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# Antifungal activities of *Carica papaya* and sodium bicarbonate against Soybean fungi

Qasim Olaitan AFOLABI[1] and Kehinde Titilope KAREEM[2*]

[1] *Department of Science Laboratory Technology, Federal College of Animal Health and Production Technology, P.M.B. Moor Plantation, Ibadan, Nigeria.*

[2]*Grain Legumes Improvement Programme, Institute of Agricultural Research and Training, Obafemi Awolowo University, P.M.B. 5029, Moor Plantation, Ibadan, Nigeria.*

[*] *Corresponding author; E-mail: kt_kareem@yahoo.com; Tel: 08024158641*

## ABSTRACT

Soybean (*Glycine max* (L.) merr.) is a well-known leguminous crop serving as a principal protein rich food source for human and livestock. The study examined the antifungal efficacy of sodium bicarbonate ($NaHCO_3$) and *Carica papaya* on fungi isolated from soybean seeds. The inhibitory effects of sodium bicarbonate ($NaHCO_3$) and leaf extracts of *Carica papaya* were tested on three fungi. *In vitro* studies were carried out on Potato Dextrose Agar (PDA) for fungal isolation from soybean seeds and for the control of the pathogens using $NaHCO_3$ and *C. papaya*. The results obtained were compared with benlate (a.i 50% Benomyl); a synthetic fungicide. *Aspergillus niger* had the highest percent occurrence (50%) followed by *F. ventricosum* (33%) and then *B. theobromae* (17%). The percent fungal inhibition was highly significant at 1% level of probability, indicating that all the antifungal agents exhibited broad spectrum inhibition against the isolates. Benlate completely (100%) inhibited the three pathogens. There was no significant difference ($P \leq 0.05$) in the inhibition of *A. niger* at 1.54 mg/ml of *C. papaya* and 3 mg/ml of $NaHCO_3$. *Fusarium ventricosum* and *B. theobromae* were best inhibited at 1.54 mg/ml of *C. papaya* with mean values of 95.63% and 95% respectively. A positive and significant correlation occurred between the inhibitions of all the fungal isolates. This implies that all the antifungal agents are capable of inhibiting the three fungal isolates. The study suggests that *C. papaya* and $NaHCO_3$ are capable of controlling seed-borne pathogens in soybean seeds.



**Keywords**: Antifungal agents, inhibition, leaf extract, mycelia growth, seed-borne fungi.

## INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is popularly known as a "miracle bean" or a "golden bean" because it is a cheap source of protein (Obatolu et al., 2007). It is an important oil seed, averaging approximately 43% protein and 20% oil by weight (Carrera et al., 2011). Soybeans are the only vegetable food that contains all the eight essential amino acids (Dudek, 2001). Soybean, bean, groundnut, chickpea, pigeonpea, cowpea, lentil, and pea are cultivated in an area of 198.7 million hectares around the world with soybean dominating 72% of production area (FAOSTAT, 2012). One of the major constraints affecting the productivity of

 
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soybean is attack by seed-borne fungi during storage, hence affecting the quality and shelf-life of the seeds. Waller (2002) reported that infected seeds act as vehicle which carries pathogens to new areas within a country and from one country to the other. Attempts to control these pathogens have led to the use of inorganic fungicides which have adverse effect on the consumers as a result of their accumulation in food. Therefore, the use of generally recognized as safe (GRAS) botanicals and sodium bicarbonate for the control of fungi are suggested alternatives to synthetic fungicides by various researchers (Amadioha, 2003).

The demand for the biological control of pathogens using vegetal extracts containing secondary metabolites has been a common practice for thousands of years (Chavez-Quintal et al., 2011). The leaves of papaya have found used in various herbal solutions; as a tumour-destroying agent, an antiseptic, as a tonic and blood purifier. The tea, prepared with the green papaya leaf promotes digestion and aids the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Ayoola and Adeyeye, 2010). Nwiloh et al. (2009) also reported that *C. papaya* leaf extracts induced a significant weight gain in albino rat used for their study. The use of alternatives which include organic materials and inorganic salts such as sodium bicarbonate for fungal control was reported by Kareem et al. (2018). Reports by Palou et al. (2001) revealed that sodium bicarbonate is readily available, cheap and poses little risk of phytotoxicity when used at concentrations of 1-4%. The objective of this study was to evaluate the efficacies of *C. papaya* and sodium bicarbonate as alternatives to synthetic fungicides used for controlling seed-borne fungi.

## MATERIALS AND METHODS

### Sources of plant materials

Soybean variety TGx 1019-2EN obtained from the Grain Legumes Improvement Programme, Institute of Agricultural Research and Training (I.A.R.&T), Obafemi Awolowo University, Ibadan was used in this study. Leaves of *Carica papaya* collected from the whole plant was washed in distilled water and air-dried between 7 to10 days. The dried leaves were ground into powder using a kitchen blender and kept in air-tight container until when needed.

### Fungal isolation and preparation of leaf extracts

Soybean seeds were surfaced disinfected in 70% ethanol for 1 min and later in 10% sodium hypochlorite solution for 30 mins. Afterwards, the seeds were rinsed in three changes of distilled water and dried on sterilized paper towel. Six to ten disinfected seeds were aseptically placed in Petri dishes containing solidified Potato Dextrose Agar (PDA) and incubated at 25±1 $^{o}$C for 4-7 days. Identification of isolated fungi was carried out using both cultural and morphological characteristics as described by Barnet and Hunter (1999).

Grinded leaves weighing about 40 g and 50 g were each soaked in 100 ml of distilled water overnight. The socked leaves were sieved with a sterile muslin cloth into 250 ml conical flasks. The residues were air-dried to constant weights and subtracted from the initial weights of the grinded leaves to obtain the actual concentrations of plant extracts in the solvent. Concentrations of 1.33 and mg/ml and 1.54 mg/ml were obtained from *C. papaya* at 40 g and 50 g of leaves respectively.

**Frequency of occurrence of isolated fungi**

Frequency of occurrence of the isolated fungi was determined by counting the number of times each fungal species appeared in the mixed culture and it was expressed in percentage of the total number of fungi that appeared on PDA.

$$Percent\ occurrence\ Frequency\ of\ occurrence\ of\ each\ fungal\ isolate = \frac{\times 100}{Total\ occurrence\ of\ all\ fungal\ isolates}$$

***In vitro* evaluation of *Carica papaya* leaf extracts and sodium bicarbonate against fungal isolates**

The *in vitro* efficacies of the leaf extracts of *C. papaya* (1.33 and 1.54 mg/ml) and sodium bicarbonate (2 and 3 mg/ml) on the isolated fungi was carried out on PDA. In addition, the efficacy of a commercial fungicide; Benlate (a.i 50% Benomyl) at 1.0 mg/ml was evaluated on the fungal isolates. About 1.0 ml of each antifungal agent was dispensed into 90 mm diameter Petri dishes and sterilized molten PDA was added to each Petri dish and swirled to allow uniform distribution of the antifungal agents. The medium was allowed to solidify and a central well of about 5 mm diameter was made using a sterilized cork borer. Actively growing margin of fungal colonies was picked with a cork borer and gently placed in the well. The control plates were inoculated with each of the fungus but no antifungal agent was added. Each test was replicated three times and the plates were incubated at room temperature. The radial mycelia growth of fungus in each plate was measured daily until the control treatment was fully covered with the mycelia. The percentage inhibition of mycelia growth was calculated using the formula:

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

Where;

Mp= Percentage inhibition of mycelia growth.

M1= Mycelia growth in control plate.

M2= Mycelia growth in treated plate.

**Statistical analysis**

Analysis of Variance (ANOVA) was carried out using SPSS statistical software version (16.0) and means were compared using Duncan Multiple Range test at 5% level of probability. Correlation analysis was carried out to determine the relationship between the percent inhibitions of the fungal species.

**RESULTS**

The fungal pathogens isolated from the seeds of soybean were *Aspergillus niger*, *Fusarium ventricosum* and *Botryodiplodia theobromae*. The mycelia of *Aspergillus niger* appeared whitish on PDA and later turned black at later stage of growth. The reverse side was yellow and furrowed. Globose conidia were characteristic of *A. niger*. *Fusarium ventricosum* has a fluffy texture with a smooth surface and mycelia colour appeared yellowish white with the reverse side looking creamy. The shape of the conidia of *Botryodiplodia theobromae* is oval to ellipsoidal and the surface colour appeared grey. The spore colour of *B. theobromae* was dark brown and the reverse side appeared grey to black (Table 1).

**Frequency of occurrence of isolated fungi**

The fungus with the highest frequency of occurrence was *A. niger* with 50% percent occurrence. This was followed by *F. ventricosum* (33%) and then *B. theobromae* taking about 17% of the total population (Figure 1).

***In vitro* evaluation of *Carica papaya* leaf extracts and sodium bicarbonate on radial growth of fungal isolates**

The effect of the antifungal agents was highly significant for fungal radial growth at P ≤ 0.01 (Table 2). Higher concentrations of $NaHCO_3$ (3.0 mg/ml) and *C. papaya* (1.54 mg/ml) reduced the mycelial growth of *A.*

*niger* with values of 17.5 mm and 18 mm respectively. The radial growth of *F. ventricosum* was significantly reduced at $P \leq 0.05$ when treated with 1.54 mg/ml of plant extract. However, 1.33 mg/ml of the plant extract did not reduce the radial growth of *F. ventricosum* as much as the two concentrations of sodium bicarbonate. The radial growth of *B. theobromae* was much more reduced at 1.54 mg/ml of plant extract (4 mm) when compared with other treatments. Benlate completely inhibited the growth of all the pathogens (Table 3).

### *In vitro* evaluation of *Carica papaya* leaf extracts and sodium bicarbonate on fungal inhibition

A summary of analysis of variance for fungal inhibition is given in Table 4. The mean square values of fungal inhibition were significant for all the fungal isolates. Benlate totally controlled the growth of the three fungi. The highest inhibition of *A. niger* was obtained at 1.54 mg/ml of plant extract and 3 mg/ml of $NaHCO_3$ with inhibition values of 77.5% and 78.13% respectively. The inhibitory effect of the bicarbonate salts was similar on *F. ventricosum* while plant extract at 1.54 mg/ml had a higher inhibition (95.63%) on *F. ventricosum*. The highest inhibition of 95% recorded from *B. theobromae* was obtained at 1.54 mg/ml while there was no significant difference ($P \leq 0.05$) in the inhibition of the other antifungal agents (Table 5).

### Correlation analysis showing the relationship between mycelial radial growth and fungal inhibition

Correlation coefficients generally revealed negative significant correlations among fungal radial growths and fungal inhibitions. The radial growth of *A. niger* was positively and significantly correlated to the radial growths of *F. ventricosum* and *B. theobromae*. Similarly, the correlation of *F. ventricosum* with *A. niger* and *B. theobromae* was positive and significant. The same trend was observed for the correlation of *B. theobromae* with the other two isolates. Also, the percent inhibition of *A. niger* was positively and significantly correlated with the percent inhibition of *F. ventricosum* (0.879**) and *B. theobromae* (0.873**) and vice-versa (Table 6).

**Table 1**: Cultural and morphological characteristics of isolated fungi.

| Characteristic | *Aspergillus niger* | *Fusarium ventricosum* | *Botryodiplodia theobromae* |
|---|---|---|---|
| Conidia shape | Globose | Oval to kidney shaped | Oval shape |
| Texture | Velvety | Fluffy | Fluffy |
| Surface | Smooth | Smooth | Smooth |
| Surface colour | White | Yellowish white | Grey |
| Spore colour | Black | White-creamy | Dark brown |
| Reverse colour | Yellow | Creamy | Grey to black |
| Zonation | Heavily furrowed on the reverse | Zonate | No zonation |
| Type of mycelium | Conidiospore | Chlamydospore | Conidiospore |
| Type of reproduction | Asexual | Asexual | Sexual |
| Septation | Septate | Septate | Septate |
| Probable organism | *Aspergillus niger* | *F. ventricosum* | *Botryodiplodia theobromae* |

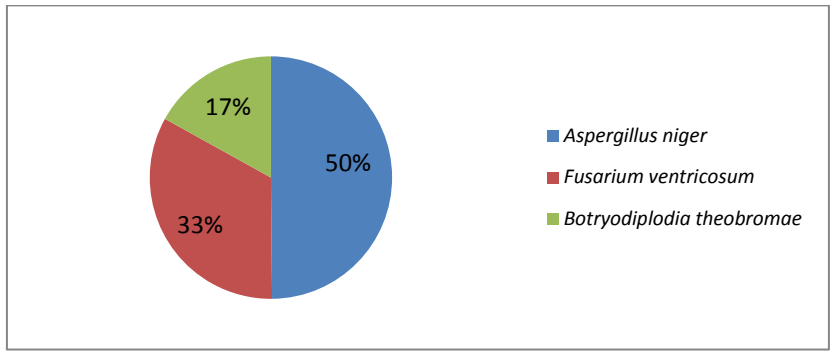


**Figure 1**: Percent occurrence of fungal isolates from soybean seed.

**Table 2**: Analysis of variance showing mean square values of radial growth of fungi after treatment with antifungal agents.

| Source | DF | *A. niger* | *F. ventricosum* | *B. theobromae* |
|---|---|---|---|---|
| Antifungal agent | 4 | 208.25** | 26.13** | 37.40** |
| Error | 5 | 7.0 | 0.36 | 1.64 |

**Significant at 1% level of probability.

**Table 3**: Effect of *Carica papaya* and sodium bicarbonate on radial growth of fungi.

| Fungicide (mg/ml) | *Aspergilus niger* (mm) | *Fusarium ventricosum* (mm) | *Botryodiplodia theobromae* (mm) |
|---|---|---|---|
| 1.0 (Benlate) | $0.0^{c}$ | $0.0^{d}$ | $0.0^{c}$ |
| 2.0 ($NaHCO_3$) | $26.5^{a}$ | $7.5^{b}$ | $10.0^{a}$ |
| 3.0 ($NaHCO_3$) | $17.5^{b}$ | $6.0^{b}$ | $9.5^{a}$ |
| 1.33 (*C. papaya*) | $23.0^{ab}$ | $9.25^{a}$ | $8.75^{a}$ |
| 1.54 (*C. papaya*) | $18.0^{b}$ | $3.5^{c}$ | $4.0^{b}$ |

Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

**Table 4**: Analysis of variance showing mean squares of fungal inhibition.

| Source | DF | *A. niger* | *F. ventricosum* | *B. theobromae* |
|---|---|---|---|---|
| Antifungal agent | 4 | 325.39** | 40.80** | 58.41** |
| Error | 5 | 10.94 | 0.56 | 2.57 |

**Significant at 1% level of probability

**Table 5**: Fungal inhibition by *Carica papaya* and sodium bicarbonate.

| Fungicide (mg/ml) | *Aspergilus niger* | *Fusarium ventricosum* | *Botryodiplodia theobromae* |
|---|---|---|---|
| 1.0 (Benlate) | $100.0^{a}$ | $100.0^{a}$ | $100.0^{a}$ |
| 2.0 ($NaHCO_3$) | $66.88^{c}$ | $90.63^{c}$ | $87.5^{c}$ |
| 3.0 ($NaHCO_3$) | $78.13^{b}$ | $92.5^{c}$ | $88.13^{c}$ |
| 1.33 (*C. papaya*) | $71.25^{bc}$ | $88.44^{d}$ | $89.07^{c}$ |
| 1.54 (*C. papaya*) | $77.5^{b}$ | $95.63^{b}$ | $95.00^{b}$ |

Means followed by the same letter are not significantly different according to Duncan's multiple range test at $P \leq 0.05$.

**Table 6**: Correlation analysis showing the relationship between mycelial radial growth and fungal inhibition.

| Fungal isolate | *A. niger*[a] | *F. ventricosum*[a] | *B. theobromae*[a] | *A. niger*[b] (%) | *F. ventricosum*[b] (%) | *B. theobromae*[b] (%) |
|---|---|---|---|---|---|---|
| *A. niger*[a] | 1 | 0.897** | 0.873** | -1.00** | -0.879** | -0.873** |
| *F. ventricosum*[a] | | 1 | 0.887** | -0.879** | -1.00** | -0.887** |
| *B. theobromae*[a] | | | 1 | -0.873** | -0.887** | -1.00** |
| *A. niger*[b] *(%)* | | | | 1 | 0.879** | 0.873** |
| *F. ventricosum*[b] *(%)* | | | | | 1 | 0.887** |
| *B. theobromae*[b] *(%)* | | | | | | 1 |

**Correlation is significant at 1% level of probability
a - radial growth, b- fungal inhibition.

## DISCUSSION

This study revealed the occurrence of mixed fungal isolates in the soybean seeds. The presence of fungi in soybean could be attributed to the ubiquitous nature of microorganisms. This is in conformity with the work of Nasir (2003) which reported the presence of 39 species of fungi from 15 genera isolated from 6-month old soybean seeds. Presence of mixed fungal species including *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Alternaria* was also reported as post-harvest molds in storage condition by Mehrotra and Aggarwal (2003).

The most frequently isolated fungus in this study was *A. niger*. This is not surprising as *A. niger* has been reported to have the highest prevalence in many literatures (Mehrotra and Aggarwal, 2003; Chavan, 2011).

The high significant effect of the antifungal agents on the fungal isolates suggests that all the antifungal agents were capable of inhibiting the growth of the three organisms *invitro*. Turkkan and Erper (2014) reported a significant difference in the inhibitory effect of sodium salts on the mycelia growth of *Fusarium oxysporum*. The antifungal activities of methanol extracts of *Desmodium adscendens* root and *Bombax buonopozense* leaves were reported by Adeniyi et al. (2013). Benlate totally inhibited the growth of all the fungal isolates. This statement agrees with the work of Cramer (2000) who reported that the treatments of seeds with fungicides such as benomyl, carbendazim, carboxin, maneb, methoxymehtyl mercury chloride, prochloraz, tebuconazole, and thiram reduced the disease of onions. Although, the leaf extracts of *C. papaya* did not inhibit the fungal isolates completely but it exhibited a significant inhibitory activities against the organisms. Several authors have reported the use of *C. papaya* for the control of seed-borne fungi in agricultural crops (Chavez-Quintal et al., 2011; Bakare et al., 2015). Sodium bicarbonate inhibited the fungal isolates at varying degrees. This statement conforms to the report of Hang and Woodams (2003) which stated that baking soda ($NaHCO_3$) or $KHCO_3$ were capable of significantly reducing the mycelia growth of *Fusarium* oxysporum by greater than 95% in Czapek Dox broth at as low as 0.2 g/100 mL.

The negative and significant correlation between fungal radial growth and fungal inhibition is an indication that the antifungal agents had the ability to inhibit the fungal isolates. The positive and significant correlation between the inhibitions of the three isolates suggests that all the antifungal agents have broad spectrum activities and a direct relationship exists between the suppression of one organism and the other organisms. This implies that any of the antifungal agents capable of suppressing the growth of one of the isolates woulds definitely

suppress the growth of the other isolates. Karavaev et al. (2002) reported the correlation between the protective action of wheat seedlings from fungi by plant extracts and the suppression of fungal development by the extracts.

## Conclusion

This study has shown the ability of *C. papaya* and sodium bicarbonate to inhibit seed-borne fungi associated with soybean. These are cheap, non-toxic, biodegradable materials and generally recognized as safe (GRAS) which could serve as alternatives to synthetic fungicides for controlling the growth of seed-borne fungi.

## COMPETING INTERESTS

The authors declare that there is no competing interest among them.

## AUTHORS' CONTRIBUTIONS

The study was a collaborative work between KTK and QOA. KTK designed the work and developed the methodology while QOA carried out the laboratory experiment. Both authors took part in writing the paper.

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