



## Article

# Citrus Extract Found Potent in the Control of Seed-Borne Fungal Pathogens of Pearl Millet—A Recommendation for Farmers' Seed Saving Systems

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**Abstract:** Seed saving is crucial to ensure seed and food security, especially in developing countries. In Ghana, about 90% of pearl millet farmers utilise farmer-saved seed for production. Such seeds usually have a low germination rate, and may carry important seed-borne pathogens. In this study, the quality of farmer-saved seeds and the potential of botanicals to control seed-borne fungi were examined. A structured questionnaire was administered to 120 farmers from Garu, Bongo and Kassana-Nankana districts in Ghana. During the survey, 60 samples of farmer-saved seed were obtained for seed quality evaluation. Aqueous extracts of neem (*Azadirachta indica*) and citrus (*Citrus sinensis*) were prepared as seed treatments compared with a control. Fourteen (14) fungal species were associated with the seed samples, but the four dominant ones were *Curvularia* spp. (62%), *Talaromyces* spp. (53%), *Aspergillus* spp. (52%) and *Exserohilum* spp. (42%). The application of citrus extract inhibited fungal incidence (15–31.7%) compared to neem extract (33.3–93.3%) and the control (96.7–98.3%). The application of citrus extract resulted in a higher (74.7–82.8%) field germination rate compared to neem extract (62.3–73.2%) and the control (65.5–69.2%). The citrus extract was the most efficient treatment in reducing the incidence of fungi and enhancing the seed germination and vigour of pearl millet.

**Keywords:** seed systems; pearl millet; farmer-saved seed; seed quality; citrus extract



**Citation:** Anafo, M.A.; Sugri, I.; Asungre, P.A.; Ankamah-Yeboah, T.; Eleblu, J.S.Y.; Danquah, E.Y. Citrus Extract Found Potent in the Control of Seed-Borne Fungal Pathogens of Pearl Millet—A Recommendation for Farmers' Seed Saving Systems. *Horticulturae* **2023**, *9*, 1075. <https://doi.org/10.3390/horticulturae9101075>

Academic Editor: Sergio Ruffo Roberto

Received: 25 August 2023  
Revised: 11 September 2023  
Accepted: 14 September 2023  
Published: 26 September 2023



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## 1. Introduction

Pearl millet (*Pennisetum glaucum* (L.)), commonly called Bajra (Hindu) or Naara (Frafra), is a key staple food crop which provides food to about 90 million people across Asia and Africa [1]. It provides good nutrition and income to farmers, thus contributing to food security and improved livelihoods among farmers [1,2]. According to Hassan et al. [3], pearl millet is widely cultivated on 30 million ha of land in Africa and Asia, and contributes to half of the global millet production. About 93% of the produce in Africa and Asia is used for food, and the remaining 7% is used for feeding livestock [4]. In northern Ghana, pearl millet is the third most important staple food crop, providing food to more than 90% of the population. The crop is cultivated in about 157,000 ha of land in Ghana, with an average grain yield of 1.05 t ha<sup>-1</sup> [5]. Out of the annual millet grain produced (236,000 t) in Ghana, about 212,827 t is utilised domestically as food [6].

Pearl millet is produced in subsistence farming systems in Africa, where the availability of improved varieties and quality seed is limited. In general, access to quality seed is vital

for sustainable crop production. The use of quality seed is estimated to contribute to 20–25% of crop productivity [7,8]. Quality seed provides good germination and good crop establishment, minimises the incidence of field diseases, and results in higher crop yields [7,8]. According to Asungre et al. [9], a lack of improved varieties and poor access to quality seed resulting in low crop yields are among the dominant constraints of pearl millet production in Ghana. The poor access to improved varieties additionally contributes to a majority (90%) of farmers saving seed for production [9]. Mostly, the farmer-saved seeds are stored in indigenous storage facilities (mud bins and barns), and under environmental conditions that reduce the seed quality.

Seed treatment helps to improve the seed quality of most crops. Both botanicals and synthetic chemicals are efficient seed treatment options. Although chemical seed treatment is efficient at improving seed quality, it has adverse effects on beneficial microorganisms and the environment [10–12]. According to Singh et al. [12], seed treatment with fungicides has been noted to show environmental pollution damage, thus suggesting the need for more ecologically friendly options. Botanical seed treatments have some fungicidal and insecticidal properties that protect seeds against pathogens and insects. Seed priming with *Melia azedarach* and neem extracts effectively controlled *Sclerospora graminicola* and enhanced seed germination of pearl millet [13].

Seed saving is crucial to ensure seed and food security, especially in developing countries [14]. About 80% of seeds available globally are farmer-saved, through the informal seed system [15]. According to Asungre et al. [9], about 84% of pearl millet farmers in Ghana save their own seed, whilst 5.6% and 7.6% source seed from friends and the local seed market, respectively, for production. Only a small fraction of farmers (2.4%) source seed from research institutions or input dealers. Overall, about 90% of pearl millet farmers in Ghana save their own seed or source seed from friends for production, which may be of poor quality and carry a number of important seed-borne pathogens. According to Kanton et al. [16], farmer-saved seed costs less compared to improved seed; contributing to why farmers save and recycle their own seed. In some cases, the saved seed is adapted to farmers' growing conditions [17]. However, farmer-saved seed in Ghana is mostly stored in poor storage facilities with no proper seed treatments, which results in seed quality decline. Therefore, seed quality assessment could help enhance the agronomic value of the seed [18]. Seed quality analysis as well as seed treatments have advantages regarding germination, crop establishment and the yield of crops.

Several plant species including *Acacia* spp., *Eucalyptus* sp., *Allium sativa* and *Azadirachta indica* have been evaluated for their potential use in the seed treatment of pearl millet. However, little research exists on the potential of citrus extract as a seed treatment for pearl millet. This study examined the seed quality of farmer-saved pearl millet and assessed the potential of two botanicals to control seed-borne fungal pathogens. Three specific objectives were studied, namely: (1) performing a survey of pearl millet seed systems and seed saving and storage practices in the Upper East region of Ghana; (2) evaluating the quality attributes and identifying seed-borne fungi associated with farmer-saved pearl millet seed; and (3) determining the efficacy of citrus and neem aqueous extracts against pearl millet seed-borne fungi, and the subsequent effects on germination and vigour.

## 2. Materials and Methods

### 2.1. Study Area

This study was conducted in the Upper East region of Ghana, which lies in the Sudan savannah agro-ecological zone. This region is located between latitude 10°15' and 10°10' N and longitude 0° and 1°4' W. It occupies 8628 square kilometres of land, with 83.7% of total households engaged in agriculture. The annual rainfall of the region is 1149 mm. The region has soils with pH ranging from 4.10 to 7.40, and it is the largest producer of pearl millet in Ghana [6,19,20].

## 2.2. Survey of Pearl Millet Seed Systems

A survey was conducted in three districts (Garu, Bongo and Kassana-Nankana Municipality) of the Upper East region of Ghana using a multi-stage sampling technique. The survey gathered information about pearl millet cultivation, seed saving and seed storage practices. A structured questionnaire was administered to 120 pearl millet farmers from six communities for their informed response. A purposive sampling technique was used to select the communities based on production data, and 20 respondents were randomly selected per community. The communities were Denugu and Duuri from Garu district, Nyaariga and Vea from Bongo district, and Mirigu and Kandiga from Kassana-Nankana Municipality.

Sixty samples of farmer-saved seed (about 1 kg each) were obtained for seed quality and seed health analysis. Certified seeds of pearl millet (WAAP-Naara and Akad-kom) were also obtained from the Savanna Agricultural Research Institute (CSIR-SARI), Manga-Bawku as standard checks for the study. The seed samples were kept in zip-lock bags and packed neatly in moisture-proof containers before transporting them to test centres. Seed quality assessment of the samples was conducted at Ghana Seed Inspection Division of the Ministry of Food and Agriculture (GSID-MoFA), Bolgatanga and CSIR-SARI (Latitude 10030' to 1108' N, and longitude 1015' W to 005' E).

## 2.3. Determination of Seed Physical and Physiological Characteristics

The following seed quality characteristics were assessed: seed moisture content, seed weight, germination percentage, seed vigour and purity percentage.

### 2.3.1. Seed Moisture Content (%)

For each sample, 250 g was poured into an electronic moisture meter (Steinlite digital moisture tester SB 900, Seedbro, Des Plaines, IL, USA) that was set to test the seed moisture of pearl millet. The seed moisture content value displayed on the moisture meter was recorded. The process was repeated thrice for each seed sample.

### 2.3.2. Physical Seed Purity (%)

A working sample of 25 g each was measured and poured onto a purity board. The seed lots were sorted into pure seed, inert matter (stones and chaff), other crop seed, and weed seed using a magnifying lens of the purity board. The percentage of each portion was recorded on a weight-by-weight basis.

### 2.3.3. Thousand Seed Weight (g)

The 1000-seed weight (g) was determined following the procedure of Bekele et al. [18]. One thousand seeds were counted from each sample, and weighed using an electronic balance (Ohaus Scout™ SPX123, Des Plaines, IL, USA). The measurements (g) were replicated thrice for each seed sample.

### 2.3.4. Standard Germination and Seed Vigour

Both standard germination and seed vigour were evaluated using the paper towel method [21]. For each sample, a working sample of 10 g of pure seed was sampled and three replicates of 100 seeds were incubated. Each 100-seed sample was put on a template board, spaced on a 2-layered moist anchor paper, and then covered with another moist anchor paper. The anchor papers were then gently rolled into towels, clipped with rubber bands and put into a transparent polythene bag to minimise moisture loss. The rolled paper towels were incubated at 30 °C with 10 h of fluorescent light exposure in a germinator for seven days [22]. The polythene bags were then removed from the germinator and the paper towels were unrolled for germination counts. The germination percentage was calculated on the basis of normal germinated seedlings. Normal seedlings included those having both a radicle and coleoptile, and no deformities. Additionally, 10 seedlings were selected along the diagonals of the anchor papers, and the shoot and radicle length of each seedling were

measured using a calliper. Seedling vigour index (I) was estimated using the mean shoot and radicle lengths as indicated below:

$$\text{Vigour index (I)} = \frac{(\text{Mean shoot length} + \text{mean radicle length}) \times}{\text{germination percentage}} \quad (1)$$

### 2.3.5. Field Germination and Vigour Test

Field germination and seedling vigour analyses were conducted on three raised beds with dimensions of 1 m wide and 13 m long. Sixty-two drilled rows spaced at 20 cm apart were created on each bed, and each drill was sown to a specific seed source. The seed beds were then labelled. Germination counts for emergence were performed at 4, 7 and 14 days after sowing.

Seedling vigour score was assessed in the context of plant height and number of days to the 3-leaf, 5-leaf, and 7-leaf stages. The plant height of selected seedlings per sample was measured at 7, 14 and 21 days after sowing using a measuring tape. The number of days to 50% flowering was also recorded.

### 2.4. Determination of Seed Health

The sanitary quality of the seed was assessed using the agar plate method. A full-strength potato dextrose agar (PDA) was prepared to serve as a medium for the culture and isolation of pathogens associated with the seeds. Primary samples of 300 seeds were reduced to working samples of 30 seeds using the spoon sample reduction method as described in the International Seed Testing Association rules on sampling [23]. The working samples were surface-sterilised with 5% bleach for two minutes and 70% ethanol for three minutes. The samples were then washed with distilled water for five minutes and blotted dry using tissue paper. With the help of a pair of forceps, 10 seeds were plated on Petri dishes in three replicates. The Petri dishes were then sealed using a masking tape, labelled and incubated at room temperature ( $25 \pm 2$  °C) for seven days. The incidence of seed-borne pathogens was evaluated using the formula below:

$$\text{Incidence (\%)} = \frac{\text{No. of seeds infected by fungi}}{\text{total number of seeds plated}} \times 100 \quad (2)$$

All the fungal species isolated from the seed samples were sub-cultured for identification. The fungi were identified based on their macro- (colony colour and growth pattern) and micro-characteristics (spore shape and colour) using a reference manual, "Illustrate Genera of Imperfect Fungi", fourth edition by Barnett and Hunter (1972), and online sources (google images). A wet mount of each fungi isolate was prepared on a slide and slip, and was observed using a compound microscope (Novex binocular microscope, K-Range). A magnification objective lens (10×) was used to observe and identify the hyphal structure, spore/conidia shape and colour of the pathogens [24].

### 2.5. Preparation of Plant Extracts

Nem and citrus extracts were prepared as seed treatments following the procedure of Gyasi et al. [25], with slight modification. Depulped fresh neem kernels (50 g) and fresh citrus peels (50 g) were washed with sterile distilled water. Each sample was grounded into paste, dissolved in 0.1 L of distilled water for one hour, and filtered using a cheese cloth to obtain a 50% (*w/v*) aqueous extract [25–27]. A 50% concentration of imidacloprid + thiram 3.5:1 was also prepared by measuring 0.125 g of the fungicide powder into a beaker and dissolved in 0.1 L of distilled water for one hour.

### 2.6. Assessment of Botanical Seed Treatments

Five treatments including aqueous extracts of citrus peels, neem kernels, fungicide–insecticide (imidacloprid + thiram 3.5:1), sterile distilled water, and a control were assessed for their impact on seed quality. The treatments were applied to 8 selected seed samples.

For each 3.5 g of seeds, 0.01 L of each treatment was applied. The samples were treated in sealed Ziplock bags for 12 h, and air-dried on a laboratory bench for six hours. The seed health test was conducted to evaluate the efficacy of the treatments against seed-borne fungi. The treated seeds were not surface sterilised in ethanol and bleach to prevent washing away the treatment. According to ISTA rules, treated seeds are usually tested without removing the treatment [23]. Three replicates of 100 seeds were counted from each treatment and used to conduct standard germination, field germination and vigour tests.

### 2.7. Data Analysis

The survey questionnaire was coded and analysed using the SPSS statistical package (20th edition). The seed quality assessment and seed treatment data were analysed using ANOVA in GENSTAT statistical package (11th edition). The count dataset was transformed using the square root data transformation method.

## 3. Results

### 3.1. Demographic Characteristics of Pearl Millet Farmers

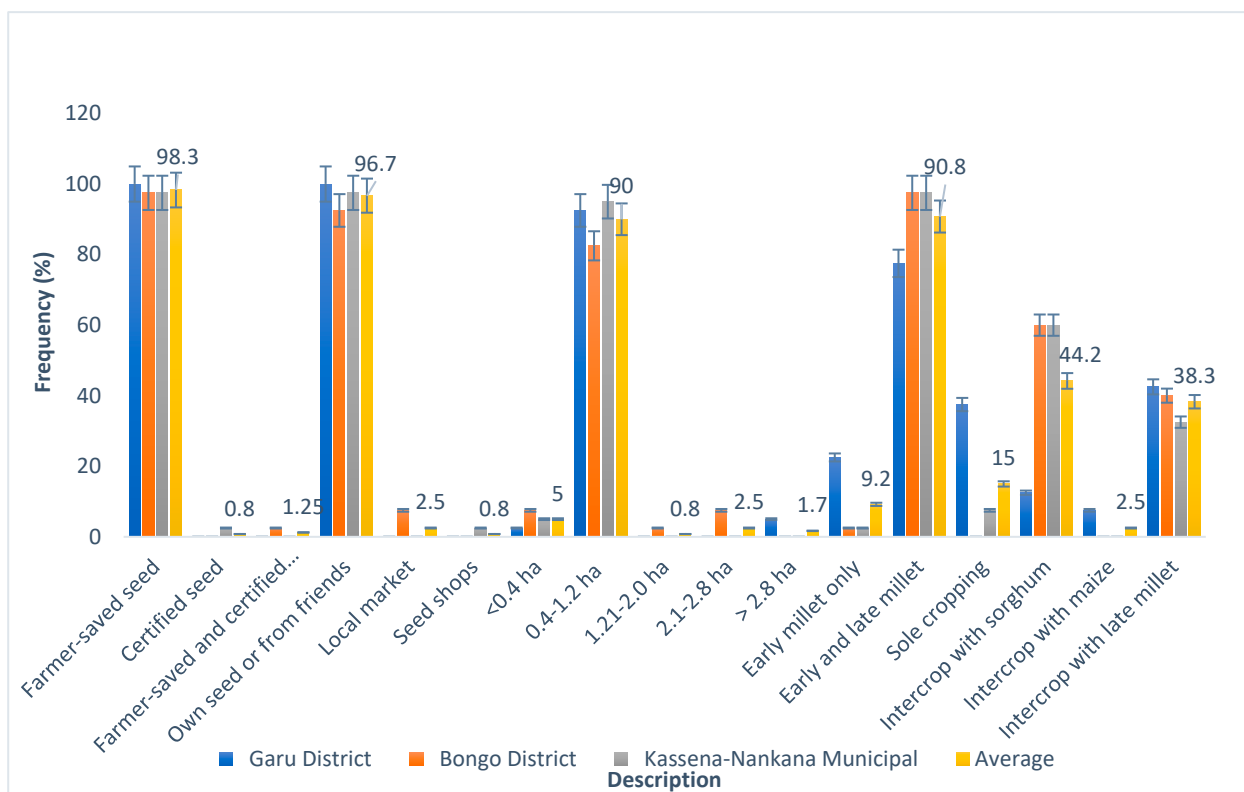
Overall, 49.2% of the respondents were males, while 50.8% were females (Table 1). The age distribution shows that 7.5% of farmers were in the 20–30-year range, 22.5% were in the 31–40- and 41–50-year ranges, 31.7% were in the 51–60-year range, and 15.8% were in above 60 years of age. About 63.3% of the farmers had no formal education, 18.3% had only primary education, 10.8% had secondary education and 7.5% had tertiary education. On average, the majority of the farmers were females in the 51–60-year age range, and most did not have any form of formal education.

**Table 1.** Demographic characteristics of pearl millet farmers in the Upper East region, Ghana.

Description		Location			Average (%)
		Garu District	Bongo District	Kassana-Nankana Municipality	
Gender	Male	50	50	47.5	49.2
	Female	50	50	52.5	50.8
Age (years)	20–30	5	7.5	10	7.5
	31–40	17.5	17.5	32.5	22.5
	41–50	22.5	17.5	27.5	22.5
	51–60	35	42.5	17.5	31.7
	>60	20	15	12.5	15.8
Education	Primary	15	5	35	18.3
	Secondary	0	10	22.5	10.8
	Tertiary	15	2.5	5	7.5
	No formal education	70	82.5	37.5	63.3

### 3.2. Pearl Millet Seed Systems

Pearl millet is mostly cultivated in a mixed or intercropped form on a small piece of land where farmers predominately use farmer-saved seed (98.3%) for production (Figure 1). The majority (96.7–99.2%) of the farmers sourced seeds from the informal seed sector such as community seed markets and neighbours. About 90.8% of farmers cultivate both early- and late-maturing pearl millets, while only 9.2% solely cultivate early-maturing millet. The dominant cropping system is intercropping early millet with sorghum (44.2%), followed by early millet–late millet intercropping (38.3%), and maize–early millet intercropping (2.5%). On average, about 90% of the farmers own between 0.4 and 1.2 hectares of farmland (Figure 1).



**Figure 1.** Characteristics of pearl millet production in the Upper East region.

Seed selection criteria employed by farmers included plants with larger panicles (0.8%), disease-free panicles (15.8), and disease-free plants with larger panicles (83.3%) (Table 2). Farmers employed several seed treatment methods including the use of ash (61.1%), orange peels (4.9%), synthetic chemicals (19.7%), lemon grass (5.8%), cut bicycle or motor tubes (1.0%), a local plant “*badokoka*” (1.9%) or a combination of two or more of these. Ash treatment was the dominant method (61.1%), whilst the lemon grass–reddish gravel combination was the least common (0.9%). Seed storage methods deployed by the respondents included hanging panicles, jute sacks, polythene bags, laminated polythene bags, clay pots, barns and container. About 51.7% of the farmers store their seed in laminated polythene bags, followed by clay pot storage (34.2%), hanging panicles (1.7%), polythene bags (1.7%) and containers (1.7%). The majority (58.3%) of the farmers did not report seed quality deterioration after storage, whilst 41.7% noticed seed quality deterioration. The dominant seed quality problems were fungal mould attack (28.3%), insect attack (11.7%), and a combination of mould and insect attack (1.7%) (Table 2).

### 3.3. Physical Seed Quality of Farmer-Saved Pearl Millet

Some physical seed quality traits such as seed moisture, physical purity and seed weight are summarized in Table 3. Seed moisture content evaluation revealed significant variation between the samples ( $p < 0.001$ ). Generally, seed samples from the Garu district had the highest moisture content (9.3%), whilst the certified seed samples had the lowest moisture content (7.4%). There was a significant variation ( $p < 0.001$ ) in seed physical purity among the samples, but the range was 91.24–98.99%. Samples from the Garu district had the lowest physical purity (91.24%), whilst the certified seed sources showed higher physical purity (99.0%). Thousand-seed weight varied significantly ( $p < 0.001$ ) among the samples. Seeds from Kassana-Nankana Municipality had the highest 1000-seed weight (14.4 g) compared to the other samples (Table 3).

**Table 2.** Pearl millet seed saving methods in the Upper East region, Ghana.

Description	Location			Average	
	Garu District	Bongo District	Kassana-Nankana Municipal		
Seed selection criteria	Larger panicles	2.5	0	0	0.8
	Disease free panicles	2.5	27.5	17.5	15.8
	Disease free with larger panicles	95	72.5	82.5	83.3
Storage method	Hanging panicles	2.5	0	2.5	1.7
	Jute sacks	12.5	5	0	5.8
	Polythene bags	5	0	0	1.7
	Laminated polythene bags	10	85	60	51.7
	Clay pots	65	7.5	30	34.2
	Barns	5	2.5	2.5	3.3
	Containers	0	0	5	1.7
Seed treatments	Ash	92.1	44.1	47.2	61.1
	Orange peels	0	14.7	0	4.9
	Synthetic chemicals	5.3	17.6	36.1	19.7
	Orange peels and ash	2.6	5.9	0	2.8
	Synthetic chemicals and ash	0	2.9	2.8	1.9
	Cut tubes	0	2.9	0	1
	Lemon grass	0	11.8	5.6	5.8
	<i>Badokoka</i>	0	0	5.6	1.9
Lemon grass and reddish gravel	0	0	2.8	0.9	
Seed quality problems	Mouldiness/fungi attack	52.5	10	22.5	28.3
	Insect attack	12.5	10	12.5	11.7
	No mould or insects	35	75	65	58.3
	Insects and mould	0	5	0	1.7

**Table 3.** Seed quality traits of samples from the Upper East region.

Seed Samples	Seed Moisture (%) *	Purity Percent (%) *	1000-Seed Weight (g) *
Garu District	9.34 ± 0.772	91.24 ± 9.55	14.07 ± 2.621
Bongo District	8.49 ± 1.120	96.61 ± 1.63	14.25 ± 1.696
Kassana-Nankana Municipal	8.84 ± 1.017	97.86 ± 1.61	14.37 ± 1.359
Certified seed	7.44 ± 0.173	98.99 ± 0.56	11.35 ± 1.054
<i>p</i> -value	<0.001	<0.001	<0.001
L.S.D	0.3078	1.748	0.0618
CV (%)	10	5.1	12.7

\* Mean ± standard deviation.

### 3.4. Seed Germination and Vigour

Assessment of standard germination in the field revealed significant differences ( $p < 0.001$ ) between the samples (Table 4). The standard germination ranged from 65.7 to 83.8%, with the certified seed showing superior performance (83.8%) compared to the Bongo (77.5%), Kassana-Nankana Municipality (73.7%) and Garu (65.7%) samples. Germination in the field ranged from 55.7 to 74.1%, with samples from the Garu district having the lowest field germination (55.7%) compared to Bongo (66.9%) and Kassana-Nankana Municipality (69.2%). The certified seed had the highest germination rate in the field (74.1%).

Seedling vigour assessment revealed significant differences between the samples for all of the vigour indicators; days to the three-, five- and seven-leaf stages, plant height at 7, 14 and 21 days, and the vigour index at seven days (Table 4). The vigour index ranged from 953 to 1394.5, with the lowest vigour index (953) recorded from the Garu district samples. Also, the Garu district samples had the highest number of days (22.1 days) to the seven-leaf stage compared to the Bongo district (21.3 days) and Kassana-Nankana Municipality (20.9 days) samples. Among all the seed sources, the certified seeds had the lowest number of days (18.8 days) to the seven-leaf stage. It was also observed that the certified seeds had

the highest mean plant height (10.5 cm), whilst the Garu district samples had the lowest mean plant height (7.8 cm) at 21 days.

**Table 4.** Germination, vigour analysis and incidence of fungal pathogens on pearl millet seed.

Seed Samples	Standard Germination (%) *	Field Germination (%) at 7 Days *	Fungi Incidence (%) at 7 Days *	Vigour Index at 7 Days	Days to			Plant Height (cm)		
					3-Leaf Stage	5-Leaf Stage	7-Leaf Stage	7 Days	14 Days	21 Days
Garu District	65.7 (8.08)	55.7 (7.28)	70.6 (8.16)	953	8.82	14.45	22.14	1.96	5.70	7.76
Bongo District	77.52 (8.79)	66.9 (8.13)	46.8 (6.09)	1395.5	8.6	14.12	21.33	2.30	6.75	9.19
Kassana-Nankana Municipal Certified seed	73.68 (8.39)	69.2 (8.26)	45.7 (6.04)	1394.5	8.15	13.74	20.9	2.14	6.05	8.28
Grand mean	75.17 (8.60)	66.5 (8.12)	54.9 (6.84)	1263	8.40	13.66	20.78	2.13	6.20	8.92
p-value	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	0.011	<0.001	<0.001
L.S.D	4.103 (0.355)	5.65 (0.46)	9.68 (0.96)	116.5	0.47	0.56	0.778	0.201	0.4549	0.755
CV (%)	15.2 (11.5)	24.6 (16.4)	49 (39)	25.6	15.4	11.5	10.4	26.2	20.4	23.5

\* Datasets were managed using square root transformation. The transformed values are presented in parenthesis.

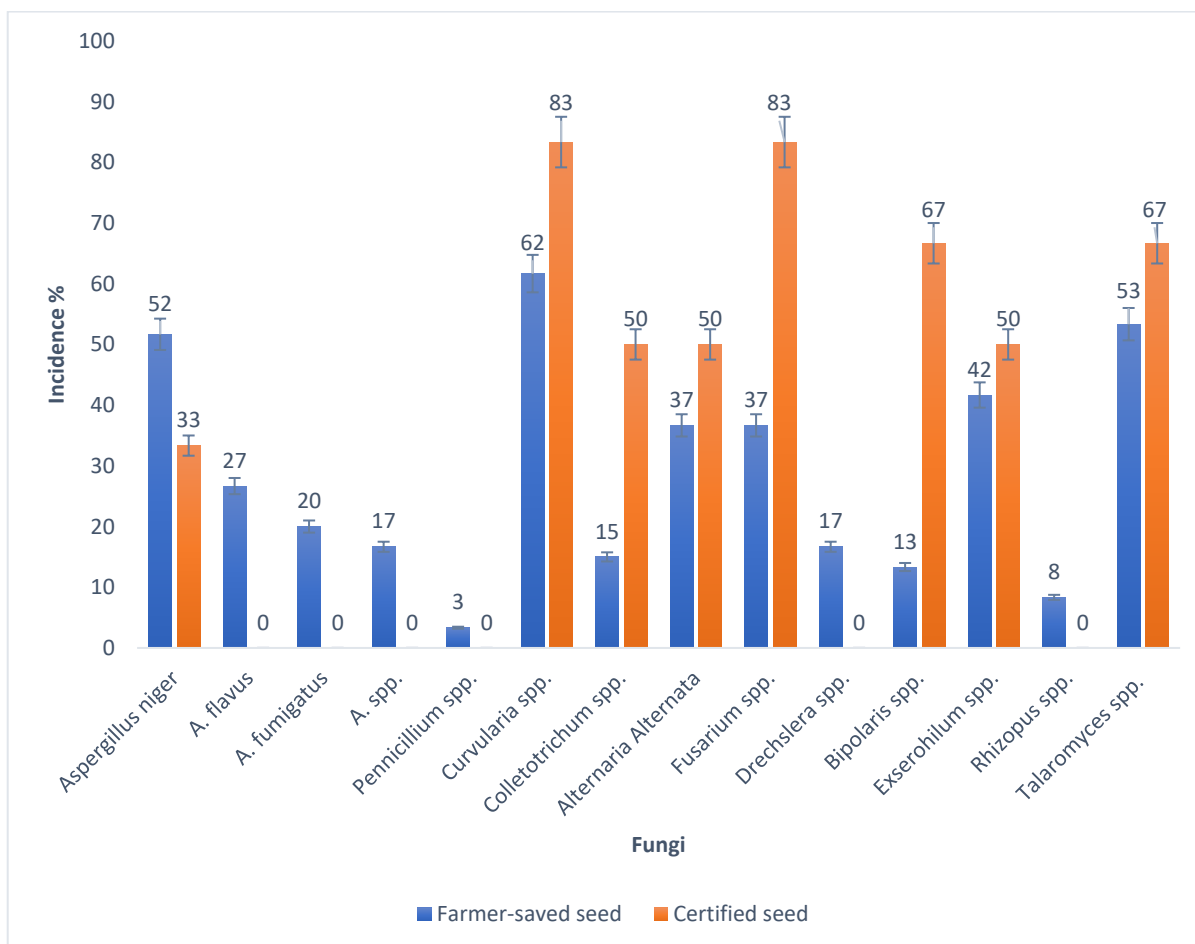
### 3.5. Seed Health Analysis

Seed health analysis revealed a total of 14 fungi associated with the stored seed, namely *Aspergillus* spp., *A. niger*, *A. flavus*, *A. fumigatus*, *Penicillium* spp., *Curvularia* spp., *Alternaria alternata*, *Colletotrichum* spp., *Fusarium* spp., *Drechslera* spp., *Bipolaris* spp., *Exserohilium* spp., *Rhizopus* spp., and *Talaromyces* spp. (Table 5). Overall, *Aspergillus niger* (70%) was the dominant pathogen in the Garu district, whilst *Penicillium* spp., *Colletotrichum* spp. and *Drechslera* spp. were the least occurring fungi in the district, with 5% incidence each. *Curvularia* spp. (60%) and *Exserohilium* spp. (60%) were the two dominant fungi associated with the Bongo district samples. Similarly, the incidence of *Curvularia* spp. was 65% in the Kassana-Nankana Municipality.

**Table 5.** Incidence of fungi on farmer-saved seed and certified seed.

Fungal Pathogen	Garu Incidence (%)			Bongo Incidence (%)			Kassana-Nankana Municipal Incidence (%)			Certified Seed Incidence (%)		
	Denugu	Duuri	Mean	Ve	Nyaariga	Mean	Kandiga	Mirigu	Mean	WAAP-Naara	Akad-Kom	Mean
<i>Aspergillus niger</i>	80	60	70	70	30	50	20	50	35	0	67	33
<i>A. flavus</i>	30	0	15	30	50	40	20	30	25	0	0	0
<i>A. fumigatus</i>	10	50	30	20	10	15	10	20	15	0	0	0
<i>Aspergillus</i> spp.	30	50	40	0	20	10	0	0	0	0	0	0
<i>Penicillium</i> spp.	0	10	5	10	0	5	0	0	0	0	0	0
<i>Curvularia</i> spp.	50	70	60	50	70	60	40	90	65	100	67	83
<i>Colletotrichum</i> spp.	0	10	5	10	20	15	30	20	25	67	33	50
<i>Alternaria alternata</i>	60	20	40	30	50	40	50	10	30	33	67	50
<i>Fusarium</i> spp.	30	20	25	60	30	45	40	40	40	67	100	83
<i>Drechslera</i> spp.	0	10	5	20	0	10	20	50	35	0	0	0
<i>Bipolaris</i> spp.	20	20	20	10	0	5	20	10	15	67	67	67
<i>Exserohilium</i> spp.	60	40	50	70	50	60	20	10	15	33	67	50
<i>Rhizopus</i> spp.	10	10	10	20	0	10	10	0	5	0	0	0
<i>Talaromyces</i> spp.	70	30	50	70	40	55	60	50	55	67	67	67

Overall, the four dominant fungi in the farmer-saved seed were *Curvularia* spp. (62%), *Talaromyces* spp. (53%), *Aspergillus* spp. (52%) and *Exserohilium* spp. (42%) (Figure 2). Also, *Curvularia* spp. (83%) and *Fusarium* spp. (83%) were dominant in the certified seed.



**Figure 2.** Overall incidence of fungi on farmer-saved seed compared with certified seed.

### 3.6. Effect of Seed Treatments on Incidence of Seed-Borne Fungi

The effect of seed treatments on the incidence of fungal pathogens revealed significant differences ( $p < 0.001$ ) between the treatments (Table 6). The application of citrus extract showed lower fungal incidence (15–31.7%) across the samples compared to imidacloprid + thiram (3.5:1) (25–56.7%), neem extract (33.3–93.3%), and the control (96.7–98.3%). The application of citrus extract led to the lowest fungal incidence (15%) in samples from Bongo district, Kassana-Nankana Municipality (21.7%), Garu district (30%), and the certified seed (31.7%) (Table 6). The application of citrus extract provided the most effect in reducing fungal incidence (24.6%) among the treatments (Figure 3).

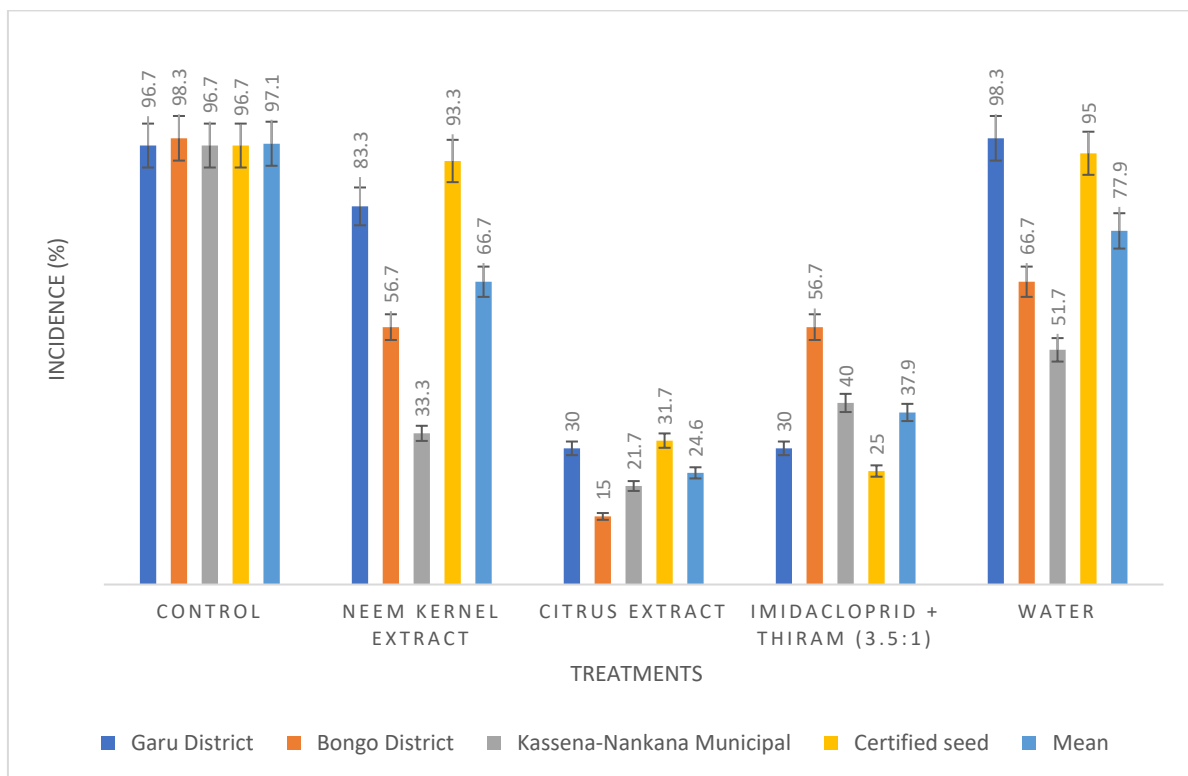
### 3.7. Effect of Seed Treatments on Germination and Vigour

The effects of seed treatments on standard germination and vigour characteristics are presented in Table 7. There was significant variation ( $p < 0.001$ ) among the treatments for standard germination in the field across the samples. The standard germination for the control ranged from 57.7 to 64.3%, whilst 83.2–87.8% standard germination was recorded for the imidacloprid + thiram (3.5:1) treatment. The imidacloprid + thiram (3.5:1) treatment had the highest mean standard germination (85.2%), followed by the citrus extract (80.8%), with the control (61.2%) recording the lowest. Germination in the field ranged from 74.7 to 82.8% and from 77.5 to 82.5% for the citrus extract and imidacloprid + thiram (3.5:1) treatments, respectively, which were higher than the germination in the field for neem extract (62.3–73.2%) and the control (65.5–69.2%).

**Table 6.** Effect of seed treatments on the incidence of fungi on pearl millet seed.

Seed Samples	Seed Treatments	Fungal Incidence (%) *
Garu District	Control	96.7 (9.82)
	Neem kernel extract	83.3 (9.11)
	Citrus extract	30 (5.18)
	Imidacloprid + thiram (3.5:1)	30 (5.18)
	Water	98.3 (9.91)
Bongo District	Control	98.3 (9.91)
	Neem extract	56.7 (7.42)
	Citrus extract	15 (3.46)
	Imidacloprid + thiram (3.5:1)	56.7 (7.38)
	Water	66.7 (7.75)
Kassena-Nankana Municipal	Control	96.7 (9.82)
	Neem extract	33.3 (4.61)
	Citrus extract	21.7 (3.52)
	Imidacloprid + thiram (3.5:1)	40 (6.24)
	Water	51.7 (6.18)
Certified seed	Control	96.7 (9.83)
	Neem extract	93.3 (9.65)
	Citrus extract	31.7 (5.47)
	Imidacloprid + thiram (3.5:1)	25 (4.97)
	Water	95 (9.74)
Grand mean		60.8 (7.25)
<i>p</i> -value		0.001
L.S.D		22.81 (2.16)
CV (%)		32.7 (26)

\* Means were managed using square root transformation with the transformed values presented in parenthesis.



**Figure 3.** Effect of seed treatments on the incidence of fungi on pearl millet seed.

**Table 7.** Effect of seed treatments on the germination and vigour of pearl millet seed.

Seed Samples	Treatment	Standard Germination (%) *	Field Germination at 7 Days (%) *	Vigour Index at 7 Days	Days to			Plant Height (cm)		
					3-Leaf Stage	5-Leaf Stage	7-Leaf Stage	7 Days	14 Days	21 Days
Garu District	Control	60.83 (7.79)	66.17 (8.12)	351	7.17	11.67	15.67	2.10	7.65	15.09
	Neem kernel extract	72.5 (8.50)	62.33 (7.88)	490	6.83	10.50	15.50	2.54	8.82	15.82
	Citrus extract	80 (8.94)	74.67(8.64)	591	6.33	10.50	14.67	2.74	8.81	17.46
	Imidacloprid + thiram (3.5:1)	87.83 (9.37)	77.5 (8.01)	784	6.17	9.67	14.17	2.72	9.54	16.47
	Water	73.33 (8.56)	70 (8.36)	625	6.5	10.33	14.83	2.79	9.56	17.14
Bongo District	Control	64.33 (8.01)	65.5 (8.09)	450	7	11.00	15.83	2.03	7.93	12.77
	Neem kernel extract	68 (8.24)	62.67 (7.90)	534	7	10.83	15.33	2.40	8.61	13.45
	Citrus extract	81.33 (9.04)	82.83 (9.10)	704	6.17	9.83	14.50	2.91	9.4	15.91
	Imidacloprid + thiram (3.5:1)	85.5 (9.23)	82.5 (9.08)	812	6.33	10.17	14.67	2.71	9.31	14.94
	Water	74.5 (8.63)	77.83 (8.82)	723	6.67	10.17	14.83	2.87	9.13	15.51
Kassana-Nankana Municipal	Control	57.67 (7.57)	66.17 (8.13)	481	7	10.50	15.50	2.21	7.89	13.85
	Neem kernel extract	70.33 (8.37)	69.83 (8.35)	636	6.5	9.83	15.17	2.72	8.77	15.46
	Citrus extract	84.17 (9.17)	81.83 (9.04)	835	6.33	9.67	14.50	2.83	8.82	16.15
	Imidacloprid + thiram (3.5:1)	83.17 (9.10)	79 (8.89)	838	6.83	9.67	14.50	2.86	9.68	16.86
	Water	69.5 (8.33)	71.83 (8.47)	594	6.5	10.00	14.83	2.82	9.05	15.8
Certified Seed	Control	62 (7.87)	69.17 (8.31)	573	7.67	10.83	15.67	2.29	8.34	14.7
	Neem kernel extract	71 (8.41)	73.17 (8.55)	583	7.33	11.00	15.50	2.45	8.67	15.85
	Citrus extract	77.67 (8.81)	81 (9.0)	859	6.33	10.00	14.50	3.21	10.47	17.4
	Imidacloprid + thiram (3.5:1)	84.17 (9.17)	80.33 (8.96)	829	6.33	9.83	14.33	2.94	9.59	17.16
	Water	65.17 (8.06)	72.17 (8.49)	618	7.33	10.33	15.33	2.76	9.1	17.58
Grand mean	73.65 (8.56)	73.33 (8.55)	646	6.72	10.32	14.99	2.65	8.96	15.77	
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.07
L.S.D	3.957 (0.23)	2.92 (0.17)	128.3	0.32	0.56	0.55	0.23	0.72	2.09	
CV (%)	9.4 (4.7)	6.9 (3.6)	34.7	8.2	9.5	6.40	15.40	14	23.2	

\* Means were managed using square root transformation with the transformed values presented in parenthesis.

Seedling vigour assessment revealed significant variation ( $p < 0.001$ ) among the treatments for the vigour index at 7 days, the days to the three-, five- and seven-leaf stages, and plant height at 7 and 14 days. However, no significant difference was observed for plant height at 21 days. The vigour index for the plants subjected to imidacloprid + thiram (3.5:1) and citrus treatments ranged from 784 to 838 and from 591 to 859, respectively, across the samples. The plants subjected to imidacloprid + thiram (3.5:1) and citrus extract treatments had higher mean vigour indexes of 815.8 and 747.2, respectively, across the samples compared to the control treatment (463.8). Plants treated with citrus extract took a fewer number of days (6.2–6.3 days) to reach the three-leaf stage compared to those treated with neem extract (6.5–7.3 days) and the control (7.0–7.7 days). A similar trend was observed from the plants treated with citrus extract for the days to the five and seven-leaf stages and plant height at 7 days. Across the samples, the application of citrus extract led to a higher plant height (2.8–3.2 cm) compared to neem extract (2.4–2.7 cm) and the control (2.0–2.3 cm) (Table 7).

#### 4. Discussion

Pearl millet is cultivated in mixed or intercropped systems on small pieces of lands (0.4–1.2 ha), and farmers (98.3%) predominately utilise farmer-saved seed. This finding is in agreement with Asungre et al. [9] that pearl millet is mostly intercropped with other cereals, and about 90% of farmers in Ghana use farmer-saved seed. Pearl millet is cultivated on land within the farming communities or around homesteads which is subject to constant reduction and fragmentation due to real estate development [28]. Land fragmentation could be a reason why pearl millet is produced as a mixed crop to facilitate maximum utilisation of the limited land. This view agrees with Mason et al. [29], who reported that the production of pearl millet is achieved in mixed cropping systems to maximise returns from the most limiting production factors (e.g., land), and to help reduce the risk of pests and diseases. According to Oljira and Girma [30], most millet and sorghum farming communities employ mixed cropping as a production strategy to mitigate biotic and abiotic stresses [30,31].

The seed saving practices in the districts were seed selection and seed storage. The seed selection criteria were based on the absence of disease infection, panicle size and seed size. This finding is in agreement with Oljira and Girma [30] that farmers consider the

panicle length, panicle weight, seed size and seed colour as key indicators of seed quality during seed selection. The common seed storage methods were hanging panicles, storage in jute bags, polythene bags, laminated polythene bags, clay pots, containers and storage in barns. Among these, laminated polythene bags and clay pots were the two dominant seed storage methods in the region under studied. The clay pots are constructed with locally available materials (mud or clay), and therefore represent a low-cost option for seed storage [32]. Farmers treat their seeds with ash, orange peels, synthetic chemicals, lemon grass, cut bicycle or motor tubes, reddish gravel, a local plant “*badokoka*” or a combination of these methods. The application of wood ash was the dominant seed treatment (61.1%) in the districts, which could be attributed to its lower cost and the little expertise required for its application compared to synthetic chemicals. This agrees with Prakash et al. [33], who reported that 78% of farmers in Karnataka, India, use ash treatment for storing food grain and seed due to its low cost. Ash contains silica, which reduces the relative humidity within the storage vessel and reduces insect and fungi multiplication [33,34].

#### 4.1. Seed Quality of Farmer-Saved Pearl Millet

Seed quality revolves around the seed moisture content, physical purity and the method of seed storage. The moisture contents of the seed ranged from 7.4 to 9.3%, and were within the recommended range of 6–12% for safe moisture content for seed storage. According to Tonapi et al. [35], storing seed at a 6–12% moisture content helps to maintain seed viability, whilst extreme limits (below 6% or above 12%) lead to seed deterioration through lipid autoxidation. It is also estimated that every 1% decrease in the seed moisture content within the physiological range doubles seed longevity [21,35]. The certified seed had the lowest (7.4%) seed moisture content, which was reflected in a high seed germination rate (Tables 3 and 4). Similarly, the relatively higher moisture content of the Garu district samples was reflected in a lower seed germination rate. Lower seed moisture contents slow seed respiration and result in slow seed deterioration, and vice versa [21,35].

Samples from Garu district also displayed lower germination rates due to the use of ash and clay pots as the dominant methods for seed treatment and storage, respectively. According to Mobolade et al. [32], seeds stored in clay pots absorb moisture from the environment when not properly sealed and kept on a concrete platform. Poor seed storage increases seed moisture imbibition, and increases the incidence of seed disease pathogens, which lead to poor germination [32,36]. This trend was also observed in the seed vigour score, where samples from the Garu district took more days to attain the seven-leaf stage compared to the certified seed. The optimum physical purity of pearl millet is 98% [17,35]. In this study, majority of the seed samples had physical purity below the recommended 98%. The low physical purity contributed to the low germination of the farmer-saved samples [21]. This requires improvements in seed cleaning operations at the farmer level to improve the seed purity. The 1000-seed weight of the samples varied significantly across the districts. The difference in 1000-seed weight could be due to the use of different pearl millet varieties in the districts. This agrees with Sugri et al. [37], who found the range of 1000 seed weight of pearl millet in Ghana to be 7.8–17.9 g among eight varieties tested.

#### 4.2. Incidence of Pathogens on Pearl Millet Seed

A total of 14 fungi were associated with the seed samples. This is consistent with other studies in which fungal pathogens were identified as being associated with pearl millet seeds [38–42]. The overall incidence of the fungi revealed that *Curvularia* spp. was the most commonly occurring fungi (62%), followed by *Talaromyces* spp. (53%), *Aspergillus niger* (52%), with *Penicillium* spp. (3%) being the least common. *Curvularia* spp. causes leaf spots and seed decay of pearl millet [43]. *Talaromyces* spp. and *Aspergillus* spp. cause seed mould, which reduces the seed germination rate [10,38,42]. The presence of these inherent seed-borne fungi thus contributed to the low seed germination rate of the farmer-saved seed.

#### 4.3. Application of Seed Treatments

The application of neem extract, citrus extract, and imidacloprid + thiram (3.5:1) treatments was effective in reducing the incidence of fungi compared to the control. However, the citrus extract provided the most effect in reducing fungal incidence (24.6%) among the treatments. Both citrus and neem extracts contain antifungal properties which hindered the sporulation of fungal inoculum on the seeds [11,44–46]. Das and Godbole [26] stated that aqueous or ethanolic extracts of neem and lemon have the potential to inhibit fungal growth due to the presence of phytochemical compounds including flavonoids, saponins, alkaloids, terpenoids and tannins in the extracts. These phytochemical compounds are reported to inhibit the sporulation and mycelia growth of fungi [26,47]. Citrus extract has a bio-stimulant effect on seed germination, and therefore enhanced the germination of pearl millet seeds in this study [45].

#### 5. Conclusions

The seed system of pearl millet is not well developed in Ghana, meaning that the majority (98.3%) of farmers utilise farmer-saved seed for production. The dominant farmer seed-saving practices found in this study were seed selection, seed treatment with ash, and seed storage in both laminated polythene bags and clay pots. The moisture contents of the seeds were within the recommended range for quality seeds; however, the physical purity of the majority of the samples was below the optimum (<98%), leading to low germination rates (<75%). Citrus extract treatment significantly reduced the incidence of seed-borne fungi, and improved the germination and vigour score of seedlings. Therefore, the citrus extract is more efficient at improving the seed quality of pearl millet.

#### 6. Recommendations

Farmers should be encouraged to use certified seeds as they showed superior qualities in terms of seed physical purity, higher germination rate, and minimum number of days to the three-, five-, and seven-leaf stages in order to minimise the risk of crop failure associated with poor germination rates. There is also a need to build the technical capacity of farmers regarding efficient seed processing and storage methods to improve the quality of farmer-saved seed.

**Author Contributions:** Conceptualization, M.A.A., J.S.Y.E., I.S. and E.Y.D.; Investigation, M.A.A., J.S.Y.E. and E.Y.D.; Methodology, M.A.A., I.S., J.S.Y.E. and P.A.A.; Supervision, J.S.Y.E., I.S. and T.A.-Y.; Data curation and analysis, M.A.A. and I.S.; Writing—original draft preparation, M.A.A.; Writing—review and editing, I.S., J.S.Y.E., T.A.-Y. and P.A.A. All authors have read and agreed to the published version of the manuscript.

**Funding:** This study was supported by the World Bank African Centres for Excellence through the West Africa Centre for Crop Improvement (WACCI, University of Ghana), and partially funded by the German Academic Exchange Service (DAAD).

**Data Availability Statement:** Data is available on request.

**Acknowledgments:** We thank Salim Lamini and Gloria Mensah for their technical assistance at the Plant Pathology Laboratory of CSIR-Savanna Agricultural Research Institute. The assistance of Adek Azantilow at Ghana Seed Inspection Directorate of MoFA, Bolgatanga is also gratefully acknowledged.

**Conflicts of Interest:** The authors declare no conflict of interest.

#### Abbreviations

ANOVA	Analysis of variance
ISTA	International Seed Testing Association
MoFA	Ministry of Food and Agriculture
CSIR-SARI	Council for Scientific and Industrial Research-Savanna Agricultural Research Institute
LSD	Least significance difference
WACCI	West Africa Centre for Crop Improvement

GSID	Ghana Seed Inspection Division
PDA	Potato dextrose agar
DAAD	German Academic Exchange Service

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