Antifungal of Cashew (*Anacardium occidentale* Linn) leaves, nutshells, and peduncle bagasse ashes extracts against sooty mould fungi (*Capnodium* sp)

W R Andayanie¹*, M Lukito¹ and F Chasanatun²

¹Department of Agrotechnology, Agriculture Faculty, Merdeka Madiun University, Jl. Serayu 79, Madiun, East Java, 63133, Indonesia
²Department of Primary Teaching, Education Faculty, Madiun PGRI University, Indonesia

*E-mail: wuye.andayanie@gmail.com*

**Abstract.** Different concentrations of cashew (*Anacardium occidentale* Linn) leaves, nutshells, and peduncle bagasse ashes extract evaluated for growth inhibition of *Capnodium* sp and the antifungal activity. Results revealed that different concentration of cashew leaves, nutshells, and peduncle bagasse ashes extracts showed values difference of absorbance (DA) ranging from 0.00 to 0.42. All the concentrations of extracts showed significant inhibition in the spore germination of sooty mould. The highest levels caused maximum inhibition in the spore germination followed by lower concentrations of cashew nut shells, peduncle bagasse and leaf extracts respectively. Cashew nut shells extract had a high level of total polyphenolic, flavonoid content, pH and low values of titratable acidity, which could be attributed to its potent antifungal activity.

1. **Introduction**

*Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae) is a type of the cosmopolitan insect pest that it can develop in tropical and subtropical climate zones. This insect produces black sooty mould fungus that grows on honeydew excretion after sucking the sap of soybean leaf. *B. tabaci* causes the reduction of soybean production by producing honeydew. The main components of honeydew are sugars, free amino acids, proteins, minerals and other organic compounds [1].

Honeydew triggered the growth of sooty mould (*Capnodium* sp) fungi on the leaf surface and caused chlorosis, which disrupts the process of leaf photosynthesis and soybean value. Several studies reported that the sooty mould could spread from one plant to another by airborne and water splash spores [2-4]. The incidence of sooty mould infection has increased tremendously; as a result, the use of insecticide synthetic against *B. tabaci*. The typical symptom of sooty mould showed the slight black spot to the dark blackspot covers the entire surfaces of the leaves with black mycelia. Therefore, sooty mould has recognized as the main plant pathogenic group.

The use of fungicides in soybean farming increases grain yields and economic profit. However, these often cause negative effects, *viz.* phytotoxicity and residues in food. More recently, the botanical insecticide used for managing *B. tabaci*, which recommended for *B. tabaci* as the low risk of the toxic residual effect [5]. Cashew nutshell extract was also reported as antiviral against *Cowpea mild mottle virus* on soybean plants and botanical herbicide on the cultivation of black grass jelly (*Mesona palustris* BL) [6-8]. Various plant extracts as inhibitory effect on the mycelial growth and conidial germination of the phytopathogenic fungus *Fusarium. Anacardium occidentale* L (Anacardiaceae) peduncle bagasse
ash and leaf extract were effective against plant pathogenic Fusarium strain [9]. The ethanolic extracts of cashew nutshell (CNS) and peduncle bagasse ash (CPBA) extract also reported as antifungal activity against Aspergillus flavus, A. fumigatus, A. niger, Curvularia sp, Colletotrichum gloeosporioides and Lasiodiplodia theobromae [10]. Compound derived from CNS and CPBA sources have shown non-phytotoxic on the germination [11,12]. The majority of studies show that CNS extract may have a toxic effect on the mycelial growth of phytopathogenic fungus. Still of these, very few have the ability of leaves, nutshells and peduncle bagasse ashes extract to suppress sooty moulds fungus on soybean. These extracts may also be potential alternatives of fungicidal for managing the sooty moulds fungus, which make them suitable fungicides for organic agriculture. The present study aimed to determine the percentage growth inhibition of Capnodium sp with leaves, nutshells and peduncles extracts of cashew (Anacardium occidentale Linn) and evaluate the antifungal activity of extracts using phytochemical screening. This research is important to recognize of the cashew tree as a good fungicidal source against Capnodium sp on soybeans. In addition, the result of this research can be potentially used as reference to best concentration of parts of the cashew tree (leaves, nut shells and apples) in the control of sooty moulds on soybean.

2. Materials and Methods

2.1. Preparation of plant extract and stock culture

Fresh leaves, nutshells, peduncle bagasse ashes samples of Anacardium occidentale L. were washed thoroughly with sterile distilled water and cut into small pieces. Each sample was dried in the oven at 36 °C for 48 h to the ground using a blender to a fine powder. Approximately, 500 g of each powder sample was macerated with n-hexane (0.5 g/ml) and stirred for one h. After that, the extracts were incubated and filtered through double-layered cheesecloth. The filtrate was evaporated using rotary vacuum evaporator at 45 °C±5 with pressure (550 mm Hg). It was made different concentration at levels of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm. The extract sterilized for 24 h using UV rays.

The Capnodium sp isolates were maintained and subcultured via spore suspension on Potato Dextrose Agar. The plates were incubated at 28°C for seven days. Spores were counted and used at a concentration of 3×10⁶ spores/ml.

2.2. Spore germination assay

Leaves, nutshells and peduncle bagasse ashes extracts of cashew (Anacardium occidentale Linn) were dipped about 2 cm apart with different concentrations on each microscopic slide (30 µl per slide) in triplicate, respectively. Then 20 µl of homogenous spore suspension from the 7 days old cultures (3×10⁶ spores/ml) was added to each 30 µl of the extract.

Antifungal activity was detected using a microtiter plate. Performed using 200 µl a reaction mixture containing: 80 µl of homogenous spore suspensions from the seven days old cultures (10⁴ spore suspensions/ml), 100 µl Czapek Dox Broth and 20 µL of plant extract were pipetted with different extract and concentrations into each well of the microtiter plate. One plate row was filled with untreated spore suspension in Czapek Dox Broth as a negative control. The optical density (OD) in each well was calculated after 48 h incubation by a Spectrostar Nano microplate reader at a wavelength of 405 nm.

The percentage of spore germination was assessed after 24 h at 15 °C by mounting 10 µl of spore suspension on a slide glass and counting the number of germinated spores using a haemocytometer grid at 4 × 10-2 mm². The percentage of spore germination was calculated for the tenth wells and averaged. Each treatment was repeated four times. The percentage of spore germination is calculated using formula:

\[
\text{% of spore germination} = \frac{\text{No. of spore germination}}{\text{Total no. of spore examined}} \times 100\% \quad (1)
\]
2.3 Determination of total phenolic and flavonoid content

The content of total phenolics (TPC) of the different concentration of leaves, nutshells, and peduncle bagasse ashes extracts of cashew was determined by the method of Emelike et al. (2017) with slight modification [13]. Exactly 1 mg of each extract dissolved in methanol solution (1 ml). Each sample (250 µl) mixed with 2.5 ml of 10 % Folin-Ciocalteu’s reagent by manual shaking for 30 s. 2.0 ml of 7 % Na₂CO₃ added. After that, the reaction mixture was shaken for 10 min on a vortex and incubated in the dark for 60 min at room temperature. The solution was mixed, and absorbance was measured against ultra-pure water as blank at λ 750 nm with a UV visible spectrophotometer (Varian Cary 100 Conc UV-Vis) Blank consists of all reagents except the extract.

The content of total phenolics was expressed as gallic acid (mg GAE·g⁻¹ dry weight) of lyophilized plant extract. The standard phenolic compound was prepared 0.00625−0.1 µg ml⁻¹, curve equation: r² = 0.9911. Total flavonoids content (TFC) was expressed as quercetin (mg QE·Mg⁻¹ dry weight) of the extract. The standard flavonoid compound curve equation: r² = 0.9951.

2.4. Determination of Titratable Acidity and pH analysis

The acidity of the sample was determined by titration of a diluted sample with a 0.02 N alkaline sodium hydroxide solution to a phenolphthalein. Phenolphthalein (1%) was used as the indicator (4 drops in 20 mL of each sample before starting the analysis) to determine the endpoint in acid-base titrations. Sodium hydroxide was added dropwise with constant swirling until the solution turned pink throughout. The volume of base required to reach the equivalence point was used to calculate the acidity of the extracts expressed in meq of sodium hydroxide per unit sample weight (meq NaOH/g). The pH value of each extract was determined with a Hamilton pH electrode sensor. All measurements were repeated twice within a period of 7 days.

2.5. Experimental design

A randomized was used for experimental design. Analysis of variance (ANOVA) formulas by Fisher’s protected LSD test at P ≤ 0.05 were used for means values. The Probability values were determined by 0.05 and 0.01 significance level, at 95 % and 99 % confidence limits respectively. The experimental data of total phenolic content, total flavonoid content, pH of leaves, nutshells, and peduncle bagasse ashes extracts obtained were expressed as an average.

3. Result and Discussions

3.1. Antifungal screening

3.1.1. Different absorbance of extracts Spore germination assay. After 48 h incubation, at the different concentration of leaves, nutshells, and peduncle bagasse ashes extracts showed values difference of absorbance of (DA) ranging from 0.00 to 0.42. These values were significantly different from the control value of DA (Table 1).

| Concentration | Difference Absorbance after 48 h incubation |
|---------------|--------------------------------------------|
|               | Control⁴                                   | Fungicide⁵ | 500 ppm | 1000 ppm | 1500 ppm | 2000 ppm |
| Cashew nutshells | 0.41 a                                    | 0.00 a     | 0.13 b   | 0.09 b    | 0.05 c    | 0.00 c    |
| Cashew leaves   | 0.40 a                                    | 0.01 a     | 0.25 a   | 0.19 a    | 0.17 a    | 0.11 a    |
| Cashew peduncle bagasse ashes | 0.42 a | 0.00 a | 0.21 ab | 0.15 ab | 0.07 b | 0.05 b |

Mean of four replicates; **: a = no treated (positive control); b = negative control; Means in the same column followed by same letter are not statistically different at P =0.05 according to the Fisher LSD Method.
In the present assay, out of the different concentration of leaves, nutshell, and peduncle bagasse ashes extracts of cashew, the CNS extract at concentration 2000 ppm showed the same trend of difference absorbance (DA) as the fungicide value. It has been either shown that plant extracts with a DA < 0.04 were not or very low conidia germination of the phytopathogenic fungus *Fusarium oxysporum* [9].

3.1.2. **Spore germination**

From the analysis of data in Table 2, we infer that the different concentration of leaves, nut shell, and peduncle bagasse ash extracts of cashew caused significant inhibition of spore germination. The highest inhibition on spore germination was obtained at 2000 ppm in leaves, nutshell, and peduncle bagasse ash extracts of cashew against sooty mould fungi. It was followed by 1500 ppm, 1000 ppm and 500 ppm of extracts. In addition, there was significant difference among treatments (\( P = 0.01 \)). The antifungal activity of extracts was better than conventional fungicide. The extract of the cashew nutshell (CNS) at 2000 ppm proved the most effective in reducing the spore germination. The fungus did not have sufficient nutrients to spore germination. Analysis of spore germination showed a high correlation between the difference of absorbance (DA) at 48 h and the percentage of spore germination observed after 24 h at concentration of 1500 ppm and 2000 ppm, respectively. The effect of CNS extract was observed on spore germination at various concentrations ranging from 3.80 to 43.94 %. The compound of CNS tended to inactivate enzymes related to the synthesis of basic metabolites of fungus and very effective in inhibiting spore germination at a concentration of 200 ppm. The control treatment was found the lowest inhibition on spore germination of sooty mould.

### Table 2. The percentage of spore germination of cashew leaves, nut shells, and peduncle bagasse ashes extracts

| Concentration | Control | 500 ppm | 1000 ppm | 1500 ppm | 2000 ppm |
|---------------|---------|---------|----------|----------|----------|
| Cashew nutshells | 96.61 (78.15) | 43.94 (46.32) | 28.20 (30.05) | 12.01 (22.19) | 3.80 (7.26) |
| Cashew leaves | 90.57 (70.32) | 81.17 (69.44) | 66.28 (49.26) | 48.26 (44.35) | 20.58 (25.19) |
| Cashew peduncle bagasse ashes | 94.74 (75.11)** | 60.05 (55.72) | 50.73 (42.80) | 35.22 (38.76) | 16.70 (19.54) |

SE.diff C.D (\( P=0.05 \)) C.D (\( P=0.01 \))

| Fungicides | 2.12 | 1.75 | 2.18 | 2.12 |
| Concentration | 0.87 | 1.59 | 2.35 | 0.87 |
| Fungicide x conc. | 1.55 | 3.06 | 3.94 | 1.55 |

Mean of four replicates; ** Figures in parentheses are arc Sin√% age transformed value and are statistically identical.

The potential effect of cashew nut shell liquid (CNSL) at a concentration of 320 μg mL-1, resulted in significant inhibition of the mycelial growth of *C. gloeosporioides* and *L. theobromae* on papaya fruit [14]. The fungus inactivated enzyme-producing energy by the toxic substance of CNSL. Therefore, after contact with conidia or mycelia did not have sufficient nutrients to mycelial growth. Additionally, the ash of cashew peduncle bagasse has also great potential to be a powerful non-toxic fungicidal agent against particular *Fusarium* species [11].

3.2. **The content of total phenolics (TPC) and flavonoid content (TFC), acidity and PH analysis**

The content of total phenolic and flavonoid content was expressed as mg GAE/g DW and mg QE/g DW, respectively. There were varied narrowly among concentration 1500 ppm and 2000 ppm) of cashew nutshell extract. Results presented in Table 3 that the average of total phenolics, flavonoids contents
were 67.28 mg GAE. g$^{-1}$, 68.03 mg GAE. g$^{-1}$ and 15.79 mg QE.g$^{-1}$, 16.06 mg QE.g$^{-1}$ of CNS extract at a concentration of 1500 ppm and 2000 ppm, respectively. While cashew nutshells extract at a concentration of 2000 ppm showed the lowest spore germination activity after 24 h incubation. The extract has a low value of acidity and high value of pH. Cashew nutshells extract with high of total phenolic, and flavonoid content generally also indicated an excellent antifungal activity against sooty mould.

### Table 3. Total phenolic content (TPC), Total flavonoid content (TFC), and pH of leaves, nut shells, and peduncle bagasse ashes extracts at the different concentrations.

| Treatments                  | Conc (ppm) | TPC mg GAE. g$^{-1}$ | TFC mg QE. mg$^{-1}$ | Acidity meq NaOH/g | pH    |
|-----------------------------|------------|----------------------|----------------------|--------------------|-------|
| No treated (positive control) | -          | -                    | -                    | -                  | -     |
| Fungicide (negative control) | -          | -                    | -                    | -                  | -     |
| Cashew nutshells            | 500        | 11.24                | 5.43                 | 0.687              | 4.17  |
|                             | 1000       | 35.62                | 9.80                 | 0.507              | 4.85  |
|                             | 1500       | 67.28                | 15.79                | 0.480              | 5.12  |
|                             | 2000       | 68.03                | 16.06                | 0.412              | 7.56  |
| Cashew leaves               | 500        | 3.05                 | 1.95                 | 0.860              | 4.15  |
|                             | 1000       | 6.99                 | 3.58                 | 0.895              | 3.87  |
|                             | 1500       | 10.40                | 5.32                 | 0.541              | 5.95  |
|                             | 2000       | 15.11                | 6.56                 | 0.598              | 5.60  |
| Cashew peduncle bagasse ashes | 500        | 18.78                | 7.12                 | 0.575              | 6.03  |
|                             | 1000       | 22.40                | 7.25                 | 0.586              | 5.91  |
|                             | 1500       | 30.89                | 9.08                 | 1.015              | 5.32  |
|                             | 2000       | 52.24                | 10.15                | 8.172              | 6.11  |

These results are in agreement with previous studies [5,9,12] which found that bioactive polyphenol compounds, singly or in combination, could not stimulate fungal and viral growth. The compounds may have destroyed of the cell membrane fungi.

### 4. Conclusion

All the concentrations of extracts showed significant inhibition in the spore germination of sooty mould. Cashew nut extract at a concentration of 2000 ppm showed the lowest spore germination activity. The extract had a high level of total polyphenolic, flavonoid content, pH and a low value of titratable acidity which could be attributed to its potent antifungal activity.

### Acknowledgments

The authors would like to thank the DRPM Ministry of Research, Technology and Higher Education of the Republic of Indonesia who has funded this research and publication through the Leading Higher

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