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Potential of Botanicals for the Disinfection of Cowpea (*Vigna unguiculata*) Seeds and Bacterial Control in Burkina Faso

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Abstract

In Burkina Faso, diverse seed sources and weak phytosanitary control compromise seed quality and promote the spread of pathogens such us *Xanthomonas* genus strains. Strengthening local capacity in diagnostics and biocontrol is essential to enhance production system resilience. To this end, cowpea seed lots (Komcallé variety) were collected from six provinces of Burkina Faso. The bacterial load of the seed lots was evaluated on working samples of 1,050 seeds per province. The presence of bacteria of the genus *Xanthomonas* in these samples strains were genotyped using the X4c/X4e PCR primers. In addition, the efficacy of *Ocimum gratissimum*, *Capsicum chinense*, *Zingiber officinale*, *Cassia nigricans* and *Piper nigrum* extracts at a concentration of 4 g L^{-1} was evaluated *in vitro* on the bacterial load of cowpea seeds. In situ, germination rate and percentage of leaf blight symptoms were used to compare the different treatments. The results showed that bacterial loads, expressed as log CFU mL^{-1}, ranged from 7.585 to 8.185 depending on the origin. The PCR was positive for seven suspected strains. Among the treatments tested, *Ocimum gratissimum* and *Capsicum chinense* extracts (4 g L^{-1}) significantly reduced residual bacterial load, with respective averages of 37 and 52 colonies, showing efficacy comparable to the bactericidal control IDEFIX (67 colonies). Under field conditions, *O. gratissimum* induced a high initial germination rate (83%) and reduced the incidence of bacterial blight symptoms (31%). Conversely, *Zingiber officinale*, *Cassia nigricans* and *Piper nigrum* promoted early germination but did not limit symptom progression.

Keywords: Bacterial; Burkina Faso; *Capsicum chinense*; Cowpea seed; *Ocimum gratissimum*

Introduction

In Burkina Faso, cowpea (*Vigna unguiculata*) is a widely cultivated and consumed legume, particularly in urban areas, where it serves as a major source of plant protein and plays a central role in daily diets (Dagorret-Bonetto *et al.* 2025). However, its production is severely constrained by bacterial diseases, which represent significant biotic factors limiting seed yield and quality worldwide. Among these pathogens, members of the genus *Xanthomonas* are of particular concern, as they cause considerable yield losses and can be transmitted from one plant generation to the next through infected seeds (Nandini and Kulkarni 2015; Zafar and Kutama 2024). In Burkina Faso, the Komcallé variety of cowpea is widely cultivated due to its strong agronomic performance particularly in terms of pod number, nodulation, and yield as well as its high nutritional value (Ouattara *et al.* 2025). Nevertheless, most farmers, especially in rural areas, obtain their seeds from local markets, which often lack adequate phytosanitary control (Windinmi *et al.* 2022). Consequently, seed health inspection and treatment remain the primary strategies for limiting the spread of bacterial diseases (Gitaitis and Walcott 2007). Despite their short-term efficacy, chemical seed treatments raise environmental, health, and economic concerns, underscoring the urgent need for alternative and sustainable disease management approaches (Singh *et al.* 2016; Warra and Prasad 2020). In this context, the use of plant-derived extracts as antibacterial agents represents a promising and eco-friendly alternative (Kanwal *et al.* 2009; Naz *et al.* 2014; Saeed *et al.* 2023). Numerous studies have demonstrated that bioactive compounds naturally present in

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plants such as essential oils, flavonoids, alkaloids, and polyphenols exhibit potent antimicrobial activity against a wide range of phytopathogenic bacteria, including *Xanthomonas* sp. (Vasinauskiene *et al.* 2006; Bajpai *et al.* 2010; Naqvi *et al.* 2022). For instance, extracts from local plants such as ginger (*Zingiber officinale*) have shown strong antibacterial efficacy against *Xanthomonas campestris* pv. *vesicatoria* in tomato seed treatments (Kebede *et al.* 2013). Owing to their natural origin, plant extracts are biodegradable and do not generate toxic residues or by-products likely to accumulate in the environment (Kumar *et al.* 2018). Thus, harnessing these botanical resources could provide sustainable, environmentally sound, and economically viable biocontrol solutions for local producers. This study aimed to assess the bacterial load of cowpea seeds from different regions, to determine their contamination by *Xanthomonas* spp., and to evaluate the efficacy of botanicals in reducing this contamination.

Materials and Methods

Experimental material details

Plant materials: The different treatments were applied to the cowpea variety KVX 442-3-25SH, commercially known as Komcallé. The choice of this variety was based on the fact that, in certain regions of Burkina Faso such as the Center-East, it is recommended for dissemination (Takougang *et al.* 2019). This variety was developed by the National Institute of Environment and Agricultural Research (INERA) of Burkina Faso. It has a semi-maturity cycle of 60 days, a potential yield of 1.8 ton ha^{-1}, and an average grain yield under farmer conditions of 750 kg ha^{-1}. In addition, five plant species were used as sources of bioprotective materials in the treatments, namely *Capsicum chinense*, *Zingiber officinale*, *Cassia nigricans*, *Ocimum gratissimum* and *Piper nigrum*. The organs collected included fruits of *C. chinense*, rhizomes of *Z. officinale*, leaves of *C. nigricans* and *O. gratissimum*, and seeds of *P. nigrum* were used to disinfect cowpea seeds before sowing. These plants are widely used in traditional medicine and agriculture for their antimicrobial, fungicidal, and insecticidal properties (Matasyoh *et al.* 2008; Zahin *et al.* 2021; Periferakis *et al.* 2023).

Chemical material: In addition to powders derived from local plant species, a conventional product, Idefix, whose active ingredient is copper hydroxide, was used as a bactericidal control. Idefix is a broad-spectrum fungicide-bactericide commonly employed to control fungal and bacterial pathogens.

Seed lot sampling

Seed samples were collected from six provinces namely Bougouriba, Houet, Ioba, Poni, Kadiogo and Boulkiemdé. These provinces are representative of two agro-climatic zones in Burkina Faso, namely the Sudanian zone (Houet, Ioba, and Poni) and the Sudano-Sahelian zone (Kadiogo and Boulkiemdé). Furthermore, the selection of the Poni, Ioba, and Bougouriba provinces was based on their proximity to Ghana, where cowpea bacterial blight has been reported (Deo-Donne *et al.* 2018). For each province, 1,000 Komcallé cowpea seeds (greater than 400 seeds, according international seed test association method) (Aveling 2014) obtained from 3 local markets were combined to form a composite lot, resulting in a total of 3,000 seeds. From each composite lot, a subsample of 1,050 seeds was taken for bacterial load quantification and isolation of *Xanthomonas* spp. An additional working sample of 420 seeds (60 per treatment) was prepared by pooling the remaining seeds from all provinces to evaluate the efficacy of biological disinfection treatments.

Assessment of bacterial load and identification of *Xanthomonas* spp

From each working lot of 1,050 seeds, a series of subsamples of decreasing size were prepared to ensure representative microbial assessment, as follows: one subsample of 500 seeds, five subsamples of 100 seeds each, and five subsamples of 10 seeds each (Taylor and Phelps 1982). Each subsample was weighed (Table 1) and placed in a sterile stomacher bag containing sterile distilled water at a ratio of 2.5 mL g^{-1} of seed (Grimault *et al.* 2014). The bags were incubated at room temperature under moderate agitation (150 rpm) for 24 h to dislodge surface-associated and internally borne bacteria. Serial tenfold dilutions (10^{-1} to 10^{-6}) were then prepared, and 50 μL of each dilution were plated on YPGA medium (yeast extract 7 g, peptone 7 g, glucose 7 g, agar 15 g per 1,000 mL sterile distilled water) supplemented with cycloheximide (50 μg mL^{-1}), and on 10% TSA medium (3 g tryptic soy broth, 15 g agar per 1,000 mL sterile distilled water). Plates were incubated at 28°C for 72 h. Colony counts on YPGA plates were used to estimate bacterial population density (log CFU mL^{-1}). Convex, pale-yellow colonies with morphological characteristics typical of *Xanthomonas* spp. on 10% TSA were selected, purified, and preserved in nutrient broth containing 50% glycerol (v/v, 2:1 ratio) for molecular characterization.

Molecular identification of *Xanthomonas* spp.

Bacterial isolates were initially cultured on YPGA medium for 72 h at 28°C and subsequently transferred to 10% Tryptic Soy Agar (TSA) medium, where they were incubated at 28°C for 24 h. Genomic DNA was extracted from 24 h bacterial suspensions adjusted to 1×10^7 CFU mL^{-1} by heat lysis at 95°C for 10 min in a Biometra thermocycler, followed by gradual cooling on ice. Polymerase chain reaction (PCR) amplification were performed in a 20 μL reaction mixture (Table 2) using specific primers p7X4c (5′-ggcaacacccgatccctaaacagg-3′)

Table 1: Quantity of water volume (mL) and seed mass (g) used for the assessment of the total bacterial load of subsamples

Provinces	Subsamples Code	Number of seeds	Weight (g)	Water volume (mL)
Houet	H500	500	84.5	211.25
Houet	H100.1	100	16.96	42.4
Houet	H100.2	100	17	42.5
Houet	H100.3	100	17.56	43.9
Houet	H100.4	100	17.01	42.52
Houet	H100.5	100	17.42	43.55
Houet	H10.1	10	1.64	4.1
Houet	H10.2	10	1.84	4.6
Houet	H10.3	10	1.75	4.37
Houet	H10.4	10	1.67	4.17
Houet	H10.5	10	1.71	4.27
Boulkiemdé	BO500	500	87.51	218.75
Boulkiemdé	BO100.1	100	17.69	44.22
Boulkiemdé	BO100.2	100	17.78	44.45
Boulkiemdé	BO100.3	100	17.33	43.32
Boulkiemdé	BO100.4	100	17.39	43.47
Boulkiemdé	BO100.5	100	17.62	44.05
Boulkiemdé	BO10.1	10	1.8	4.5
Boulkiemdé	BO10.2	10	1.76	4.4
Boulkiemdé	BO10.3	10	1.77	4.42
Boulkiemdé	BO10.4	10	1.74	4.35
Boulkiemdé	BO10.5	10	1.7	4.25
Bougouriba	B500	500	83.28	208.2
Bougouriba	B100.1	100	16.79	41.97
Bougouriba	B100.2	100	17.02	42.55
Bougouriba	B100.3	100	16.47	41.17
Bougouriba	B100.4	100	16.57	41.42
Bougouriba	B100.5	100	17.09	42.72
Bougouriba	B10.1	10	1.71	4.27
Bougouriba	B10.2	10	1.6	4
Bougouriba	B10.3	10	1.61	4.02
Bougouriba	B10.4	10	1.62	4.05
Bougouriba	B10.5	10	1.67	4.17
Ioba	I500	500	69.37	173.42
Ioba	I100.1	100	14.97	37.42
Ioba	I100.2	100	16.56	41.4
Ioba	I100.3	100	13.45	33.62
Ioba	I100.4	100	14.08	35.2
Ioba	I100.5	100	13.55	33.87
Ioba	I10.1	10	1.48	3.7
Ioba	I10.2	10	1.47	3.67
Ioba	I10.3	10	1.75	4.37
Ioba	I10.4	10	1.66	4.15
Ioba	I10.5	10	1.63	4.07
Kadiogo	K500	500	82.27	205.67
Kadiogo	K100.1	100	16.34	40.85
Kadiogo	K100.2	100	17.52	43.8
Kadiogo	K100.3	100	17.35	43.37
Kadiogo	K100.4	100	15.44	38.6
Kadiogo	K100.5	100	17.24	43.1
Kadiogo	K10.1	10	1.46	3.65
Kadiogo	K10.2	10	1.4	3.5
Kadiogo	K10.3	10	1.36	3.4
Kadiogo	K10.4	10	1.67	4.17
Kadiogo	K10.5	10	1.33	3.32
Poni	P500	500	97.32	243.3
Poni	P100.1	100	19.91	49.77
Poni	P100.2	100	18.77	46.92
Poni	P100.3	100	18.9	47.25
Poni	P100.4	100	19.32	48.3
Poni	P100.5	100	17.79	44.47
Poni	P10.1	10	1.87	4.67
Poni	P10.2	10	1.91	4.77
Poni	P10.3	10	2.35	5.87
Poni	P10.4	10	1.77	4.42
Poni	P10.5	10	1.85	4.62

and p7X4e (5′-cgccggaagcacgatcctcgaag-3′), generating a 730 bp fragment (Audy *et al*. 1996). The PCR conditions consisted of an initial denaturation at 94°C for 3 min; followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 30 s, and extension at 72°C for 1 min

Table 2: Composition of the reaction mixture used for PCR amplification of the *hrpB* gene

Composition	Volume (μL)
Tampon 5´ Go Taq (Promega)	4
dNTP (2,5 m*M*)	1.6
X4E (20 μM)	0.5
X4C (20 μM)	0.5
Go Taq 5 U μL^{-1}	0.08
Boiled bacterial suspension	5
Sterile distilled water	8.32

30 s; with a final extension at 72°C for 10 min. Amplified PCR products (5 μL) were separated by electrophoresis on 1.5% agarose gel prepared in 1× TAE buffer and visualized under a UV transilluminator gel imaging system. Xanthomonas citri pv. fuscans strain CFBP 7767 served as the positive control.

Seed treatment

Plant materials were dried in the dark at room temperature and ground into a fine powder, which was stored in the shade for one week. For each treatment, a crude extract was prepared by macerating the dried powder in sterile distilled water at a ratio of 50 g of powder per 5 kg of seeds. The detailed ratios of treatments were as follows: *C. chinense*/CN (0.04 g powder: 10.24 g seeds: 25.60 mL water), *Z. officinale*/PJ (0.04 g: 10.75 g: 26.87 mL), *C. nigricans*/CN (0.04 g: 10.89 g: 27.22 mL), *O. gratissimum*/OG (0.04 g: 10.08 g: 25.20 mL) and *P. nigrum*/PN (0.04 g: 10.81 g: 27.02 mL). Sixty Komcallé seeds were immersed in each extract and incubated at room temperature under gentle agitation (150 rpm) for 12 h. Following incubation, seeds were drained and air-dried at room temperature for 12 h before further evaluation. Untreated seeds and seeds treated with Idefix (50 g per 5 kg of seeds) served as negative (TNT) and positive (TB) controls, respectively. The treated seeds were used to evaluate the efficacy of plant extracts *in vitro* and *in vivo*, summarizes the main characteristics of the biological treatments applied.

***In vitro* extracts efficacy on residual bacterial load**

For this purpose, 30 seeds per treatment were evenly placed on LPGA medium supplemented with cycloheximide (50 µg mL^{-1}) to inhibit fungal growth. Seeds were arranged in Petri dishes at 10 seeds per dish, with three independent replicates per treatment. The dishes were incubated at 28°C for 72 h in a bacteriological incubator and bacterial growth around each seed was visually assessed to enumerate colonies.

***In situ* efficacy of extracts on germination and bacterial blight**

A field trial was conducted at Farako-Bâ in Houet province, western Burkina Faso. The site experiences a Sudanian-

Guinean climate (Guinko 1984), with two distinct seasons: a rainy season from June to October and a dry season from November to May. Annual rainfall ranges from 800 to 1,000 mm over 75–85 days, with a mean annual maximum temperature of 33°C. The experiment covered a total area of 5.70 m × 4.00 m and was arranged in a completely randomized block design with three blocks. Each block contained six elementary plots measuring 0.60 m × 1.00 m, randomly assigned to one treatment per plot, and separated by 0.30 m. Seeds were sown at a rate of ten seeds per treatment per block (five seeds per row), with 0.20 m spacing between holes and 0.30 m between rows, ensuring uniform plant development and minimizing intra-specific competition. Germination rate (G) was assessed on 4th days after sowing using the formula:

$$G\,(\%) = \frac{NG}{NS} \times 100 \qquad (1)$$

Where, NS = 10 seeds sown per plot. Two weeks post-sowing, the first trifoliate leaves were collected, and areas showing symptoms of bacterial blight were quantified using ImageJ and Ilastik software (Foucher *et al.* 2022). A total of 12 leaves were analyzed per treatment.

Statistical analysis

Data were recorded in Excel and analyzed with RStudio 2025.05.1 Build 513. Bacterial populations were compared using the Kruskal–Wallis test followed by *Dunn's* test with Bonferroni correction. The effect of treatments on bacterial colonies and symptom percentages was assessed by one-way ANOVA followed by Tukey's HSD test. Germination rates were analyzed with the *Kruskal–Wallis* test and Bonferroni-adjusted pairwise comparisons.

Results

Bacterial load and *Xanthomonas* spp. infection of seeds

The analysis of bacterial population distribution, expressed in log CFU/mL, detected on cowpea seeds collected from six provinces in Burkina Faso revealed a significant variation in bacterial loads depending on their origin ($P = 0.0175$). The highest average loads were observed in the Houet (8.185 log CFU mL^{-1}) and Ioba (8.063 log CFU mL^{-1}) provinces, which were statistically comparable to those recorded in the Boulkiemdé, Kadiogo and Poni provinces (Fig. 1). The Bougouriba province, on the other hand, had the lowest average load (7.585 log CFU mL^{-1}), significantly lower than those of Houet and Ioba. Overall, five out of six provinces showed similar average bacterial loads on the Komcallé cowpea variety. Furthermore, 21 bacterial strains suspected to belong to *Xanthomonas* spp. were isolated, distributed as follows: Boulkiemdé (8), Houet (5), Kadiogo (6), and Ioba (2). The identity of 7 isolates as X. citri pv. fuscans or X. phaseoli pv. phaseoli was confirmed by PCR. These are: 2 from Boulkiemdé, 3 from Kadiogo and 2 from Houet (Fig. 2).

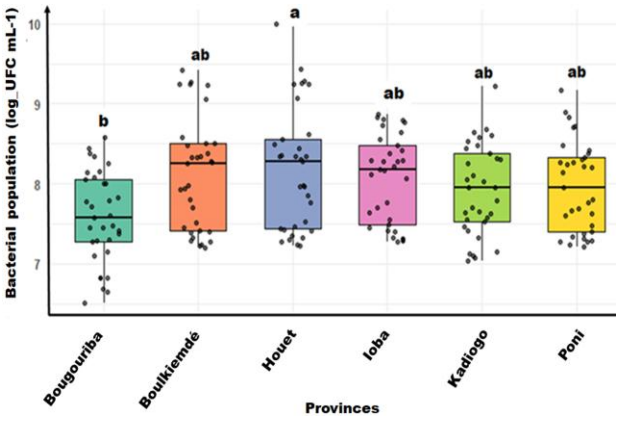

Fig. 1: Bacterial population (Log UFC mL^{-1}) of Komcallé seeds variety from six provinces of Burkina Faso. Different letters above the boxes indicate significant differences between provinces according to Dunn's test ($P < 0.05$) following a Kruskal–Wallis test. Boxes represent the interquartile range (IQR), the horizontal line inside each box indicates the median, and dots represent individual data points

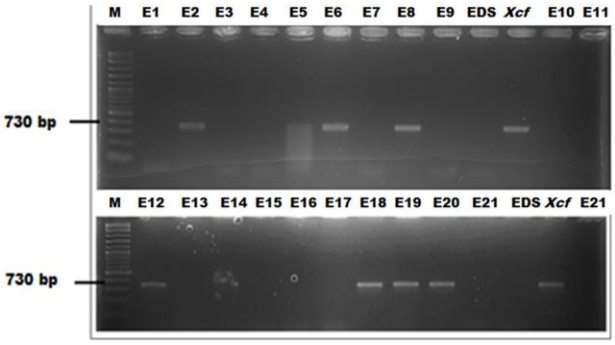

Fig. 2: Agarose gel electrophoresis (1.5%) of PCR products amplified with *hrp*B-specific primers. Lane M: 1 kb DNA ladder; Lane *Xcf*: positive control (*Xanthomonas citri* pv. *fuscans* strain; Lane EDS: negative control (sterile distilled water)

Effect of treatments on the residual bacterial load of seeds

Statistical analysis revealed a significant difference in residual bacterial load depending on the treatment applied. Cowpea seeds treated with *O. gratissimum* and *C. chinense* showed the lowest mean numbers of residual bacterial colonies, with 37 and 52 colonies respectively (Fig. 3). These results were comparable to those obtained with the bactericidal control (67 colonies on average) and were significantly better than the untreated control. Thus, *O. gratissimum* and *C. chinense* emerged as the most effective biological treatments, significantly reducing the residual bacterial load of seeds, unlike the other extracts tested, whose effectiveness remained similar to that of the controls.

In situ effects of the applied treatments

Analyses revealed that the treatments significantly influenced the mean germination rate of seeds at T4. Seeds treated with *C. nigricans* and *O. gratissimum* showed the highest mean germination of 83%, which was statistically comparable to that obtained with *Z. officinale* and *C. chinense* (Fig. 4a). Treatments with *P. nigrum* and the bactericidal control showed intermediate mean rates of 60% and 53%, respectively, while the untreated control displayed the lowest germination rate (≈ 50%). Regarding the percentage of bacterial blight symptoms observed on trifoliate leaves, differences between treatments were also significant. Treatment with *C. nigricans* exhibited the highest proportion of symptoms, with a mean of 55%, significantly higher than that observed with *O. gratissimum* (≈ 30%) (Fig. 4b). Other treatments, including the bactericidal and untreated controls, showed intermediate values with no significant differences among them.

Discussion

The results of this study confirm that cowpea seeds marketed across several provinces in Burkina Faso harbor high bacterial loads. This observation is consistent with previous reports from Nigeria, where substantial bacterial colonization, particularly by *Xanthomonas axonopodis* pv. *vignicola*, was detected on the seed coat and hilum (Claudius-Cole *et al.* 2016). Significant variations in bacterial loads according to seed provenance may be attributed to differences in post-harvest handling, storage conditions, and local phytosanitary practices, as previously suggested in Benin (Anago *et al.* 2021). The Houet and Ioba provinces, which exhibited the highest bacterial loads, could therefore be prioritized for the implementation of preventive seed disinfection strategies. Moreover, the similarity of bacterial loads observed in five out of six provinces highlights the ease of seed movement between regions in the absence of strict phytosanitary control. The PCR with several seed isolates confirmed their affiliation with the genus Xanthomonas, demonstrating that the dissemination of bacteria such as X. citri pv. fuscans is closely linked to their capacity to survive within seeds (Darrasse *et al.* 2018). *In vitro* evaluation of biological treatments showed that extracts of *O. gratissimum* and *C. chinense* significantly reduced residual bacterial loads, with efficacy comparable to copper-based chemical treatment. This highlights the potent antibacterial and antivirulence activities of *C. chinense* extracts, confirming their potential as plant-derived antimicrobial agents (Menezes *et al.* 2022). The antibacterial activity of *O. gratissimum* is attributed to its high content of eugenol and other phenolic compounds capable of disrupting bacterial membranes (Pandey 2017). Furthermore, phytochemical screening of *O. gratissimum* leaves revealed the presence of flavonoids, tannins, alkaloids, and other secondary metabolites, providing a mechanistic basis for its

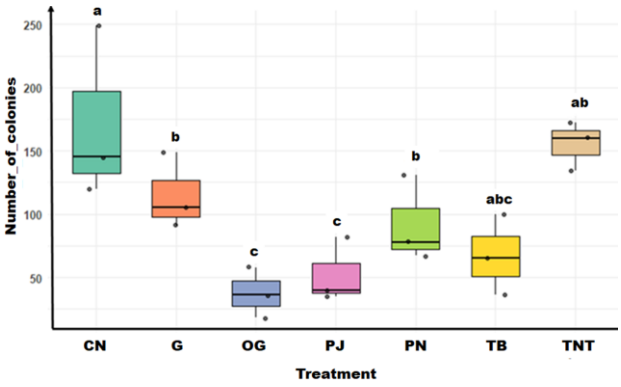

Fig. 3: Effect of treatments on the number of bacterial colonies. Different letters above the boxes indicate significant differences between treatments according to Tukey's test ($P < 0.05$) following a one-way ANOVA. Boxes represent the interquartile range (IQR), the horizontal line inside each box indicates the median, the black dot represents the mean, and dots correspond to individual data points

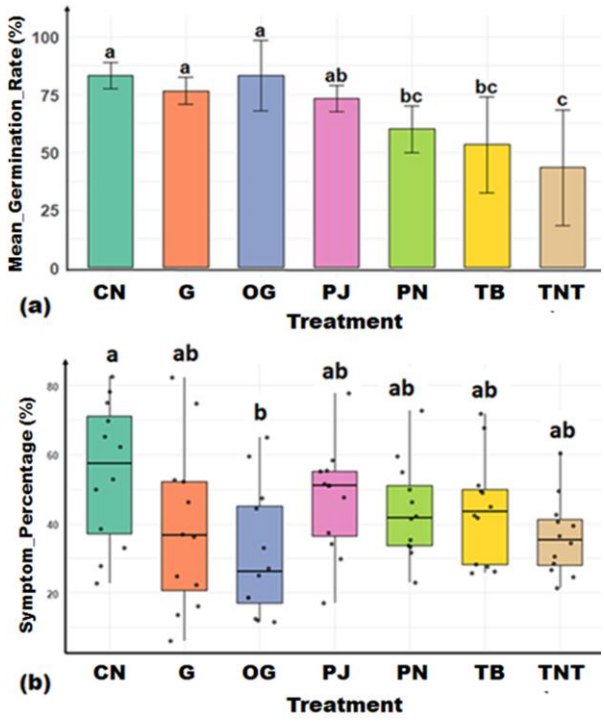

Fig. 4: *In situ* effects of treatments applied to Komcallé seeds (CN: *C. nigricans;* G: *Z. officinale*; OG: *O. gratissimum;* PJ: *C. chinense;* PN: *P. nigrum;* TB: Idefix*;* TNT: untreated seeds): **(a)** Mean germination rates per treatment. Bars represent the mean germination rate (%) for each treatment. Error bars indicate standard deviations. Letters above the bars correspond to statistically distinct groups determined by a Kruskal–Wallis test (*P* value = *0.03115*), followed by pairwise comparisons with Bonferroni correction (significance level α = 0.05). **(b)** Mean percentage of bacterial blight symptoms on the first trifoliate leaves 14 days after emergence for each treatment. Letters indicate statistically different groups according to Tukey's HSD test (Pr(>F) = 0.0408) after one-way ANOVA

antibacterial activity (Eghosa 2025). Notably, the superior field performance of *O. gratissimum* suggests not only antimicrobial effects but also potential biostimulant or seed-vigor enhancing properties. Comparable *in situ* stimulation of seed vigor and root development has been documented for plant-derived biostimulants enriched in polyphenols and terpenoids (Katam *et al.* 2022; Martínez-Lorente *et al.* 2024). In fact, the combination of *O. gratissimum* extracts and chitosan nanoparticles present in the soil has demonstrated antioxidant and antibacterial activities, suggesting their potential as biostimulants (Onyebuchi and Kavaz 2019). This dual role warrants further investigation, particularly to determine whether specific metabolites contribute simultaneously to germination and early seedling growth. This biostimulant effect may be attributed to the modulation of antioxidant enzyme activities and the induction of systemic resistance, as previously reported for essential oil-based biocontrol formulations (Silva *et al.* 2025). In contrast, extracts such as those from *C. nigricans* and *Z. officinale*, which improved germination without offering protection against disease symptoms, confirm that enhanced germination does not necessarily translate into increased resistance to pathogens (Mondal and Bose 2014).

Conclusion

This study concluded that the Komcallé cowpea variety carries a consistently high bacterial load across the most provinces of Burkina Faso, representing a potential source of *Xanthomonas* dissemination through seeds. Nonetheless, certain local plants, particularly *O. gratissimum* and *C. chinense*, showed promising potential as sustainable alternatives to synthetic chemical treatments. Their efficacy, observed both *in vitro* and *in situ*, highlights their possible role in reducing reliance on synthetic pesticides and promoting integrated disease management strategies adapted to West African agricultural systems. However, further multi-year and multi-location trials remain essential to validate their effectiveness and ensure their successful integration into sustainable farming practices.

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Author Contributions

NES, BO and IW designed the experiment, analyzed the data and drafted the first version of the manuscript. ZOD, AD, LB and KK contributed to manuscript revisions. IW supervised the entire research work.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability

All data generated or analyzed in this study are included in this article and can be made available upon request.

Ethics Approval

Not applicable for this paper.

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