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Effects of antifungal plant extracts on improving maize seedling emergence and plant growth

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Abstract

Maize seeds are usually contaminated with seed-borne fungi which cause seed deterioration and seedling death. Seed treatment using chemical fungicides is a common practice, although fungicides can reduce seed longevity and lead to residues, resistance, and environmental pollution. This study was conducted to determine the effects of plant extracts (PEs; *Azadirachta indica*, *Coffea Arabica*, and *Zingiber officinale*) on seedling emergence and seedling vigour of certified (CS) and farmer-saved (FSS) seeds of maize. Seeds treated with water-extracted PEs had significantly higher seedling emergence with 85.8% and 61.7% for FSS and CS, respectively, while ethanol-extracted PEs had 6.7% and 7.5% seedling emergence for FSS and CS, respectively. The minimum mean number of days to the first emergence was less for seeds treated with water-extracted PEs (3.0 and 3.3 for FSS and CS, respectively) than those treated with ethanol-extracted PEs (6.3 and 7.0 for FSS and CS, respectively). According to this study, water-extracted PEs are potential candidates in seed treatment, because they have fewer adverse effects on seedling emergence and vigour. Although other studies mention PEs extracted using organic solvents to be the best, the study's recommendation is to ensure that organic solvents are completely removed from PE solutions before using them.

Keywords: anti-fungal, plant extracts, seed-borne fungi, seed germination, seed treatment, seedling vigour

Introduction

Maize (*Zea mays* L.) is the most important food crop in Tanzania and is produced throughout the country under diverse environments (URT, 2017). Maize production is mostly under rain-fed conditions with minimal use of certified seeds (CS) and other agricultural inputs (Baijukya *et al*., 2020; Mghweno *et al*., 2020). Maize is predominantly grown by small holder farmers who commonly use farmer-saved seeds (FSS) from previous seasons (Msuya and Stephano, 2010; Mghweno *et al*., 2020; Kansiime *et al*., 2021). This is due to the low cost and timely accessibility of those seeds (Etten *et al*., 2017; SAT, 2019; Mghweno *et al*., 2020). Unfortunately, FSSs are normally infected by pathogens, especially pathogenic fungi, including *Fusarium verticillioides* (Nirenberg) (Tsedaley, 2016). These pathogens have effects on seed germination and plant growth. Therefore, it is important to make interventions before sowing. In managing seed-borne pathogens/diseases, seed treatments have been a common practice of seed companies, mostly involving synthetic chemical fungicides (Hubert *et al*., 2015). Such fungicides are not adequately available in smallholder farming systems and, where available, they are too expensive for smallholder farmers. When used, chemical fungicides can reduce seed longevity, leave residues that are harmful to human health and can also be toxic to non-target organisms (Mbega *et al*., 2012; Hubert *et al*., 2015). Due to growing consciousness of the hazardous side effects of these chemicals, more emphasis is being given to the use of safe and eco-friendly biocontrol agents (Lalitha *et al*., 2010; Gyasi *et al*., 2020). Recently, the efficacy of several biocontrol agents including PEs has been evaluated. Several plants have already emerged as potential candidates for managing plant diseases, including neem (*Azadirachta indica* A. Juss.) and ginger (*Zingiber officinale* Roscoe). Different solvents have been used to prepare botanical extracts for use as bio- pesticides to improve efficiency. The current study aimed to determine the efficacy of selected antifungal plant extracts (obtained by using different extraction solvents) in improving seed germination, seedling emergence and plant growth.

Materials and methods

Study area and duration

The field study was conducted at the Crop Museum of Sokoine University of Agriculture (SUA), Tanzania; is located at 6° 49' 27'' S, 37° 39' 48'' E, and 525 m a.s.l., where the climateis sub-humid tropical. The soil is sandy clay loam with a pH of 5.61. There was no significant variation in weather conditions between the two growing seasons over which experiments were conducted. In general, the area experiences a bimodal rainfall pattern with short rains (October-December) and long rain (March-May). Annual rainfall ranges from 750-1050 mm. Planting was done at the beginning of the long rainy season. Temperature moderation exists due to the effects of the Uluguru mountain; but is also dependent on the season. For the long rainy season, the average temperature ranges from 23.5 to 26.5°C.

Maize seeds

Two categories of STAHA maize seed sources were used: farmer-saved seeds (FSS) sourced from smallholder farmers of the Mvomero district, Morogoro Region, and untreated certified seeds (CS) from the Agricultural Seed Agency (ASA), Morogoro Region.

Preparation of plant materials and PEs (Bio-fungicides)

Fresh neem leaves, ginger rhizomes and dry coffee beans were the raw materials used in preparing PEs. Neem leaves and ginger rhizomes were washed, then cut into small pieces and air-dried for a month. The three dried plant materials were separately ground into powder using a milling machine (Foss Tecator Cyclotec 1093 Sample Mill) and then sieved using 1 mm-mesh sieve (Hasan *et al*., 2005; Akinbode and Ikotun, 2008; Hubert *et al*., 2015). 50 g powder of each type of plant material was separately dissolved in 100 ml of sterile distilled water and 70% ethanol to give 50% w/v in a 500 ml conical flask. The mixture was thoroughly stirred and left in a refrigerator at 25-28°C for 24 hours (Nduagu *et al*., 2008; Zida *et al*., 2008). The extracts were separately filtered through a muslin cloth and re-filtered again through Whatman No. 1. filter paper into a sterile 500 ml beaker. For ethanol-extracted bio-fungicides, the filtrates obtained were concentrated by evaporation of the solvent (ethanol) in a water bath at 50°C according to (Rauha *et al*., 2000). Then the obtained solutions were collected into sterilized conical flasks and stored at 25-28°C until use (Mamiro and Royse, 2004).

Experimental design and treatment application

Preliminary laboratory and screen-house studies (experiments laid down in completely randomized design) were carried out for phytosanitary seed testing and bio-efficacy assays of the selected plant extracts against seed-borne fungi and improvement of seed germination. Afterward, the field experiment was conducted as a factorial in a randomized complete block design with three replicates to test the effects of plant extracts in improving seedling emergence and seedling vigor of seeds artificially inoculated with *Fusarium verticillioides.* The three factors were: (A) seed source (FSS or CS); (B) fungicide (one of the three PEs, Apron Star® 42WS or distilled water); and (C) extraction solvent (ethanol or distilled water). Mono-cropping system was used and repeated twice (two consecutive years) with uniform adoption of standard agronomic practices. The spacing used was 750 mm × 300 mm. Plot size was 2.55 m × 3 m with a space of 1 m between replicates and 0.5 m between plots. The number of plants per plot was 40 making a total of 800 plants per replicate and 2400 plants in the whole experiment.

Preliminary seed testing

Preliminary seed health testing of the two seed sources was done (ISTA, 2019). *Fusarium verticillioides*, *Aspergillus flavus*, *A. niger*, *Penicillium* spp., *Rhizopus* spp., and *Curvularia* spp. were detected from all the seed samples.

Isolation of fungus

Due to its significant effect of causing seed deterioration, field epidemics, and production of mycotoxins, *Fusarium verticillioides* was purposely isolated and used in the bio-efficacy assay.

In-vitro assay

A preliminary *in-vitro* assay to evaluate the efficacy of the plant extracts on *Fusarium verticillioides* was done, in which the pure fungal cultures were inoculated at the center of bio-fungicide-amended potato dextrose agar in Petri dishes. Three types of bio-fungicides were evaluated independently: neem, ginger and coffee each extracted by water or by ethanol. The mycelial growth inhibition due to bio-fungicides were recorded. Ethanol-extracted bio-fungicides showed significantly higher inhibitory effect on *F. verticillioides* than the water-extracted bio-fungicides.

Inoculation and treatment of inoculated seeds with plant extracts (PE)
The pure cultured and harvested fungal spores of the previously isolated *F. verticillioides* were spray inoculated (1×10^5 spores ml^{-1}) to all the seed samples (Namai and Ehara, 1986). Inoculated seeds were dried in the laminar flow chamber on three layers of blotter papers in Petri dishes for two hours and then incubated to confirm the presence of the pathogen. The inoculated seeds were treated with well-prepared bio-fungicides. The 50% w/v of previously made ethanol-extracted and water-extracted PEs were used. Maize seeds that were pre-inoculated with *F. verticillioides* were placed in a beaker and each PE suspension was added until all the seeds were totally immersed. The seeds were gently stirred using a glass rod to ensure complete immersion and even distribution. To lessen exterior contamination, beakers with already inoculated seeds were enclosed with aluminium foil. The beakers were then placed at 25°C for 20 hours. Thereafter, the seeds were dried on sterile blotter papers for two hours in the laminar flow chamber (Hubert *et al*., 2015). Untreated seeds and seeds treated with chemical fungicide, Apron Star® 42WS (200 g kg^{-1} Thiamethoxam, 200 g kg^{-1} Metalaxyl –M, 20 g kg^{-1} Difenoconazole) were maintained as negative and positive controls, respectively.

In-vivo assays
Treated seeds were sown in the sand media contained in plastic containers. Data on seed germination, shoot length and seedling weight were collected.

Field experiment
Treated seeds were planted to evaluate the effectiveness of PEs in improving seedling emergence and vigor by protecting seeds against *F. verticillioides*. The following data were collected: seedling emergence 3 and 9 days after planting, seedling height (cm), leaf width (cm), leaf length (cm), and fresh weight (g) (CIMMYT, 1985). Collected data were processed to calculate the emergence percentage based on the number of seeds sown and leaf area as the product of leaf length and width.

Data analysis
Data analysis was performed based on the factorial experiment' arrangement in an RCBD analysis of variance (ANOVA) model. GenStat (16th version, VSN International) was the software used. Means were separated by Tukey's test at $P < 0.05$. The statistical model used was:

$Y = \mu + A + B + C + AB + AC + BC + ABC + \varepsilon$ .... iii where μ is grand mean, ε is a random error term, factor A = seed source, B = fungicide, and C = extraction solvent.

Results

Seedling emergence
Results on the effect of fungicides on the number of days to first seedling emergence showed a highly significant effect ($P < 0.001$) for both growing seasons (though results

from season 1 did not differ significantly from those of season 2). A lower mean number of days to first seedling emergence was observed for seeds treated with extracts of ginger, coffee, neem, and water (negative control), while seeds treated with Apron Star® 42WS (positive control) had a significantly higher mean number of days to first seedling emergence (figure 1A).

Interaction between fungicide and extraction solvent had a highly significant effect ($P < 0.001$ for both growing seasons) on first seedling emergence. Neem × ethanol, coffee × ethanol, and ginger × ethanol resulted in lower first counts than the rest, which had similar responses (figure 1B).

The effects of interaction between seed source, fungicide, and extraction solvent were significant on final seedling emergence ($P = 0.006$ for both growing seasons). Lower percentages of final counts were observed from both CS and FSS interacted with ethanol-extracted bio-fungicides while higher percentages were from seeds treated with water, Apron Star® 42WS, and water-extracted bio-fungicides (figure 1C).

Seedling vigour

Interaction between fungicides and extraction solvent had a significant effect on plant height ($P = 0.002$ for both growing seasons). Neem × water, ginger × water, coffee × water, water × water, and Apron Star® 42WS × water led to higher mean plant heights than the interactions of bio-fungicides with ethanol (figure 2A).

Seed source and extraction solvent had significant effects ($P = 0.002$ and $P < 0.001$. respectively) on plant fresh weight for both growing seasons. FSS had a higher plant fresh weight than CS. In contrast, the water as the extraction agent resulted in higher plant fresh weight than ethanol (figure 2B).

Interaction between seed source, fungicide, and extraction solvent had significant effects on leaf area ($P = 0.047$ for both the growing seasons). Though the differences were small, seeds treated with water-extracted bio-fungicides, water, and Apron Star® 42WS had higher leaf area than the other treatments (figure 2C).

Discussion

Plant extracts (PEs) with antifungal effects have been widely advocated as alternatives to synthetic fungicides for the management of seed-borne fungal diseases. In this study, the effects of antifungal PEs on seedling emergence and growth of both certified and farmer-saved maize seeds were significantly dependent on the type of extraction solvent used to prepare such extracts (figures 1 and 2). The best seedling emergence, final emergence, and growth performance were observed for seedlings from seeds treated with water-extracted plant extracts. In contrast, ethanol-extracted PEs and chemical fungicide (Apron Star® 42 WS; a positive control) might have contained phytotoxicity or growth inhibitory effects perhaps due to ethanol residues in the PE solutions due to incomplete evaporation. This agrees with previous studies (Zida *et al*., 2008; Hubert *et al*., 2015) which reported ethanol and Apron Star® 42 WS to be responsible for reduced seedling emergence.

The performance of farmer-saved seeds (FSS) was superior to that of certified seeds (CS), possibly due to differences in seed size; the FSS were larger in size compared to CS,

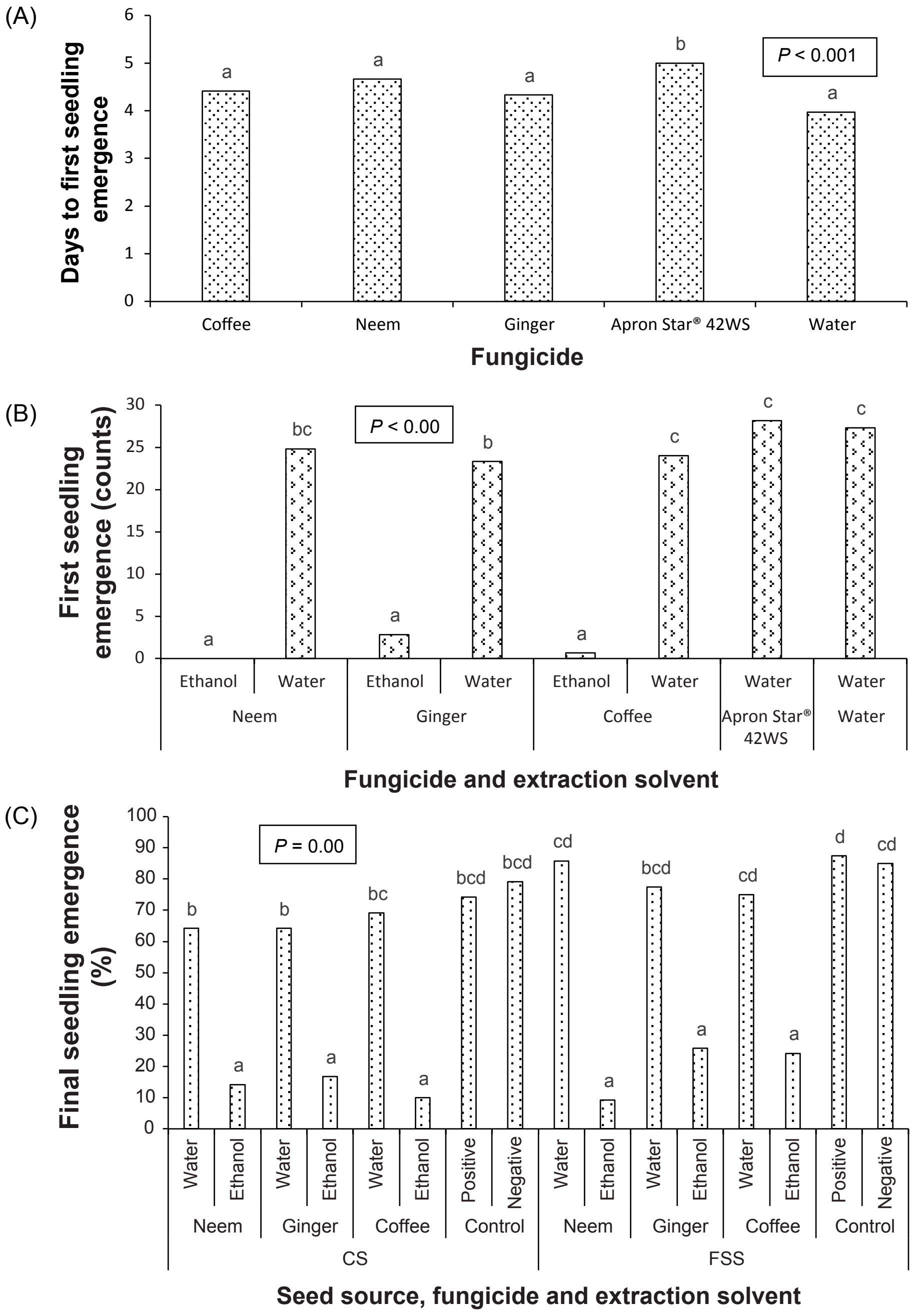

Figure 1. Seedling emergence. (**A**) effects of fungicides on the number of days to first seedling emergence; (**B**) effects of the interaction between fungicides and extraction solvents on first seedling emergence; (**C**) effects of the interaction between seed source, fungicides, and extraction solvents on final seedling emergence.

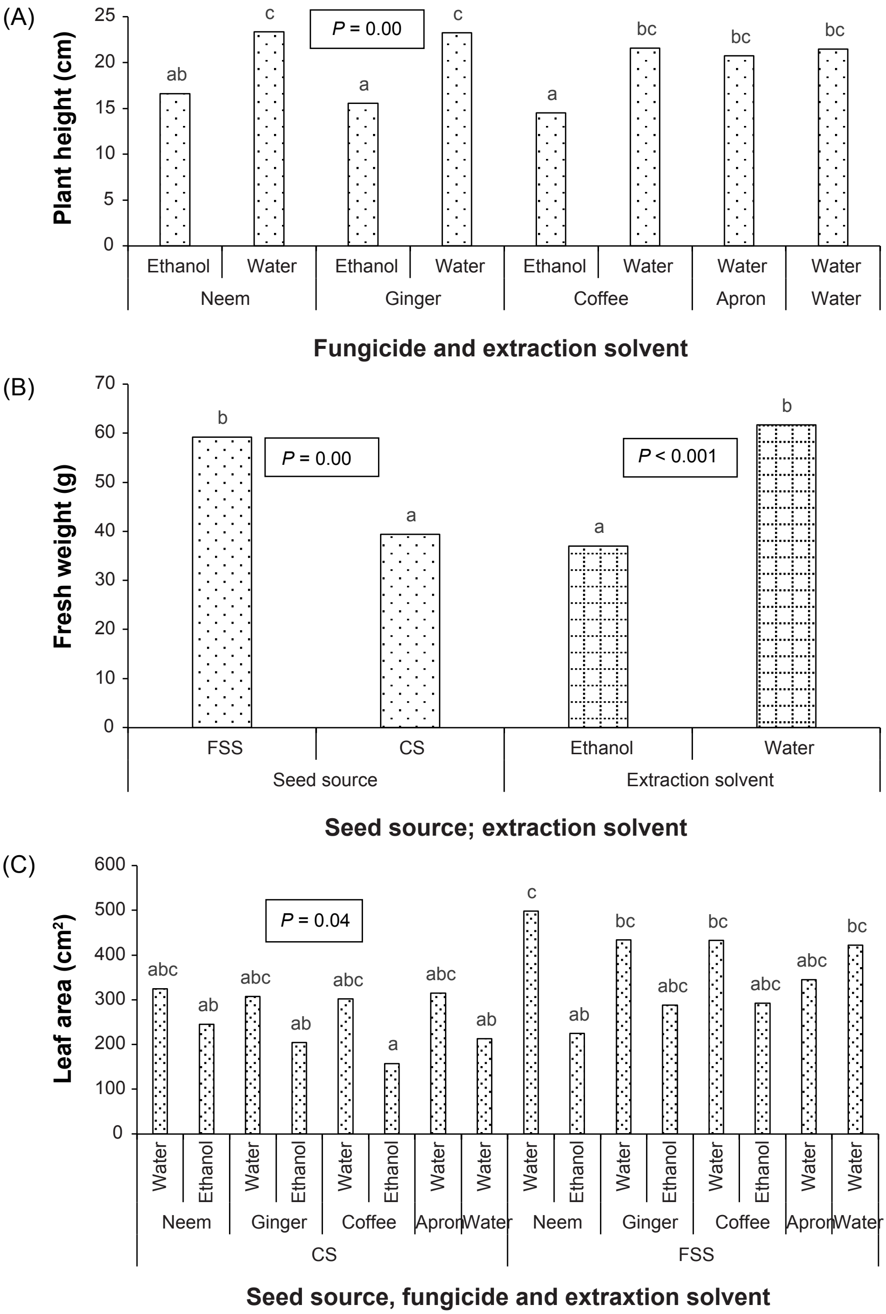

Figure 2. Seedling vigour. (**A**) effects of the interaction between fungicides, and extraction solvents on plant height; (**B**) effects of seed source, and extraction solvents on fresh weight; (**C**) effects of the interaction between seed source, fungicides, and extraction solvents on leaf area.

meaning that FSS had considerably higher amounts of reserved food (storage reserves) available for early growth (personal observation). Well-started plants tend to have superior growth characteristics including plant height and leaf area. Previous studies found that plant height correlates positively with seed mass and is a major determinant of a plant's ability to compete for light (Moles *et al*., 2009; Wright *et al*., 2017). Seedlings from seeds treated with water-extracted PEs grew more vigorously than those treated with ethanol-extracted PEs. This might be related to the negative impacts caused by ethanol residues in the PEs. Ethanol residues could be responsible for poor seedling growth due to seedling chlorosis (field observation). On the other side, bio-actives from PEs were assumed to have positive effects on promoting seedling growth. These are consistent with the study by Keta *et al*. (2019) which reported higher amounts of bioactive compounds in plant extracts. These bioactive compounds not only manage seed-borne fungi which could otherwise limit seedling growth; but also stimulate seed growth of seedlings (Mbega *et al*., 2012). Observation from measurements found that plants with larger leaf areas had higher fresh weights. This proportional relationship between plant fresh weight and plant leaf area imply that foliar water content and large leaf surface area are fairly important in capturing light responsible for photosynthesis which contributes significantly to plant growth and yield (Milla and Reich, 2007; Ullah *et al*., 2013; Huang *et al*., 2019).

Conclusions and recommendations

Maize seeds treated with water-extracted PEs from *Zingiber officinale*, *Azadirachta indica*, and *Coffea arabica* had significantly higher emergence percentages and vigorous seedlings than those treated with ethanol-extracted PEs. The ability of these PEs to improve seedling emergence without causing negative effects on seedling vigour by managing seed-borne fungi; points to their potential use against seed-borne fungi. The study also found that seedling emergence and seedling vigour for maize seeds treated with ethanol-extracted PEs were poor. This may be due to incomplete ethanol evaporation. Further studies are recommended to identify bioactive compositions contained in PEs responsible for managing seed-borne diseases and stimulating plant growth, as well as their mechanism of action. Nonetheless, proper methods of removing extraction solvents from the extracted PEs are needed, since PEs extracted using ethanol or other organic solvents have been reported to contain higher percentages of bioactive compounds (hence highly effective in managing pathogens) than those extracted using water.

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