



## RESEARCH ARTICLE

### EFFICACY OF PALM KERNEL BUNCH EXTRACT AGAINST CUCUMBER DAMPING-OFF CAUSED BY SCLEROTIUM ROLFSII SACC

\*Jacinta, N. Akalazu

Department of Plant Science and Biotechnology at Imo State University in Owerri, Nigeria

#### ARTICLE INFO

##### Article History:

Received 16<sup>th</sup> June, 2023

Received in revised form

17<sup>th</sup> July, 2023

Accepted 20<sup>th</sup> August, 2023

Published online 30<sup>th</sup> September, 2023

##### Key Words:

Phytochemicals, *Sclerotium rolfsii*, Pathogenicity, Fungi, Cucumber.

#### ABSTRACT

*Sclerotium rolfsii* Sacc. is a very damaging plant pathogen that infects more than 500 plant species, resulting in substantial reductions in crop productivity. A field and laboratory studies were conducted at the Imo State University, Owerri Botanical garden and Department of Plant Science and Biotechnology laboratory, to determine the effectiveness of oil palm fruit bunch extract against *S. rolfsii*. The pathogenicity test revealed that cucumber plants injected with *S. rolfsii* had symptoms of seedling damping-off. The mycelial growth suppression was most pronounced at a concentration of 2.5% (15.4mm), whilst the least inhibition was recorded at a concentration of 0.5% (28.32mm). The lowest number of sclerotia (140.12) was observed at a concentration of 2.5% extract, followed by a concentration of 1.5% (165.02), in comparison to the control (318.03). The disease severity was most pronounced in Week 4, (41.48%), while the lowest severity was recorded in Week 1 (0.47%). Sterile water (Sw), showed the most severe disease symptoms, followed by the oil palm bunch extract, while mancozeb had the least severe disease symptoms. The study proposes that application of oil palm fruit bunch waste can efficiently address cucumber damping-off disease, hence encouraging an eco-friendly and sustainable management of cucumber seedling damping-off disease.

Copyright © 2023, Jacinta, N. Akalazu. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

## INTRODUCTION

The cucumber, (*Cucumis sativus* L.), belongs to a cucurbitaceous family. Cucumber is a low-calorie food and highly nutritious vegetable that is abundant in vitamins A, B, and C, composed of about 96% water, 3% carbs, and 1% protein, and essential minerals like manganese, copper, iron, calcium, and potassium (Szalay 2017). Regrettably, cucumber is vulnerable to contamination from various fungi present in the soil, leading to the occurrence of damping-off and root rot diseases, that inevitably reduce the quality and productivity of the crop (Kumar et al., 2018). Fungal diseases account for approximately 70-80% of agricultural productivity losses (Deresa and Diriba, 2023). *Sclerotium rolfsii* is a soil-borne necrotrophic fungus that affects more than 500 different host plants worldwide (Hollowell and Shew, 2001; Galgóczy et al., 2023). It causes significant damage by inducing crown blight, stem canker, damping-off of seedlings, and fruit rots in plants (Rafi et al., 2017; Shoaib et al., 2016). The pathogen's prolific proliferation and ability to create durable and resistant survival structures called sclerotia make it the primary obstacle in the effective cultivation of cucumber (Derbalah et al., 2012; Kumar et al., 2018).

\*Corresponding author: Jacinta, N. Akalazu,  
Department of Plant Science and Biotechnology at Imo State University in Owerri, Nigeria.

Sclerotia undergo germination, resulting in vigorous infections under elevated temperatures (27 to 35 °C) and humid conditions (Shoaib et al., 2016). The control measures have achieved very limited effectiveness, mostly because of the wide range of hosts, rapid development, and capacity to generate numerous sclerotia that can endure in the soil for several years. The Oil Palm tree (*Elaeis guineensis* Jacq.), belongs to the class Angiospermae and belongs to the family Arecaceae. The oil palm industry generates large amounts of waste, such as empty fruit bunches (EFB), palm kernel shells (PKS), mesocarp fiber (MF), oil palm fronds (OPF), and oil palm trunks (OPT) (Rakhmawati et al., 2023). Empty fruit bunch (EFB) is a type of waste that is generated after removing the fruit. Indonesia processed around 49 million tonnes of palm oil in 2020, which led to the generation of almost 19.6 million tonnes of waste biomass (Rakhmawati et al., 2023). In Nigeria for example total empty fruit bunch generated annually is more than 2.5 million tonnes (Uchegbulam et al., 2022). Moreover, inappropriate disposal of this waste can result in serious implications for the environment, and human health, and depletion of the bioactive components (Gillani, et al., 2015). Presently, to mitigate the worldwide threats to plant health and achieve the objective of ensuring food security in a dynamic ecological context, there is a growing emphasis on exploring phytochemicals as a viable substitute for synthetic pesticides in the control of plant diseases. The multiple benefits associated with plant extracts, include their enhanced safety profile, simple biodegradability, environmentally sustainable

nature, and reduced toxicity (Omara *et al.*, 2020). Effective control of *Sclerotium rolfsii*, characterized by a vast host range, prolific development, and ability to produce a high number of sclerotia that may stay in the soil for several years remains a serious challenge. Enhancing crops' capacity to suppress pathogenic attacks before or soon after crop establishment is an important component of the comprehensive approach to disease management. The study intends to investigate the efficiency of palm kernel bunch extract against cucumber damping-off caused by *Sclerotium rolfsii* with regards to mycelial growth inhibition and sclerotia generation, as well as the decrease in damping-off severity for long-term disease management in Nigeria.

## MATERIALS AND METHODS

### Study site

**Owerri:** The study was conducted in Imo state University, Owerri, botanical garden in the humid tropical region of Nigeria located from longitude 3° E to 12°E and latitude 4°N to 9°N, (Ogungbenro and Morakinyo, 2014; Akinsanola *et al.*, 2016). The humid rainforest agroecological zones of Nigeria are noted for the country's major agricultural production (Dania *et al.*, 2019).

**Isolation and identification of *Sclerotium rolfsii*:** Soil samples were taken from infected cucumber plants. The isolates were cultured using the suspension-dilution technique on Potato Dextrose Agar (PDA) medium, and their pathogenicity was assessed by checking the signs of damping off and yellowing of the cucumber seedling.

**Field Experiment and Experimental Design:** Certified cucumber seeds were obtained from the Imo State Agricultural Development Programme, Owerri. Cucumber seeds were reprimed for 10 hours before sowing. *Sclerotium rolfsii* suspension strains were collected from 14-day-old cultures, and palm fruit bunch extract was measured at various concentrations(0.5%, 1.0, 1.5, and 2.5%). Pots were sterilized, filled with loam soil, and planted with three cucumber seeds. After 10 days, palm fruit bunch extract was administered along with Mancozeb treatments, and weekly water application was repeated four times using a randomized complete block design.

**Severity of cucumber disease:** The severity of cucumber disease was examined at different doses of the palm extract (0.5, 1.0, 1.5, and 2.5%), with a grading system ranging from 1 to 5. The ranking was determined based on obvious symptoms such as yellowing and damping off on both the leaves and stem.

**Preparation of the Fresh oil palm bunch extract:** Fresh oil palm bunch extract waste was collected and properly cleansed with distilled water. The extract was made by macerating the bunch and extracting it with ethanol. The extract was then passed through 4-ply muslin cloth and filtered through Whitman's filter paper No.41. The concentrations of 0.5, 1.0, 1.5, and 2.5% were prepared and assessed in vitro against mycelial growth and sclerotial generation of fungus (*S. rolfsii*) by poisoned food approach.

**Pathogenicity Test:** The isolated *R. sclerotium* was subjected to an assessment of their capacity to initiate seedling damping-off and yellowing of cucumber seedlings that were in a healthy state. The *S. Rolfsii* were inoculated on cucumber seedlings through the use of knife-induced injuries, as described by (Chaudhary *et al.* 2020).

The pathogenicity of *R. sclerotium* on the inoculated cucumber seedling was determined by checking the signs of damping off and yellowing of cucumber seedling.

**Antifungal sensitivity:** Fresh oil palm waste ethanol extract, was then filtered through Whitman's filter paper No.41, and stored at 40C for further analysis. Different concentrations of the extract(0.5, 1.0, 1.5, and 2.5%) were prepared and tested against mycelial growth and sclerotial generation of *S. rolfsii* in vitro. The extract was added to chickpea seed meal agar (CSMA) and inoculated with active mycelium of *S. rolfsii*. The radial mycelial growth and sclerotial production were measured after 25 days of inoculation, as described by (Ismael, and Latif 2019), with modifications. The formula for computing inhibition percentage is expressed as follows:

$$\text{Inhibition (\%)} = \frac{dc - de}{dc} \times 100.$$

Where "dc" represent the diameter of the control mycelium, and "de" represent the diameter of the mycelium treated to extracts.

**Data and statistical analysis:** All the collected data were subjected to an analysis of variance (ANOVA) using MINITAB 20 software programme and significant differences between the means are achieved using the Turkey,s HSD test ( $p < 0.05$ ).

## RESULTS

**Pathogenicity test:** The experimental results revealed that cucumber inoculated with *S.rolfsii* showed evidence of seedling damping off. Indications of damping off were noticed in the form of a dark, water-soaked, gelatinous decaying of stem and root tissues below the soil.

**The mycelial growth inhibition and sclerotia formation:** The analysis of variance comparison on the effects of oil palm fruit bunch extract, the positive control, (mancozeb), and sterile distilled water on the inhibition of *S.rolfsii* mycelial growth and sclerotia production demonstrated a statistically significant difference ( $p \leq 0.05$ ) during the duration of the study. Mancozeb displayed the best efficiency in mycelial growth inhibition and sclerotia production in *S.rolfsii*, this was followed by oil palm fruit bunch extract treatment while distilled water treatments had the least inhibitory effect on the fungal pathogen, (Table 1). The data imply that there is an inverse association between the concentrations of extract and the inhibition of mycelial growth and sclerotia generation by *S.rolfsii*. The largest mycelial growth inhibition was recorded on 2.5% (15.4mm), whereas the lowest inhibition was observed on 0.5% (28.32mm), (Table1). Minimum number of sclerotia (140.12) was developed at 2.5% extract concentration followed by 1.5% concentration (165.02) as compared to control (318.03) (table1).

**Table 1. Diameter of crude extracts' inhibition zones (mm) against *Sclerotium rolfsii* and Sclerotial Production**

Treatments	Mycelial growth(mm)	No. of Sclerotial Production
Palm fruit Bunch extract concentrations.		
0.5%	28.32(0.36)b	240.02(0.096)b
1.0%	25.65(0.12 )c	186.30(0.3)c
1.5%	20.44(0.42)d	165.02(0.48)d
2.5%	15.40(0.20)e	140.12(0.192)e
Sw.(1 ml)	86.43(0.32 )a	318.03 (0.47)a
Mancozeb (1000 µg/ml)	5.06(1.42 )f	23.00(0.38)f
F- value	24929.38	13415.43
P- value	0.002	0.001

Means that do not share a letter are significantly different ( $p \leq 0.05$ ). Values in brackets are standard deviation.

**Table 2. Effect of Oil palm fruit bunch extract at different concentrations on percentage disease severity from weeks 1 to 4 after inoculation**

Treatments	WK 1	WK 2	WK 3	WK 4	F-value	P-value
SW(1 ml)	1.41(0.06)a	22.40(0.53)a	50.60(0.36 )a	80.82(0.42)a	24929.38	0.001
0.5(%)	0.403(0.03)b	14.32(0.10 )b	43.43(0.38 )b	66.23(0.25)b	13415.43	0.002
1.0	0.43(0.06)b	10.40(0.10 )c	30.07(0.12 )c	54.3(0.40)b	11691.62	0.000
1.5	0.23(0.05)c	4.04(0.05)d	14.40(0.20)d	23.37(0.32 )c	9096.37	0.000
2.5	0.07(0.14)c	1.27(0.21)c	12.97(1.42 )d	15.50(0.10 )d	356.96	0.001
Mancozeb(1000 µg/ml).	0.03(0.05)d	0.83(0.50 )c	5.43(0.32 )e	8.70(0.36)e	431.80	0.000

Means that do not share a letter within a column in a treatment are significantly different (Turkey,s HSD test ( $p < 0.05$ )).Numbers in brackets are  $\pm$  standard deviation.

In general, all the oil palm fruit bunch extract concentrations were better than the sterile water in suppressing the mycelial development of *S. rolfsii*. Means that do not share a letter are significantly different ( $p \leq 0.05$ ). Values in brackets are standard deviation.

**Disease severity:** Week 4 exhibited the most notable disease severity (41.48%), the least severity was observed on week one (0.47%) (Table 2). The negative control, sterile water(Sw) exhibited the highest disease severity, this was followed by oil palm bunch extract while mancozeb had the lowest disease severity across the monitoring periods (Table 2). The disease severity showed a linear decline as the concentrations increased, (Table 2).

## DISCUSSION

**Pathogenicity test:** The obtained result in the present study showed that *S. rolfsii*, is a common fungus in the rhizosphere of the cucumber plants and is pathogenic to the cucumber plant in the pathogenicity tests. The result is in agreement with those recorded by (Haikal-Nahed,2007; Jinghua et al., 2008), who reported that *S. rolfsii* is considered one of the most important soil-borne pathogens which cause cucumber root-rot diseases. Meanwhile, Yu et al.,2023 reported that *S. rolfsii* destroyed peanut cells by producing infection cushions, secreting a large amount of oxalic acid, cellulose and polygalacturonase (Yu et al., 2023). The result of pathogenicity test was consistent with reports of (Jinghua et al., 2008; Elagamey et al., 2020), who stated that Cucumber is susceptible to infection by several soil-borne fungi, causing damping-off and root rot diseases which, of course, affect the quality and productivity of the crop.

**Mycelial growth inhibition and reduction in sclerotia production:** The inhibition of *Sclerotium rolfsii* mycelial growth and reduction in scler0tia production reported in this study can be attributed to the presence of antifungal chemicals found in the oil palm empty fruit bunches extract. The findings are consistent with (Sholahuddin et al., 2023), who reported

that oil palm empty fruit bunches contain lignin, which exhibits antioxidant and antimicrobial activities. Reports have shown the antimicrobial activities of lignin against *S. aureus*, *E. coli*, *B. thermosphacta* and *P. fluorescens* (Alzagameem et al., 2019). Meanwhile, Lakshmi et al., (2022) reported that some phytochemicals (Piperine, Reserpine, and  $\beta$ -Sitosterol) exhibited significant reduction in mycelial inhibition, sclerotial development, ooze formation, maturity, sclerotial number, and germination of *S. rolfsii*.

**Disease severity:** The decreased severity of the disease seen in this study may be attributable to antifungal chemicals present in the palm bunch, which inhibited the production of pathogenic enzymes (Yu et al.,2023).

## CONCLUSION

The findings of this study demonstrate the inhibitory activity of oil palm fruit bunch extract against *S.rolfsii* mycelial growth, and sclerotia production, and reduction of the cucumber disease severity, therefore qualify the bunch extract to be exploited in the development of natural fungicides. for ensuring food security, promoting environmental safety, and mitigating substantial economic losses within the agricultural industry.

## ACKNOWLEDGEMENTS

The authors are grateful to the journal editors, and the anonymous reviewers.

## REFERENCES

- Akinsanola, AA, Ogunjobi, KO, Ajayi, VO, Adefisan, EA, Omotosho, JA and Sanogo, S (2016) Comparison of five gridded precipitation products at climatological scales over West Africa. Meteorology and Atmospheric Physics 129, 669–689.

- Alzagameem A, Klein SE, Bergs M, Do XT, Korte I, Dohlen S, Hüwe C, Kreyenschmidt J, Kamm B, Larkins M, et al. (2019) Antimicrobial Activity of
- Barnett, H.L. and Hunter, B.B. (2006) Illustrated genera of imperfect fungi. 4th Edition, The American Phytopatological Society, St. Paul Minnesota.
- Choudhary, M., Jat, H. S., Datta, A., Sharma, P. C., Rajashekhar, B., and Jat, M. L. (2020). Topsoil bacterial community changes and nutrient dynamics under cereal based climate-smart agri-food systems. *Front. Microbiol.* 11, 1812.
- Dania, VO, Fadina, OO, Ayodele, M and Kumar, PL (2019 ) Distribution and virulence of fungal species isolated from yam (*Dioscorea* spp.) tubers in three agroecological zones of Nigeria. *International Journal of Pest Management* 66, 252–261.
- Derbalah, A. S., Dewir, Y. H., and El-Sayed, A. E. N. B. (2012). Antifungal activity of some plant extracts against sugar beet damping-off caused by *Sclerotium rolfsii*. *Annals of Microbiology*, 62, 1021-1029. Deresa EM, Diriba TF. Phytochemicals as alternative fungicides for controlling plant diseases: A comprehensive review of their efficacy, commercial representatives, advantages, challenges for adoption, and possible solutions. *Heliyon*. 2023 Feb 17;9(3)
- Elagamey, E.; Abdellatef, M.A.E.; Kamel, S.M.; Essa, T.A. *Fusarium oxysporum* isolates collected from the same geographical zone exhibited variations in disease severity and diversity in morphological and molecular characters. *Egypt. J. Phytopathol.* 2020, 48, 43–57.
- Galgóczy, L., Bácsi, A., Homa, M., Virág, M., Papp, T., & Vágvölgyi, C. (2011). Invitro antifungal activity of phenothiazines and their combination with amphotericin B against different Candida species. *Mycoses*, 54.
- Gillani, M., Khan, M., and Atiq, M. (2015). Health and economic implication of solid waste dumpsites: A case study hazar khwani peshawar. *FWU J. Soc. Sci.*, 9(2), 40–52.
- Haikal-Nahed, Z. (2007) “Improving biological control of Fusarium root-rot in cucumber (*Cucumis sativus* L.) by allelopathic plant extracts,” *International Journal of Agriculture and Biology*, vol. 9, pp. 459–461, 2007.
- Hollowell JE, Shew BB (2001) Yellow Nutsedge (*Cyperus esculentus* L.) As a host of *Sclerotinia minor*. *Plant Disease* 85: 562.
- Ismael, T. K., and Latif, A. A. (2019). Antifungal Activity of Some Plant Extracts. *Journal of Oral and Dental Research*, 6(2).
- Jinghua, Z. , Chang, W. , Xu, W. Hanlian, and T. Shuge,( 2008 ) “Allelopathy of diseased survival on cucumber fusarium wilt,” *Acta Phytophylacica Sinica*, vol. 35, pp. 317–321.,
- Khanzada, S. A., Iqbal, S. M., & Akram, A. (2006). In vitro efficacy of plant leaf extracts against *Sclerotium rolfsii* Sacc. *Mycopathologia*, 4(1), 51-53.
- Kumar, Ritesh, Ghatak, Abhijeet and Bhagat, Arun P. (2018). Assessing fungicides for seedling protection of cucumber to collar rot disease caused by *Sclero- tium rolfsii*. *Internat. J. Plant Protec.*, 11(1) : 10-17.
- Lakshmi, N., Basha Shaik, A., Paramita Pal, P., Begum Ahil, S., Vittal, R., Naik, S., ... and Sagar Bokka, V. (2022). Piperine, Reserpine and β-Sitosterol Attenuate Stem Rot (*Sclerotium rolfsii* Sacc.) of Groundnut by Inducing the Secretion of defense Enzymes and Phenolic Acids. *Chemistry & Biodiversity*, 19(4),
- Ogungbenro, SB and Morakinyo, TE (2014) Rainfall distribution and change detection across climatic zones in Nigeria. *Weather and Climate Extremes* 5–6, 16.
- Omara, R.I.; Essa, T.A.; Khalil, A.A.; Elsharkawy, M.M. (2020) A case study of non-traditional treatments for the control of wheat stem rust disease. *Egypt. J. Biol. Pest Control.* , 30, 1–12.
- Sana N, Shoaib A, Javaid (2016) Antifungal potential of leaf extracts of leguminous trees against *Sclerotium rolfsii*. *Africa Journal of Traditional Complementary and Alternative Medicine*. 12;13(5):54-60.
- Sholahuddin, S., Arinawati, D.Y., Nathan, V.K. et al.(2023) Antioxidant and antimicrobial activities of lignin-derived products from all steam-exploded palm oil mill lignocellulosic biomass waste. *Chem. Biol. Technol. Agric.* 11, 5
- Soares JVC, Bentes JLS, Gasparotto L (2017). Reação de genótipos de *Capsicum* spp. à podridão do colo (*Sclerotium rolfsii*). *Summa Phytopathologica* 43(1):58-59.
- Szalay, J .(2017) Cucumbers: Health Benefits and Nutrition Facts..
- Tang W, Kuang J, Qiang S (2015) The Pathogenicity of *Sclerotium rolfsii* on *Cyperus difformis* and its Potential Host Specificity among the Genus *Cyperus*. *J Plant Pathol Microbiol* S3: 002. doi:10.4172/2157-7471.S3-002
- Wavare, Shivaji and Gade, R. and Shitole, Amol. (2017). Effect of Plant Extracts, Bio Agents and Fungicides against *Sclerotium rolfsii* Causing Collar Rot in Chickpea. *Indian Journal of Pharmaceutical Sciences*. 79. 513-520.
- Uchegbulam, I., Momoh, E. O., & Agan, S. A. (2022). Potentials of palm kernel shell derivatives: A critical review on waste recovery for environmental sustainability. *Cleaner Materials*, 6, 100154.
- Yu, D., Song, W., Wang, Q., Kang, Y., Lei, Y., Wang, Z., ... & Yan, L. (2023). The quantity of OA and activity of cellulase and polygalacturonase are involved in variation of virulence in *Sclerotium rolfsii*. *Oil Crop Science*, 8(2).