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Short Communication
Potential of Leaf Decoctions in Germinability Improvement and Protection Against Fungal Pathogens on *Abelmoschus esculentus*

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Abstract

Background and Objective: Accomplishing fast and uniform seedling development is strategic for crop vigour because delayed germination regularly open seedlings to unfavorable ecological conditions and soil-borne diseases. This study was aimed at assessing the germinability enhancement and protective potentials of leaf decoctions of four selected plants (*Chromolaena odorata*, CO; *Nauclea latifolia*, NL, *Ipomoea asavifolia*, IP and *Moringa oleifera*, MO) in comparison to hydrogen peroxide (HP), normal saline suspended cells of *Pseudomonas aeruginosa* (PA) and water (control) against selected fungal pathogens (*Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium* and *Aspergillus niger*) on *Abelmoschus esculentus* (okra). **Materials and Methods:** Surface sterilized seeds of *Abelmoschus esculentus* were soaked in the fungal pathogens for 1 h before being steeped in a given concentration of the respective decoctions for another 1 h and then planted on wet blotter for 7 days while monitoring the germination parameters. **Results:** The highest germination rates of 64.23 and 64.29% were observed when the seeds were primed with 5-fold dilution of CO and IP, respectively. However, germination was highest (64.28%) at 2-fold and 5-fold dilutions when primed with MO while HP-primed seed gave the germination rate was 92.86%. For seeds primed with the PA, germination rate of 71.42% was observed in the undiluted and 5-fold dilutions. The optimum soaking time was observed to be between 3 and 6 h, when the seeds were primed with the different decoctions. Following pre-treatment of the seeds with the fungal pathogens prior to priming with the different osmotica, germination rate reduced to 50%. This is significant compared to no growth observed in infected seeds without priming. **Conclusion:** Priming with decoctions of CO, IP and MO as well as HP and PA significantly improve germinability and confer protection against fungal infection. Thus, scale-up to field trials using CO, IP and MO decoctions, as well as HP and PA as bio-fungicide prior to planting for protection of okra seeds, seems justified as a sustainable alternative to the use of chemical fungicides.

Key words: Leaf decoctions, seed priming, fungal pathogens, germination index

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fungal pathogens attached to seeds or within seeds constitute a considerable challenge to plant growth and development and have been implicated in poor seed quality[1-3]. Because the majority of crop diseases are seed borne and are caused by microbial pathogens, thus leading to poor seed quality and decreased yields in many crops, seed treatment is therefore vital to the improvement of seed quality, which can lead to significant increase in crop yield[4-6].

It is indicated that seed borne pathogens are extensively controlled by synthetic chemicals which are effective. However, owing to their non-biodegradability and residual toxicity, the use of chemicals for seed treatment is no longer encouraged[7]. Besides, the use of chemicals is expensive for rural farmers and can also affect the rhizosphere microbial population, which are beneficial to the ecosystem[8]. Due to the drawback of chemical treatment, the search for alternative methods for the control of pre and post-harvest crop diseases has been on-going in recent years[9].

It is important to explore new approaches for the prevention and treatment of seeds that are less expensive, non-chemical and eco-friendly in the control of both pre and post-harvest infections[9,10]. In recent years, the use of plants components for seed protection has gained prominence. This is because, apart from their effectiveness in preventing pathogens, some of these plants are known to enhance vigor and other germinability indices in plants. Apart from their abundance, medicinal plants are said to be safe and effective in the treatment of seed-borne infections[11]. Several studies aimed at investigating the deployment of different extracts of plants and their compounds in the control of fungal pathogens have shown the promising potential of these extracts in the inhibition of fungal growth. Therefore, this study was aimed at exploring the potential of the leaf decoctions of four selected plants in germination enhancement and seed protection of *Abelmoschus esculentus*, commonly known as okra against fungi pathogen.

MATERIALS AND METHODS

Source of the test seeds: The seeds used for the study were that of okra (*Abelmoschus esculentus*). The seeds were obtained from an agro-product store in Omu-Aran, Kwara State, Nigeria. Prior to use, the seeds were surface-sterilised with sodium hypochlorite (5% v/v) for 5 min.

Preparation of plant decoctions: A total of four respective plant leaves were used for the study. The plants, which were *Chromolaena odorata* (CO), *Nauclia latifolia* (NL), *Ipomoea asavifolia* (IP) and *Moringa oleifera* (MO) were identified at the Herbarium Services Unit of the Department of Plant Biology, University of Ilorin, Nigeria.

The respective plant leaves were collected fresh in December, 2016, washed with tap water to removes sand and other dirt before blending with an electronic blender. The blended leaves were then mixed with known quantity of water (750 g/3 L of water for the CO, 216 g/6 L of water for the NL, 727 g/3 L of water for the IP and 451 g/3 L of water for the MO) and allowed to stand on for 24 h. After standing for 24 h, the respective water-leave mixtures were filtered with filter paper. The filtrates were stored in aliquots and kept in the refrigerators until when needed. The total volume of filtrate recovered was 3.5, 5.5, 2.9 and 3.1 L for the CO, NL, IP and MO, respectively. In this study, the respective filtrates obtained were referred to as decoctions.

Determination of effective concentration: To determine the effective concentration of the respective decoctions for germination and vigor enhancement of the test seeds, different concentrations (0, 1:1, 1:2, 1:3, 1:4 and 1:5 decoction-water dilutions) were prepared. A total of 10 surface-sterilised seeds in triplicate setups were steeped in 10 mL of the respective dilutions for 1 h after which 7 seeds were planted in plastic cups containing blotters for 7 days while monitoring the germination parameters.

Three categorises of controls were used in the study. A control experiment that contained seeds that were steeped in tap water, control with 30% hydrogen peroxide (HP) and control with *Pseudomonas aeruginosa* cells (PA) suspended in normal saline solution (0.98% w/v NaCl) at similar dilutions.

Determination of optimum soaking time: For the determination of the optimum soaking time, 60 surface-sterilised seeds in triplicate setups were steeped in 60 mL of known concentrations of the respective decoctions and the controls for 6 h. At every 1 h, for the 6 h duration, 7 seeds were withdrawn from each setup and treated as described earlier.

Determination of protective potential of the decoctions: A total of four fungal cultures (*Aspergillus fumigatus*, *Aspergillus flavus*, *Fusarium* and *Aspergillus niger*) were used for the study. The fungal isolates were first grown in sabouraud dextrose agar plants to obtain pure culture before being transferred to 100 mL of sterile sabouraud dextrose broth in conical flasks and grown for 72 h. At the expiration of the growth period, the broth containing the respective fungi

were centrifuged at 5000 rpm for 10 min, to separate the cells from the supernatant. The cells were then suspended in sterile distilled water and refrigerated until when needed.

To determine the protective potential of the decoctions and the control setups, the surface sterilised seeds were first steeped in the respective fungal suspensions for 1 h, after which they were transferred to known concentrations of respective decoctions and the controls for another 1 h. After the steeping period, the seeds were planted and monitored, as described earlier. Setups that involved steeping in the fungal suspensions for the 2 h duration before planting served as control.

Statistical analysis: All statistical analyses were carried out using the SPSS Statistical Software (Version 20.0). For comparison of means, the One-Way Analysis of Variance (ANOVA) was used at probability level of 0.05.

RESULTS

When the okra seeds were primed at the different concentrations of the *Chromolaena odorata* (CO) decoctions, highest germination of 64.23% was observed at 5-fold diluted concentration. The germination index was best (63.38) with the 5-fold dilution while the least germination time (5.1 days) was observed with the 2-fold dilution (Fig. 1). In the NL-primed seeds, the percentage germination was highest (78.42%) in the undiluted and 2-fold dilutions. The germination index was highest (82.59) in seeds that were primed with the undiluted decoction. The least germination time of 5.0 days was observed in seeds primed the 1-fold diluted decoction (Fig. 1). For seeds that were primed the IP decoctions, the 0-fold and 5-fold dilutions gave the highest percentage germination of 64.29% while the 1-fold primed seeds gave the highest germination index (53.68) and germination time (5.18 days). When the different concentrations of the *Moringa oleifera* (MO) decoctions were used for priming, percentage germination was highest (64.28%) in the 2-fold decoction primed seeds while the germination index was highest (64.15) in the 5-fold diluted decoction. The lowest germination time (4.97 days) was observed in seeds primed with the 1-fold diluted decoction (Fig. 1). In the HP-primed seeds, the highest percentage germination (92.86%) was observed in the 1-fold diluted solution. The highest germination index and germination time values of 104.19 and 4.97 days were observed in seeds primed in the 1-fold dilute solution and the undiluted solution, respectively (Fig. 1). In the PA primed seeds, the highest germination of 71.42% was observed in the undiluted and 5-fold dilutions, respectively. Germination index was highest (81.34) in the 5-fold primed seeds while germination time was least (5.04 days) in the 3-fold diluted osmotica (Fig. 1).

When investigating the effect of priming duration on the germinability of the okra seeds, percentage germination was observed to be highest at 6 h for CO, NL, IP, MO and HP except for PA where the highest percentage germination was

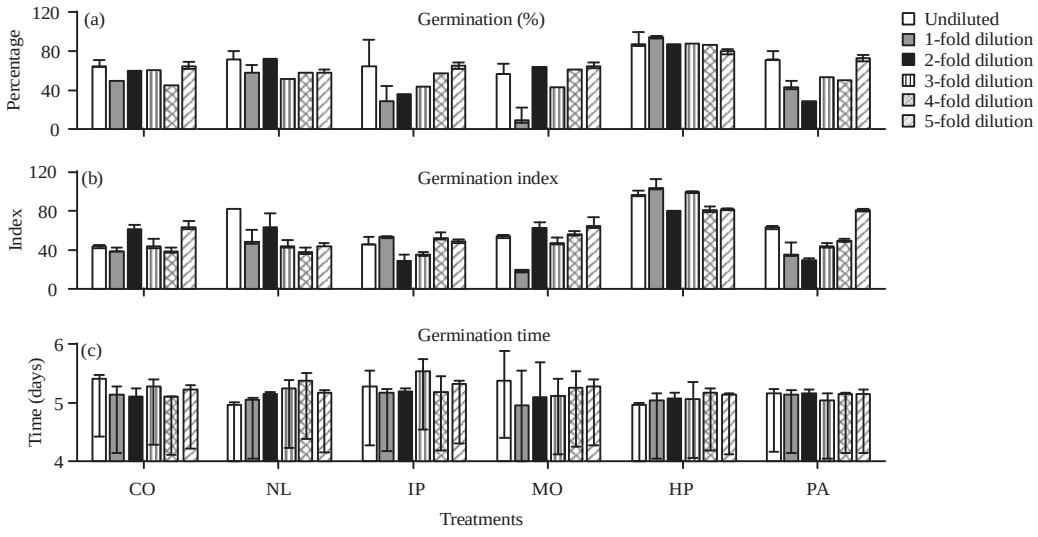

Fig. 1(a-c): Effect of different concentration of the leaf decoctions on the germination parameters of the okra seeds when primed in different concentrations of the decoctions

CO: *Chromolaena odorata*, NL: *Nauclea latifolia*, IP: *Ipomoea asavifolia*, MO: *Moringa oleifera*, HP: Hydrogen peroxide and PA: *Pseudomonas aeruginosa*

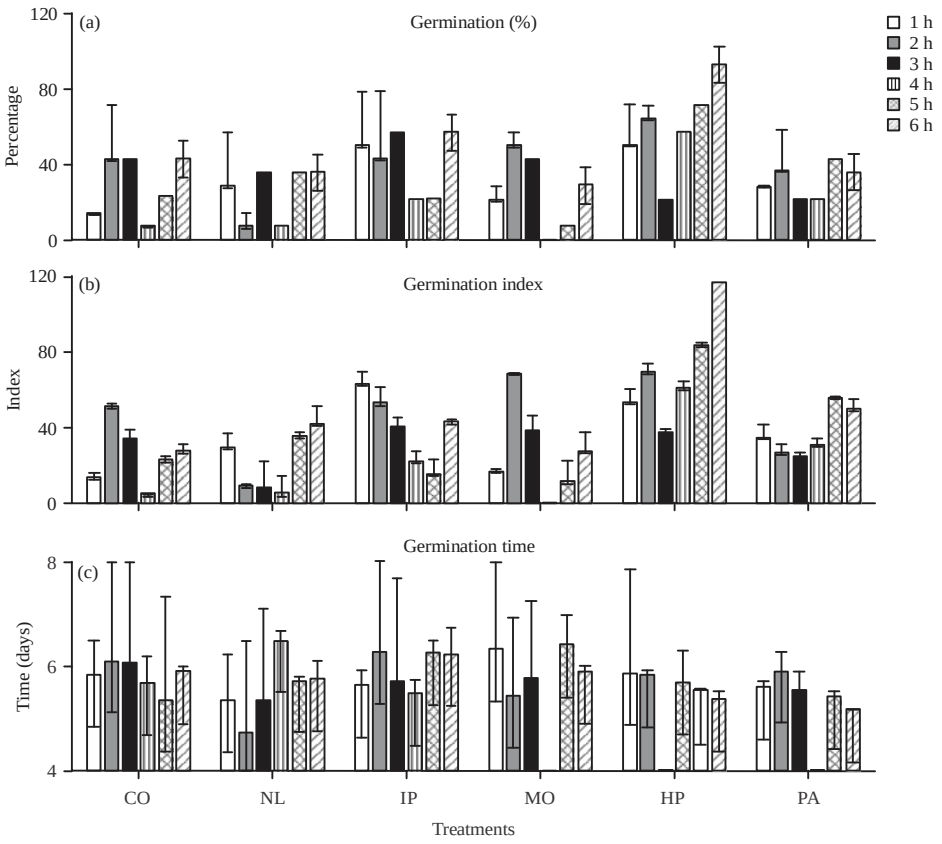

Fig. 2(a-c): Effect of soaking time on the germination parameters germinability of the okra seeds when primed at different soaking durations in the decoctions

CO: *Chromolaena odorata*, NL: *Nauclea latifolia*, IP: *Ipomoea asavifolia*, MO: *Moringa oleifera*, HP: Hydrogen peroxide and PA: *Pseudomonas aeruginosa*

recorded after 5 h. Germination index was highest at 2 h for CO, 6 h for NL, 1 h for IP, 2 h for MO, 6 h for HP and 5 h for AP. Germination time was fastest at 5 h for CO, 2 h for NL, IP and MO, 6 h for HP and PA (Fig. 2).

In seeds that were first infected with a liquid culture of *Aspergillus fumigatus* before priming with the different treatments, the HP-treated seeds gave the highest percentage germination 71.43%, while the least (14.29%) was recorded for NL. Germination index was observed to be highest (78.92) in the IP-treated seeds and lowest (9.36) in the NL-treated seeds (Fig. 3).

For the case of seeds that were infected with the *Aspergillus flavus* before priming with the respective treatments, the IP and MO-treated seeds gave the highest percentage germination 57.14%, while the least (14.29%) was recorded for CO. Germination index was observed to be highest (72.87) in the MO-treated seeds and lowest (18.89) in the CO-treated seeds (Fig. 4).

When the seeds were infected with the *Fusarium* sp. before priming with the respective treatments, the HP-treated

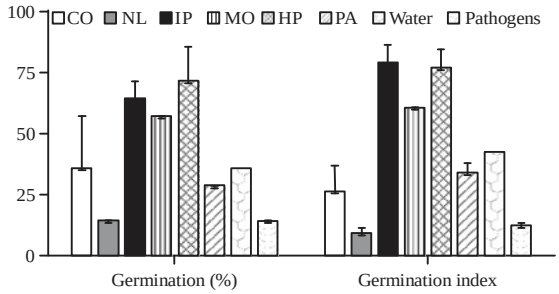

Fig. 3: Protective potential of the decoctions against seeds infected with the *Aspergillus fumigatus cultures*

CO: *Chromolaena odorata*, NL: *Nauclea latifolia*, IP: *Ipomoea asavifolia*, MO: *Moringa oleifera*, HP: Hydrogen peroxide and PA: *Pseudomonas aeruginosa*

seeds gave the highest percentage germination 71.43%, while the least (42.86%) was recorded for NL. Germination index was observed to be highest (80.08) in the IP-treated seeds and lowest (20.32) in the PA-treated seeds (Fig. 5).

In seeds that were infected with the *Aspergillus niger* before priming with the respective treatments, the HP-treated

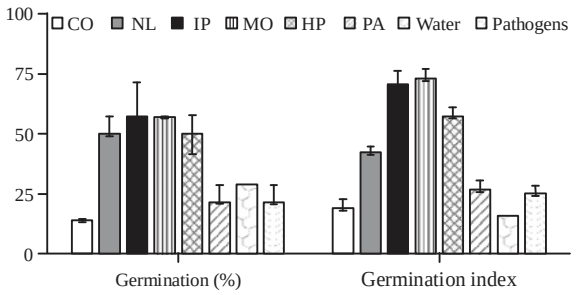

Fig. 4: Protective potential of the different treatment solutions against seeds infected with the *Aspergillus flavus cultures*

CO: *Chromolaena odorata*, NL: *Nauclea latifolia*, IP: *Ipomoea asavifolia*, MO: *Moringa oleifera*, HP: Hydrogen peroxide and PA: *Pseudomonas aeruginosa*

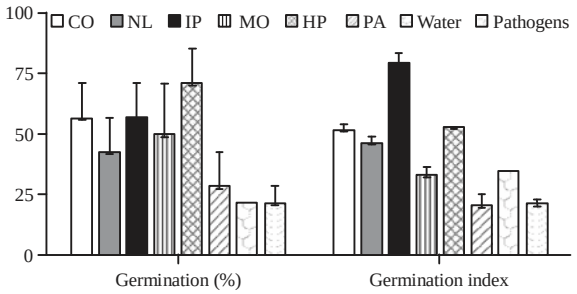

Fig. 5: Protective potential of the decoctions against seeds infected with the *Fusarium* sp.

CO: *Chromolaena odorata*, NL: *Nauclea latifolia*, IP: *Ipomoea asavifolia*, MO: *Moringa oleifera*, HP: Hydrogen peroxide and PA: *Pseudomonas aeruginosa*

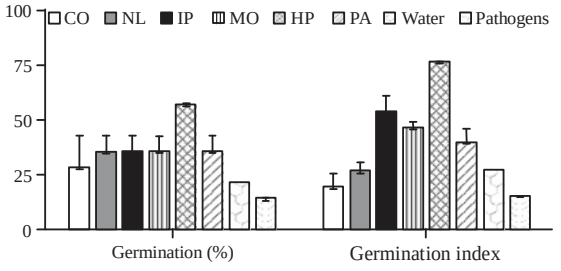

Fig. 6: Protective potential of the decoctions against seeds infected with the *Aspergillus niger*

CO: *Chromolaena odorata*, NL: *Nauclea latifolia*, IP: *Ipomoea asavifolia*, MO: *Moringa oleifera*, HP: Hydrogen peroxide and PA: *Pseudomonas aeruginosa*

seeds gave the highest percentage germination 57.14%, while the least (28.57%) was recorded for CO. Germination index was observed to be highest (76.73) in the IP-treated seeds and lowest (19.45) in the CO-treated seeds (Fig. 6).

DISCUSSION

When compared with the water control setup, all the leaf decoctions used in this study were observed to enhance germinability of the okra seeds, although highest germination was observed at different concentrations for the different decoctions. In a study on the effect of *Chromolaena odorata* on the growth and biomass accumulation of *Celosia argentea,* Ilori *et al.*[12] indicated higher shoot height, leaf area , fresh and dry weights of plants that were treated with aqueous extracts, when compared with those of the control regime, thus showing an indication that aqueous extract of *Chromolaena odorata* has the potential of enhancing the growth *Celosia argentea*.

In this study, higher concentrations of the decoctions were observed to reduce percentage germination and increase germination time. A similar observation has been recorded by Mousavi *et al.*[13]. The increase in the degree of inhibition observed at higher concentrations of some of the decoctions may be attributed to the likelihood of increased concentrations of allelochemicals that may be present in the decoctions[14]. Mousavi *et al.*[13] has indicated increase in allelopathic effect of different concentrations of alfalfa was highly significant for germination while there were significant decreases in germination rate and mean germination time with increasing concentration of the extracts on seedling growth of wheat. In this investigation, a remarkable decline in germination percentage was observed at some concentrations of the decoctions used for priming. Reports of different concentrations of leaf extracts causing significant inhibitory effects on germination has been recorded by earlier investigators in similar studies[15,16].

The potential of different concentrations of the leaf extracts of *Moringa oleifera* as a seed priming agent to increase the germination rate and plant vigor of three kinds of grass (*Cenchrus ciliaris*, *Panicum antidotale* and *Echinochloa crus-galli*) has been reported by previous investigators[17]. In their finding, although all of the priming strategies were observed to show remarkable priming potential, as compared to the control, matripriming and priming with 1:30 dilution was observed to show greater efficiency[17]. In a study on the effect of seed priming with plant leaf extracts on growth characteristics and root rot disease in tomato, seeds that were primed with different extracts showed improved germination and decreased germination time was recorded in all treatments at certain concentrations[16]. It is opined that during priming, decrease in germination time and an increase in

germination percentage may be due to changes in both the chemical and physiological nature of the embryo of the seed and other related structures[18].

Data from this study revealed the highest germination and lowest germination time at priming durations between 2-4 h in most of the treatments. This observation corroborates the observation of Murungu[19]. In the report on the effects of seed priming and water potential on seed germination and emergence of wheat (*Triticum aestivum* L.) varieties in laboratory assays and in the field, Murungu[19] reported that at relatively higher water potentials, primed seeds were observed to show higher germination than seeds that were not primed. It is hypothesized that when seeds are not primed, their susceptibility to moisture stress conditions is greater[20]. In a study of seed quality enhancement through seed priming in pigeonpea (*Cajanuscajan* (L.) Mill sp.), seed and seedling quality characteristics were reportedly enhanced with 2% leaf extract at the soaking duration of an hour[21].

CONCLUSION

This present study concludes that leaf decoctions of *Chromolaena odorata*, *Nauclea latifolia*, *Ipomoea asavifolia* and *Moringa oleifera* are viable primers that could enhance germination potential of and protect against selected fungal pathogens of *Abelmoschus esculentus*. These decoctions are not only organic but are cheap, readily available and friendly to the environment.

There is however the need to explore their potential as effective foliar spray and soil additives that could enhance yield of plants, which is the subject of our further studies. In addition, it is also vital to test these decoctions more deeply so as to identify further their use, when compared with priming agents that are of synthetic origin. Harnessing such findings could lead to greater productivity

SIGNIFICANCE STATEMENT

Results from this study is of potential benefit to low income farmers who may not be able to afford the luxury of the expensive chemical fungicides in prevention of postharvest spoilage of seeds and seedling. In addition, the significant protection afforded by these plant decoctions indicate their potentials as alternative to chemical fungicides which have most often been reported with some environmental risks.

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