

RESEARCH ARTICLE

Efficacy of aqueous leaf extracts of neem, bitterleaf and their mixture for prevention of smut and red rot diseases of sugarcane

Ganiyu S. Olan

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Abstract

Sugarcane is the main source of sugar. Sugar production is greatly threatened by smut and red rot diseases caused by *Sporisorium scitamineum* and *Colletotrichum falcatum*, respectively. Although there are recommended chemical and cultural control measures for these dreaded diseases, the control measures have serious shortcomings as a result of which there is need to evaluate the efficacy of aqueous leaf extracts of neem and bitterleaf as a control measure, the aim of this study. Sugarcane setts were inoculated with mycelial suspensions of *Sporisorium scitamineum* and *Colletotrichum falcatum*.

The inoculated cane setts meant for pre-planting treatment with 30 g/100 ml leaf extracts of neem, bitterleaf and their mixture were soaked in each of the extracts for 30 minutes, dried, and then planted in plastic buckets containing sterilized soil.

The inoculated cane setts for post-planting treatment were also planted in plastic buckets containing sterilized soil and sprayed fortnightly with 30 g/100 ml leaf extracts of neem, bitterleaf, and their mixture. All the plastic buckets were labeled appropriately and allowed to stand for 3 months, the incubation period for smut and red rot diseases of sugarcane. Pictures of the seedlings that sprouted from each of the inoculated cane setts showed the efficacy of 30 g/100 ml leaf extracts of neem, bitterleaf, and their mixture in preventing the occurrence of these diseases, with the post-planting fortnight spraying being more efficient. It is therefore recommended for prevention of smut and red rot diseases of sugarcane.

Keywords: *Colletotrichum falcatum*; fortnight; Post-planting; Pre-planting; *Sporisorium scitamineum*

Dept of Plant Biology, University of Ilorin, Nigeria

*Correspondence authors: Olan.gs@unilorin.edu.ng

Introduction

Sugarcane, *Saccharum officinarum* L., a member of the Andropogoneae tribe in the family Poaceae is cultivated mainly for its sweet juice which has many nutritional, health and economic benefits (Anonymous 2018; Mohamed et al. 2019; Hussien et al. 2023). Production of sugarcane is threatened by diseases caused by some fungi, bacteria, viruses, Rickettsia-Like Organisms (RLO) and phytoplasmas in different parts of the world (Rott and Comstock 2015; Bassey and Wada 2024).

Smut (caused by *Sporisorium scitamineum* (Sydow) Piepenbring, Stoll & Oberwinkler) and red rot (caused by *Colletotrichum falcatum* Went) are among the most dreaded sugarcane diseases worldwide (Hossain et al. 2020; Hesham et al. 2023).

Research has recently focused on using medicinal plant parts to control plant and animal diseases due to their secondary metabolites, which possess antimicrobial properties. These metabolites include alkaloids, anthraquinones, cardiac glycosides, cyanogenic glycosides, tannins, and polyphenols (Iwu 2000).

Studies have shown that leaf extracts from *Azadirachta indica* L. and *Melia azedarach* L. (Meliaceae family) exhibit strong antifungal activity against *Macrophomina phaseolina*, a pathogen causing charcoal rot disease in various plant species (Javaid and Rehman 2011).

Additionally, neem leaf extracts have been found to inhibit the growth of *Penicillium digitatum*, with increased inhibition observed at higher concentrations (Suleiman 2011). Control of smut and red rot diseases of sugarcane using synthetic fungicides have enabled the pathogens to produce new resistant pathological races or strains (Ghazanfar and Kamran 2016).

The synthetic fungicides also have harmful side effects on humans, other animals and the environment.

The use of hot water or air for the control of these diseases can also cause burns if not carefully done. Therefore, this study focuses on exploring the potential of natural, biodegradable products like plant extracts for prevention, which is the primary objective of this research.

Materials and Methods

Soil sterilization and preparation of aqueous leaf extracts of neem and bitterleaf

Topsoil was collected from the Botanical Garden of the University of Ilorin and sieved to remove dirt. The clean soil was sterilized in an autoclave at 121° C and 15 psi pressure (Fawole and Oso 2007). The sterilized soil after each round of sterilization in the autoclave was emptied on a clean tarpaulin and allowed to cool. Aqueous leaf extracts of 30% (w/v) concentration of neem, bitterleaf and their mixture were prepared following the methods of Olahan et al. (2022). They were stored in the refrigerator prior to their use as botanicals in this study.

Preparation of mycelial suspensions of *Ustilago scitamineum* and *Colletotrichum falcatum*

The procedures of Bhuiyan et al. (2009) were adopted for mycelial suspension preparation. A known weight (5 g) of each of the stock cultures of *S. scitamineum* and *C. falcatum* maintained on PDA was added to 2 liters of sterile distilled water separately. Two drops of Tween® 20 were added to each of them after which each of the propagules was blended separately in an electric blender (SMB 2977 model). Volume of each of the mycelial suspensions was increased to 5 liters with sterile distilled water and then stored in a refrigerator at 10° C prior to their usage (Fawole and Oso 2007).

In vivo assessment of the efficacy of aqueous leaf extracts of neem, bitterleaf and their mixture on the pathogens of smut and red rot diseases of sugarcane

Powdered leaves (100 g) of neem (*Azadirachta indica*) and bitterleaf (*Vernonia amygdalina*) as well as equal proportion (50 g each) of the powdered leaves of *A. indica* and *V. amygdalina* were soaked separately in 3 liters of distilled water inside three small plastic buckets for 48 hours. Each of the leaf extracts was sterile-filtered thereafter and stored in a labeled sterile container inside a refrigerator set at 10° C prior to its usage as a botanical (Okigbo and Mbeka 2005).

Commercial sugarcane germplasms, variety Co.6217, susceptible to the smut and red rot diseases were collected from Wuya in Gbako Local Government Area of Niger State and the identity was authenticated at the Herbarium Section of the Department of Plant Biology, University of Ilorin with the voucher number UILH/001/2019/475. They were cut into twenty – eight (28) cane setts of 12 cm in length each using a sharp sterile cutlass. Each cane cutting had at least one node. Twenty-eight 4-liter plastic buckets were prepared by piercing the bottom of each one and filling them with cool, sterile soil.

The 28 cane setts were divided into two sets of 14 cane setts each. A set of 14 cane setts was inoculated by soaking them in the inoculum suspension of *Sporisorium scitamineum* for 30 minutes, while the second set of 14 cane setts was also inoculated by soaking them in the inoculum suspension of *Colletotrichum falcatum* for 30 minutes as well. They were removed from the separate inoculum suspensions and air-dried separately for 30 minutes on different portions of a new polythene sheet. The air-dried inoculated cane setts were subjected to the procedures of Ram et al. (2009) under an aseptic environment provided by a U.V. light with little modifications as follows:

a. Two of the cane setts inoculated with *Sporisorium scitamineum* were planted separately in two of the plastic buckets and labeled as Control S, while two of the cane setts inoculated with *Colletotrichum falcatum* were planted in separate plastic buckets and both plastic buckets labeled as Control C.

b. Two each of the remaining cane setts inoculated with *Sporisorium scitamineum* and thereafter dried were soaked in the aqueous neem leaf extract, the aqueous bitterleaf leaf extract and the aqueous mixed leaf extract separately for an hour each, and then air-dried for 30 minutes on a new polythene sheet. They were then planted in different soil-filled plastic buckets labeled as Pre-Planting S Neem, Pre-Planting S BL and Pre-Planting S Mixed, respectively.

c. Two each of the remaining cane setts inoculated with *Colletotrichum falcatum* and thereafter dried were soaked in the aqueous neem leaf extract, the aqueous bitterleaf leaf extract and the aqueous mixed leaf extract separately for an hour each, and then air-dried for 30 minutes on a new polythene sheet. They were then planted in different soil-filled plastic buckets labeled as Pre-Planting C Neem, Pre-Planting C BL and Pre-Planting C Mixed, respectively.

The remaining six cane setts inoculated with mycelial suspension of *Sporisorium scitamineum* and *Colletotrichum falcatum* were used as follows:

a. Two each of the cane setts inoculated with *Sporisorium scitamineum* and later air-dried were planted in 3 soil-filled plastic pots labeled as Post-Planting S Neem, Post-Planting S BL and Post-Planting S Mixed, respectively.

b. Two each of the cane setts inoculated with *Colletotrichum falcatum* and later air-dried were planted in 3 plastic pots labeled as Post-Planting C Neem, Post-Planting C BL and Post-Planting C Mixed, respectively.

All the inoculated cane setts planted in the soil-filled plastic buckets labeled as Post-Planting S Neem and Post-Planting C Neem were sprayed with freshly prepared aqueous neem leaf extract fortnightly as from the second week after planting for 3 months, the incubation period for smut and red rot diseases of



sugarcane. Similarly, all the inoculated cane setts planted in the soil-filled plastic buckets labeled as Post-Planting from the second week after planting for 3 months, while all the inoculated cane setts planted in the soil-filled plastic buckets labeled Post-Planting S Mixed and Post-Planting C Mixed were sprayed with freshly prepared aqueous mixed leaf extract fortnightly as from the second week after planting for 3 months.

The labeled soil-filled plastic buckets were arranged accordingly on a concrete platform inside the screen house within the Unilorin Botanical Garden and adequately watered daily, while an inorganic fertilizer (Granulated Urea 46:0:0) was applied moderately once per month (Wada et al. 2008).

The seedlings that sprouted from some of the treated cane setts planted in the soil-filled plastic buckets were observed for the incidence of either of the two diseases (smut and red rot diseases of sugarcane) whose pathogen was used to inoculate the cane setts at the end of the third month after planting. Their respective pictures were taken with a digital camera.

Results

The seedlings from the inoculated cane setts planted in the plastic buckets labeled Control S and Control C showed symptoms of the diseases caused by their respective inoculant (mycelial suspensions of *Sporisorium scitamineum* and *Colletotrichum falcatum*),

S BL and Post-Plant C BL were sprayed with freshly prepared aqueous bitterleaf leaf extract fortnightly as i. e. smut whip and red rot, respectively (Fig. 1a and 1b). The aqueous leaf extracts of neem, bitterleaf, and their mixture prevented the incidence of smut and red rot diseases on the seedlings from the pre – and post – planting leaf – treated artificially-inoculated cane setts in this study (Figs 2–7).

The seedlings from the artificially-inoculated cane setts subjected to post-planting foliar application of the aqueous leaf extracts, however, looked healthier than those that sprouted from artificially-inoculated cane setts subjected to pre-planting treatment, when compared with Controls S and C respectively (Figs. 2 - 7).

Fig.2a shows the healthy seedlings that sprouted from the cane setts inoculated with *S. scitamineum* and then given pre-planting treatment with aqueous leaf extract of neem, while Fig. 2b shows the healthy seedlings from the cane setts inoculated with *S. scitamineum* and then given post-planting treatment with aqueous leaf extract of neem. Fig. 3a shows the healthy seedlings from the cane setts that were artificially inoculated with mycelial suspension of *C. falcatum* and subjected to pre-planting treatment with aqueous leaf extract of neem, while Fig 3b shows the seedlings from the cane setts that were artificially inoculated with mycelial suspension of *C. falcatum* and then subjected to post-planting treatment with aqueous leaf extract of neem.

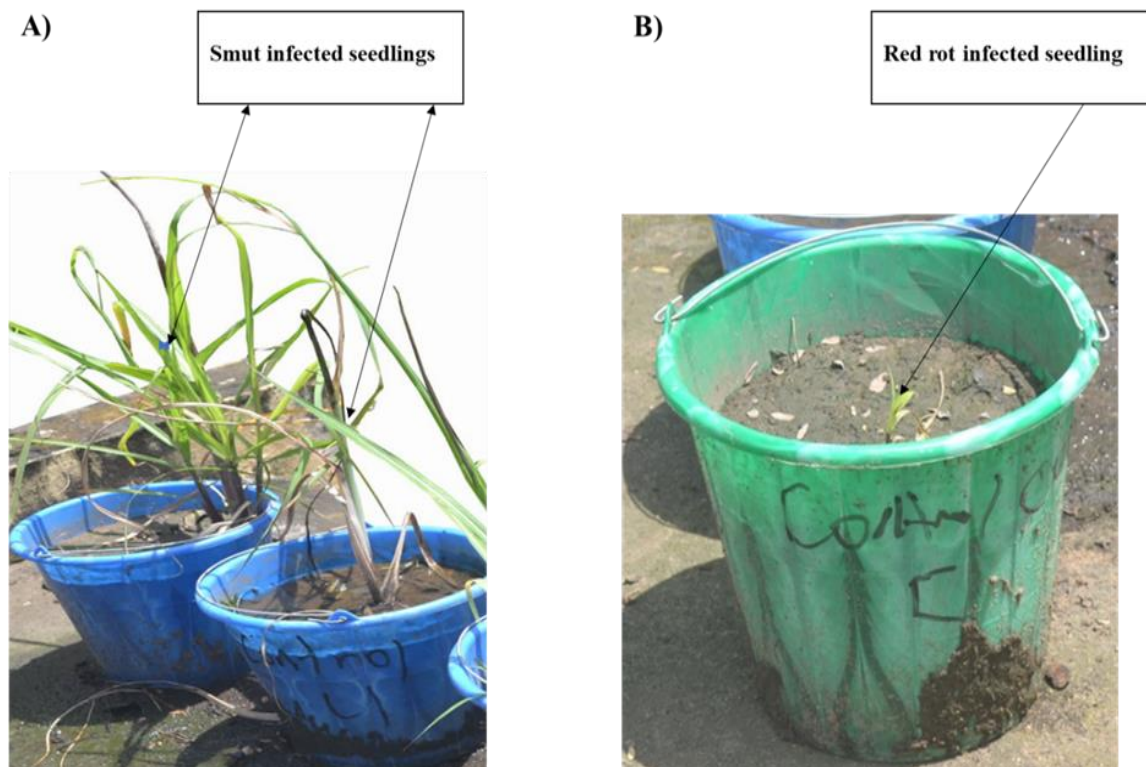


Fig. 1. Cane seedlings exhibiting symptoms of smut disease (A), and red rot disease (B).

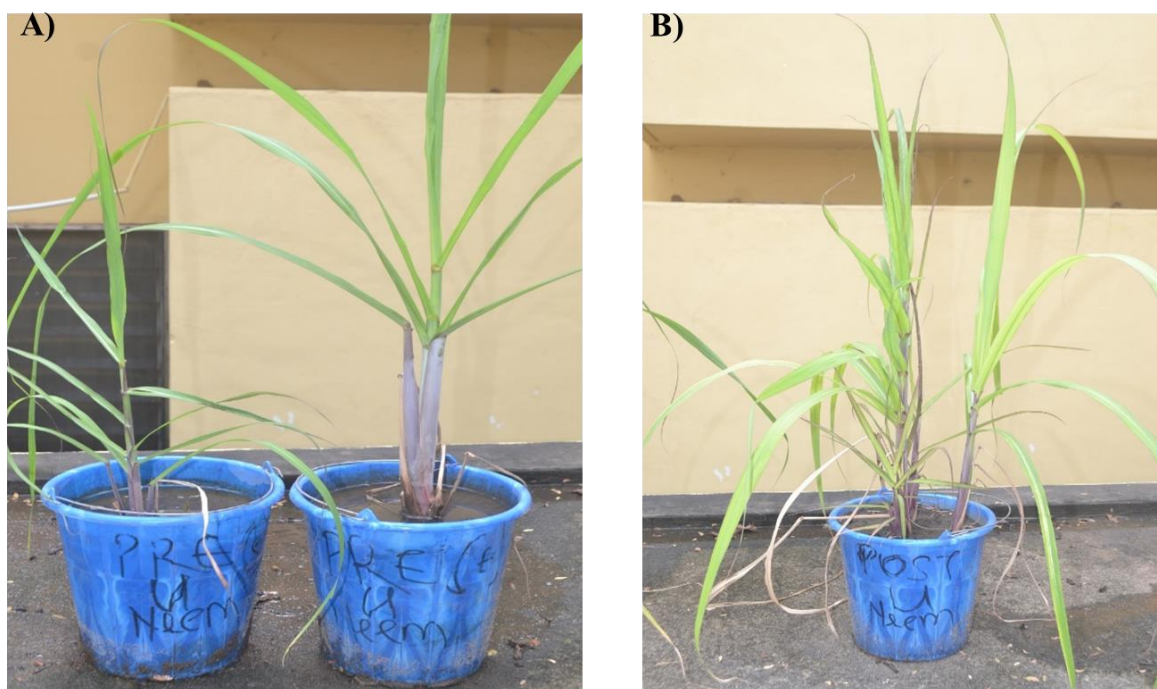


Fig. 2. Seedlings from cane setts inoculated with mycelium suspension of *S. scitamineum* and subjected to pre-planting treatment (a) and post-planting treatment (b) with aqueous leaf extract of neem.

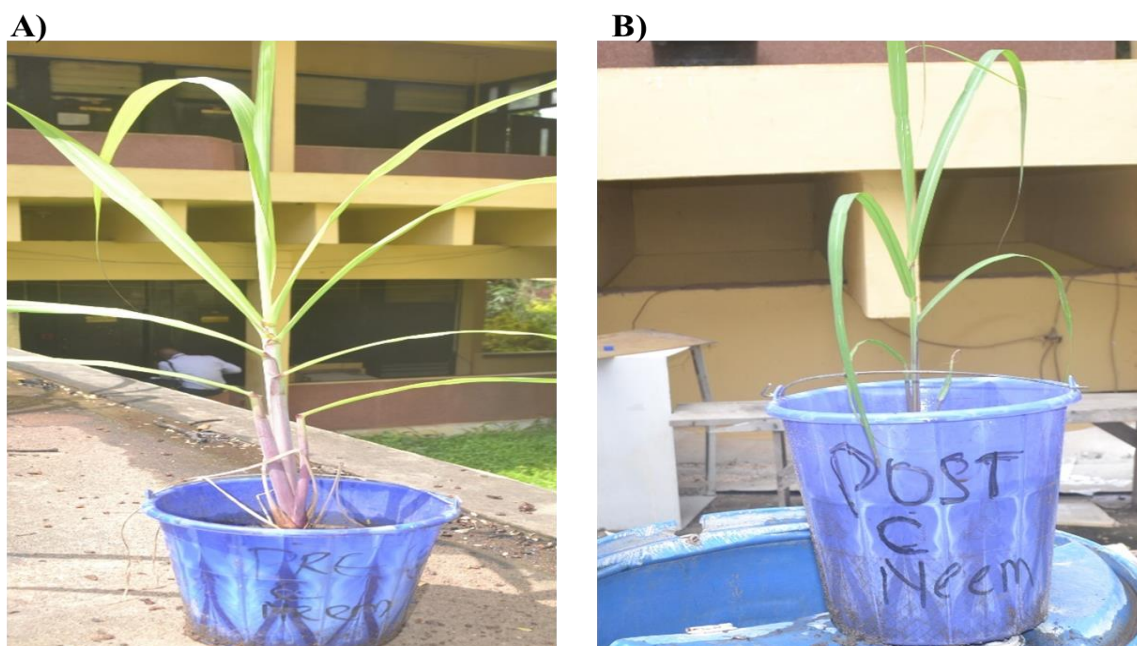


Fig. 3. Healthy seedlings from the cane setts that were artificially inoculated with a mycelial suspension of *C. falcatum* and subjected to pre-planting (A) and post-planting (B) treatments with aqueous leaf extract of neem.

Figure 4 shows the healthy seedlings from the cane setts inoculated with mycelial suspensions of *S. scitamineum* and thereafter subjected to pre- and post-planting treatments with aqueous leaf extract of bitterleaf exhibiting no infections, while Fig. 5 shows healthy seedlings from cane setts inoculated with mycelial suspensions of *Colletotrichum falcatum* subjected to pre- and post-planting treatments with aqueous bitterleaf leaf extract, while Fig. 6 shows the healthy seedlings from

the *Sporisorium scitamineum* –inoculated cane setts subjected to pre- and post-planting treatments with aqueous mixed leaf extract. Fig. 7a shows the healthy seedlings from *Colletotrichum falcatum*-inoculated cane setts subjected to pre-planting treatment with the aqueous mixed leaf extracts, while Fig. 7b shows the healthy seedlings that sprouted from *Colletotrichum falcatum*-inoculated cane setts subjected to post-planting treatment.



Fig. 4. Healthy seedlings from the cane setts inoculated with mycelial suspensions of *S. scitamineum* and thereafter subjected to pre- and post-planting treatments with aqueous leaf extract of bitterleaf, exhibiting no infections.

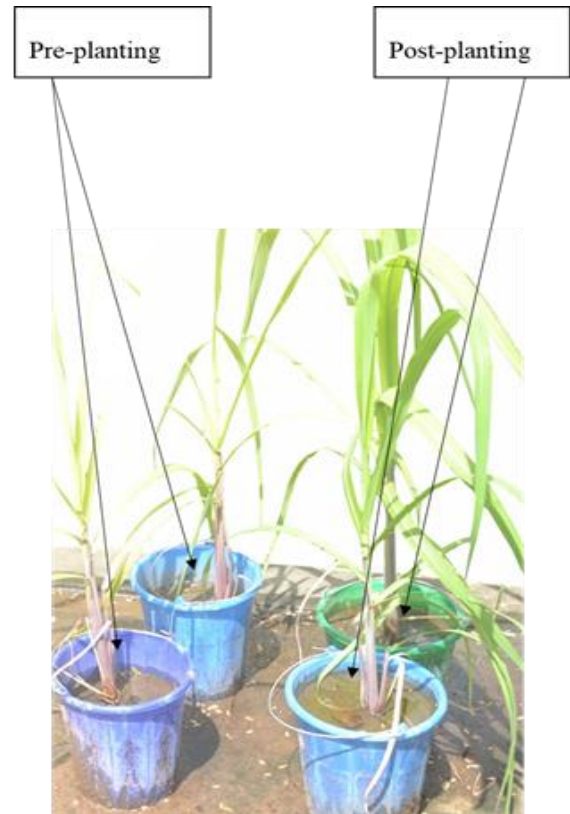


Fig. 5. Healthy seedlings from cane setts inoculated with mycelial suspensions of *Colletotrichum falcatum* and subjected to pre- and post-planting treatments with aqueous leaf extract of bitterleaf.

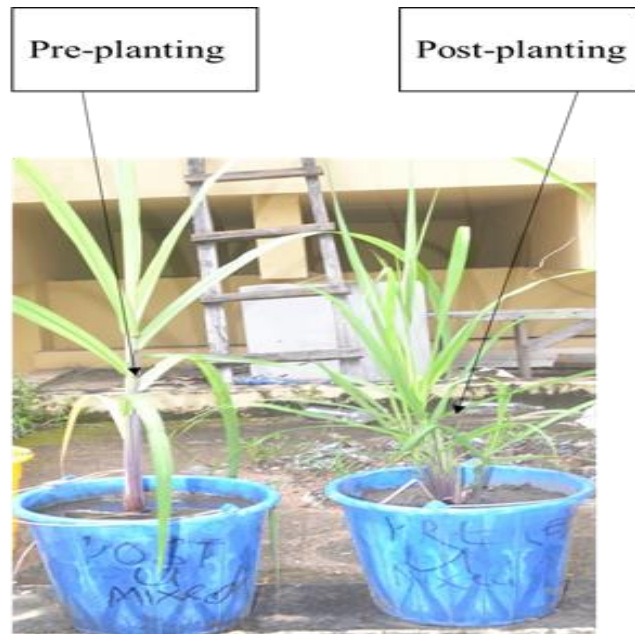


Fig. 6. Healthy seedlings from the *Sporisorium scitamineum*-inoculated cane setts subjected to pre- and post-planting treatments with aqueous mixed leaf extract

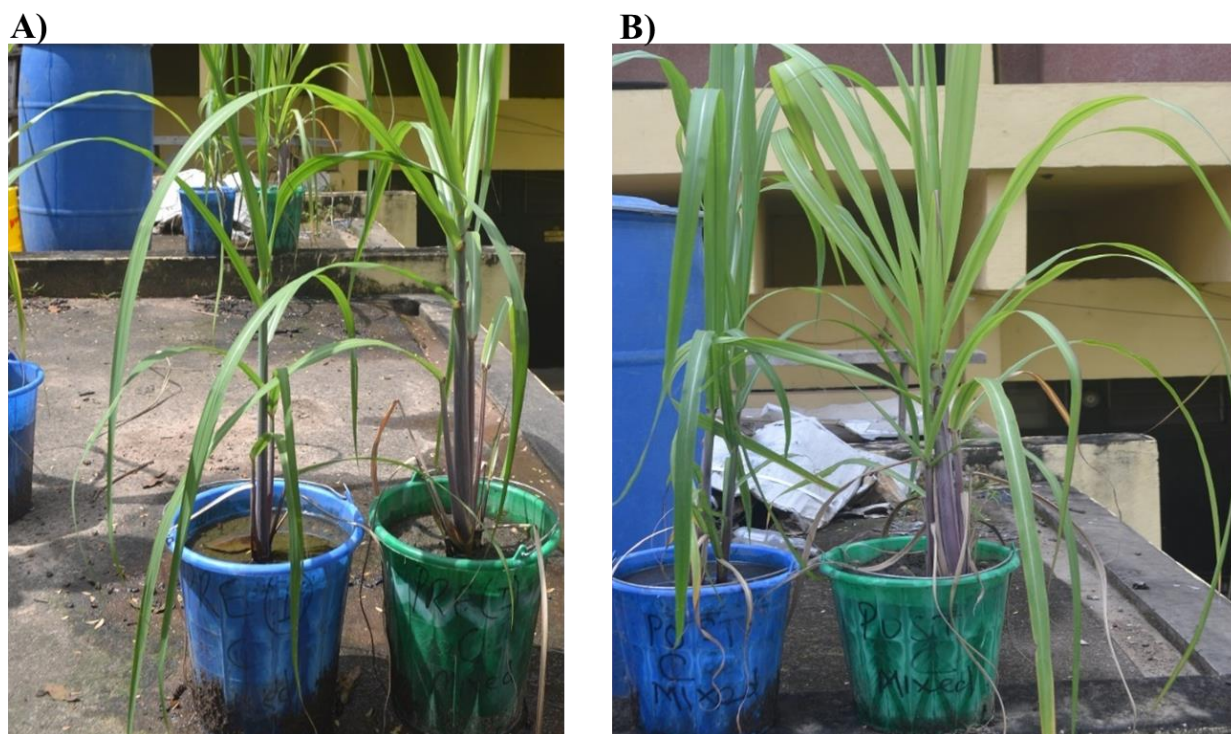


Fig. 7. Healthy seedlings from *Colletotrichum falcatum*-inoculated cane setts subjected to pre-planting (A) and post-planting (B) treatments with the aqueous mixed leaf extracts.

Discussion

The aqueous leaf extracts of neem, bitterleaf and their mixture showed remarkable positive *in vivo* activity against the pathogens of smut and red rot diseases of sugarcane in this study. The seedlings that sprouted from buds of the cane setts inoculated with mycelial suspensions of the pathogens and treated with the aqueous leaf extracts of neem, bitterleaf, and their mixture did not exhibit any of the symptoms of the two diseases, compared with the Control. Efficacy of the mixed leaf extracts was however, more prominent than that of either the neem or bitterleaf aqueous leaf extracts.

These observations tallied with results of the *in vitro* antifungal activity of the aqueous and ethanolic leaf extracts of the test plants on cultures of *S. scitamineum* and *C. falcatum* as reported by Olahan et al. (2020) and Olahan et al. (2022) respectively. Also, Iyayi and Ohimain (2021) reported that the mixture of neem and bitterleaf leaf extracts, regardless of the extractant has higher phytochemical content compared to that of the individual leaf extracts. Secondary metabolites such as alkaloids, flavonoids, terpenoids, triterpenoids, saponins, and phenolics contained in the extracts of neem and bitterleaf leaves, have been reported to possess antimicrobial and allelopathic properties which the plants containing them use as protection from diseases and damage (Saxena et al. 2013).

The observations presented in Plates 1 to 11 agree with some earlier reports on the *in vivo* activity of extracts of some medicinal plants on some plant pathogens. Leaf extracts of *Calendula officinalis* and *Solanum nigrum* suppressed the incidence of smut disease on cane setts artificially inoculated with *U. scitamineum* and then dipped in the leaf extracts before planting in a study conducted by Ram et al. (2009). Koch and Roberts (2014) reported that crude extracts of aerial parts of *Agapanthus africanus* was comparable with thiram (a systemic fungicide). Yavuz and Arslan (2013) concluded that ethanolic and aqueous leaf extracts of *Capsicum annum* L., *Helianthus annuus* L., *Juglans regia* L., *Sinapis arvensis* L., and *Solanum lycopersicon* could become natural alternatives to synthetic fungicides for control of the diseases caused by *Botrytis cinerea*, Pers.Fr., *Fusarium culmorum* (W.G. Smith) Sacc., *F. solani* (Mart), *Macrophomina phaseolina* (Tassi), and *Rhizoctonia solani* Kuhn. These results hint at a natural, eco-friendly approach to managing sugarcane diseases, with potential for large-scale field applications. However, further research is needed to verify the extracts' effectiveness across various sugarcane varieties and growth conditions, as well as to determine optimal application rates and timing.

Additionally, identifying the active compounds responsible for bioactivity and exploring their commercial potential will be crucial to harnessing the full potential of these extracts in sugarcane disease control.

Conclusions

Based on the observations made in this study, fortnight spraying of 30 g/100 ml aqueous mixed extract of neem and bitterleaf leaves is recommended for the control of smut and red rot diseases of sugarcane.

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