Original Research Article

Invitro and *Invivo*Control of Fungal Cobweb Disease of *Pleurotusostreatus*(Jacq), using Organic Materials

ABSTRACT

Aim: Assessment of *Invitro*And *Invivo*Control Of Fungal Cobweb Disease of*Pleurotusostreatus*(Jacq), using organic materials
Study Design: This study was designed to control the disease by using plant materials as organic control agent; both in vitro and in vivo
Methodology: in *Pleurotusostreatus*. In the *in vitro* test; food poison method was used while the *in vivo* test was undertaken by inoculation of fully colonized substrate bags of *Pleurotusostreatus* infected with cobweb disease with crude extracts of *Piper guineense* and lime juice as treatment stock.
Results: The results indicated *P. guineense* inhibition of the growth of the pathogen at 22mm while lime juice did not show any impact on the growth of the pathogen. Additionally, *P. guineense* inhibited the growth of the pathogen in fully colonized but infected substrates bags of *P. ostreatus*invivo; such that the treated samples grew and developed the fruiting bodies of the mushroom, 3 days after treatment; whereas the one treated with lime juice developed the cobweb disease.
Conclusion: The study showed that efficacy of *P. guineense* extract on the pathogen was possibly due to the bioactive chemicals such as phenols, tannins, flavonoid etc which concentration in the plant material occurred in surplus. These may have interfered with the molecular targets of the pathogen and caused it to loose cellular integrity and leakage of cell content. Alternatively, the study revealed that lime juice was ineffective; possibly because of the solvent used for it's preparation which could not enhance the release of the essential bioactive chemicals in lime juice for anti fungals. Consequently, this study recommends the use of *P. guineense* to mushroom farmers against fungal cobweb disease of oyster mushrooms.

Keywords: Invitro, Invivo, cobweb disease, *Pleurotusostreatus,* bioactive chemicals, *piper guineense*, lime juice.

INTRODUCTION

Mushrooms are macrofungi with distinctive fruiting bodies of higher fungi such as Basidiomycota and Ascomycota; epigeous or hypogeous. They are large enough to be seen with naked eyes and picked by hand. Mushrooms have been used as food by mankind, since the stone age [1][2][3]. Mushrooms are a rich source of nutrients, particularly proteins, minerals, vitamins as well as bioactive constituents such as phenolic compounds, terpenes, steroids and polysaccharides [4]. They are known for good quality amino acids, vitamin B complex, sodium, potassium, iron and dietary fibres. They are considered as the primary natural sources of ergosterol or provitamins [5][6]. Mushrooms are also accredited with medicinal benefits imbedded with pharmacological effects such as antiviral, antioxidant, antitumoral, hypo-cholesterolemic and hypoglycemic[7]. Edible mushrooms are also reported to be found effective in reducing stress, cholesterol, asthma, diabetes, cancer and insomnia etc [8][9].
A Fungal cobweb disease of cultivated mushroom is commonly reported to cause dramatic mushroom crop failure and chemical control of the disease is undesirable due to residual effects.
Mushroom survival and multiplications lie on a number of factors which may act singly or have interactive effects among themselves [10][11].

Comment [o1]: Avoid needless use of initial capital
Comment [o2]: What disease?
Comment [o3]: italicise
Comment [o4]: italicise
Comment [o5]: use “effectiveness”
Comment [o6]: delete
Comment [o7]: summarise
Comment [o8]: use initial cap for each word
Comment [o9]: antitumor
Comment [o10]:
Comment [o11]: effects
Comment [o12]: diabetes mellitus
Comment [o13]: delete
Comment [o14]: rephrase sentence

Intensive cultivations of edible mushrooms can often be affected by some microbes such as some fungi,bacterialetcthat rather frequently cause dramatic production loss. These infections are facilitated by the particular conditions under which the mushroom cultivation is carried out; such as warm temperatures, high humidity,carbondioxide levels. Mushrooms, like all other crops are also affected adversely by a large number of biotic and abiotic agents/factors. Among the biotic agents are fungi, bacteria, viruses, nematodes, insects and mites that cause damage to mushrooms directly or indirectly [12].

A number of harmful fungi are encountered in compost and casing soil during cultivation of mushrooms. Many of these acts as cobweb disease, competitor molds, thereby adversely affecting spawn run; whereas others attack the fruit bodies at various stages of the crop growth producing distinct disease symptoms. Most times, there is complete crop failure depending upon the stage of infection, quality of compost and environmental conditions [4][13][14].

Cobweb disease of mushrooms is caused by several species of a fungal genus,*CladobotryumNees emend* (Syn. *DactyliumNees*). They correspond to the conidial or asexual stage of species from the *Hypomyces*(Fries) L. R. Tulaone (Ascomycota, Hypocreales, Hypocreacea).
It is reported to appear at the end of a crop cycle; first as small white circular patches that appear on casing soil or basiciomas. They quickly spread by the grey-white mycelium that resembles a spider web [15]. As the mycelium sporulate producing masses of spores that are easily released when physically disturbed mainly by watering or picking operations; air currents from air-conditioning systems also sufficiently cause strong mobilization on the harmful spores [16]. Once released, the conidia spread through mushroom facilities by air currents to form secondary colonies on casing layers [5].

Cobweb disease is reported to be caused by several species of fungi in the genus *Cladobotryum. which* is known as one of the most infectious pathogen of mushrooms.
Its infections leads to the formation of patches of white cobweb-like mycelium [17][18]. As the disease develops, the first symptom is the appearance of white patches on the basidiomsand then spreads quickly by means of fine gray white mycelium that resembles spider web. Fruiting bodies that are severely infected show discolouration and rottening. As the disease advances, dry spores begin to be released from the mycelium and spread to other basidiomes with the help of various agents such as air current,sprinklers etc [16].

Prevention of cobweb disease is very paramount to preclude the emergence of the disease and reduce its impact peradventure it has already occurred. Regular cleaning of the mushroom house; lowering the RH in the cropping house and increase in air circulation in the cropping house will go very far to preclude the occurrence of cobweb disease. These are possible based on the report of Sharma [19].

In order to control or manage cobweb disease, a method employed should prevent dispersion of conidia as reported above; hence this is the main way leading to the infection [20][21][22][23].

The end of the crop cycle is very important for the removal of any residual disease. The wet conidia of *Cladobotryum*species could be destroyed at 45^0C for at least 3 mins; but they resist higher temperatures of about 100^0C when dry. However, the pathogenic mycelium is susceptible to a 15 mins, (ie 40^0C for 15 mins) when dry [21]. Although thermal disinfection at the end of a crop cycle by cooking out (about 65 – 70^0C for 9 – 12 hours) still stands as one of the most important strategy to ensure the mushroom crop is very disinfected.
There are reports that some farmers still employ chemical control strategy over cobweb disease. No matter how effective a chemical may be, its application is undesirable because of its residual impact and hazardous effect on consumers.

Due to consumer and the environmental concerns; there is a very strong pressure to reduce or preclude the use of chemical pesticides. This has led to the intensification of organic control in Agriculture, for safe alternatives by the use of plant materials.
This then calls for safe alternatives possibly with plant materials.

Comment [o15]: are often
Comment [o16]: delete
Comment [o17]: and
Comment [o18]: delete
Comment [o19]: and
Comment [o20]: use the word “them”
Comment [o21]: on
Comment [o22]: of
Comment [o23]: which
Comment [o24]: and
Comment [o25]: delete
Comment [o26]: delete
Comment [o27]: min
Comment [o28]: min
Comment [o29]: use “that is”
Comment [o30]: well
Comment [o31]: delete
Comment [o32]: to control

Interestingly, it is reported that many plants with bioactive potentialscan act as organic or biofungicides. Compost tea from spent mushroom substrate and essential oils from plants have been tested as alternatives. Many aromatic plants with bioactive chemicals are better proven as safe alternatives. Plant materials such as (Lime juice), *Piper guineense, Xylopiaaethiopica,* Trametes and Sclerotia powder etcalso have been reported to contain some bioactive constituents [24][25][26][27].
However, it is reported by Inga and Alexander [28] that the tested natural preventatives are *Oreganumvulgare,* essential oils, carvacrol, thymoleugenol and trans-cinnamaldehyde. These are the most tested organics with good antifungal effects against some storage fungi. In the other hand, Ibukun *et al*., [29] also submitted that the potency of lime juice is being enhanced by the type of solvent used, which indicates that there are some active ingredients in the lime juice that have antimicrobial/antifungal effect which will not be released except when lime fruit is used in conjunction with a particular solvent.
Ibunkun *et al* [29] reported that the potency of lime juice is dependent on the type of solvent used to extract it. And that there are some solvent that can enhance the release of bioactive chemicals for antifugals in lime juice.
These plants have been reported to contain bioactive compounds such as tannins, flavonoids, essential oils and phenolic acids etc [30]. Davidson [31] reported that tannins affect fungal pathogens directly on the cell membrane by metal depletion.

Harris and Dennis [32][33] reported that terpenoid type of bioactive chemical caused the zoospores of *Phytophthora*spp to develop cytoplasmic granulations and disruptions of the pathogen's plasma membrane and leakage of cellular contents.
According to the report of Cowan [30], tannins can bind to the pathogen's protein enzymes to inhibit the enzyme and cause substrate deprivations; while alkaloids can interact into the cell wall for disruption and cause leakage of cellular contents. He also suggested that flavonoids and phenolic acid bind to adhesion and complex with the fungal cell wall and inactivates fungal enzymes.

Consequently, the aim of this study is to isolate, and identify the fungal species responsible for cobweb disease in *P. ostreatus;*as well as proffer strategy for the management of the disease in vivo and in vitro using plant materials.

Comment [o33]: potential
Comment [o34]: delete
Comment [o35]: remove the bracket
Comment [o36]: delete
Comment [o37]: delete
Comment [o38]: rephrase the sentence
Comment [o39]: delete
Comment [o40]: delete
Comment [o41]: rephrase the sentences
Comment [o42]: was

MATERIALS AND METHODS

The study was carried out in the laboratory of the Department of Plant Science and Biotechnology of Rivers State University. And the sample was procured from Dilomat Mushroom farm located in the campus. The samples were fully colonized or ramified mushroom substrates of *Pleurotusostreatus,* infected with fungal cobweb disease obtained from the Dilomat mushroom farms and taken to the laboratory for study.

Plate 1: Fully Colonized Mushroom Substrate Infected with Cobweb Disease

Sample Preparation

A 10g quantity of the infected substrates was obtained mechanically using a sterile inoculating loop into a test tube; from which the infectious stock was prepared for inoculation.

Preparation of Normal Saline for Serial Dilution

A quantity of 8.5g of analytical salt (NaCl)was dissolved in 1 litre of distilled water. The diluent was sterilized in an autoclave at 121^0C Psi for 15 minutes [34].

Comment [o43]: min

Serial Dilution/Inoculation

9ml of normal saline was dispensed into different test tubes, then 10 fold serial dilution was made, in which 1ml from the stock solution was transferred from 10^{-1} to 10^{-3} and also transferred direct.

Comment [o44]: Exactly 9 mL

Media Preparation (Sabouraud Dextrose Agar – SDA)

This was prepared according to the manufacturer's prescription, by weighing 65gm of SDA powder and dissolving in 1 litre distilled water. Mass volume relationship was used to compute the actual required measurements. The mixture was shaken vigorously and sterilized by autoclaving at 121^{0}C Psi for 15 mins. Antibiotic was added to prevent bacterial contaminations on cooling, it was dispensed into sterile Petri dishes [35].

ORGANIC MATERIALS AS CONTROL AGENT

The plant materials used are lime juice and dry seeds of *Piper guineense*. The crude extracts of the pulp of lime and the dried seeds of *P. guiueense* were made and used as food poison into the agar wells of the SDA.

In Vitro Test by Agar well Diffusion Method

The crude extract of plant material,lime juice was used as a control agent of the disease: Inoculum was collected with inoculation needle and added to already prepared broth: The agar plate surface was then inoculated by spreading a volume of the microbial inoculum over the entire agar surface and a cork borer was used to create wells on the agar plate and the plant extracts were poured into the agar wells and incubated for 48 hours. This was observed to determine the possible inhibitions by the organic extract.

In Vivo Test

Two fully ramified or colonized *P. ostreatus* mushroom substrate bags which were infected with cobweb disease were inoculated with the plant extracts separately per treatment (lime juice and the extract of *Piper guineense*) in the PSB laboratory. A sterilized knife by flaming red-hot and cooking was used to create holes on the mushroom substrates. 5ml each of the extracts were placed per treatment into each of the holes and incubated. The treated substrate bags were kept away from the rest uninfected substrates. These were observed daily for possible results.

Comment [o45]: What was prepared?

Comment [o46]: delete

Comment [o47]: min

Comment [o48]: were

Comment [o49]:

Plate 2: Infected and Colonized Mushroom Substrates, also Treated with Plant Extracts

Phytochemical Screening of the Plant Materials
The phytochemical screening of the plant materials used as control agent for various bioactive chemicals were conducted using standard procedures as prescribed by Soforora [36]; Trease and Evans [37].

Test for Alkaloids
Extracts of the plant materials were dissolved separately, each in dilute Hydrochloric acid (Hcl) and filtered. The filtrates were treated with Mayer's reagent (Potassium mercuric iodide. Formation of a yellow coloured precipitate was unindication of the presence of alkaloids.

Test for Terpenoids
Salkowski method was used to determine the presence of terpenoids. The crude extracts were separately shaken with 2ml chloroform; followed by addition of concentrated 2ml of H_2SO_4 along the sides of the test tube. A reddish – brown colour of the interface indicated the presence of alkaloids of terpenoids.

Test for Tanins
Two methods were used to test for tanins;
(a) To a 10ml freshly prepared 10% KOH in a beaker; 0.5ml of each of the extracts were added and shaken to dissolve. A dirty precipitate observed indicated the presence of tanins.
(b) 0.5ml of the extracts were boiled in 10ml water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or blue black colouration.

Test for Saponins
A 0.5ml of the extracts were added to 5ml distilled water in a test tube and the solutions shaken vigorously and then were observed for a stable persistent froth. The frothings were mixed each with 3 drops of olive oil and shaken vigorously, after which the experiments were observed for the formation of an emulsion.

Test for Steroids
1ml of the extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence, an indication of the presence of steroids.

Test for Flavonoid
Two methods were used to test for flavonoids.
(a) A portion of the extracts was heated with 10ml of ethyl acetate over a steam bath for 3 minutes; the mixtures were filtered and 4ml of the filtrates were shaken with 1ml of ethyl acetate over a steam bath for 3 minutes; the mixtures were filtered and 4ml of the filtrates were shaken with 1ml of dilute ammonia solution. A yellow colouration confirms the presence of flavonoids.
(b) Dilute 5ml ammonia was added to a portion of an aqueous filtrate of the extracts. Then 1ml concentration H_2SO_4 was added. A yellow colouration indicated the presence of flavonoids.

The extracts were stirred each with 10ml distilled water and then filtered. A few drops of 5% ferric chloride was added. Black or blue-green colouration or precipitate was the confirmatory test for phenols.

Results and Discussion
The results of this study are presented on plates 3 – 5 and table 1.

Comment [o50]: h
Comment [o51]: HCl
Comment [o52]: an indication
Comment [o53]: delete
Comment [o54]: Tannins
Comment [o55]: tannins
Comment [o56]: tannins
Comment [o57]: About
Comment [o58]: Exactly I mL
Comment [o59]: Flavonoids
Comment [o60]: concentrated
Comment [o61]: Table

The results of the invitro test showed that the treatment of the sample with the crude extract of *Piper guineense* in the agar wells inhibited the growth of the cobweb propagules with inhibition growth measurement of 22mm; while the lime juice did not show any impact on the disease propagule in vitro.
The in vivo results also showed that lime juice could not inhibit the growth of the cobweb disease after treatment. However, treatment with *P. guineense* inhibited the growth of the cobweb disease in vivo and allowed the growth and development of the mushroom fruiting bodies; 3 days after treatment. This is shown in Plate 3.

Plate 3: Petri dishes bearing agar wells and showing inhibitory growth of cobweb propagule by*Piper guineense*

Plate 4: Showing no impact on the disease propagule by lime juice treatment and no inhibitory effect

Plate 5: Successful treatment of cobweb disease with *P. guineense* and showing growth of mushroom fruit bodies, 3 days after treatment

The results of the phytochemical screening test is presented on Table 1. The results revealed that alkaloid and flavonoids are absent in both *P. guineense* and lime juice used as control agents against the fungal cobweb disease of the study sample. It also revealed that tanins and saponins are present in the seed extract of *P. guineense*, but absent in lime juice, although it contained terpenoids which was shown absent in *Piper.* However, the phytochemical results also revealed surplus of steroids and phenol in both plant extracts.

Comment [o62]: were

Comment [o63]: delete

Comment [o64]: tannins

Table 1: Showing Phytochemical Screening of *P. guineense* and lime juice for the treatment of cobweb disease in *Pleurotusostreatus.*

S/NO	Phytochemicals	*Piper guineense*	Lime juice
1.	Alkaloid	_	_
2.	Terpenoids	_	+
3.	Tannins	+	_
4.	Saponins	+	_
5.	Steroids	++	++
6.	Flavonoids	_	_
7.	Phenol	+++	+++

The results of this study indicated that the plant extract of *Piper guineense,* used as food poison in agar wells had an impact of inhibition on the fungal cobweb pathogen, with a growth inhibition of 22mm.

Alternatively, the lime juice used in same pattern as in above did not have any inhibitory or destructive impact on the spread of the pathogen.
However, the invivo result showed that *P. guineense* seed extract administered on fully colonized substrate bags of *Pleurotusostreatus*inhibited the growth and development of the cobweb disease and gave room to the growth of the mushroom fruiting bodies; 3 days after treatment. The result of the phytochemical screening of the plant extracts used indicated that alkaloids and flavonoids were absent in the plant extracts; while tannins and saponins were absent in the lime juice; although steroids and phenols occurred in surplus quantities of the two plant extracts.
It is possible that *P. guineense* was efficacious because of the presence of the bioactive chemicals contained therein. The bioactive chemicals may have acted by inhibiting cutinases and lacases of the pathogen; to conform to the report of Nmom and Ajuru [22]; who reported that saponins and tannins inhibit cutinases and lacases of pathogenic fungi. It is also likely that the bioactive compounds may have acted directly on the fungal cell membrane by metal depletion, in line with the suggestions of Davidson [31]; that tanins affect fungal pathogens directly on the cell membrane by metal depletion. It was also possible that the extract of *P. guineense* interfered with the molecular targets of the pathogen's tissues. Since steroids and phenols occurred in surplus quantity in the *P. guineense* extract. It could be that they interfered with the fungal membrane integrity and possibly complexed with the sugar residues of the bioactive chemicals. This seems to clearly agree with the report of Keykens*et al* [38] that; steroidal glycol alkaloids interfere with fungal membrane integrity and complexes with the sugar residues of saponin molecules with the pathogen.
Most importantly, the bioactive compounds may have also acted by causing the fungal spores to develop cytoplasmic granulations and disruptions of the plasma membrane; thereby leading to leakage of cellular contents; inaccordance with the submissions of Harris and Dennis [32][33]. The presence of tannins may have caused its binding to the pathogen's protein enzyme and as a result, inhibited its enzymatic actions. This is also in line with the report of Cowan]30]. Also in line with his report, is that, it is likely that the chemicals of the flavonoids and phenolic acid may have bound to adhesion and complexed with the fungal cell wall and inactivated the pathogen's enzymes.

For the ineffectiveness of the lime juice in vitro and in vivo; it is obvious as was reported earlier in this study, that lime juice is not one of the reportedly tested natural preservatives, as was reported by Inga and Alexander [28]. Additionally, it is possible that the solvent used in this study for juicing is not such that could release, the bioactive chemicals in the lime. This therefore implies that the potency of the lime juice in this study was not enhanced due to the type of solvent used to juice the lime. This implies that, if the right solvent was used, there would have been an enhancement of the release of accompanying bioactive chemicals for antifungals; as was suggested by Ibukun*et al.* [29].

Comment [o65]: for
Comment [o66]: delete
Comment [o67]: in
Comment [o68]: effective
Comment [o69]: in them
Comment [o70]: use "this"
Comment [o71]: tannins
Comment [o72]: is
Comment [o73]: use "this agrees"
Comment [o74]: delete

Conclusion

The results of this study establishes that *Piper guineense* extract inhibited the growth of the fungal cobweb pathogen; Cladobotryummycophiliumat 22mm invitro and also inhibited the growth of the pathogen in fully colonized substrate bags of *Pleurotusostreatus;* such that the treated samples grew and developed the mushroom fruiting bodies; 3 days after treatment whereas lime juice did not show any impact on the growth of the pathogen, invitro and in vivo. The shows that treatment of cobweb disease with the seed extract of *P. guineense* is effective.

This study also has established that the efficacy of the extract of *P. guineense* was possible due to the bioactive chemicals; such as Phenols, tannins, flavonoids etc which concentrations in the plant material occurred in surplus. These may have interfered with the molecular targets of the pathogen and caused it to loose integrity and cell leakage. Alternatively, the study also has established that lime juicing which could not enhance the release of essential bioactive chemicals for antifungals could do better if an appropriate juice is used.

Conclusively, fungal cobweb disease of oyster mushroom can be managed, using organic material as a good alternative to field inventory to enhance agricultural sustainability of oyster mushroom cropping.

Comment [o75]: delete

Comment [o76]: delete

Comment [o77]: delete

Comment [o78]: This

Comment [o79]: p

Comment [o80]: delete

Comment [o81]: thus causing

Comment [o82]: solvent

Comment [o83]: rephrase sentence

REFERENCES

1. Chang, S. T. and Miles, P. G. (1992). Mushrooms biology, a new discipline. *Mycologist* 6,: 64 – 65. Doi: 10. 1016/S0209-915 x (09) 804 49 – 7.

2. Fasidi, L. O., Kadiri, M., Jonathan, S. G., Adenipekun, C. O. and Kufuriji, O. O. (2008). Cultivation of edible tropical mushrooms.

3. Nmom, F. W. (2021). Basic Mycology.Prisnet Digital Solutions.

4. Belletini, M. B., SebasliaoBelletini; Fernanda, A. Fiorda, Alexsandra, C. Pedro, Fabiane Bach, Miriam, F., Fabela – Moron, Rosemary Hofmann-Ribani (2018). Diseases and pests noxious to pleurotus spp. Mushroom crops. 50(2): 216 – 226. Doi: 10.10 – 16/J. ram 2017.08. 009.

5. Adie, B. (2000). *The biology and epidemiology of the cobweb disease pathogen; (Cladobotryum*spp*) infecting the cultivated mushroom* (*Agaricusbisporus*). Ph.D Thesis, Imperial College, University of London.

6. Ghimire, A., Krishna Ray Pandey; Yagya Raj Joshi and SobitaSubedi (2021). Major fungal contaminants of mushrooms and their management. *International Journal of Applied Sciences and Biotechnology.* 9(7): 80 – 93. Doi: 10.3126/ijasbt.v 912.37573.

7. Cheung, P. (2010). The nutritional and health benefits of mushrooms. *Nutritional Bulletin* 35(4): 292 – 299.

8. Wani, B. A., Bodha, R. and Wani, A. (2010). Nitritional and medicinal importance of mushrooms, 12(1): 1 – 16. Doi: 10.1615/intjmedmushor. Vol. 12.11.10.

9. Joseph, M. (2021). Types of edible mushrooms. Retrieved from www.nutrition advance.com: http://www.nutrion advance.com.

10. Kim, K., Choi, B., Lee, L., Lee, H., Kwon, S., Oh, K., Kim, A. Y. (2011). *Bioproduction of mushroom Mycelium of AgaricusBisporus* by commercial submerged fermentation for the production of meat. 91: 109.

11. Belletini, M. B. and Fioda, F. A. (2016). Production pests and diseases in mushroom, Pleurotusspp crops. *GuarapuavaApprehendere,*pp: 152.

12. Kim, M. K., Lee, Y. H. and Cho, K. M. (2014). Fungicide sensitivity and characterization of cobweb disease on a Pleurotuseryrgii mushroom crop caused by *Cladobotrymycophilum. Plant Pathol J.* 30: 82 – 89. https://doi.org/10.5423 PPJ.OA.09.2013.0098.

13. Kim, S. W., Kim, M. G., Kim, J., Lee, H. S., Ro, H. S. (2008). Detection of the mycovirus OMSV in the edible mushroom; *Pleurotusostreatus,* using an SPR biosensorchip. *J. Virol Methods.*

14. Bruno, G. L., De Corato, U., Rana, G. L., De. Luca, P., Pipoli, V., Lops, R., Scarola, L., Mannerucci, F. Piscitelli, L; and Cariddi, C. (2015). Suppressive of white vinegar and steam – exploded liquid waste against the causal agents of *Pleurotuseryngii yellowing.* Crop Prof.; 70: 61 – 79.

15. Carrasco, J., Navarro, M. J., Sant, M., Dianez, F. and Gea, F. J. (2016). Incidence, identification and pathogenicity of *CladobotryumMycophilum;* causal agent of cobweb disease on *Agaricusbisporus* mushroom crops in spain. *Ann Appl. Biol.* 168: 214 – 224.

16. Adie, B., Gorgan, H., Archer, S. and Hills, P. (2006). Temporal and special dispersal of Cadobotryum conidia in the controlled environment of a mushroom growing room. *Appl. Environ Microbiol.* 72: 7212 – 7217. http:// doi.org/10.1128/AEM. 01369 – 06.

17. Carrasco, J. Navarro, M. J., Santos, M., Dianez, F. and Gea, F. J. (2017). Effect of five fungicides with different modes of action on cobweb disease (*C. mycophilium*) and mushroom yield *Ann Appl Biol.* 171: 162 – 169.

18. Royse, D. J., Baars, J. and Tan, Q. (2017). Current overview of mushroom production in the world. Technology and Applications. In: Edible and medicinal mushrooms. Doi: 10.1002/9781119149446.ch2.

19. Sharma, S. (1994). Cobweb disease of button mushroom (*Agaricusbisporus*) in Korea.J. Gen… Thompson. J. D., Higgins, D. G. and Gibson T. J.

20. Adie, B. and Grogan, H. (2000). The Liberation of cobweb (*Cladobotryummycophilium*) conidia within a mushroom crop. Proc. 15th Int. Cong on the Science and Cultivation of edible fungi, pp: 595 – 600. Maastricht, Netherlands, 15 – 19 May.

21. Fletcher, J. T. and Gaze, R. H. (2008). Mushroom pest and disease control: A colour handbook, I sted, Manson publishing Ltd. Academic Press. Sandiego, C. A. USA.

22. Nmom, F. W. and Ajuru, M. G. (2019). Efficacy of crude leaf extracts of *Ficusexasperata*(vall) in the control of powdery mildew on *Vernonia*amydalina (Del).

23. Nmom, F. W. and Ajuru, M. G. (2020). Plant bioactive chemicals for antifungal and biofungicidal potencies. *Int. J. of Adv Academic Research* (Sc. Tech & Engineering). ISSN: 2488-9849.

24. Potocnik, I., Vukojevic, J., Stajic, M. Rekanovic, E., STepanovic, M., Milijasevic, A. and Todorvic, B. (2010). Toxicity of Fungicide Timorex 66 EC to *Cladobotryumdendroides and Agaricusbisporus*. *Crop Prot* 29: 29: 290-294. http:/doi.org/10.1016/j.cropro. 2009.07.016.

25. Kosanovic, D., Polocnik, I., Duduk, B., Vukojevic, J., Stajic, M., Rekanovic, E. and MilliJasevic, Marcic, S. (2013). Trichoderma species on *Agaricusbisporus*farms in serbia and their biocontrol. *Annals of Applied* Biology, 163(2): 218 – 230. Doi:10.1111/aab.12048.

26. Gea, F. J., Carrasco, J., Dianez, F., Santos, M. and Navarro, M. J. (2014). Control of dry bubble disease (*Lecanicilliumfungicita*) in button mushroom (*Agaricusbisporus*) by spent mushroom substrate tea. *Eur. J. Plants Pathol* 138: 711 – 720. http://doi.org/10.1007/s 10658-013-0344-y.

27. Geosel, A., Szabo, A., Akan, O., Szarvas, J. (2014). Effect of essential oils on mycopathogens of *Agaricusbisporus. Proc. 8th conf. of Mushroom Biology and Mushroom Products;* PP: 530 – 535. Mushroom Society of India (Solan) (eds). New Delli India.

28. Inga S. and Alexander P. (2018) Antifungal activity of selected natural preservatives against food-borne molds, penicillium and A. Westerdarn. *FEM Microbiology letter* Vol. 361, Issue 13 FRY 125.

29. Ibukun A., Adrenipekun T., Adelowutan T., Ogunsanya T. and Odugeni T. (2007). *Afri. J. CAM* 4 (2): 185 – 190

30. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Microbiology Reviews,* 12: 564 – 582.

31. Davidson, P. M. (1997). Chemical perspectives and natural anti-microbial compounds. In: Rai*et al.* (eds). Naturally occurring bioactive compounds. Elsevier Sci Ltd, pp: 423 – 467.

32. Harris, J. E. and Dennis, C. (1976). Antifungal activity of Post-infectional metabolites from potato tubers. *Physiol. Plant Pathol,* 9:155 – 165.

33. Harris, J. E. and Dennis, C. (1977). The effect of post inflectional potato tuber metabolites and surfactants on zoospores of Oomycetes. *Physiol. Plant Pathol.* 9: 163 – 169.

34. Prescott's Microbiology by Joanne Willey; Lenda M. Sherwood and Christopher J. Wool-Verton (2011). (World Cat. Org).

35. Cheesebrough, M. (2005). Preparation of reagent and culture media. District laboratory practice in tropical countries, Cambridge University Press, U. K.: 394 – 401.

36. Sofowora, A. (1989). Medicinal Plants and traditional medicine in Africa Spectrum Books ltd (2nd edn): 26 – 100.

37. Trease, G. E. and Evans, W. C. (2002). Phytochemicals. In: Pharmacognosy (15th edn). Sanders Publishers, London.

38. Keykens, E. A. J., Deurije, T., Van Denboom, C., Dewaard, P., Chipiri, V., Jogen, W. M. F. and De Kruijff, B. (1995). Molecular basis of glycoalkaloids induced membrane disruption. *Biochemistry Biophysics Acta*1240: 216 – 228.