

## EVALUATION OF *Tridax procumbens* and *Lantana camara* on *Khaya senegalensis* A. Juss SEEDS INFESTED BY MYCOFLORA

\*<sup>1</sup> Olubiyi, L. U., <sup>1</sup> Adegeye, A. O. and <sup>2</sup>Olorunnibe, V. N.

<sup>1</sup> Department of Forest Production and Products, University of Ibadan, Nigeria

<sup>2</sup>Department of Crop Protection and Environmental Biology, University of Ibadan, Nigeria

Corresponding e-mail: [ukamaka.olubiyi@yahoo.com](mailto:ukamaka.olubiyi@yahoo.com), Phone No. 08062262852

### SUMMARY

Tree seedlings production are often constrained due to loss of seed viability caused by mycoflora. Evidence revealed that plant extracts could effectively controlled seed mycoflora. This study was conducted to investigate the association of mycoflora with *Khaya senegalensis* seeds and evaluate *in-vitro*, the effect of *Tridax procumbens* and *Lantana camara* leaves extract on its control. Qualitative and quantitative phytochemical analysis of the plant extracts was carried out following standards procedure. Blotter test (ISTA-1966) method was used to detect and identify mycoflora on the seeds and plants extracts were evaluated *in-vitro* on detected fungi. Data collected were analysed using descriptive statistics and ANOVA. Means were separated using Duncan Multiple Range Test at  $p=0.05$  level of significant. From the results *T. procumbens* leaf extract contained tannins (11.90mg/g), saponins (0.08mg/g), flavonoid (0.07mg/g) and alkaloids (0.02mg/g) while that of *L. camara* contained tannins (9.33mg/g), saponins (0.12mg/g), flavonoids (0.06mg/g) and alkaloid (0.05mg/g). Six levels of fungal occurrence; *Trichoderma sp.* (6), *Collectotricum sp.* (38), *Aspergillus terreus* (26), *Aspergillus flavus* (15), *Aspergillus niger* (6) and *Curvularia sp.* (9) were detected and identified. There was significant difference in mean areas of *Collectotricum sp.*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus* and *Curvularia sp.* mycelia growth at  $p=0.05$ . The mean area of *Trichoderma sp.* growth on Benomyl and other plant extracts except for *L. camara* at 50% concentration were not significantly different from each other at  $p=0.05$ . The level of mycelia inhibition of the plant extract increased with increasing concentrations. This study showed that *T. procumbens* and *L. camara* leaf extracts has the potential to control damage caused by fungi on *K. senegalensis* seeds.

**Keywords:** *Khaya senegalensis*, Plant extracts, Seeds, Mycoflora, Phytochemical.

*Khaya senegalensis* (Desr.) A. Juss is an important indigenous, overexploited tropical forest tree classified as vulnerable (IUCN, 2017). It belongs to the mahogany family which are known for their durability and high economic value for woodwork and timber market. The over exploitation and limitations associated with proliferating tropical hardwood species such as *K. senegalensis* has

resulted in habitat loss in its natural range (Ky-Dembele *et al.*, 2011; Adjonou *et al.*, 2014). Regeneration of *K. senegalensis* is generally poor as its seeds lose viability easily coupled with diseases incidence usually at the nursery stage (Nikiema and Pasternak, 2008; Apetorgbor and Roux, 2015; Habashy *et al.*, 2016).

Diseases on tree seedlings at the nursery stage are mostly caused by pathogens associated with seeds (Fraedrich, 2009). Plant pathogens such as fungi, bacteria, nematodes, and viruses incite plant diseases and 85% of these is credited to fungi (Pernezny *et al.*, 2017). These pathogens cause severe damage to seed as well as transmit disease such as rot, blight, pre- and post-emergence damping-off diseases to emerging seedling (Saxena and Saxena, 2012). Seeds are potential harbour of a wide variety of mycoflora consisting of pathogenic and saprophytic microorganisms (Utobo *et al.*, 2011). Mycoflora cause seed deterioration, loss of quality, affect viability and reduce germination resulting in the production of abnormal seedlings (Paul, 1989; Bateman and Kwasna, 1999; Khanzada *et al.*, 2002).

Over the years, chemical fungicide of different kinds has been used to control mycoflora associated with forest tree seeds (Khanzada *et al.*, 2002; Habashy *et al.*, 2016). However, these agents have been reported to have negative effects on beneficial microbial population present in the ecosystem. Hence, there is the need to use natural plant disease controlling agents. Natural plant agents are important sources of new agrochemicals for control of pathogens (Gulter, 1988; Tripathi and Dubey, 2004). Plant extracts have been reported to show antifungal activities against a wide range of fungi (Abd-Alla *et al.*, 2001; Masoko *et al.*, 2007). Seed treatment using plant extracts against pathogens have been found to increase germination and ensure uniform seedling emergence and survival (Ezeobi, 2010; Ahmed *et al.*, 2013). It also can protect seeds and seedlings from early season disease and insect pests (Sridhar *et al.*, 2013). The use of plant extracts remains the most common source of antimicrobial agents and this has always been of great interest to scientists because they are relatively cheap, less toxic, and environmentally friendly as well as been effective

on targeted organisms (Ekpo and Banjoko, 1994; Rahman *et al.*, 2008). Botanicals such as *Azadirachta indica* A. Juss, *Lens arietinum* L and *Lathyrus sativus* L has been found to be a near alternative. However, there is the need to explore more plants as alternatives for the control of seed-borne fungi.

The production of healthy seedlings and the ultimate survival of seedlings is a crucial step in the establishment of plantation. Hence, the knowledge of seed-borne fungi associated with *K. senegalensis* and the development of specific eco-friendly effective control measures is necessary.

## MATERIALS AND METHODS

**Seed collection:** Pods of *Khaya senegalensis* were collected from a fruiting mother tree located at Obaluku, Cross River State, Nigeria. The seeds were collected after opening the pod and later stored in an aerated paper bag at room temperature ( $28 \pm 2$  °C) before use.

**Preparation of extracts:** Fresh and tender leaves of *Tridax procumbens* and *Lantana camara* collected from the Millennium Park, faculty of Renewable Natural Resources, University of Ibadan were identified and authenticated at the herbarium in the Department of Botany, where a voucher specimen already existed. The leaves were thoroughly washed in running water and air-dried for two weeks. The leaves were further dried in oven at 45°C for 8 hours. Leaf powder was prepared by pulverizing the dried leaves using an electric grinder. Then the leaf powder was passed through a 25 mm diameter sieve to obtain fine and uniform powder.

For serial dilution for 100 % concentration, leaf powder, 100 g was weighed into conical flask and 100 ml distilled water was added to form a ratio of 1:1 w/v. The mixture was then corked with cotton wool and foil paper and agitated by shaking for 15 minutes to enhance proper mixing of the solvent and powder, and then boiled for 40 minutes at 40°C in a water bath. The content was allowed to cool for 20 minutes and then filtered using Whatman No.1 filter paper. This procedure

was used to prepare for 50% and 70% concentration, by adding 50 g and 70g of leaves powder in 100 ml of distilled water. All filtrates were concentrated at 40 °C using a rotary evaporator.

### Phytochemical analysis of extracts

Phytochemical analysis of *Lantana camara* and *Tridax procumbens* leaf extracts was carried out using standard procedures as described by Edeoga *et al.* (2005).

### Qualitative analysis

**Test for Saponins:** The powdered sample, 2 g was boiled in 20ml of distilled water in a water bath and filtered. An aliquot of 10 ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The froth was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

**Test for flavonoids:** Diluted ammonia solution, 5 g was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated  $H_2SO_4$ . A yellow colouration observed in each extract indicates the presence of flavonoids.

**Test for steroids:** Acetic anhydride, 2ml was added to 0.5 g ethanoic extract of each sample with 2 ml  $H_2SO_4$ . The colour changes from violet to blue or green in the samples indicating the presence of steroids.

**Test for terpenoids:** For each extract, 5ml was mixed in 2 ml of chloroform, and 3 ml of concentrated  $H_2SO_4$  was carefully added to form a layer. A reddish-brown colouration of the interface was formed to show positive results for the presence of terpenoids.

**Test for Cardiac glycosides:** For each extract, 5ml was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under-laid with 1 ml of concentrated

H<sub>2</sub> SO<sub>4</sub> . A brown ring of the interface indicated a deoxy-sugar characteristic of cardenolides. A greenish ring formed gradually throughout thin layer indicated a positive result.

**Test for Tannins:** For each extract, 2 ml was dissolved in 2ml of distilled water, then 2 to 3 drops 5% ferric chloride (FeCl<sub>3</sub>) was added and observed for brownish green precipitate or a blue-black coloration indicating a positive result.

**Test for Emodins:** For each extract, 2 ml was added to 2 ml of NH<sub>4</sub> OH and 3ml of benzene. A red coloration was observed as a positive result.

**Test for Coumarins:** For each extract, 2 ml was added to 3 mL NaOH (10%), a yellow coloration indicated the presence of Coumarins.

**Test for Alkaloids:** For each extract, 2 ml was added to a few drops of Hager's reagent. Yellow precipitate indicated the presence of alkaloids.

### **Quantitative Analysis**

**Determination of alkaloids:** This was carried out using the method of Harbone (1973). Leaves sample, 5 g was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for four hours. This was filtered and the extract concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate collected and washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed which is the alkaloid.

**Determination of tannins:** This was done using the method of Van-Burden and Robinson (1981). Leaves sample, 5 mg was weighed into a 50 ml plastic bottle. Distilled water, 50 ml was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and

made up to the mark. Then, 5 ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl<sub>3</sub> in 0.1 M HCl and 0.008 M potassium ferro-cyanide. The absorbance was measured at 120 nm within 10 minutes.

**Determination OF saponins:** The method of Obdoni and Ochuko (2001) was adopted. Leaves sample, 25 g was put into a conical flask and 100 cm<sup>3</sup> of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered, and the residue re-extracted with another 200 ml 20% ethanol. The combined extract was reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 ml funnel and 20 ml of di-ethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 ml of n-butanol was added. The combined n-butanol extract was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight which is the saponins content.

**Determination of flavonoids:** The method by Boham and Kocipai-Abyazan (1994) was used in this experiment. Leaves sample, 10 g was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

**Detection of mycoflora using blotter test method:** The detection of seed mycoflora was carried out following the blotter test method as described by ISTA (2001). Sterile blotting papers were soaked in distilled water and three layers of these paper were placed in each Petri dish. One hundred seeds of *K. senegalensis* were surface sterilized with 10% sodium hypochlorite for 1 minute and rinsed with three changes of distilled water. Then after, the seeds (5 each) were placed into Petri dishes using flamed sterilized forceps and incubated in a sterile environment for seven

days at temperature of  $28 \pm 2$  °C and alternating 12/12 h day/night. After seven days of incubation, the plated seeds were examined under a stereoscopic binocular microscope for the presence of fungi. Emerging fungi on blotter were subcultures on Saboraud Dextrose Agar (SDA) plate for identification following the keys outlined by Gilman (1957), Subramanian (1971) and Barnett and Hunter (1972).

**In-vitro evaluation of plant extracts on mycoflora:** Following the method of Ky-Dembele et al. (2011). Solidified leaf extract in Saboraud Dextrose Agar (SDA) was prepared by dissolving 6.5 g of SDA in 100 ml, 70 ml and 50 ml plant leaves extracts separately before sterilization and dispensed into Petri dishes and allowed to cool. Benomyl amended media was prepared by first dissolving 0.1 g of the powder in 250 ml of distilled water to get the recommended dose of 5mg/ml. Before sterilization, 16.25 g of SDA powder was added per 250 mL of benomyl solution. Treatments include: Benomyl (T1), *L. camara* (50% concentration) (T2), *L. camara* (70% concentration) (T3), *L. camara* (100% concentration) (T4), *T. procumbens* (50% concentration) (T5), *T. Procumbens* (70% concentration) (T6), *T. Procumbens* (100% concentration) (T7) in SDA media and Control (T8). The solidified amended agar media was inoculated with the test fungi and incubated for three days at  $28 \pm 2$  °C until non-amended petri dishes was filled with mycelial growth. The radial growth of mycelium in each petri dishes was recorded at 6 days interval, till the control plate was fully covered with the fungal mycelium and the per cent inhibition of fungal growth was estimated. Data was collected on the frequency of occurrence of mycoflora, mycelia growth and inhibition of associated fungi. The experiment was replicated twenty times.

$$\text{Frequency} = \frac{\text{Number of seeds with pathogen}}{\text{Total number of seeds}} \times 100\% \dots\dots\dots 1$$

$$\text{Average Area} = \pi r^2 \dots\dots\dots 2$$

$$\text{Percentage Inhibition} = \frac{\text{Average control area} - \text{Average treatment area}}{\text{Average control area}} \times 100\% \dots\dots\dots 3$$

### Data analysis

Data collected were analysed using descriptive and analysis of variance (ANOVA). Duncan Multiple Range Test (DMRT) was used to compare differences in the treatment means at 5% probability level.

### RESULTS

Results on the qualitative and quantitative phytochemical compounds present in the crude leaves extracts of *Lantana camara* and *Tridax procumbens* are presented in Tables 1 and 2. Phytochemical compounds present are saponins, tannin, flavonoids, alkaloids, steroids, cardiac glycoside, and terpenoids while phenol, anthocyanin, Coumarins, phytosterol and emodins were absent in the extracts of *Lantana camara*. Similarly, *Tridax procumbens* aqueous extracts contained saponins, tannin, flavonoids, alkaloids, cardiac glycoside. Aqueous crude extract of *L. camara* contained 11.90 mg/g, 0.08 mg/g, 0.07 mg/g and 0.02 mg/g of tannins, saponins, flavonoids and alkaloids while *T. procumbens* contained 9.33 mg/g, 0.12 g/g, 0.06 mg/g and 0.05 mg/g of tannins, saponins, flavonoids and alkaloids respectively.



**Table 1:** Qualitative phytochemical compounds in the leaves *Lantana camara* and *Tridax procumbens*

| S/N | Phytochemicals    | <i>L. camara</i> extract | <i>T. procumbens</i> extract |
|-----|-------------------|--------------------------|------------------------------|
| 1   | Saponins          | +                        | +                            |
| 2   | Tannin            | +                        | +                            |
| 3   | Flavonoid         | +                        | +                            |
| 4   | Alkaloid          | +                        | +                            |
| 5   | Steroid           | +                        | —                            |
| 6   | Cardiac glycoside | +                        | +                            |
| 7   | Terpenoids        | +                        | —                            |
| 8   | Phenol            | —                        | —                            |
| 9   | Anthocyanin       | —                        | —                            |
| 10  | Coumarins         | -                        | -                            |
| 11  | Phytosterol       | —                        | +                            |
| 12  | Emodins           | —                        | —                            |

**Table 2:** Quantitative phytochemical compounds in the leaves of *Lantana camara* and *Tridax procumbens*

| Leaves Extract       | Tannin mg/g | Saponins mg/g | Flavonoid mg/g | Alkaloid mg/g |
|----------------------|-------------|---------------|----------------|---------------|
| <i>L. camara</i>     | 11.90       | 0.08          | 0.07           | 0.02          |
| <i>T. procumbens</i> | 9.33        | 0.12          | 0.06           | 0.05          |

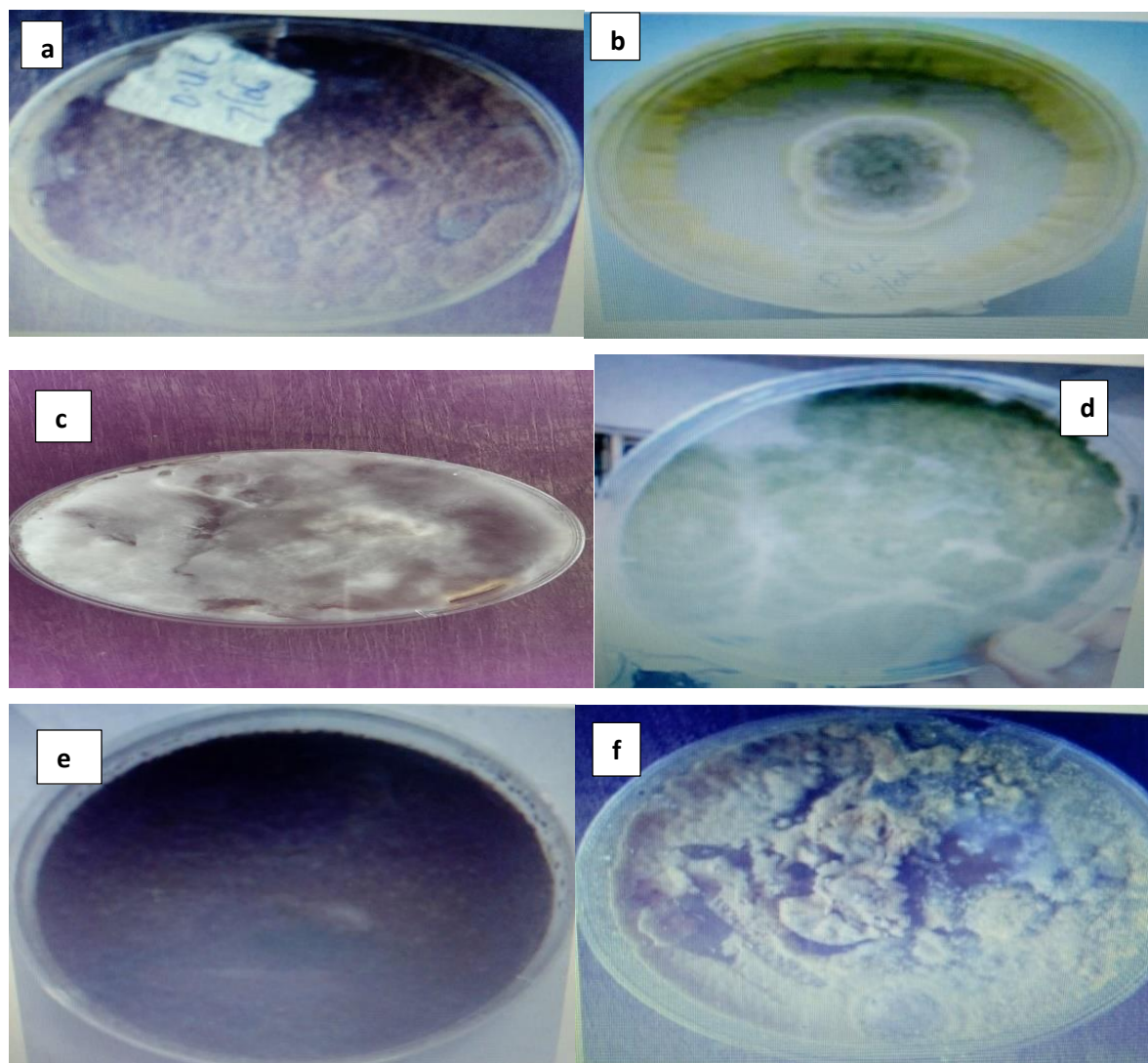
**Detection and Identification fungi in *K. senegalensis* seeds**

Seed-borne fungi were detected on the *K. senegalensis* seeds five days after incubation. Pure cultures of the fungi were identified according to their cultural properties, morphological and microscopical characteristics. A total of six fungi were observed growing on the sterilized seeds. These include *Trichoderma* sp., *Collectotricum* sp., *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus niger* and *Curvularia* sp. *Collectotricum* sp. had the highest frequency of occurrence

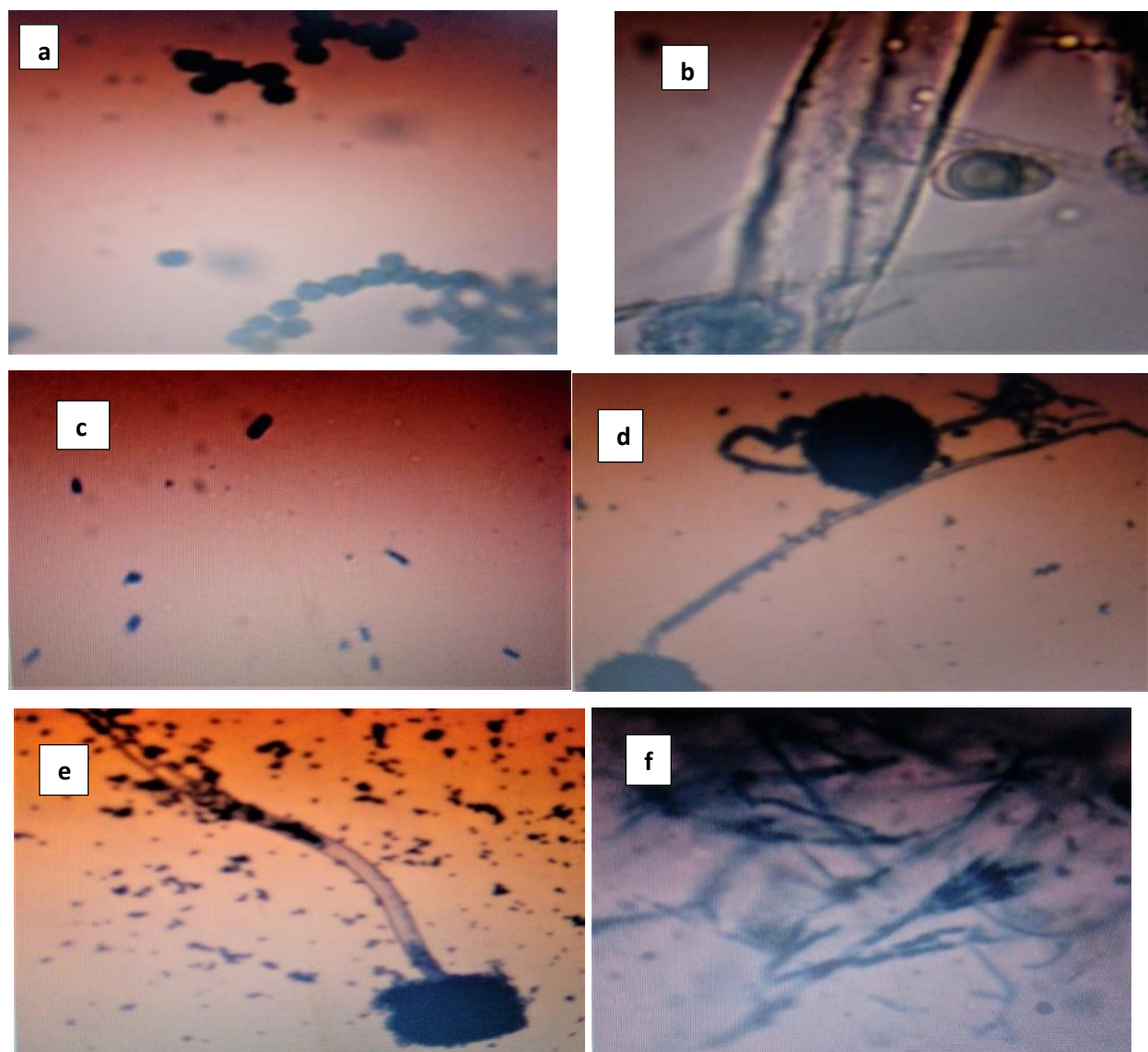
(38%) while *Trichoderma sp.* and *Aspergillus niger* had the lowest frequency of occurrence of 6% (Table 3). Pure culture of *Trichoderma sp.* detected showed initial creamy substance which later turned golden yellow as it matured. *Collectotricum sp.* detected was white fluffy and woolly, its appearance was initially bright but later turned dull and dry as it matured. *Aspergillus niger* detected had a characteristic carbon black colour. It grew rapidly covering the Petri dish within three days of inoculation. Detected *Aspergillus terreus* appeared brown in colour but later fade as it aged, and its spores were chain like. The pure culture of *Curvularia sp.* detected produced a woolly suede-like colony which darkened with maturity (Plate 1). Micrographs of the respective fungi are presented in Plate 2.

**Table 3:** Frequency of occurrence of fungi on *Khaya senegalensis* seeds

| S/N | Fungi                      | Frequency of occurrence (%) |
|-----|----------------------------|-----------------------------|
| 1   | <i>Trichoderma sp.</i>     | 6                           |
| 2   | <i>Collectotricum sp.</i>  | 38                          |
| 3   | <i>Aspergillus niger</i>   | 6                           |
| 4   | <i>Aspergillus terreus</i> | 26                          |
| 5   | <i>Aspergillus flavus</i>  | 15                          |
| 6   | <i>Curvularaia sp.</i>     | 9                           |



**Plate 1:** Pure cultures of isolated fungi from *K. senegalensis* seeds on SDA plates showing *Aspergillus terreus* (a), *Curvularia* sp. (b), *Colletotricum* sp. (c), *Aspergillus flavus* (d), *Aspergillus niger* (e) and *Trichoderma* sp. (f)



**Plate 2:** Micrographs of isolated fungi from *K. senegalensis* seeds on SDA plates showing *Aspergillus terreus* (a), *Curvularia* sp. (b), *Colletotricum* sp. (c), *Aspergillus flavus* (d), *Aspergillus niger* (e) and *Trichoderma* sp. (f)

**Effect of plants extracts on *K. senegalensis* seed mycoflora.**

Results from this study are presented in Table 4 and Figure 1, mycelia growth of isolated fungi in *K. senegalensis* seeds showed significant difference in all the treatments at  $p = 0.05$ . However, there was no significant difference in mycelia growth of *Trichoderma sp.* for treatments, T1, T3, T4, T5, T6 and T7 at  $p=0.05$ . Control (T8) had the highest mean area value of 16.91 cm<sup>2</sup> while Benomyl (T1) had the lowest mean area value of 0.06 cm<sup>2</sup> of mycelia growth of *Trichoderma sp.* Between the plant extracts, T2 had the highest mean area value (6.71cm<sup>2</sup>) while T7 had the lowest mean area value of 0.31cm<sup>2</sup>. Benomyl (T1) had the highest percentage inhibition (99.9%) on *Trichoderma sp.* while the lowest was recorded for T2 (60.3%) (Figure 1).

The effect of the plants extracts on mycelia growth of *Colletotrichum sp.* showed that T4 had the lowest mean area value (12.60cm<sup>2</sup>) while T2 had the highest mean area value of 42.59cm<sup>2</sup>. Treatment, T4 had the highest percentage inhibition (75.1%) among all the treatments. Mycelia growth of *Aspergillus niger* showed that T8 had the highest mean area value (56.22cm<sup>2</sup>) followed by T2 (54.42cm<sup>2</sup>) and T5 (35.13cm<sup>2</sup>) while benomyl had the lowest mean area value (0.07cm<sup>2</sup>). Percentage inhibition of the extracts on *A. niger* showed that T4 and T7 had inhibition percent of 68.3% and 63.8% respectively. Mycelia growth of *Aspergillus terreus* ranged between 55.19 cm<sup>2</sup> (T5) and 0.06cm<sup>2</sup> (T1). Treatment, T2, T3, T4, T6, T7 and T8 had mean area values of 45.51cm<sup>2</sup>, 53.24cm<sup>2</sup>, 50.56cm<sup>2</sup>, 49.44cm<sup>2</sup>, 32.40cm<sup>2</sup> and 54.25cm<sup>2</sup> respectively. Treatment, T1 inhibited the growth of *A. terreus* by 99.9% while T7 inhibited by 40.3%.

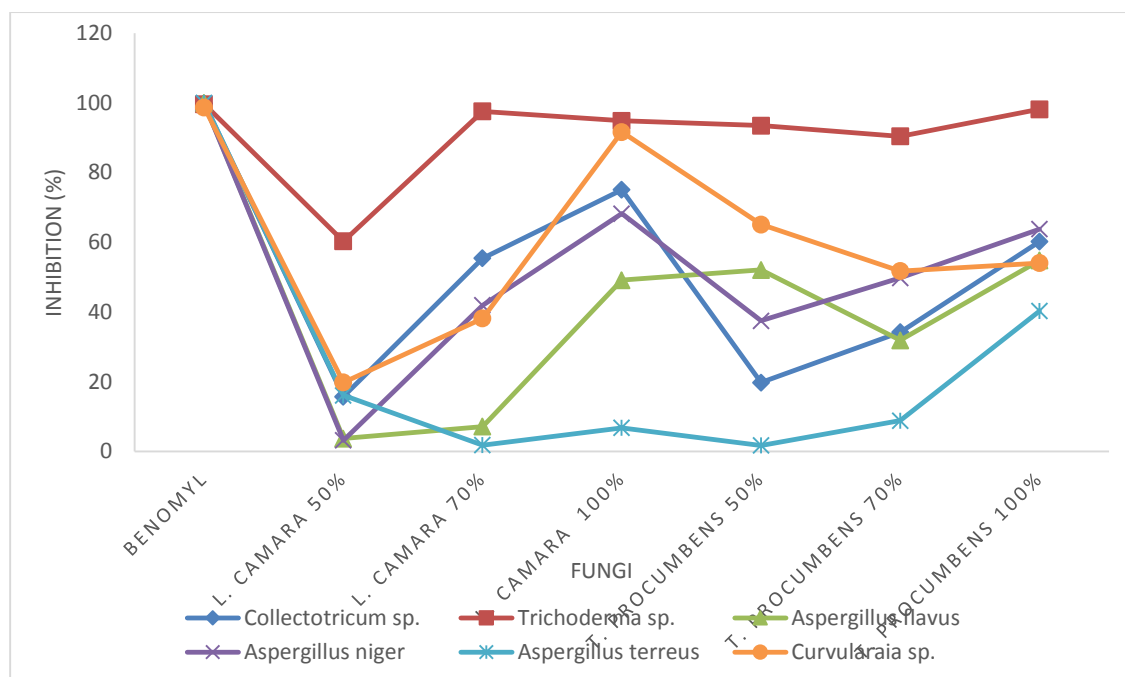
For *A. flavus*, the highest mycelia growth was observed for T8 (55.30cm<sup>2</sup>), followed by T2 (53.23cm<sup>2</sup>). Treatment T1 had mean area value of 0.054cm<sup>2</sup>. Treatment T2 had percentage inhibition of 4% while T4 and T7 had percentage inhibition values of 49.1% and 54.8% respectively. Treatment, T8 had the highest mean area value of 4.79cm<sup>2</sup> for *Curvularia sp.* mycelia growth followed by T2 (3.84cm<sup>2</sup>) while T1 had the lowest mean area value (0.062 cm<sup>2</sup>). Treatment, T4 had the highest inhibition percent (91.6%) when compared to other plants extracts.

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**Table 4:** Effect of *L. camara* and *T. procumbens* extracts on *Khaya senegalensis* seed-borne mycoflora

| Treatments                       | Mean Area(cm <sup>2</sup> ) |                           |                          |                            |                           |                       |
|----------------------------------|-----------------------------|---------------------------|--------------------------|----------------------------|---------------------------|-----------------------|
|                                  | <i>Trichoderma</i> sp.      | <i>Collectotricum</i> sp. | <i>Aspergillus niger</i> | <i>Aspergillus terreus</i> | <i>Aspergillus flavus</i> | <i>Curvularia</i> sp. |
| Benomyl (T1)                     | 0.06a                       | 0.05a                     | 0.07a                    | 0.06a                      | 0.054a                    | 0.062a                |
| <i>L. camara</i> (50%) (T2)      | 6.71b                       | 42.58ef                   | 54.42e                   | 45.51c                     | 53.23d                    | 3.84cd                |
| <i>L. camara</i> (70%) (T3)      | 0.41a                       | 22.52c                    | 32.64d                   | 53.24c                     | 51.33d                    | 2.96bc                |
| <i>L. camara</i> (100%) (T4)     | 0.87a                       | 12.60b                    | 17.85b                   | 50.56c                     | 28.12b                    | 0.40a                 |
| <i>T. procumbens</i> (50%) (T5)  | 1.10a                       | 40.55de                   | 35.13d                   | 55.19c                     | 26.50b                    | 1.67b                 |
| <i>T. Procumbens</i> (70%) (T6)  | 1.62a                       | 33.24d                    | 28.23cd                  | 49.44c                     | 37.65c                    | 2.31b                 |
| <i>T. Procumbens</i> (100%) (T7) | 0.31a                       | 20.12bc                   | 20.35bc                  | 32.40b                     | 24.99b                    | 2.20b                 |
| Control (T8)                     | 16.91c                      | 50.54f                    | 56.22e                   | 54.25c                     | 55.30d                    | 4.79d                 |
| F-value                          | 125.60                      | 33.20                     | 33.10                    | 35.50                      | 48.10                     | 13.34                 |

Means with the same letters across column are not significantly different at p=0.05



**Figure 1:** Effect of plant leaves extracts on inhibition of seed-borne associated mycoflora on *Khaya senegalensis* seed.

## DISCUSSION

The aqueous leaf extract of *L. camara* from this study was found to contain saponins, tannin, flavonoid, alkaloid, steroid, cardiac glycoside and terpenoids. This finding agrees with the reports of Saxena and Saxena (2012) and Sunil and Sevak (2016) who discovered same metabolites in *L. camara*. The aqueous extracts of *T. procumbens* contain 6 bioactive constituents (tannins, saponins, flavonoids, cardiac glycosides, phytosterol and alkaloids). This corroborated the reports of Mir et al., (2016) and Saikh (2018) that flavonoids, pholabatannins, resins, lipids and fats, phenolic compounds, saponins, steroids, tannins and terpenoids were present in *T. procumbens*. From this study, *T. procumbens* leaves extract had more alkaloids and saponins than *L. camara* while the reverse is the case for tannin and flavonoid.

Six fungi; *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Collectotricum sp.*, *Trichoderma sp.* and *Curvularia sp.* was detected and identified on *K. senegalensis* seeds used for the study. Fungi such as *Aspergillus sp.* has been reported to be predominately present in trees seeds. This corroborated the reports of Adegeye (1990), Dick and Vanner (2008) and Suelen *et al.* (2012) that most isolated seed-borne pathogens of forest tree species are fungi, many of which are *Aspergillus sp.*, *Diplodia sp.*, *Penicilium sp.*, *Fusarium sp.*, *Pestalotia sp.*, *Trichoderma sp.*, *Curvularia sp.*, *Alternaria sp.*, *Macrophomina sp.*, *Rhizopus sp.*, and *Collectotricum sp.* *In vitro* antifungal evaluation showed the efficacy of *L. camara* and *T. procumbens* aqueous leaves extracts in controlling the test fungi growth as well as the zone of inhibition. Leaves extracts at 100% concentrations performed better in inhibiting some of the detected fungi than extracts at 50 % and 75 % concentration. However, this was not true for some fungi at 100 % concentration. These findings disagreed with report of Ahmed *et al.* (2013) that plants extract at higher concentration were more effective in reducing the incidence of seed-borne mycoflora. Similarly, Ogbabor *et al.* (2007) also reported that an increase in the concentration of plants extracts increases the zone of inhibition of mycelial growth of *Collectotricum sp.* This could be due to the characteristics of fungi and the antifungal properties of the plant extracts as well as the plant parts used. *Tridax*

*procumbens* at 50% concentration induced the mycelia growth of *Aspergillus terreus*. This corroborated the findings of Ky-Dembele *et al.* (2011) that *Jatropha curcas* and *Vernonia amygdalina* leaves extract at low concentration (10% and 25%) induced significant increase in the mycelial diameter of *Collectotricum sp.* While a particular fungus may be inhibited at a low concentration, similar concentration may stimulate the growth of another fungi. This could be due to the ability of certain fungi to breakdown plant bioactive substances for growth and development. In this study, leaf extracts used reduced the growth of *Trichoderma sp* drastically. This supports the findings by Jain *et al.* (2015) that leaf extract of *T. procumbens* inhibited the mycelia growth of *Trichoderma reesei* as well as other microbes. *T. procumbens* and *L. camara* have strong antifungal activity against *Trichoderma sp.* The plant extracts also exhibited significant inhibition on *Collectotricum sp.* The high concentration of the extracts had much effect on the area of mycelia growth. The growth of *Aspergillus flavus* was inhibited by the leaf extracts of *T. procumbens* at 100% concentration with 54.8% inhibition. This result disagreed with the findings of Jindal and Kumar (2012) that leaf extracts of *T. procumbens* does not inhibit mycelia growth of *A. flavus*. The inhibition zone of *A. flavus* by *L. camara* (100%) on *A. flavus* was highly obvious more potent in this study. It has been reported that *Aspergillus flavus* and *Aspergillus fumigatus* was inhibited by leaf extracts of *L. camara* (Rabia and Asghari, 2013).

## CONCLUSION

From this study, it can be concluded that certain fungi are associated with the seeds of *Khaya senegalensis* and that the use of leaf extracts of *L. camara* and *T. procumbens* can be used for it control. However, there is the need to evaluate more plants and plant parts for the management of seedborne fungi. Leaf extracts of *L. camara* and *T. procumbens* has antifungal properties capable of inhibiting mycelia growth and survival of seed-borne fungi and can be used for seed pre-treatment. The presence of specific bioactive compounds such as tannin, flavonoid, saponins, and alkaloid could be responsible for the antifungal properties of the leaf extract of *L. camara* and *T. procumbens*.



## REFERENCES

1. **Abd-Alla, M. S., Atalla, K. M. and El-Sawi, M. A. M. 2001.** Effect of some plant waste extracts on growth and aflatoxin production by *Aspergillus flavus*. *Annals Agricultural Science* 46; 579-592.
2. **Adegeye, A. O. 1990.** Pathological aspects of seed-borne mycoflora of seed of *Pinus caribaea* Morelet var *Hondurensis*. Ph.D. Thesis. Department of Forest Resources Management, University of Ibadan.139pp.
3. **Adjonou, K., Nuto, Y., Bosu, P. P., Adu-Bredu, S., Kokutse, A. D. and Kokou, K. 2014.** Natural distribution of *Nauclea diderrichii* (Rubiceae) in semi deciduous forest of Togo (West Africa) and Implementation of Integrated Silviculture. *American Journal of Plant Sciences* 5: 1220-1235.
4. **Ahmed, M., Hossain, M., Hassan, K. and Dash, C. K. 2013.** Efficacy of different plant extract on reducing seed-borne infection and increasing germination of collected rice seed sample. *Universal Journal of Plant Science* 1. (3):66-73.
5. **Apetorgbor, M. M. and Roux, J. 2015.** Diseases of plantation of forestry trees in Southern Ghana. *International Journal of Phytopathology* 4. (1): 05-13.
6. **Barnett, H. L. and Hunter, B. B. 1972.** Illustrated Genera of Imperfect Fungi. Burgess. Pub. Co., Minnesota, USA. p. 241.
7. **Bateman, G. L. and Kwasna, H. 1999.** Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. *Applied Soil Ecology* 13, 271-282. [http://dx.doi.org/10.1016/S0929-1393\(99\)00040-2](http://dx.doi.org/10.1016/S0929-1393(99)00040-2).
8. **Boham, B. A. and Kocipai-Abyazan, R. 1994.** Flavonoids and condensed tannins from leaves of Hawaiian *Vaccinium vaticulatum* and *V. calycinium*. *Pacific Science* 48: 458-463.
9. **Dick, M. and Vanner, A. L. 2008.** Nursery diseases MD. Forest Pathology in New Zealand 16.1-19.
10. **Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. 2005.** Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology* 4. (7): 685-688.

11. **Ekpo, E. J. A. and Banjoko, K. M. 1994.** Efficacy of Fersanasan D and wood ash in the control of seed-borne fungi, pre-emergence mortality and seedling blight of Maize. *Discovery and Innovation* 6. (1):84-88.
12. **Ezeobi, U. L. 2010.** Pathological studies and biological control of seed-borne mycoflora of *Terminalia ivorensis* A. Chev. BSc. Project. Department of Forest Resources Management, University of Ibadan.
13. **Fraedrich, S. W. 2009.** Seed-borne pathogens and strategies to eliminate and reduce their presence on tree seeds. Exotic Forest Pests Online Symposium 08.1-7.
14. **Gillman, J. C. 1957.** A Manual of soil fungi. Iowa State Univ. Press.
15. **Gulter, H. C. 1988.** Natural products and their potential in agriculture: In Gulter, H.C. (Ed.) Biologically Active Natural Products: Potential use in agriculture. American Chemical Soc, Washington, pp.1-2.
16. **Habashy, S. H. R., Abd El-Mageed, M. H., Fawzy R. N., Eid Kh. E. and El-Sheme, H. S. A. 2016.** Efficiency of some fungicides, plant extracts, chemical inducers, and plant hormones on the management of damping-off and root rot diseases of *Khaya senegalensis* under greenhouse conditions. *International Journal of Scientific and Engineering Research* 7(4):930-944.
17. **Harborne, J. B. 1973.** Phytochemical methods, London. Chapman and Hall, Ltd. 49-188.
18. **International Seed Testing Association (ISTA) 2001.** International Rules for Seed Testing. Rules Amendments. *Seed Science and Technology* 29. (2):1 – 127.
19. **International Union for Conservation of Nature (IUCN). 2017.** IUCN Red list of Threatened species. Retrieved 30 May 2017 from [www.iucnredlist.org](http://www.iucnredlist.org). 63.
20. **Jain, A., Rao, D. V. and Batra, A. 2015.** A study on antimicrobial potential of *Tridax procumbens* (L.) against clinical isolates. *International Journal of Pharmaceutical Sciences and Research* 6. (3): 1330-1335.

21. **Jindal, A. and Kumar, P. 2012.** Antimicrobial activity of alkaloids of *Tridax procumbens* against human pathogens. *International Journal of Pharmaceutical Sciences and Research* 3. (9): 3481-3485.
22. **Khanzada, K. A., Rajput, M. A., Shah, G. S., Lodhi, A. M. and Mehboob, F. 2002.** Effect of seed dressing fungicides for the control of seed borne mycota of wheat. *Asian Journal of Plant Science* 1, 441-444. <http://dx.doi.org/10.3923/ajps.2002.441.444>.
23. **Ky-Dembele, C., Tigabu, M., Bayala, J., Savadogo, P., Bossism, I. J. and Oden, P. 2011.** Clonal Propagation of *Khaya senegalensis*: The effects of stem length, leaf area, auxins, smoke solution, and stock plant age. *International Journal of Forestry Research*. 281269:1-10.
24. **Masoko, J., Picard, J. N. and Eloff, P. 2007.** The antifungal activity of twenty-four southern African Comretum species (Combretaceae). *South Africa Journal of Botany* 73, 173-183. <http://dx.doi.org/10.1016/j.sajb.2006.09.010>.
25. **Mir, A. S., Dar, M. A., Mir, S., Ahmad, S. M. and Chitale, G. 2016.** Analysis of photochemistry and antimicrobial activity of *Tridax procumbens* Linn. *Chemical Sciences Journal* 7. (2): 1-4.
26. **Nikiema, A. and Pasternak, D. 2008.** “*Khaya senegalensis* (Desr.) A. Juss,” In plant resources of tropical Africa, D. Louppe, A. A. Oteng-Amoako, and M. Brink, (Eds.) PROTA Foundation, Wageningen, the Netherlands. 339–344. 66
27. **Obdoni, B. O. and Ochuko, P. O. 2001.** Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. *Global Journal of Pure and Applied Science* 8. (6): 203-208.
28. **Ogbebor, N. O., Adekunle A. T., and Enobakhare D. A. 2007.** Inhibition of *Colletotrichum gloeosporioides* (Penz) Sac. Causal organism of Rubber (*Hevea brasiliensis* Muell. Arg.) Leaf spot using plant extracts. *African Journal of Biotechnology* 6. (3): 213-218.
29. **Paul, Y. S. 1989.** Seed borne mycota of soybean and its control in Himachal Pradesh. *Indian Journal of Mycology and Plant Pathology*. 119; 235-257.

30. **Pernezny, K., Elliott, M., Palmateer, A. and Havranek, N. 2017.** Guidelines for identification and management of plant disease problems: Part II. Diagnosing Plant Diseases caused by fungi, bacteria, and viruses. Plant Pathology Department, UF/IFAS Extension. Original publication date February 2008. Revised June 2014 and March 2017.
31. **Rabia, N. and Asghari, B. 2013.** Phytochemical screening, antioxidants, and antimicrobial potential of *Lantana camara* in different solvents. *Asian Pacific Journal of Tropical Diseases* 3. (6): 480-486.
32. **Rahman, M. M. E., Ali, M. E., Ali, M. S., Rahman, M. M. and Islam, M. N. 2008.** Hot water thermal treatment for controlling seed-borne mycoflora of maize. *International Journal of Sustainable Crop Production*. 3. (5):5-9.
33. **Rao, T. V., Rajeswari, B., Prasad, A. L. and Keshavulu, K. 2015.** Seed transmission studies on seed-borne fungi of soybean. *International Journal of Scientific and Research Publications* 5. (10): 1-6. 68.
34. **Saxena, M., and Saxena, J. 2012.** Phytochemical screening of *Acorus calamus* and *Lantana camara*. *International Research Journal of Pharmacy*. 3. (5):324-326.
35. **Shaikh, S. S. 2018.** Preliminary phytochemical qualitative and quantitative screening of *Tridax procumbens* L. *International Journal of Chemical and Physical Sciences* 7: 213-217.
36. **Sridhar, S., Kumar, S. A., Thooyavathy, R. A. and Vijayalakshmi, R. 2013.** Seed treatment techniques. Centre for Indian Knowledge Systems, Chennai Revitalising Rainfed Agriculture Network 1-14. <http://www.ciks.org> (assessed 23rd July 2018).
37. **Subramanian, C. V. 1971.** Hypomycetes. Indian Council of Agricultural Research, New Delhi.
38. **Suelen, S. R., Álvaro, F. S., Antônio, C. N. and Yoshiko, S. K. 2012.** Detection, transmission, and pathogenicity of fungi on *Blepharocalyx salicifolius* (H. B. K.) Berg. Seeds. *SciELO Revista Brasileira de Sementes* 34. (1):1-5. 70.

39. **Sunil, H. G. and Sevak, B. G. 2016.** Preliminary Phytochemical and TLC Profiling of Lantana camara leaf extracts. *Journal of Chemical and Pharmaceutical Research*, 8. (5): 614-617.
40. **Tripathi, P. and Dubey, N. K. 2004.** Exploitation of natural products as an alternative strategy to control post-harvest fungal rotting of fruit and vegetables. *Post-harvest Biology and Technology* 32, 235-245.
41. **Utoho, E. B., Ogbodo, E. N. and Nwogbaga, A. C. 2011.** Seed borne mycota associated with rice and their influence on growth of Abakaliki, Southeast agro-ecology, *Nigeria. Libyan Agricultural Research Centre Journal International* 2, 79-84.
42. **Van-Burden, T. P. and Robinson, W. C. 1981.** Formation of complexes between protein and Tannin acid. *Journal for Agriculture and Food Chemistry* 1: 77- 82.
43. **Yago, J. I., Roh, J., Bae, S., Yoon, Y., Kim, H. and Nam, M. 2011.** The effect of seed-borne mycoflora from sorghum and foxtail millet on germination and disease transmission. *Mycobiology* 39. (3):206-218.

