Management of Seed-Borne Fungi in Cowpea Using Leaf Extracts and Sodium Bicarbonate

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ABSTRACT: The study investigated the in-vitro antifungal activities of sodium bicarbonate (NaHCO₃) and leaf extracts of Vernonia amygdalina (bitter leaf) and Azadirachta indica (neem) on fungal species isolated from two cowpea cultivars; IFE BROWN and Drum (a local cultivar). Concentrations of 1.95 mg/ml and 1.43 mg/ml were used for A. indica while 1.45 mg/ml and 1.15 mg/ml were used for V. amygdalina. Sodium bicarbonate was evaluated on all the isolates at 2.0 mg/ml and 3.0 mg/ml. The inhibitory effects of these antifungal agents were compared with benlate (a synthetic fungicide) on Potato Dextrose Agar. Benlate totally inhibited all the fungal isolates. Candida species isolated from the two cowpea cultivars were greatly inhibited by the antifungal agents while Trichoderma species isolated from IFE BROWN was the least inhibited. The inhibitory effects of NaHCO₃ and A. indica on Candida spp. were not significantly different and values ranged from 74.5% to 84%. The two concentrations of A. indica had better inhibitory effect on Pythium spp. than NaHCO₃, Vernonia amygdalina at 1.45 mg/ml inhibited A. niger and Candida spp. by 83.75% and 87.5% respectively while NaHCO₃ at 2.0 mg/ml inhibited Rhizopus stolonifer isolated from Drum by 75%. The study concludes that the two plant extracts and sodium bicarbonate had broad spectrum activities on fungal species and are therefore recommended as alternatives to toxic and synthetic fungicides.

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Cowpea (Vigna unguiculata L. Walp.) is regarded as the most essential legume crop for poor people in developing countries (Valenzuela and Smith, 2002; Langyintuo et al., 2003). It is a major source of indigenous plant protein in Nigeria (Ndem and Sowemimo, 2004) and 40% of the people daily protein requirements is supplied by cowpea (Muleba et al., 1997; Egho, 2009). Some important local names of cowpea include “niebe”, “wake” and “ewa” other common names of cowpea are “southern pea”, “black eye pea”, “field pea”, “pink eyes” and “crowder” (Singh et al., 2002). The production and storage of cowpea are hindered by an array of pests and diseases which affect yield, quality, nutritional value and viability. Lack of adequate storage and transport facilities have led to severe postharvest losses experienced in developing countries (Sharma et al., 2009), thus, making the environment more conducive for the growth of storage fungi. Furthermore, most of these fungi produce toxic metabolites, called mycotoxins, which constitute health problems to humans, animals and plants (Reverberi et al., 2010).

Various synthetic fungicides used on cowpea have some adverse effects such as toxic residue, development of resistant strain, high cost and toxicity to mammals because some of them contain heavy metals (Azher, 2009). Hence, there is need for eco-friendly alternatives to seed dressing chemical fungicide in the control of seed-borne fungi. Such alternatives include the use of organic materials such as plant extracts and inorganic salts such as sodium bicarbonate. Plant materials are safe for use in the environment and are inexpensive. Therefore, they can be exploited as suitable alternatives to the expensive, toxic and environmentally unsafe synthetic fungicides (Isman, 2006; Akunne et al., 2013). The leaf of neem (Azadirachta indica) and its constituents have been reported to exhibit immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalaria, antifungal, antibacterial, antioxidant, antimutagenic and anticarcinogenic properties (Subapriya and Nagini, 2005). The aqueous extract of neem has powerful chemotherapeutic and antiviral activities (Hassan et al., 2010). The seed extract of neem was reported to retard the growth of two fungi;
**Materials and Methods**

Two varieties of cowpea namely IFE BROWN and Drum were used in this study. IFE BROWN was obtained from the Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan while Drum (a local cultivar) was obtained from Apatap market, Ibadan, Nigeria. Seeds were surface sterilized according to the method of Mohammadi and Sivritepe (2007) with modifications using 70% ethanol for 1 min and later 10% hypochlorite solution for 30 min. Then the seeds were rinsed in five changes of distilled water and dried on sterilized paper towel. Seed-borne fungal pathogens were isolated from cowpea seeds on Potato Dextrose Agar (PDA) using Agar plate method as described by Anon (1993). The isolated fungi were identified using cultural appearance and microscopic characteristics (Ogbulie et al., 2001).

Leaves of *Azadirachta indica* and *Vernonia amygdalina* were collected from the whole plants, washed in distilled water and air dried between 7 to 10 days. The leaves were ground into powder using a kitchen blender. Approximately 40 g and 50 g of the pulverized leaves were soaked in 100 ml of distilled water overnight. After soaking, the mixture was sieved into a 250 ml conical flask using a sterile muslin cloth. The plant residues were dried, weighed and subtracted from the initial weights of the pulverized leaves to obtain the actual concentrations of plant extracts. Concentrations of 1.95 mg/ml and 1.43 mg/ml were obtained from *Azadirachta indica* at 40 g and 50 g of leaves respectively while 1.45 mg/ml and 1.15 mg/ml were obtained from 40 g and 50 g of *Vernonia amygdalina* leaves respectively.

**Frequency of occurrence of isolated fungi:** The frequency of occurrence of the isolated fungi was determined by counting the number of times each fungal species appeared in the mixed culture. Percent occurrence of each of the fungal species was calculated as shown below:

\[
PC = \frac{FFI \times 100}{TFI}
\]

Where PC = % occurrence; FFI = frequency of occurrence of each fungal isolate; TFI = total occurrence of all fungal isolates.

**Evaluation of the efficacies of plant extracts and sodium bicarbonate against fungal isolates:** The inhibitory effects of the leaf extracts of *Azadirachta indica* at 1.95 mg/ml and 1.43 mg/ml were tested against fungal species isolated from IFE BROWN variety while *Vernonia amygdalina* at 1.45 mg/ml and 1.15 mg/ml were tested on the fungal species isolated from Drum. Similarly, sodium bicarbonate was evaluated on all the isolates at 2.0 mg/ml and 3.0 mg/ml. Benlate (a.i 50% Benomyl); a synthetic fungicide was also tested on the fungal isolates. Approximately 1.0 ml of each antifungal agent was dispensed into 90 mm diameter Petri dishes using sterile syringe. Sterilized molten PDA was dispensed into the Petri dishes and swirled to allow even distribution of the antifungal agents. Agar was allowed to solidify and each plate was inoculated with actively growing margin of fungal colonies using a sterilized 5 mm diameter cork borer. There were three replicates and plates were incubated at room temperature with radial mycelia growth measured daily until the control treatment (plate without antifungal agent) was fully covered with the mycelia. The mycelia growth was determined using the formula adopted from Oyedeji and Kareem (2016).

\[
Mp = \frac{M1 - M2 \times 100}{M1}
\]
Where: Mp= Percentage inhibition of mycelia growth; M1= Mycelia growth in control plate; M2= Mycelia growth in treated plate.

Statistical analysis: Data obtained were subjected to statistical analysis using Statistical package for social sciences (SPSS) and means were compared using Duncan Multiple Range test at 5% level of probability.

RESULTS AND DISCUSSION
Frequency of occurrence of isolated fungi: Four fungal species were isolated from the seeds of IFE BROWN and they include Candida spp., Pythium spp., Rhizopus stolonifer and Trichoderma spp. The frequency of occurrence of all the isolated fungi was not significantly different from each other. The occurrence frequency of Pythium spp., Candida spp. and Trichoderma spp. was 3.0 while that of Rhizopus stolonifer was 4.5 (Table 1). This corroborates the research of Makun et al. (2012) which reported the incidence of seed-borne fungi in cowpea survey carried out in Niger State, Nigeria.

Rhizopus stolonifer, Aspergillus niger, and Candida spp. were the fungal species isolated from the local cultivar; Drum. Results revealed that Aspergillus niger and Rhizopus stolonifer had frequency of occurrence of 1.5 while Candida spp. had a frequency of occurrence of 1.0. The percent occurrence of Candida spp. was 25.0% while Aspergillus niger and Rhizopus stolonifer had percent occurrence of 37.5% (Table 1). The occurrences of seed-borne fungi in cowpea have been reported by several authors with Aspergillus spp. being reported as the most frequently occurred (Popoola et al., 2003; Makun et al., 2012; Shahnaz et al., 2015).

Inhibition of fungi by leaf extracts and sodium bicarbonate: The highest inhibited organism by the antifungal agents was Candida spp. isolated from Drum and IFE BROWN at 80.25% ± 5.1 and 83.5% ± 2.9 respectively. This was followed by Aspergillus niger (79.01% ± 5.2) isolated from Drum while the least inhibited organism was Trichoderma spp. (57.5% ± 7.2) isolated from IFE BROWN (Table 2). Fallik et al. (1997) reported that the inhibitory effect of sodium bicarbonate on microorganisms could be due to the reduction of cell turgor which causes collapse and shrinkage of hyphae and spores resulting in fungistasis. The fungicidal properties of aqueous extract of V. amygdalina against Fusarium in-vitro was reported by Suleiman et al. (2008). Fungal growths were totally inhibited by benlate while A. indica and sodium bicarbonate at different concentrations also inhibited the growth of the fungi isolated from IFE BROWN but not as much as benlate. This result is not surprising because benlate is a synthetic fungicide and its effect on microorganisms is highly toxic. The inhibition of B. theobromae by three synthetic fungicides has been reported by Oyedeye and Kareem (2016).

There was no significant difference in the activity of sodium bicarbonate and A. indica on Candida spp. However, A. indica at 1.95 mg/ml and 1.43 mg/ml controlled Pythium spp. better than sodium bicarbonate at the two concentrations. Rhizopus stolonifer was inhibited by 70% and 67.75% at 1.95 mg/ml and 3.0 mg/ml of A. indica and 3.0 mg/ml of sodium bicarbonate respectively. The highest inhibition (53%) of Trichoderma spp. was by 2.0 mg/ml of sodium bicarbonate (Table 3).

### Table 1: Occurrence of fungi isolated from the seeds of IFE BROWN and Drum

| Pathogen              | IFE BROWN % occurrence | Frequency of occurrence | Drum % occurrence | Frequency of occurrence |
|-----------------------|------------------------|-------------------------|------------------|------------------------|
| Candida spp.          | 3.0                    | 22.22 ± a                | 1.5 ± a          | 37.5 ± a               |
| Pythium               | 3.0                    | 22.22 ± a                | NI               | NI                     |
| Rhizopus              | 4.5                    | 33.33 ± a                | 1.5 ± a          | 37.5 ± a               |
| Trichoderma           | 3.0                    | 22.22 ± a                | NI               | NI                     |
| Aspergillus niger     | NI                     | NI                      | 1.0 ± a          | 25.0 ± a               |

Means followed by the same letter along the columns are not significantly different according to Duncan’s Multiple Range Test (DMRT). NI= not isolated

### Table 2. Descriptive statistics of antifungal agents on fungal isolates

| Variety  | Fungal species   | Mean  | S.E | Range  |
|----------|------------------|-------|-----|--------|
| Drum     | Aspergillus niger| 79.0  | 4.0 | 53.75  |
|          | Rhizopus stolonifer| 73.99| 5.2 | 56.30  |
|          | Candida species  | 80.25 | 5.1 | 56.25  |
| IFE BROWN| Candida species  | 83.50 | 2.9 | 25.50  |
|          | Phythium         | 70.50 | 6.3 | 50.50  |
|          | Rhizopus stolonifer| 69.95| 5.6 | 53.00  |
|          | Trichoderma species| 57.50| 7.2 | 59.00  |

S.E = standard error

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The fungitoxic activity of water and alcoholic extracts of A. indica against the causal organism of rice blast has been reported by Amadioha (1999) while Nigro et al. (2006) obtained positive results in the control of B. cinerea on table grapes, using calcium chloride, sodium bicarbonate and other carbonate salts. In the local cultivar; Drum; Aspergillus niger was best inhibited (83.75%) by V. Amygdalina at 1.45 mg/ml followed by its inhibition (73.75%) at 3.0 mg/ml of NaHCO₃. Sodium bicarbonate inhibited Candida spp. by 74.38% and 59.38% at 2.0 and 3.0 mg/ml respectively (Table 4). The inhibition of fungal isolates by sodium bicarbonate and extracts of A. indica suggests that both antifungal agents have broad spectrum activities. The result agrees with the earlier study of Karabulut et al. (2003) which reported that sodium bicarbonate effectively controlled postharvest diseases of grapes. In addition, the efficacy of V. amygdalina amongst other plant extracts against seed-borne fungi isolated from African yam bean was reported by Nwachukwu and Umechuruba (2001).

**Conclusion:** The presence of seed-borne fungi was evident in this study. Although, the synthetic fungicide used was able to inhibit the growth of the organisms completely but its use by farmers cannot be recommended because of the adverse effects on human health and the environment. The use of sodium bicarbonate and leaf extracts of A. indica and Vernonia amygdalina obviously inhibited the growth of the pathogens and are therefore, recommended for sustainable food production and food security.

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