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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 ($NaHCO_3$) and leaf extracts of *Vernonia amydgalina* (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. amydgalina.* 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 $NaHCO_3$ 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 $NaHCO_3$. *Vernonia amydgalina* at 1.45 mg/ml inhibited *A. niger* and *Candida* spp. by 83.75% and 87.5% respectively while $NaHCO_3$ 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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Key words: Cowpea, eco-friendly, fungicide, inhibition

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;

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Gaeumannomyces graminis and *Typhula inccarnata* (Coventry and Allan, 2001).

Vernonia amygdalina, a member of the Asteraceae family has been used traditionally to treat sexually transmitted diseases such as gonorrhea and malaria in rift valley and western parts of Kenya (Erasto *et al.,* 2007). The multiple health benefits of *Vernonia amygdalina* include anticancer (Sweeney *et al*., 2005), anti-diabetic (Nwanjo, 2005), antioxidant (Erasto *et al.,* 2007) amongst others. The aqueous extract of this plant has been found to have cell growth inhibitory effects in prostate cancer cell line (Adebayo *et al*., 2014). The leaves and the roots are used traditionally in phytomedicine to treat fever, kidney, heart diseases and stomach discomfort (Farombi and Owoeye, 2011).

There is a growing interest in the use of sodium bicarbonate ($NaHCO_3$) and potassium bicarbonate ($KHCO_3$) for the control of numerous fungal diseases in plants (Karabulut *et al*., 2003; Smilanick *et al*., 2006). Bicarbonates are widely used in the food industry (Lindsay, 1985), and therefore, have no negative effect on the environment, plants or humans. Ziv and Zitter (1992) reported the ability of bicarbonates to suppress several fungal diseases of cucumber plants. Spraying plants with $NaHCO_3$ solution provided good control of several plant diseases (Janisiewicz and Peterson, 2005). Therefore, this study evaluated the inhibitory effects of the leaf extracts of two plants (*Azadirachta indica* and *Vernonia amygdalina*) and an inorganic salt; sodium bicarbonate on fungal species isolated from cowpea seeds.

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 Apata market, Ibadan, Nigeria. Seeds were surfaced 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 mins. 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 to10 days. The leaves were ground into powder using a kitchen blender. Approximately 40 g and 50 g of the pulverized leaves were soaked in 100ml 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 1.95mg/ml and1.43mg/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 2.0mg/ml and 3.0mg/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 determine using the formula adopted from Oyedeji and Kareem (2016).

$$Mp = \frac{\mathrm{M1} - M2 \times 100}{M1}$$

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 tugor which causes collapse and shrinkage of hyphae and spores resulting in fungistasis. The fungicidal properties of aqueous extract of *V. amydgalina* 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 Oyedeji 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 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

	IFE BROWN		**Drum**	
Pathogen	Frequency of occurrence	% occurrence	Frequency of occurrence	% occurrence
Candida spp.	3.0[a]	22.22[a]	1.5 [a]	37.5 [a]
Pythium	3.0[a]	22.22[a]	NI	NI
Rhizopus	4.5[a]	33.33[a]	1.5 [a]	37.5 [a]
Trichoderma	3.0[a]	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*	79.01	4.0	33.75
	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

Table 3. Percentage inhibition induced by *Azadiracta indica* and sodium bicarbonate on fungi isolated from IFE BROWN

Concentration of antifungal agents (mg/ml)	*Candida* spp.	*Pythium* spp.	*Rhizopus stolonifer*	*Trichoderma* spp.
1.95 (*A. indica*)	84.0b	78.0b	70.0b	47.0bc
1.43 (*A. indica)*	79.0b	75.0b	65.0bc	40.5c
2.0 (NaHCO3)	74.5b	50.0c	47.0c	53.0b
3.0 (NaHCO3)	80.0b	49.5c	67.75b	47.0bc
1.0 (Benlate)	100.0a	100.0a	100.0a	100.0a

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

Table 4. Percentage inhibition induced by *Vernonia amydgalina* and sodium bicarbonate on fungi isolated from Drum

Concentration of antifungal agents (mg/ml)	*Aspergillus niger*	*Candida* spp.	*Rhizopus stolonifer*
1.45 (*V. amydgalina*)	83.75b	87.5ab	72.5b
1.15 (*V. amydgalina*)	66.25d	80.0abc	66.25b
2.0 (NaHCO3)	71.28cd	74.38bc	75.0b
3.0 (NaHCO3)	73.75c	59.38c	56.22b
1.0 (Benlate)	100.0a	100.0a	100.0a

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

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 $NaHCO_3$. 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. amydgalina* 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 amydgalina* obviously inhibited the growth of the pathogens and are therefore, recommended for sustainable food production and food security.

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