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Research Article

Evaluation of three plant species to control black scurf disease of Irish potato (*Solanum tuberosum* Linn.)

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Abstract

Irish potato (*Solanum tuberosum*) is an important worldwide food crop and one of the most popular in Nigeria. Its abundance and successful yield have been immensely affected by black scurf disease caused by *Rhizoctonia solani*. Harnessing a cost-effective management of this pathogenic fungus, three botanical species *Acalypha wilkesiana*, *Moringa oleifera* and *Carica papaya* leaves, each at concentrations of 0 mg ml^{-1} (control), 25 mg ml^{-1}, 50 mg ml^{-1} and 75 mg ml^{-1} were evaluated *in vitro*. The plant leaf extracts were prepared using methanol and were evaluated for their toxicity using agar well diffusion method. The fungus was isolated from spoilt Irish potato with black scurf symptoms. The results showed the presence of some phytochemicals in leaf extract of each of the plants tested. The three leaves extract independently inhibited mycelial growth of *R. solani*. The potency of all the plant extracts increased with the increase in concentration. The highest concentration (75 mg ml^{-1}) of *M. oleifera* and *C. papaya* evaluated, gave the highest inhibitory effect of 0.81 mm and 1.63 mm respectively, which were not significantly different ($p > 0.05$), but was obviously different from *A. wilkesiana* (2.81 mm). Furthermore, *M. oleifera* extract gave the highest percentage of mycelial growth inhibition of the fungus in all grades of the concentrations evaluated, whereas *A. wilkesiana* showed the least. The leaves of the three species are therefore recommended for *in vivo* control of this fungus, owing to their proven efficacy and to their cheap availability.

Keywords: disease control; fungicides; phytochemicals; potato; *Rhizoctonia solani*; scurf disease

Introduction

Irish potato (*Solanum tuberosum* L.) belonging to the family Solanaceae is an essential non grain food crop, grown in around 150 countries spread across both temperate and tropical regions in the world, ranked top in terms of total production with over 365 million tonnes per year, after maize, wheat and rice (FAOSTAT, 2018), Nigeria being the seventh biggest producer in Africa (FAOSTAT, 2018). The tuber is the most important part of the Irish potato plant and it is an excellent source of carbohydrates, protein and vitamins (Jansky *et al*., 2019).

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With its current growing rate of cultivation, Irish potato is still affected with some pathogens causing diseases such as: *Fusarium* wilt, early blight, late blight, black scurf, etc. (Shainidze *et al*., 2016). Black scurf disease caused by *Rhizoctonia solani* is an important disease of potato that is soil and tuber-borne (Garbeva *et al*., 2008). It affects roots, stolon, stems and tubers. It further devalues the product and cause reduction in the market value (Kapsa, 2008). Disease symptoms include leaf blights, leaf spots, damping-off, rots on roots, shoots and fruits, canker lesions on sprouts and stolons, sclerotial diseases (Chang and Chou, 2007).

Control of black scurf has depended on some cultural practices, multiple applications of fungicides and development of cultivars tolerant to this disease (Kapsa, 2008). However, repeated application of fungicides could lead to reduced efficacy of the fungicides due to a gradual loss of sensitivity in the target pathogen population (Farrar *et al*., 2002). Therefore, the search for alternative to chemical products such as the use of natural biocides of plant origin is the most promising outlet for a safe and sustainable agriculture (Govindarajan *et al*., 2008).

The medicinal importance of plant materials typically results from the combination of secondary products present in plants (Adebola *et al*., 2019). These products are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, which are synthesized and deposited, in specific parts or in all parts of the plant (Liu, 2014; Wink *et al*., 2015). Their activity is probably due to their ability to complex with extracellular and soluble proteins (Chan *et al*., 2012; Chen *et al*., 2013).

Phytochemicals have been previously reported to reduce disease incidence of plants and consequently increase its yield (Larkin and Griffin, 2007; Adebola *et al*., 2019). It is on this note that this research evaluates the inhibitory effects of leaf extracts of acalypha (*Acalypha wilkesiana*), moringa (*Moringa oleifera*) and pawpaw (*Carica papaya*) for the control of *R. solani*, the causative organism of black scurf in Irish potato.

Materials and Methods

Collection of materials

Spoilt Irish potatoes with black scurf symptoms were collected at Mobil fruits and vegetables market in Minna, Nigeria. The leaves of the three different plant species: *A. wilkesiana*, *M. oleifera* and *C. papay* were collected from the wild. These plant samples were taken to the Department of Plant Biology, Federal University of Technology, Minna, Nigeria for respective use at the laboratory and proper authentication at the herbarium.

Preparation of plant extracts

This was done as previously described by Adebola *et al*. (2019) with little modification. Fresh leaves of *A. wilkesiana*, *M. oleifera* and *C. papaya* were washed with sodium hypochlorite and rinsed with distilled water. They were dried at room temperature for 14-16 days and the dried leaves were separately homogenized into powdered form. One hundred grams (100 g) of each plant were separately heated in 400 ml of methanol using a Soxhlet apparatus for 4 hrs at a temperature not exceeding the boiling point of the solvent, to extract the antimicrobial active compounds. Filtrations to remove residue was done using double layer muslin cloth followed by another stage of filtration using Whattman filter paper (No. 1). The filtrate was then separately concentrated *in vacuo* using rotary evaporator to 10% of the original volume at 37 °C - 40 °C. The final concentration to dryness was done by evaporating to dryness in water bath at 60 °C. The collected extracts were then used for anti-fungal activities (Jensen, 2007).

Phytochemical screening of the plant leaves

Samples of the three plant leaves were screened for the presence of phytochemical active compounds using the method described by Obasi *et al*. *(*2010).

Isolation and identification of the fungus

Spoilt Irish potatoes were sterilized in 1% sodium hypochlorite solution for about 60 seconds (Dimka and Onuegbu, 2010). These were then rinsed in three successive changes of sterile distilled water and blotted dry with sterile filter paper. Small segments of tissues from the margins of black scurf lesions were cut out with a sterile scalpel and plated on Potato Dextrose Agar (PDA) in Petri dishes. The plates were incubated at room temperature (27±2 °C) for 7 days (Jonathan *et al*., 2017). Developing fungal colonies were sub-cultured continuously on fresh PDA plates to obtain pure culture of the isolate. Fungal isolate was identified based on morphological and microscopic examinations, previously adopted by Adebola *et al*. (2019).

Screening plant extracts for antifungal activity

Two hundred millilitres of Potato Dextrose Agar (PDA) in Petri dishes were inoculated with the isolated and identified pathogen. After 72 hours, wells of 5.0 mm diameter were cut from the inoculated plate using sterile cork borer. The cut agar discs were carefully removed by the use of sterile forceps. Each well was filled separately with different plant extracts. Control treatments were set up by introducing Sterile Distilled Water (SDW).

Three replicates of each extracts at 25%, 50% and 75% concentrations were made. The plates were allowed to stand for one hour at 4 °C in the refrigerator, to allow for diffusion of the extracts into the PDA. The plates were then incubated at 27±2 °C at 24 hours (Okigbo and Ogbonaya, 2006).

The inhibition percentage was determined as:

$$\frac{\mathrm{R}_1 - R_2}{R_1} \times 100$$

(Where R_1 = radial growth of pathogen in control and R_2 = the radial growth of pathogen in plant extract treatment).

Pathogenicity test

Healthy Irish potatoes were swabbed with cotton wool soaked in 1% Mercuric Chloride and then washed twice in distilled water. Holes were created in the tubers by using 5 mm diameter cork-borer and the plug was pulled and replaced with 5 mm diameter mycelia disc containing the isolated fungus. Control consists of sterilized 5 mm PDA disc placed in the holes of the healthy tubers. The plug was carefully pulled and the wounded area sealed with Vaseline to prevent extraneous infection. Three replications were prepared for each treatment. Inoculated tubers were incubated for 4 weeks at 27±2 °C. Inoculated Irish potato tubers were later observed for black scurf development.

Data analysis

Statistical analysis of inhibition of mycelia growth were subjected to one-way analysis of variance (ANOVA) using Statistical Package for Social Science (SPSS) version 17.0 and means were separated according to Duncan's Multiple Range Test (DMRT) at 5% probability level.

Results

Isolation and identification of causative fungus in black scurf disease of Irish potato

Figure 1 shows the observed colony of *R. solani* on a PDA plate (A), as well as its photomicrograph (B). The colony appeared brown at the seventh day of incubation. Its hyphae were partitioned into individual cells by septum, while mycelia formed a right-angled branching.

Pathogenicity test

The result of the pathogenicity test implicated *R. solani* as the causative organism with lesion found on the potato tubers. The black mass (BM) showing on Figure 2 C and D are symptoms of *R. solani*. (Figure 2).

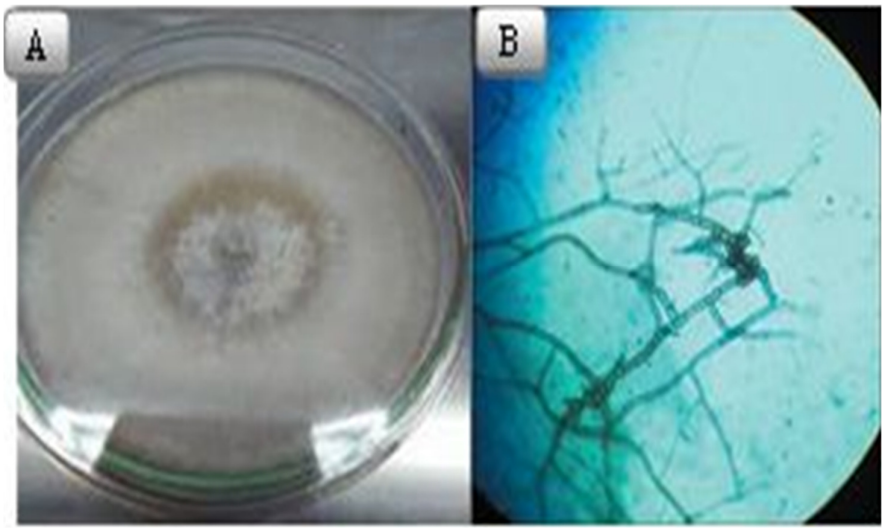

Figure 1. Morphology of *Rhizoctonia solani.* **A**: Pure culture of *R. solani* isolated from diseased Irish tuber. **B**: Photomicrograph of *R. solani*

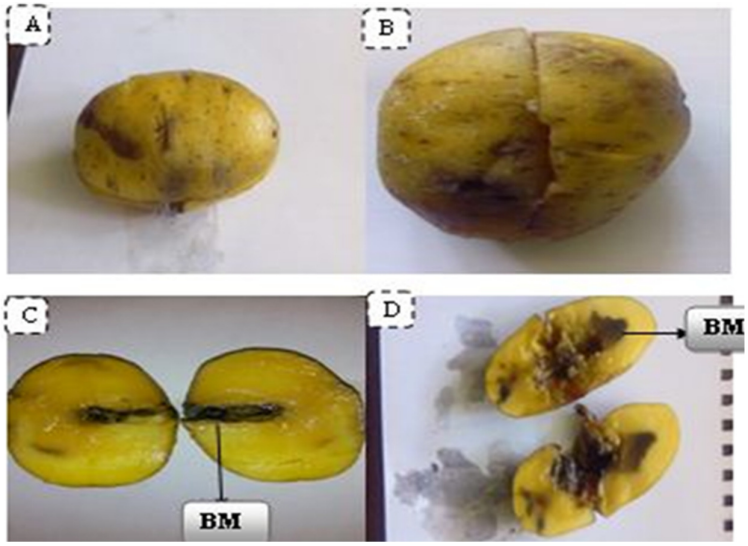

Figure 2. Scurfy Irish potato. Irish potato inoculated with *R. solani* after 5 days (A), 10 days (B), 18 days (C) and 28 days (D)

Phytochemical screening

Phytochemical screening revealed the presence of flavonoid, saponin, alkaloid and terpenes in all the three species, while steroid and phenols were not present in *Carica papaya* and *Acalypha wilkesiana.* Only steroid was not found in *Moringa oleifera.* Tannin was found in *C. papaya* and *M. oleifera*, but was absent in *A. wilkesiana* (Table 1).

Table 1. Phytochemical constituents of the three aqueous leaf extract tested

Phytochemicals	*C. papaya*	*M. oleifera*	*A. wilkesiana*
Tannin	+	+	_
Flavonoid	+	+	+
Saponin	+	+	+
Steroid	_	_	_
Alkaloid	+	+	+
Terpenes	+	+	+
Phenols	_	+	_

Note: + = Present, _ = Absent

In vitro inhibitory effect of C. papaya aqueous leaf extract on the growth of R. solani

The *in vitro* inhibitory effect of aqueous leaf extract of *C. papaya* on the growth of *R. solani* in Irish potato is presented in Table 2. At day 1, there was no significant difference ($p > 0.05$) between the fungal growth in the treatments 25 mg ml^{-1} (0.10±0.06) and 50 mg ml^{-1} (0.13±0.09). There was significant difference ($p < 0.05$) however between the 75 mg ml^{-1} treatment (0.00±0.00), which had no fungal growth, and the control treatment (0.30±0.06) that showed the highest fungal growth. At day 4, the fungal growth was significant ($p \leq 0.05$) and showed the highest growth in mycelia for the control treatment (1.91±0.07) compared with the treatments 75 mg ml^{-1} (1.17±0.12) and 25 mg ml^{-1} (1.53±0.14).

At day 6, the extract in the treatment 75 mg ml^{-1} (1.31±0.10) has the lowest fungal growth when compared with the control treatment (3.62±0.07) that was observed to have the highest fungal growth. Moreover, there was no significant ($p >0.05$) growth between the treatments with 25 mg ml^{-1} (2.50±0.22) and with 50 mg ml^{-1} (2.61±0.06).

At day 7, the extract in the treatment with 75 mg ml^{-1} (1.63±0.10) significantly ($p <0.05$) reduced the fungal growth, while the highest fungal growth was recorded in the control group (4.13±0.07). There was also significant difference ($p < 0.05$) in the treatment with 25 mg ml^{-1} (3.50±0.06) and with 50 mg ml^{-1} (2.81±0.05) (Table 2).

Table 2. *In vitro* inhibitory effect of *C. papaya* aqueous leaf extract on the mycelial growth of *R. solani*

Treatment mg ml^{-1}	Day 1 (mm)	Day 2 (mm)	Day 3 (mm)	Day 4 (mm)	Day 5 (mm)	Day 6 (mm)	Day 7 (mm)
25	0.10 ± 0.06^{b}	0.65 ± 0.03^{a}	1.11 ± 0.11^{b}	1.53 ± 0.14^{b}	2.01 ± 0.21^{b}	2.50 ± 0.22^{b}	3.50 ± 0.06^{c}
50	0.13 ± 0.09^{b}	0.67 ± 0.10^{b}	1.32 ± 0.10^{b}	1.87 ± 0.05^{c}	2.24 ± 0.04^{b}	2.61 ± 0.06^{b}	2.81 ± 0.05^{b}
75	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.50 ± 0.17^{a}	1.17 ± 0.12^{a}	0.68 ± 0.04^{a}	1.31 ± 0.10^{a}	1.63 ± 0.10^{a}
Control	0.30 ± 0.06^{c}	0.75 ± 0.03^{b}	1.22 ± 0.09^{b}	1.91 ± 0.07^{c}	2.75 ± 0.06^{c}	3.62 ± 0.06^{c}	4.13 ± 0.07^{d}

Values followed by the same superscript letter on the same column are not significantly different at $p > 0.05$
Values are presented in mean ± standard error of three replicates.

In vitro inhibitory effect of A. wilkesiana aqueous leaf extract on the mycelial growth of R solani

The *in vitro* inhibitory effect of aqueous leaf extract of *A. wilkesiana* on the growth of *R. solani* in Irish potato is presented in Table 3. At day 1, the leaf extract significantly ($p < 0.05$) inhibited the fungal growth in the treatment with 25 mg ml^{-1} (0.03±0.03), while the highest fungal growth was recorded in the control treatment (0.03±0.06). The leaf extract showed no significant difference ($p > 0.05$) with the treatment 50 mg ml^{-1} (0.13±0.09) and 75 mg ml^{-1} (0.10±0.06).

At day 2, the control treatment (0.75±0.03) showed no significant difference ($p > 0.05$) with the other treatments of 25 mg ml^{-1} (0.87±0.05), 50 mg ml^{-1} (0.77±0.08) and 75 mg ml^{-1} (0.66±0.10).

At days 6 and 7, the fungal growth was significantly ($p > 0.05$) high in the control treatments (3.62±0.06) and (4.1 ±0.07) respectively, when compared with the fungal growth in their respective 75 mg ml^{-1} treatments (2.54 0.03) and (2.81±0.05). However, the extract slightly inhibited the fungal growth with the treatment of 50 mg ml^{-1} (3.14±0.09) and (3.27±0.05) respectively, showing significant difference ($p > 0.05$) with the treatments of 25 mg ml^{-1} (3.37±0.07) and (3.76±0.03) respectively.

Table 3. *In vitro* inhibitory effect of *A. wilkesiana* aqueous leaf extract on the mycelial growth of *R. solani*

Treatment mg ml^{-1}	Day 1 (mm)	Day 2 (mm)	Day 3 (mm)	Day 4 (mm)	Day 5 (mm)	Day 6 (mm)	Day 7 (mm)
25	0.03 ± 0.03^{a}	0.87 ± 0.05^{a}	1.58 ± 0.62^{c}	2.16 ± 0.21^{b}	2.83 ± 0.05^{c}	3.37 ± 0.07^{c}	3.76 ± 0.03^{c}
50	0.13 ± 0.09^{b}	0.77 ± 0.08^{a}	1.38 ± 0.07^{b}	2.31 ± 0.23^{b}	2.59 ± 0.09^{b}	3.14 ± 0.09^{b}	3.27 ± 0.04^{b}
75	0.10 ± 0.06^{b}	0.66 ± 0.10^{a}	1.32 ± 0.12^{b}	1.92 ± 0.05^{a}	2.24 ± 0.04^{a}	2.54 ± 0.03^{a}	2.81 ± 0.05^{a}
Control	0.30 ± 0.06^{c}	0.75 ± 0.03^{a}	1.22 ± 0.09^{a}	1.87 ± 0.07^{a}	2.75 ± 0.06^{b}	3.62 ± 0.06^{d}	4.13 ± 0.07^{d}

Values followed by the same superscript letter on the same column are not significantly different at $p > 0.05$
Values are presented in mean ± standard error of three replicates.

In vitro inhibitory effect of M. oleifera aqueous leaf extract on the mycelial growth of R. solani

The *in vitro* inhibitory effect of aqueous leaf extract of *M. oleifera* on the growth of *R. solani* in Irish potato is presented in Table 4. At day 1, there was no significant difference ($p > 0.05$) with absence of fungal growth in the treatments with 75 mg ml^{-1} (0.00±0.00) and 50 mg ml^{-1} (0.00±0.00). The control treatment (0.30±0.06) however was significant ($p < 0.05$) and showed the highest fungal growth. At days 4 and 5, the extract inhibited the fungal growth significantly ($p < 0.05$) with the treatment of 75 mg ml^{-1} (0.20±0.07) and (0.44±0.07) respectively. Also, the fungal growth was significant ($p < 0.05$) and showed the highest fungal growth in the treatment with 25 mg ml^{-1} at the respective days, (2.05±0.03) and (2.55±0.06), showing no significant difference ($p > 0.05$) with the fungal growth in the control treatment (1.87±0.07) and (2.75±0.06) respectively.

At day 7, the leaf extract inhibited the fungal growth greatly in the treatment with 75 mg ml^{-1} (0.81±0.11), having significant difference ($p < 0.05$) when compared to the fungal growth in the control treatment (4.13±0.07).

Table 4. *In vitro* inhibitory effect of *M. oleifera* aqueous leaf extract on the mycelial growth of *R. solani*

Treatment mg ml^{-1}	Day 1 (mm)	Day 2 (mm)	Day 3 (mm)	Day 4 (mm)	Day 5 (mm)	Day 6 (mm)	Day 7 (mm)
25	0.09 ± 0.00^{b}	0.87 ± 0.05^{c}	1.49 ± 0.04^{c}	2.05 ± 0.03^{c}	2.55 ± 0.06^{c}	2.80 ± 0.06^{c}	3.14 ± 0.03^{c}
50	0.00 ± 0.00^{a}	0.20 ± 0.06^{b}	0.68 ± 0.27^{b}	0.88 ± 0.19^{b}	1.47 ± 0.20^{b}	1.85 ± 0.10^{b}	2.14 ± 0.03^{b}
75	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.03 ± 0.03^{a}	0.20 ± 0.07^{a}	0.44 ± 0.07^{a}	0.67 ± 0.07^{a}	0.81 ± 0.11^{a}
Control	0.30 ± 0.06^{c}	0.75 ± 0.03^{c}	1.22 ± 0.09^{c}	1.87 ± 0.07^{c}	2.75 ± 0.06^{c}	3.62 ± 0.06^{d}	4.13 ± 0.07^{d}

Values followed by the same superscript letetr on the same column are not significantly different at $p > 0.05$
Values are presented in mean ± standard error of three replicates.

Percentage mycelial growth inhibition of R. solani of the three plant leaf extracts

The percentage mycelial growth inhibition of *R. solani* of the three-plant leaf extracts that causes black scurf in Irish potato is presented in Table 5. *C. papaya* leaf extract at 75 mg ml^{-1} concentration significantly ($p < 0.05$) inhibited the fungal mycelial growth at 60.73% when compared with the 25 mg ml^{-1} concentration of the leaf extract, that inhibited the mycelial growth by 16.67%. The fungal mycelial growth having *A. wilkesiana* leaf extract with the 25 mg ml^{-1} concentration was slightly inhibited (10.53%), having significant difference ($p < 0.05$) when compared with the 75 mg ml^{-1} that prevented the fungal mycelial growth to 35.57%. The fungal mycelial growth was 79% significantly ($p < 0.05$) inhibited by the 75 mg ml^{-1} concentration of the *M. oleifera* leaf extract when compared to the 25 mg ml^{-1} concentration of the leaf extract that inhibited the fungal mycelial growth by 25.17%.

Table 5. Percentage mycelial growth inhibition of *R. solani* by the three plant leaf extracts

Sample	*C. papaya*	*A. wilkesiana*	*M. oleifera*
25%	16.67 ± 1.36^{b}	10.53 ± 0.71^{b}	25.17 ± 0.84^{b}
50%	33.13 ± 1.24^{c}	21.77 ± 1.08^{c}	48.97 ± 0.84^{c}
75%	60.73 ± 2.11^{d}	35.57 ± 0.83^{d}	79.00 ± 1.44^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Values followed by the same superscript letter on the same column are not significantly different at $p > 0.05$
Values are presented in mean ± standard error of three replicates.

Discussion

The macroscopic and microscopic observations reported in the present study were similar to those described by Sneh *et al*. (1996) and Sirari *et al*. (2015), who observed similar structure in the root of wheat. The branching pattern of the mycelia observed was similar to earlier report of Kyle *et al*. (2014).

Blackish mass of sclerotia found during the pathogenicity test was indicative of the fungus and was previously reported by Sirari *et al*. (2015), who identified the sclerotia as black patches. This mass may be the resultant effect of the fungus, which would consequently discolour the tubers and reduce the yield of the crop.

Findings from the present investigation on the antifungal activities of the aqueous extract of *C. papaya, M. oleifera* and *A. wilkesiana* against the growth of *R. solani* has shown that the aqueous extract of these plants contain certain inhibitory bioactive components which triggered significant reduction in the daily mycelial growth of the pathogen. Previous studies have similarly reported *C. papaya* and *M. oleifera* to contain

a wide range of bioactive secondary metabolites which include terpenoids, alkaloids, phlobatannins, tannins, saponins, phenols, quinones, lecitins, polyphenols, glycosides, flavonoids, polypeptides and steroids (Edeoga *et al*., 2005; Enyiukwu and Awurum, 2013).

Furthermore, the inhibitory effect of *C. papaya* observed in the hereby study was similar to the previous report of Ebele (2011) who had similar work on the use of *C. papaya* in the control of the rot of pawpaw fruit caused by fungi.

Also, *M. oleifera* that was observed to have the highest inhibitory potential in the current study was stressed by previous works. Abdull *et al.* (2014) and Mishra *et al.* (2011) have revealed that *M. oleifera* is a multipurpose tree with a whole wide range of applications. They also found out that the leaves contain a variety of phytochemicals. As a result of these bioactive compounds found in Moringa extracts, they have to inhibit the growth of the pathogens studied.

The present study has shown that maximum inhibition is obtainable as the concentration of the extracts used increases. This observation is similar to the findings recorded by Adebola *et al*. (2019), who found that fungicidal efficacy of leaf extracts increased with increased concentration. Such findings could imply that an increase in the concentration of the extract of leaves may result in an increase in the bioactive antifungal component of the solution that may inhibit physiological processes in *R. solani* and consequently restrict the mycelial growth of the fungus. A similar conclusion was previously reported by Tijani *et al*. (2012) who opined that the efficacy of plant extracts depends on the nature and quantity of the active ingredients it contains.

Conclusions

Findings from the present study have shown that the aqueous leaf extracts of *C. papaya*, *M. oleifera* and *A. wilkesiana* have the potential to inhibit *R. solani* that causes black scurf disease of Irish potato. Out of the three plants studied, *M. oleifera* recorded the highest percentage inhibitory effect on the growth of the the studied fungus. Therefore, these could be independently used by farmers as cost-effective alternatives to environmentally hazardous and expensive chemical control, consequently reducing the cost of production and improving production of Potato in developing countries. *In vitro* study at higher concentrations of these plant extracts should be tried against *R. solani*. Tests for *in vivo* efficacy of these three medicinal plant extracts on the control of black scurf disease of Irish potato should be carried out.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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