	0.44 a	2.24 a	8.59 d
Azadirachta indica	0.42 bc	0.55 b	9.22 b	23.74 b	0.40 b	20.47 a	0.33 b	1.29 b	8.88 bc
Dysphania ambrosioides	0.40 bc	0.48 b	8.14 b	22.93 bc	0.33 b	19.16 b	0.27 bc	1.10 b	9.10 ab
Lippia javanica	0.37 c	0.48 b	8.13 b	23.72 b	0.38 b	20.32 a	0.23 c	1.12 b	8.79 cd
Control + Mancolaxyl	0.43 b	0.50 b	8.39 b	22.96 bc	0.35 b	20.27 a	0.32 b	1.27 b	9.21 a
Control + Bioderma	0.39 bc	0.48 b	8.05 b	24.88 a	0.31 b	20.71 a	0.25 c	1.22 b	9.12 ab
R^2 (%)	0.08	0.36	0.36	0.06	0.05	0.03	0.08	0.23	0.05
F	9.76	61.01	61.01	7.24	5.96	3.39	9.65	32.79	6.65
Pr > (*F*)	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.005	< 0.0001	< 0.0001	< 0.0001

Data shown are means. Analysis of Variance (n = 6) followed by Fisher's LSD test where treatments in the same column with different letters are significantly different at the 95% confidence interval

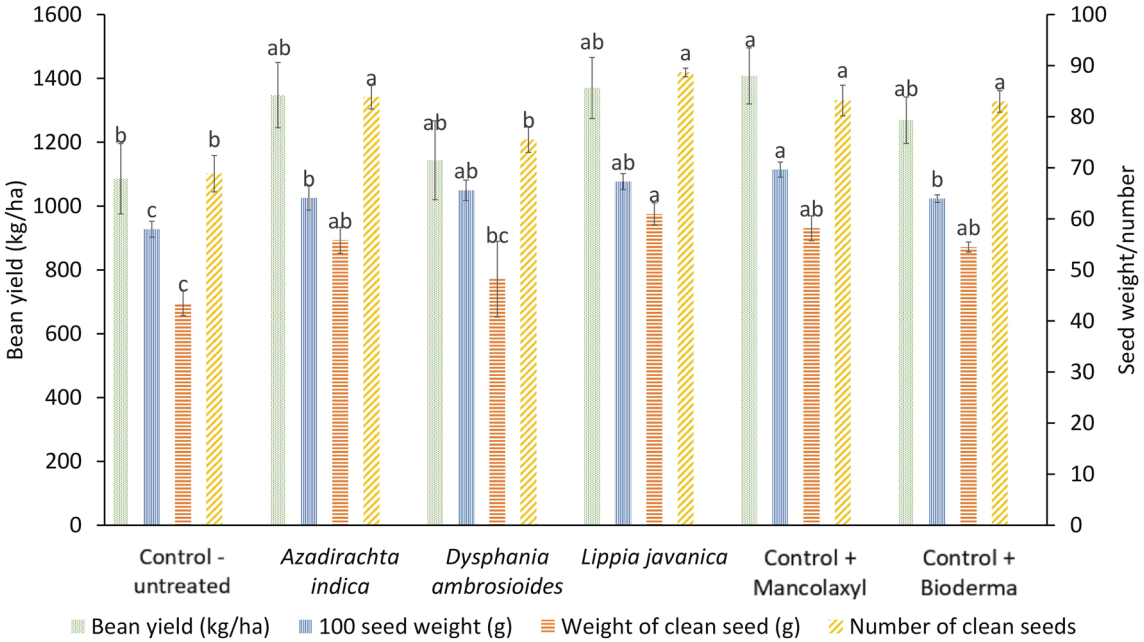

Fig. 6 Impact of botanical treatments on common bean seed yield and the quality of the seed produced in a farmer field trial. The bean plants were initially infected with the fungal pathogen anthracnose, *C. boninense* and subsequently treated with botanical extracts. Analysis of Variance followed by Fisher's LSD test where treatments in the same column with different letters are significantly different at the 95% confidence interval, $P < 0.0001$

the tropics. These three species are often cultivated and promoted for other reasons across sub-Saharan Africa, making their use sustainable as supply can potentially be increased relatively quickly to meet the increasing demand. Further, the chemistry of these three species is well-known, and where they have undergone some safety evaluations and have uses as teas and spices (Boeke et al. 2004; Braga et al., 2021; Brahmachari 2004; Kasali et al., 2021; Maroyi, 2017; Singh and Pandey 2022; Viljoen et al. 2005). The bioactive insecticidal properties of *L. javanica* have been attributed to perialdehyde (Kamanula et al., 2017) which is also known to possess antifungal properties (Chen et al. 2020) and, therefore, may account for the activity reported here. Further bioactive compounds in *L. javanica* include limonene, terpinen-4-ol, and artemisia ketone (Endris et al., 2015), non-polar compounds that often associate with essential oils and have to be extracted in ethanol (Amaral et al. 2020). Thus, these compounds may also account for the activity of the extract. Other antifungal compounds found in *A. indica* include nimbidin, gedunin, and cyclic tri- and tetra- sulphides (Gupta et al. 2019), all of which would be extracted in ethanol. Antifungal properties in *D. ambrosioides* have been attributed to p-cymene, ascardiole (Stappen et al. 2018), and α-terpinene (Dagni et al. 2022), monoterpenes that should be extracted in ethanol. Phytochemical constituents, such as terpenes, ketones, and phenols, are often toxic to fungal pathogens including *Colletotrichum* spp. (Dagni et al. 2022; Endris et al., 2015). The modes of action of specific plant compounds are often not well studied but have been linked to breaking down fungal cell walls and cell organelles (Jiang et al. 2023; Prakash et al. 2022), inhibiting spore germination, reducing mycelial growth and germ tube elongation, delaying sporulation, and inhibiting protein synthesis (Yoon et al. 2013).

The screenhouse trial using potted beans showed that, if left untreated, *C. boninense* could severely impact the growth and development of common bean when infected at the seed stage. Infected seed used for planting the next crop is often how anthracnose is transmitted (Shao and Teri 1985; Sharma et al. 2008), and the untreated control plants in this trial generally had a much lower percentage of clean seeds (76%) compared to the botanical and fungicide treated plants (89–98%). Bean seed yield from botanical and fungicide treatments was 50% more than the yield from untreated control plants. This may be partly attributed to anthracnose infection interfering with photosynthesis (Bassanezi et al. 2001; Lopes and Berger 2001). Similar research using similar methodologies has evaluated botanical extracts against *Colletotrichum* spp. on legume crops (Ganiyu et al. 2018; Masangwa et al., 2013; Vasuki et al., 2020). These studies

confirm that many plant extracts with known activities against insect pests may also be effective in controlling fungal pathogens.

The farmer field trial showed statistically significant differences between the untreated control and all other treatments for all measured infection and plant growth parameters. The differences between untreated and treated beans in the field trial were not as great as observed in the screenhouse trial. For example, the difference in the average number of pods per plant was about 3 pods more per plant in treated beans compared to untreated beans in the screenhouse trial; the average number of pods per plant was about 1 pod more per plant in treated beans compared to untreated beans in the field trial. Such observations are commonly reported when comparing trials with a high degree of parameter control, such as laboratory or screenhouse, vs. trials where controlling parameters is more difficult, such as field and farmer participatory trials (Bugeme et al. 2015; Paparu et al. 2008). Although this difference in pod number may seem small, the field trials showed that bean crop harvests were about 350 kg/ha higher when treated with *A. indica* or *L. javanica* compared to the untreated control. Larger scale trials at the farm level could help validate these results for a number of different legume crops affected by anthracnose disease (Dias et al. 2016; Rao et al., 2020).

Although it is widely accepted that *C. lindemuthianum* is the main species affecting common bean production (Padder et al. 2017), the isolates identified in northern Tanzania is *C. boninense*. Although our research suggests this is the first report of *C. boninense* infecting common bean, this is perhaps not surprising as *C. boninense* is known to infect a diverse range of host plants, including coffee, mango, pepper, tomato, and avocado, all of which grow in the area. An analysis of the inherent genetic diversity argues that *C. boninense* should be considered a species complex (Damm et al. 2012). Cross-infection in different crops is well-known for several species of *Colletotrichum* (Freeman et al., 2013). Further work is planned to determine whether *C. boninense* is co-infecting other major crops in the area such as coffee.

Conclusions

Bean anthracnose can be successfully controlled using extracts of plant materials that are also being used to control insect pests (Ratto et al. 2022). The crude extracts of plants often contain several different bioactive chemicals, thereby exploiting the natural capacity of plants to defend themselves against a range of pests and diseases (Khan and Javaid, 2020b). The use of such crude botanical extracts also has other benefits for crop production where their application acts as foliar fertilisers (Mkindi et al., 2020), thereby increasing crop yields through controlling insect pests and fungal pathogens and increasing plant resilience through access to key nutrients. The use of botanical extracts facilitates the conservation of biological control in the ecological system as the plant extracts have less impact on natural enemies, leaving them to prey on pests after the use of botanical treatment (Tembo et al., 2018). These multiple benefits of using locally available natural resources in crop protection and production reduce reliance on the import of synthetic inputs, increasing the resilience of smallholder farmers to promote local circular economies (Belmain et al., 2022). The outcomes of our research show that prospects for using locally available resources to control anthracnose on common beans are credible.

Methods

Study site

Laboratory and screenhouse trials were conducted at the Nelson Mandela African Institution of Science and Technology (NMAIST) in Arusha, Tanzania. Fieldwork was carried out in a smallholder farming community in the Lyamungo-Mulama Ward, Hai District, Kilimanjaro Region (latitude 3° 23′ 16″–3° 38′ 35″ S longitude 37° 24′ 25″–38° 35′ 15″ E). Ethical clearance for the work was approved through the NMAIST ethics committee, and permission for field work was granted by the Hai District office and the farmers where trials were based (COSTECH 2021-181-NA-2021-061). The area is at an elevation of 700–1500 m above sea level, temperature ranges from 15 to 30 °C, and mean annual rainfall ranges from 500 mm at lower elevations to 2000 mm at higher elevations, with the most rain occurring from March to July. In this area, common bean and maize are the main agricultural crops, alongside the significant production of banana and coffee. As temperatures are relatively cool during the growing season, common bean is prone to anthracnose epidemics and is considered by local farmers as one of the major crop production constraints.

Botanicals preparation

Plant materials were selected based on their local use for pest control by farmers, their local abundance, low toxicity to non-target organisms, and published evidence pointing to their antifungal properties (Lengai et al. 2020). Plants were collected from locally growing sources around the district. Leaf material was collected from *A. indica*, *D. ambrosioides*, *L. javanica*, *A. muricata*, *C. papaya*, *Ipomoea batatas,* and *O. gratissimum*. Leaves + fruits of *X. caffra* and leaves + flowers of *T. minuta* were collected as well as tubers of *A. sativum* and *Z. officinale*.

Herbarium specimens were deposited at the Department of Sustainable Agriculture and Biodiversity Ecosystem Management, Nelson Mandela African Institution of Science and Technology. All plant materials were washed with water and dried in the shade at ambient temperature. Dried materials were ground to powder, passed through a sieve (10 mm mesh), and stored in plastic bags in dark, dry conditions until use.

To create solvent extracts, plant powder (100 g) was placed in a flask containing 500 mL of 97% ethanol and mixed for 24 h at ambient temperature. The solution was then filtered through cheesecloth followed by filter paper (Whatman #2). Extracts were left to evaporate down to a volume of 10 mL, then placed in sealed glass vials within a refrigerator (4 °C) until required. Water extracts were made by placing 100 g of each botanical in 1 L of sterile distilled water and mixed at ambient temperature for 24 h, making a 10% w/v solution. Water solutions were similarly filtered, with extracts then stored within a refrigerator (4 °C) until required.

Fungal pathogen preparation

The anthracnose pathogen was collected, isolated, and cultured in the laboratory by first collecting locally growing bean plants with symptoms of infection (e.g., black lesions and spots, dark streaking veins). A total of 30 samples were collected where small pieces of infected leaves and stems from each were disinfected by placing them in 70% ethanol for 1 min, then moved to a 3.5% sodium hypochlorite solution for 2 min and then rinsed in 70% ethanol for 30 s. The infected plant tissues were then serially rinsed using three beakers containing deionized water for 3 min in each beaker (Perfect et al. 1999). Tissues were blotted dry and added to fresh culture medium potato dextrose agar (PDA) containing streptomycin (Sigma) at 50 mg/L. The mixture was plated on petri dishes sealed with parafilm and incubated at room temperature (24 ± 2 °C) in constant dark for seven days (Masangwa et al., 2013). Culture isolates were confirmed to be species *Colletotrichum boninense* using morphological and molecular methods. DNA extractions from pure cultures were performed using the Macherey–Nagel DNA extraction kit following the manufacturer's guidelines (Gadaga et al., 2018). This was followed by conventional PCR amplification targeting the Internal Transcribed Spacer region (ITS) using the forward primer ITS1F (5′-CTTGGTCATTTAGAGGAA GTAA-3′) and reverse primer ITS4 (5′-TCCTCCGCT TATTG ATATGC-3′) (Martiniano-Souza et al., 2021). The amplicons were purified for Sanger Sequencing using ZR-96 DNA Sequencing Clean-up Kit™ (Zymo, USA), and sequenced in both forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) using the ABI 3730xl Genetic Analyzer (Applied Biosystems, Thermo Fisher Scientific).

Laboratory evaluation of botanical extracts to inhibit fungal growth

Petri dishes (9 cm diam.) were first part-filled with PDA mixed with 4 mL of each ethanol or water extract (10% w/v) of each of the 11 botanical species (*A. sativum*, *A. muricata*, *A. indica*, *C. papaya*, *D. ambrosioides*, *I. batatas*, *L. javanica*, *O. gratissimum*, *T. minuta*, *X. caffra*, and *Z. officinale*). Then 20 mL of plain PDA medium at 40–45 °C was added to each petri dish (24 mL total PDA) and gently shaken to distribute the botanical extract throughout the PDA to realise an extract concentration of 0.2 mL/mL PDA. Two positive control treatments were made in a similar way using a synthetic fungicide (Mancolaxyl 72 WP, comprised of metalaxyl 8% + mancozeb 64% WP; Bio Agro Chemicals) and a commercially available biofungicide (Bioderma WP, comprised of the fungus *Trichoderma viride*; Tanzania Crop Care Ltd.). Unamended PDA (adding 4 mL water or ethanol to 20 mL PDA) was used as the negative control.

A 5 mm diameter plug of *C. boninense* from 7-day-old fungal culture was placed in the centre of each Petri dish containing the botanical and control treatments, 14 treatments in total with 3 replicates using a completely randomised design. The Petri dishes were sealed with parafilm and incubated at room temperature (24 ± 2 °C). The evaluation was performed by measuring (mm) the inhibition zones of mycelial growth after 3, 6, and 9 days of incubation. The formula for calculating mycelial growth diameter's proportional inhibition was used (Giamperi et al., 2020).

$$\text{Mycelial growth inhibition}(\%) = \frac{dc - dt}{dc} \times 100$$

where (dc) is the average diameter of the fungal colony of the negative control (unamended PDA) and (dt) is the diameter of the fungal colony grown in the presence of the botanical treatments or positive controls.

The percentage of mycelial growth inhibition by treatment was calculated, and the average percentage of mycelial growth inhibition was used in rating the effectiveness of each botanical extract. The effectiveness of treatments was categorized as inhibiting radial mycelial growth of *C. boninense* by giving them a score (Bogumił et al. 2013), where 1 = Low antagonistic activity (I < 51%), 2 = Moderate antagonistic activity (I = 51–59%), 3 = High antagonistic activity (I = 60–75%), and 4 = Very high antagonistic activity (I > 75%). Where (I) is the inhibiting radial growth (Muthomi et al., 2017; Yang et al. 2012).

Laboratory evaluation of botanical extracts to inhibit spore germination

The impact of treatments on fungal spore germination was performed using water extracts containing 0.05% Tween-20 to aid dispersion. For each treatment, 10 µL of the extract was placed in an Eppendorf tube and mixed with 10 µL spore suspension of *C. boninense* spores (1×10^4 spores/mL). For the negative control, the spore suspension was mixed with an equal volume of water only containing 0.05% Tween-20. From each Eppendorf, 10 µL of the treated spore suspension was placed in another tube and incubated in a humidified chamber at 25 °C for 24 h, with 14 spores assessed per treatment in a completely randomized design (CRD) with 3 replicates per treatment. After incubation, each 10 µL treated spore suspension was mixed with 10 µL of trypan blue dye. From this, 10 µL was transferred to a haemocytometer slide and observed under 40× magnification with a phase-contrast microscope to record spore germination (Quintana-Rodriguez et al. 2015). A spore was recorded as having germinated when the length of the germ tube and the length of the spore were at least the same or when there were multiple germ tubes from the same spore (Pasche et al. 2004). Ten different focus areas were scored on each slide. The percentage of spore germination inhibition was calculated as:

$$\text{Spore germination inhibition (\%)} = \frac{NSGC - NSGT}{NSGC} \times 100$$

where (NSGC) number of spores germinated in the control, (NSGT) number of spores germinated in the treatment.

Impact of botanical extracts on potted common bean plants inoculated with *C. boninense*

Bean seeds were artificially inoculated with *C. boninense* spores (Masangwa et al., 2013). Bean seeds of the variety 'Lyamungo 90' were first soaked in distilled water for 30 min. A sterilised needle was used to make a small hole into the cotyledon of each seed. The punctured seeds were then mixed with a solubilised (using a few drops of Tween-20) suspension of a 10-day-old culture of *C. boninense* (Bigirimana and Höfte 2008). After 4 h in the dark, the suspension was left to drain for about 2 h. The bean seeds were then placed onto moist paper towels in a sealed container and left overnight to permit fungal growth. Infected seeds were separately drenched in 10% (w/v) water extracts of *L. javanica*, *A. indica,* and *D. ambrosioides,* as well as positive controls of the synthetic fungicide (Mancolaxyl), the biofungicide (Bioderma) and the untreated negative control where infected seeds were drenched in distilled water, providing six treatments in total. Treated seeds were retained in the dark at 25°C for 24 h. Eight seeds of each treatment were planted in pots (28 cm diameter) filled with moist sterilized compost, with six pots per treatment. All agronomic practices were standardised for optimal bean plant growth (Mandiriza et al. 2018). Measurements were recorded weekly on plant growth parameters and infection of different plant parts. Disease severity data were collected weekly starting from the seventh day after planting (Masangwa et al., 2013). Assessment of severity was based on a scale of 0–5 (Stavely 1985), where 0 = no disease, 1 ≤ 20%, 2 = 21–40%, 3 = 41–60%, 4 = 61–80%, 5 = 81–100% leaf area infected (Muthomi et al., 2017).

Impact of botanical extracts on common bean planted in the field

Clean seeds of the 'Lyamungo 90' bean variety were planted in a field at Mulama Ward, Hai District over the cropping season of April–July, 2022. The field experiment contained six treatments each with six replicates in a randomized complete block design (Gaudencia et al., 2020). Common beans were planted in 36 plots each with six rows and 80 plants in every row. Each plot consisted of 3 m in width and 8 m in length, and one plot contained 480 bean plants. The distance between one plot and another plot was 2 m. Four seeds were planted in each hole and thinned to two plants, and 15 days from planting, 15 plants were marked with tags starting in the centre with four rows for the data collection on plant growth and disease progression. All agronomic practices required for common bean production were observed. *C. boninense* inocula were sprayed on whole plant parts using a knapsack sprayer at the start of the first leaves emerging on day 7 after planting (Gillard et al. 2012). Treatments consisted of 3 botanical species (*L. javanica*, *A. indica,* and *D. ambrosioides*), two positive control treatments (Mancolaxyl and Bioderma), and a negative soapy water (0.1% Tween-20) only treatment. Plant species were extracted in soapy water (0.1% Tween-20) and applied at 10% (w/v) using an application volume of 1.44 L per 24 m^2 plot (600 L/ha) (Gil et al. 2019) where positive control treatments were applied as per label instructions. Each treatment was sprayed on all bean plants in each plot replicate using a knapsack sprayer at the first sign of anthracnose appearance, which was 14 days after sowing. Plot sampling to discover infected plants was carried out using a zigzag (Z) sampling strategy. Plants with anthracnose symptoms were counted, and the percentage of disease incidence per plot was calculated (Kiptoo et al., 2020). Disease and plant growth data were collected on 15 pre-marked plants starting 14 days after disease emergence, where recorded information included the number of infected pods per plant, the height of the plant, seeds per pod, and the number of pods per plant. At harvest,

bean pods were left to dry in a ventilated space. Seed yield harvested from every plot was calculated in tons per hectare. The seed weight was recorded from one hundred randomly selected seeds (Amin et al., 2014).

Statistical analysis

All data were subjected to an analysis of variance (ANOVA) at $P < 0.05$. Values were separated using the Fisher's Least Significant Difference test at the 95% confidence interval. All statistical analyses were performed using Xlstat version 17.01 (Addinsoft, Paris, France).

Acknowledgements

Authors acknowledge the contributions of research assistants from the Department of Sustainable Agriculture, Biodiversity and Ecosystem Management, The Nelson Mandela African Institution of Science and Technology for their help in assisting field and screenhouse activities and the Tanzania Coffee Research Institute for field access.

Author contributions

SRB, PCS, and AGM conceived the work; TMK, SRB, PCS, and AGM designed the methods; TMK carried out the field work; TMK and SRB analysed the data; TMK and SRB wrote the original manuscript; All authors read and revised the manuscript and agreed to the published version of the manuscript.

Funding

This research was supported by a Grant (20-034) from the McKnight Foundation Global Collaboration for Resilient Food Systems Research Programme.

Availability of data and materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Declarations

Ethical approval and consent to participate

Ethical clearance for the work was approved through the Nelson Mandela African Institution of Science and Technology ethics committee, and permission for field work was Granted by the Hai District office and by the farmers where trials were based (COSTECH 2021-181-NA-2021-061).

Consent for publication

All authors have approved and consented to publication.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 3 November 2023 Accepted: 29 February 2024
Published online: 11 April 2024

References

Amaral KD, Gandra LC, Souza DJ, Della Lucia TMC. Deleterious action of azadirachtin against the mutualistic fungus of leaf-cutting ants. J Basic Microbiol. 2020;60:931–7. https://doi.org/10.1002/jobm.202000541.

Amin M, Teshele J, Tesfay A. Evaluation of bioagents seed treatment against colletotrichum lindemuthianum, in haricot bean anthracnose under field condition. Res Plant Sci. 2014;2:22–6. https://doi.org/10.12691/plant-2-1-5.

Andrade Pinto JM, Souza EA, Oliveira DF. Use of plant extracts in the control of common bean anthracnose. Crop Prot. 2010;29:838–42. https://doi.org/10.1016/J.CROPRO.2010.03.006.

Bassanezi RB, Amorim L, Filho AB, Hau B, Berger RD. Accounting for photosynthetic efficiency of bean leaves with rust, angular leaf spot and anthracnose to assess crop damage. Plant Pathol. 2001;50:443–52. https://doi.org/10.1046/j.1365-3059.2001.00584.x.

Belmain SR, Tembo Y, Mkindi AG, Arnold SEJ, Stevenson PC. Elements of agro-ecological pest and disease management. Elementa. 2022. https://doi.org/10.1525/elementa.2021.00099.

Bigirimana J, Höfte M. Bean anthracnose: inoculation methods and influence of plant stage on resistance of *Phaseolus vulgaris* cultivars. J Phytopathol. 2008;149:403–8. https://doi.org/10.1111/j.1439-0434.2001.tb03870.x.

Boeke SJ, Kossou DK, Van Huis A, Van Loon JJA, Dicke M. Field trials with plant products to protect stored cowpea against insect damage. Int J Pest Manag. 2004;50:1–9. https://doi.org/10.1080/09670870310001619282.

Bogumił A, Paszt LS, Lisek A, Trzciński P, Harbuzov A. Identification of new *Trichoderma* strains with antagonistic activity against *Botrytis cinerea*. Folia Horticult. 2013;25:123–32. https://doi.org/10.2478/fhort-2013-0014.

Braga TM, Rocha L, Chung TY, Oliveira RF, Pinho C, Oliveira AI, et al. *Azadirachta indica* A. Juss. in vivo toxicity—an updated review. Molecules. 2021. https://doi.org/10.3390/molecules26020252.

Brahmachari G. Neem—an omnipotent plant: a retrospection. ChemBioChem. 2004;5:408–21. https://doi.org/10.1002/cbic.200300749.

Bugeme DM, Knapp M, Ekesi S, Chabi-Olaye A, Boga HI, Maniania NK. Efficacy of *Metarhizium anisopliae* in controlling the two-spotted spider mite *Tetranychus urticae* on common bean in screenhouse and field experiments. Insect Sci. 2015;22:121–8. https://doi.org/10.1111/1744-7917.12111.

Cannon PF, Damm U, Johnston PR, Weir BS. Colletotrichum—current status and future directions. Stud Mycol. 2012;73:181–213. https://doi.org/10.3114/sim0014.

Chen L, Qu S, Yang K, Liu M, Li Y, Keller NP, et al. Perillaldehyde: a promising antifungal agent to treat oropharyngeal candidiasis. Biochem Pharmacol. 2020;180:114201. https://doi.org/10.1016/j.bcp.2020.114201.

Dagni A, Hegheș SC, Suharoschi R, Pop OL, Fodor A, Vulturar R, et al. Essential oils from *Dysphania* genus: traditional uses, chemical composition, toxicology, and health benefits. Front Pharmacol. 2022;13:1024274. https://doi.org/10.3389/FPHAR.2022.1024274/BIBTEX.

Damm U, Cannon PF, Woudenberg JHC, Johnston PR, Weir BS, Tan YP, et al. The Colletotrichum boninense species complex. Stud Mycol. 2012;73:1–36. https://doi.org/10.3114/sim0002.

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu PD, et al. The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol. 2012;13:414–30. https://doi.org/10.1111/j.1364-3703.2011.00783.x.

Dias MD, Pinheiro VF, Café-Filho AC. Impact of anthracnose on the yield of soybean subjected to chemical control in the north region of Brazil. Summa Phytopathol. 2016;42:18–23. https://doi.org/10.1590/0100-5405/2114.

Endris A, Asfaw N, Bisrat D. Chemical composition, antimicrobial and antioxidant activities of the essential oil of *Lippia javanica* leaves from Ethiopia. J Essent Oil Res. 2015;28:221–6. https://doi.org/10.1080/10412905.2015.1108880.

Freeman S, Horowitz-Brown S, Afanador-Kafuri L, Maymon M, Minz D. Colletotrichum: host specificity and pathogenicity on selected tropical and subtropical crops. Acta Hortic. 2013;975:209–16.

Gadaga SJC, Siqueira CdS, Machado JdC. Molecular detection of *Colletotrichum lindemuthianum* in bean seed samples. J Seed Sci. 2018;40:370–7. https://doi.org/10.1590/2317-1545v40n4192761.

Ganiyu SA, Popoola ARR, Yussuf TFF, Owolade OFF, Gbolade JOO. Management of anthracnose disease of cowpea with three plant leaf extracts for enhanced grain yield in Abeokuta, Nigeria. Niger Agric J. 2018;49:1–7.

Gaudencia JK, Miriam GK, Lexa GM, Oliver KK. Morphological traits associated with anthracnose (*Colletotrichum lindemuthianum*) resistance in selected common bean (*Phaseolus vulgaris* L.) genotypes. Afr J Plant Sci. 2020;14:45–56. https://doi.org/10.5897/AJPS2019.1909.

Giamperi L, Bucchini AEA, Ricci D, Tirillini B, Nicoletti M, Rakotosaona R, et al. *Vepris macrophylla* (Baker) I. Verd essential oil: an antifungal agent against phytopathogenic fungi. Int J Mol Sci. 2020;21:2776. https://doi.org/10.3390/ijms21082776.

Gil E, Campos J, Ortega P, Llop J, Gras A, Armengol E, et al. DOSAVIÑA: tool to calculate the optimal volume rate and pesticide amount in vineyard spray applications based on a modified leaf wall area method. Comput Electron Agric. 2019;160:117–30. https://doi.org/10.1016/j.compag.2019.03.018.

Gillard CL, Ranatunga NK, Conner RL. The control of dry bean anthracnose through seed treatment and the correct application timing of foliar fungicides. Crop Prot. 2012;37:81–90. https://doi.org/10.1016/j.cropro.2012.02.009.

González AM, Yuste-Lisbona FJ, Paula Rodiño A, De Ron AM, Capel C, García-Alcázar M, et al. Uncovering the genetic architecture of colletotrichum lindemuthianum resistance through QTL mapping and epistatic interaction analysis in common bean. Front Plant Sci. 2015. https://doi.org/10.3389/fpls.2015.00141.

Gupta A, Ansari S, Gupta S, Narwani M, Gupta M, Singh M, et al. Therapeutics role of neem and its bioactive constituents in disease prevention and treatment. J Pharmacogn Phytochem. 2019;8:680–91.

Gutiérrez-Moreno K, Ruocco M, Monti MM, de la Vega OM, Heil M. Context-dependent effects of trichoderma seed inoculation on anthracnose disease and seed yield of bean (*Phaseolus vulgaris*): ambient conditions override cultivar-specific differences. Plants. 2021. https://doi.org/10.3390/plants10081739.

Isman MB. Botanical insecticides in the twenty-first century—fulfilling their promise? Annu Rev Entomol. 2020;65:233–49. https://doi.org/10.1146/annurev-ento-011019-025010.

Jiang H, Zhong S, Schwarz P, Chen B, Rao J. Antifungal activity, mycotoxin inhibitory efficacy, and mode of action of hop essential oil nanoemulsion against *Fusarium graminearum*. Food Chem. 2023;400:134016. https://doi.org/10.1016/j.foodchem.2022.134016.

Kamanula JF, Belmain SR, Hall DR, Farman DI, Goyder DJ, Mvumi BM, et al. Chemical variation and insecticidal activity of *Lippia javanica* (Burm. f.) Spreng essential oil against *Sitophilus zeamais* Motschulsky. Ind Crops Prod. 2017;110:75–82. https://doi.org/10.1016/j.indcrop.2017.06.036.

Kasali FM, Tusiimire J, Kadima JN, Agaba AG. Ethnomedical uses, chemical constituents, and evidence-based pharmacological properties of *Chenopodium ambrosioides* L.: extensive overview. Futur J Pharm Sci. 2021. https://doi.org/10.1186/s43094-021-00306-3.

Khan IH, Javaid A. In vitro biocontrol potential of trichoderma pseudokoningii against macrophomina phaseolina. Int J Agric Biol. 2020a;24:730–6. https://doi.org/10.17957/IJAB/15.1494.

Khan IH, Javaid A. Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina*. Int J Agric Biol. 2020b;24:441–6. https://doi.org/10.17957/IJAB/15.1457.

Kiptoo GJ, Kinyua MG, Kiplagat OK, Matasyoh LG. Incidence and severity of anthracnose (Colletotrichum lindemuthianum) on selected common bean (*Phaseolus vulgaris* L.) Genotypes. Am J Agric Res. 2020. https://doi.org/10.28933/ajar-2019-09-2705.

Lengai GMW, Muthomi JW, Mbega ER. Phytochemical activity and role of botanical pesticides in pest management for sustainable agricultural crop production. Sci Afr. 2020;7:e00239. https://doi.org/10.1016/j.sciaf.2019.e00239.

Lopes DB, Berger RD. The effects of rust and anthracnose on the photosynthetic competence of diseased bean leaves. Phytopathology. 2001;91:212–20. https://doi.org/10.1094/PHYTO.2001.91.2.212.

Madjouko MA, Tchameni SN, Tchinda ES, Dongmo Jazet PM, Kamsu PN, Medzue Souop Kamga VA, et al. Inhibitory effects of essential oils from *Ocimum basilicum* and *Ocimum gratissimum* on *Colletotrichum musae*: the causal agent of bananas anthracnose. J Phytopathol. 2019;167:257–64. https://doi.org/10.1111/jph.12793.

Mandiriza G, Kritzinger Q, Aveling TAS. The evaluation of plant extracts, biocontrol agents and hot water as seed treatments to control black rot of rape in South Africa. Crop Prot. 2018;114:129–36. https://doi.org/10.1016/j.cropro.2018.08.025.

Marak T, Mahapatra S, Das T, Das S. Integrated application of botanicals, fungicides and bio-agents against anthracnose (*Colletotrichum truncatum*) of Green gram to improve benefit cost ratio. Arch Phytopathol Plant Prot. 2021;54:468–83. https://doi.org/10.1080/03235408.2020.1841533.

Maroyi A. *Ximenia caffra* Sond. (Ximeniaceae) in sub-Saharan Africa: a synthesis and review of its medicinal potential. J Ethnopharmacol. 2016;184:81–100. https://doi.org/10.1016/j.jep.2016.02.052.

Maroyi A. Lippia javanica (Burm. f.) Spreng.: traditional and commercial uses and phytochemical and pharmacological significance in the African and Indian Subcontinent. Evid-Based Complement Altern Med. 2017. https://doi.org/10.1155/2017/6746071.

Martiniano-Souza MdC, Gonçalves-Vidigal MC, da Costa AF, Vidigal Filho PS, Martins VdSR, Vaz Bisneta M, et al. Virulence and genetic diversity of *Colletotrichum lindemuthianum* and resistance of local common bean germplasm to anthracnose in Pernambuco State, Brazil. Eur J Plant Pathol. 2021;159:727–40. https://doi.org/10.1007/s10658-020-02179-3.

Masangwa JIG, Aveling TAS, Kritzinger Q. Screening of plant extracts for antifungal activities against *Colletotrichum* species of common bean (*Phaseolus vulgaris* L.) and cowpea (*Vigna unguiculata* (L.) Walp). J Agric Sci. 2013;151:482–91. https://doi.org/10.1017/S0021859612000524.

Masangwa JIG, Kritzinger Q, Aveling TAS. Germination and seedling emergence responses of common bean and cowpea to plant extract seed treatments. J Agric Sci. 2017;155:18–31. https://doi.org/10.1017/S0021859616000113.

Mkenda P, Mwanauta R, Stevenson PC, Ndakidemi P, Mtei K, Belmain SR. Extracts from field margin weeds provide economically viable and environmentally benign pest control compared to synthetic pesticides. PLoS ONE. 2015. https://doi.org/10.1371/journal.pone.0143530.

Mkindi AG, Coe R, Stevenson PC, Ndakidemi PA, Belmain SR. Qualitative cost-benefit analysis of using pesticidal plants in smallholder crop protection. Agriculture. 2021;11:1007. https://doi.org/10.3390/agriculture11101007.

Mkindi AG, Tembo YLB, Mbega ER, Smith AK, Farrell IW, Ndakidemi PA, et al. Extracts of common pesticidal plants increase plant growth and yield in common bean plants. Plants. 2020. https://doi.org/10.3390/plants9020149.

Mohammed A. An overview of distribution, biology and the management of common bean anthracnose. J Plant Pathol Microbiol. 2013. https://doi.org/10.4172/2157-7471.1000193.

Muthomi J, Fulano AM, Wagacha JM, Mwangombe AW. Management of snap bean insect pests and diseases by use of antagonistic fungi and plant extracts. Sustain Agric Res. 2017;6:52. https://doi.org/10.5539/sar.v6n3p52.

Naqvi SF, Javaid A, Khan IH. Fungicidal activity of stem extract of *Chenopodium murale* L. against the pathogen of Fusarium wilt of tomato. Allelopathy J. 2023;59:69–80. https://doi.org/10.26651/ALLELO.J/2023-59-1-1432.

Padder BA, Sharma PN, Awale HE, Kelly JD. Colletotrichum lindemuthianum, the causal agent of bean anthracnose. J Plant Pathol. 2017;99:317–30.

Paparu P, Dubois T, Gold CS, Niere B, Adipala E, Coyne D. Screenhouse and field persistence of nonpathogenic endophytic *Fusarium oxysporum* in *Musa* tissue culture plants. Microb Ecol. 2008;55:561–8. https://doi.org/10.1007/s00248-007-9301-7.

Pasche JS, Wharam CM, Gudmestad NC. Shift in sensitivity of *Alternaria solani* in response to QoI fungicides. Plant Dis. 2004;88:181–7. https://doi.org/10.1094/PDIS.2004.88.2.181.

Perfect SE, Hughes HB, O'Connell RJ, Green JR. Colletotrichum: a model genus for studies on pathology and fungal-plant interactions. Fungal Genet Biol. 1999;27:186–98. https://doi.org/10.1006/fgbi.1999.1143.

Prakash B, Singh PP, Kumar A, Gupta V. Botanicals for sustainable management of stored food grains: pesticidal efficacy, mode of action and ecological risk assessment using computational approaches. Anthr Sci. 2022;1:62–79. https://doi.org/10.1007/s44177-022-00016-2.

Quintana-Rodriguez E, Morales-Vargas AT, Molina-Torres J, Ádame-Alvarez RM, Acosta-Gallegos JA, Heil M. Plant volatiles cause direct, induced and associational resistance in common bean to the fungal pathogen *Colletotrichum lindemuthianum*. J Ecol. 2015;103:250–60. https://doi.org/10.1111/1365-2745.12340.

Radice M, Maddela NR, Scalvenzi L. Biological activities of zingiber officinale roscoe essential oil against *Fusarium* spp: a minireview of a promising tool for biocontrol. Agronomy. 2022;1:2. https://doi.org/10.3390/agronomy12051168.

Rao S, Bhattiprolu S, Kumari V, Gopal A, Kumar P, Rani P. Cross infectivity studies of *Colletotrichum* spp, causing Anthracnose in different beans. Andhra Agric J. 2020;67:70–5.

Ratto F, Bruce T, Chipabika G, Mwamakamba S, Mkandawire R, Khan Z, et al. Biological control interventions and botanical pesticides for insect pests

of crops in sub-Saharan Africa: a mapping review. Front Sustain Food Syst. 2022;6:309. https://doi.org/10.3389/fsufs.2022.883975.
Shao FM, Teri JM. Yield losses in *Phaseolus* beans induced by anthracnose in Tanzania. Trop Pest Manag. 1985;31:60–2. https://doi.org/10.1080/09670878509370947.
Sharma PN, Sharma OP, Padder BA, Kapil R. Yield loss assessment in common bean due to anthracnose (*Colletotrichum lindemuthianum*) under sub temperate conditions of North-Western Himalayas. Indian Phytopath. 2008;61:323–30.
Singh P, Pandey AK. Dysphania ambrosioides essential oils: from pharmacological agents to uses in modern crop protection—a review. Phytochem Rev. 2022;21:141–59. https://doi.org/10.1007/s11101-021-09752-6.
Stappen I, Tabanca N, Ali A, Wanner J, Lal B, Jaitak V, et al. Antifungal and repellent activities of the essential oils from three aromatic herbs from western Himalaya. Open Chem. 2018;16:306–16. https://doi.org/10.1515/chem-2018-0028.
Stavely JR. The modified Cobb scale for estimating bean rust intensity. USDA Publications, Reports of Bean Improvement Cooperative and National Dry Bean Council Research Conference, vol. 28. 1985. p. 31–2
Stevenson PC, Belmain SR, Isman MB. Pesticidal plants: From Smallholder Use to Commercialisation. Basel: MDPI; 2020. https://doi.org/10.3390/books978-3-03928-789-5.
Tembo Y, Mkindi AG, Mkenda PA, Mpumi N, Mwanauta R, Stevenson PC, et al. Pesticidal plant extracts improve yield and reduce insect pests on legume crops without harming beneficial arthropods. Front Plant Sci. 2018. https://doi.org/10.3389/fpls.2018.01425.
Tsala R, Ngatsi PZ, Temegne NC, Lontsi SLD, Tueguem WNK, Nsangou ANK, et al. Efficacy of aqueous extract of the seeds of *Annona muricata* L. in the control of late blight (*Phytophthora infestans*) of tomato (*Lycopersicon esculentum* Mill.) in the field. J Mod Agric Biotechnol. 2022. https://doi.org/10.53964/jmab.2022006.
Vasuki E, Rajinimala N, Kannan R, Sabarinathan KG. Bioefficiency of botanicals against *Colletotrichum lindemuthianum* causing anthracnose in blackgram. Int J Curr Microbiol Appl Sci. 2020;9:460–6. https://doi.org/10.20546/ijcmas.2020.908.054.
Viljoen AM, Subramoney S, van Vuuren SF, Başer KHC, Demirci B. The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. J Ethnopharmacol. 2005;96:271–7. https://doi.org/10.1016/j.jep.2004.09.017.
Yang MM, Xu LP, Xue QY, Yang JH, Xu Q, Liu HX, et al. Screening potential bacterial biocontrol agents towards *Phytophthora capsici* in pepper. Eur J Plant Pathol. 2012;134:811–20. https://doi.org/10.1007/s10658-012-0057-7.
Yesuf M, Sangchote S. Survival and transmission of *Colletotrichum lindemuthianum* from naturally infected common bean seeds to the seedlings. Trop Sci. 2007;47:96–103. https://doi.org/10.1002/TS.202.
Yoon M-Y, Cha B, Kim J-C. Recent trends in studies on botanical fungicides in agriculture. Plant Pathol J. 2013;29:1–9. https://doi.org/10.5423/PPJ.RW.05.2012.0072.