

Control of Pathogenic Fungi on *Pleurotus tuber-regium* culturesOyelakin AO¹., Fasidi IO²., Odebode AC³., Jonathan SG³ and Babalola BJ³.

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Abstract: The inhibitory effect of the pesticides was tested against the growth of the pathogenic fungi associated with cultures of the *Pleurotus tuber-regium*. *Aspergillus niger*, *A. flavus*, *Trichoderma* sp. and *Mucor* sp. were isolated in the mycelia culture plates of *P. tuber-regium*. The pesticides applied were copper sulphate, benlate (benomyl), formalin, ridomil, (metalaxyl), demosan, tecto-60, brestan and kocide. The results obtained indicated that copper sulphate, formalin, brestan and kocide at 50 ppm and 100 ppm showed highly inhibitory effect on the sporulation and mycelia growth of the pathogenic fungi. The effect of CuSO₄ on *A. niger* is significantly different from benlate, nacl, ridomil, kocide, na₂s₂o₄, formalin, tecto-dust and demosan while brestan shows the highest significant effect on *A. niger* and significantly different from CuSO₄. NaCl, Tecto-Dust and Demosan produced high significant effect on *A. flavus* while the effect were non significantly different from each other. The tecto-dust effect on *Trichoderma* sp is significantly different from formalin on *Trichoderma* sp. The effect of Tecto-dust is higher Formalin, Tecto-Dust and Demosan *Mucor* sp and significantly different from all other pesticides used. There is a high significant effect on *A. niger* at the concentration of 200ppm. The effect of the concentration at 100, 200 and 250ppm are non-significantly different for *A. flavus* but significant different from 0 concentration. The effect of the concentration at 50, 100, 150, 200 and 250ppm were non significantly different on *Trichoderma* sp but significantly different from concentration at 0 ppm, also the same effect was recorded for *Mucor* sp. The effect of pesticides is highly (p<0.01) significant for *A. flavus*, *Trichoderma* sp and *Mucor* sp but non significant for *A. niger*. The effect of concentration on the *A. niger* is significant (p<0.05) but highly significant for *A. flavus*, *Trichoderma* sp and *Mucor* sp. *A. niger* shows significant relation to *A. flavus* and *Trichoderma* sp but non significantly correlated with *Mucor* sp and negatively related to concentration. The results in this study showed the usage of chemicals to control contaminating fungi.

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1. Introduction

Mushrooms also known as achloropylous organisms with a distinct fruiting body which can be easily distinguished by the sporocarps are subject to a number of diseases caused by fungi and bacteria. The mushroom also has to struggle against the 'competitors' or 'weed fungi' which reduce the amount of food available and may produce toxic substances retarding the growth of the mushroom crop Jonathan *et al.*, (2012). Edible fungi including mushrooms, truffles, morels and puffballs are protein-rich, delicious vegetable of which world demand recently is increasing sharply Jonathan *et al.*, (2013). Different wastes (substrates) were used to cultivate this functional food. (Jonathan SG, Babalola BJ 2013). Cultivated mushrooms are subjected to attack by several pathogenic fungi during different stages of storage. Fungi can be propagated by transferring any part of this fungus to a fresh substratum but this normal development of a thallus commences with the germination of a spore

(Alexopolus *et al.*, 1996; Oluranti *et al.*, 2012). On reaching a favourable substratum, the spore swells, brings out one or more germ tubes. The germ tube elongates and in most cases, become richly branched, forming a network of hyphae collectively known as a mycelium, which may penetrate the substratum, grow on the surface or may be partly submerged and partial aerial. Jonathan *et al.*, (2013). A good substratum for mushrooms growing consists of plant materials, water, manure and or fertilizers. Fasidi *et al.*, (2008) stated that commercial growers prefer the temperature of 9°C to 13°C since mushrooms are firmer and less likely to be diseased or attacked by insects when grown within this range. However, Outside techniques can be used in Mushroom cultivation, although Indoor growing techniques can also be employed. Outside cultivation require low investment, but there is little control over environmental factors. Stability of yield and spreading of production is possible when the mushroom house is air-conditioned but very

expensive to maintain. Outside cultivation is very cheap and reliable, but dependent on natural conditions. Resources of the farmers availability of technology, price of product and costs of growing determine the investment. A number of conservation methods have been developed, differing according to the aimed market and available resources and infrastructure. Canning, air-drying, brining (salting), freeze-drying and freezing are the methods employed in mushroom conservation. The activities of microorganisms are the most important cause of spoilage. To thrive on mushroom cultures, invading pathogens require organic matter, water with certain vapour pressure, certain temperature, pH and sometimes free oxygen Gbolagade, (2005). Almost every method of preservation is therefore, aimed at avoiding or stopping the development of these organisms.

Pesticides are one of the means to reduce the loss in mushroom cultivation to diseases. It is known to be fast and effective by applying coppamata, containing zinc, manganese, copper and iron salts, but the carbamates have largely been superseded by benomyl. These new materials, has proved remarkably effective against *Dactylium* sp., and *Verticillium* sp. PCNB (penta chloronitrobenzene) is sometimes employed as a dust to combat *Dactylium* sp. and *Botrytis* sp. Prompt attention to localized areas can be effective, but generally weekly dustings should not be given until after the first flush, for it is believed it can depress production. Promising results are reported from a week 100 ppm spray immediately after casing, followed after the first flush has been picked by occasional spraying with a much stronger (500 – 1,000 ppm) solution. The use of a 15% hypochlorite powder to control isolated patches of parasitic infection has been developed by Change (1987). His trials against *Dactylium* sp. in particular were very successful; it has been advised against *Sporendonema* sp. A much used fungicide in Japan and Taiwan is Panmush. The active ingredient is 2 (4 Thiazolyl) - benzimidazole. Other names include mertect – 340, pen mush Y101, tecto 45, tecto 60.

Most of the works on the cultivation and control of diseases of mushrooms were from another part of the world. In Nigeria, there is no available literature on control measure of these diseases despite the fact that utilization of mushroom cultivation is increasing rapidly in the country and mushroom farming is becoming more popular due to the economic problem facing Nigerians. Therefore the present study is aimed at evaluating the inhibitory effects of pesticides on the sporulation and growth of pathogenic fungi associated with mushroom culture.

2. Materials and Methods

2.1: Source of Fungi and Identification:

The cultures of *Pleurotus tuber regium* used for this work were obtained from the Mycology and Biotechnology Unit, University of Ibadan, Ibadan, Nigeria.

A 3 day old culture plate of the pathogen isolates were obtained by repeated sub-culturing on Potato dextrose agar (PDA). After sub-culturing for two or three times, morphological and microscopic examinations of the cultures were used to identify the organisms (Kuforiji OO, Fasidi IO 1998). Further confirmation of the identification of the pathogenic fungi was carried out at the Seed Health Unit, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

2.2: Laboratory test:

The pesticides tested on the pathogenic fungi were mainly: Brestan: 60% Triphenyltin acetate, Demonsan (Chloroneb): 65% 1, 4 dichloro – 2, 5-dimethoxyl benzene, Formalin: 40% formaldehyde, Tecto – 60:60%, 2, 4 thiazoly benzimidazole, Copper sulphate: 100% CuSo₄.7H₂O active ingredient, Kocide: 60% active ingredient, Ridomil: 58% phenylamides Acylalanines, Sodium metabisulphite: 1.25gl LH₂O in 2,500 ppm, Sodium chloride: 1.25gl LH₂O in 2,500 ppm. The weight of each pesticides was calculated to give definite concentration in parts per million (ppm) of its active ingredients of pesticides prepared.

The pesticides were added aseptically to the spore suspension on the PDA plates and the mycelia in the liquid medium in the flask at different concentration levels. A PDA plate and liquid medium flask free of pesticides were used as control. All experiments were carried out in triplicate.

2.2.1: Effect of pesticides on spore germination of pathogen isolates

The spore suspensions of each of the isolates obtained from the substrate samples, which had grown on P.D.A. for 7 days was carried out by adding sterile distilled water to the pure culture of the isolates. The cultures were gently rubbed with sterile inoculating needle in the petri-dish for the spores' detachment. A set of Petri dish containing sterilized PDA medium free of pesticides was used as control. The number of spores of the isolates used as inocula was estimated with haemocytometer under the microscope to contain 2 x10⁴ spores per ml. Pesticides were added to the spore suspension at varying concentration levels and was transferred into the incubator at 25±2⁰C for 24 hours. Microscopic examination was carried out on a grease free slide after 24 hours for the formation of germ-tube.

2.2.2: Effect of pesticides on mycelia growth of the isolates in liquid medium

The liquid medium employed consist of $MgSO_4 \cdot 7H_2O$ – 0.5g, KH_2PO_4 – 1.0g, KNO_3 – 3.0g, $K_2HPO_4 \cdot 7H_2O$ – 1.0g, $FeSO_4 \cdot 7H_2O$ – 0.01g, Glucose – 20g/L of solution.

The solution was measured and different concentration levels dispensed into each of the conical flasks and autoclaved at 130°C for 15 minutes. The pesticides used were added aseptically to the sterile liquid medium in the flask. A set of conical flasks containing sterilized liquid medium free of pesticides was used as control. The content of each flask was inoculated with 5mm actively growing mycelia agar disc of 7 days old cultures of the test isolates grown on PDA.

The flasks were incubated at 30°C for 6 days, the mycelia mats were harvested at the end of the 6th day. It was oven dried at 80°C for 24 hours and allowed to cool in the desiccators after which weighing was carried out.

2.2.3: Effect of Pesticides on *Pleurotus tuber-regium*

The liquid medium containing single disc (5mm dia.) of 5 day-old culture of *P. tuber-regium* was cultured and replicated five times in conical flasks according to varying concentration levels. Five pesticides found to be more effective out of the ten pesticides used previously were selected. The culture flasks were incubated at 25±2°C for 5 days. Mycelia mats were harvested and oven dried at 80°C for 24 hours after which weighing.

2.3: Statistical analysis

The experiment was set up in a complete randomized design. Analysis of variance was used to analyze differences between the pesticides effect on the spore production and growth of pathogenic fungi. A general linear model option of the analysis system SPSS version 16.0 was used to perform the ANOVA. Duncan's multiple range test at $P < 0.05$ level was used for means separation (Winer 1971).

Table 1: Effect of pesticides on four fungi isolated

| PESTICIDES | <i>A. niger</i> | <i>A. flavus</i> | <i>T. sp</i> | <i>Mucor sp</i> |
|---|-----------------|------------------|--------------|-----------------|
| CuSO4 | 0.03b | 0.04d | 0.01e | 0.06bc |
| BENLATE | 0.32ab | 0.17e | 0.02e | 0.03f |
| NaCl | 0.48ab | 0.09a | 0.04cd | 0.05cde |
| RIDOMIL | 0.06ab | 0.08ab | 0.063ab | 0.05bcde |
| KOCIDE | 0.067ab | 0.07b | 0.065ab | 0.06bcd |
| NA ₂ S ₂ O ₄ | 0.67ab | 0.40cd | 0.027de | 0.07b |
| FORMALIN | 0.72ab | 0.05c | 0.067b | 0.05def |
| TECTO-DUST | 0.08ab | 0.09a | 0.08a | 0.10a |
| DEMOSAN | 0.92ab | 0.09a | 0.06bc | 0.04ef |
| BRESTAN | 0.11a | 0.48cd | 0.40cd | 0.06bcde |

Means with the same letter in the same column are not significantly different at $P < 0.05$ using Duncan's Multiple Range Test (DMRT)

Table 2: Effect of Concentration on fungi isolates from *Pleurotus tuber-regium*

| Concentration | <i>A. niger</i> | <i>A. flavus</i> | <i>Trichoderma sp</i> | <i>Mucor sp</i> |
|---------------|-----------------|------------------|-----------------------|-----------------|
| 0 | 0.10ab | 0.96a | 0.07a | 0.08a |
| 50 | 0.40b | 0.04c | 0.04b | 0.04b |
| 100 | 0.06ab | 0.060b | 0.04b | 0.06b |
| 150 | 0.04b | 0.04c | 0.04b | 0.05b |
| 200 | 0.11a | 0.06b | 0.05b | 0.05b |
| 250 | 0.05ab | 0.06b | 0.04b | 0.06b |

Means with the same letter in the same column are not significantly different at $P < 0.05$ using Duncan's Multiple Range Test (DMRT)

Table 3: Mean Square of pesticides and concentration on *A. niger*, *A. flavus*, *Trichoderma sp* and *Mucor sp*

| Source of variation | df | <i>A. niger</i> | <i>A. flavus</i> | <i>Trichoderma sp</i> | <i>Mucor sp</i> |
|---------------------|----|-----------------|------------------|-----------------------|-----------------|
| Pesticides | 9 | 0.004ns | 0.004** | 0.003** | 0.002** |
| Concentration | 5 | 0.009* | 0.004** | 0.002** | 0.002** |
| Error | 45 | 0.004 | 0.00 | 0.00 | 0.00 |
| Total | 60 | | | | |
| Corrected Total | 59 | | | | |

*, ** significant at $P < 0.05$ and $P < 0.01$ respectively ns= Non significant

Table 4: Correlation matrix among four fungi isolated from *Pleurotus tuber-regium*

| | <i>A. niger</i> | <i>A. flavus</i> | <i>Trichoderma sp</i> | <i>Mucor sp</i> | Concentration |
|-----------------------|-----------------|------------------|-----------------------|-----------------|---------------|
| Pesticides | -0.38ns | -0.00ns | -0.00ns | 0.14ns | 0.00ns |
| <i>A. Niger</i> | 0.29* | 0.30* | 0.18ns | -0.35ns | |
| <i>A. flavus</i> | 0.66** | 0.49** | -0.23* | | |
| <i>Trichoderma sp</i> | 0.55** | -0.24* | | | |
| <i>Mucor sp</i> | -0.23* | | | | |

*, ** significant at $P < 0.05$ and $P < 0.01$ respectively ns= Non significant

Table 5: Determination of ED₅₀ on spore germination.

| No | Organism/ Pesticides | <i>A. Niger</i> | <i>B. Flavus</i> | <i>Trichoderma sp.</i> | <i>Mucor sp</i> |
|----|---|-----------------|------------------|------------------------|-----------------|
| 1 | CuSO ₄ | 135 | 120 | 140 | 100 |
| 2 | NA ₂ S ₂ O ₄ | 170 | EFF | 155 | 140 |
| 3 | Nacl | 90 | 130 | 130 | 115 |
| 4 | Formalin | 150 | 175 | 105 | 90 |
| 5 | Benlate | 80 | EFF | 85 | 130 |
| 6 | Demosan | 70 | 50 | 90 | 120 |
| 7 | Ridomil | 110 | 100 | 150 | 145 |
| 8 | Tecto-60 | 150 | 115 | 130 | 140 |
| 9 | Brestan | 100 | 150 | EFF | 50 |
| 10 | Kocide | 50 | EFF | 85 | 151 |

Table 6: Determination of ED₅₀ on spore germination.

| 13 | Organism/ pesticides | <i>A. Niger</i> | <i>B. Flavus</i> | <i>Trichoderma sp</i> | <i>Mucor sp.</i> |
|----|---|-----------------|------------------|-----------------------|------------------|
| 1 | CuSO ₄ | VEFF | 100 | EFF | NEFF |
| 2 | NA ₂ S ₂ O ₄ | 290 | 240 | 145 | NEFF |
| 3 | Nacl | 100 | 265 | NEFF | NEFF |
| 4 | Formalin | 155 | 250 | 205 | 150 |
| 5 | Benlate | VEFF | VEFF | 150 | 90 |
| 6 | Demosan | 225 | 170 | 150 | NEFF |
| 7 | Ridomil | 100 | 150 | 150 | 140 |
| 8 | Tecto-60 | NEFF | NEFF | NEFF | NEFF |
| 9 | Brestan | EFF | EFF | EFF | EFF |
| 10 | Kocide | EFF | 100 | 130 | EFF |

EFF=Effective, NEFF=Not Effective, VEFF= Very Effective

3. Results and Discussion

3.1: Effect of pesticides on spore germination of fungal isolates

Result in table 3 shows that the spore germination of *A. niger* was inhibited at 50 and 100 ppm using brestan, kocide, benlate, sodium chloride. Also, at 150 and 200 ppm, there was inhibition of the spores of *A. niger* using ridomil, formalin, copper sulphate, sodium metabisulphite and tecto-60. Germination of *A. flavus* spores was effectively inhibited at 50, 100, 150, 200 and 250 ppm using sodium metabisulphite, benlate and kocide. The spores were inhibited between 100 and 150 ppm using copper sulphate, formalin, brestan, ridomil, sodium chloride and tecto-60.

Spore germination of *Trichoderma sp.* was successfully inhibited using brestan (table 5). At 50 ppm, ridomil inhibited the spore germination of the organism. Spore germination of *Mucor sp.* was inhibited between 50 and 100 ppm using brestan, formalin and copper sulphate. At 150 ppm, it was inhibited by benlate, kocide, demosan, sodium chloride, tecto-60 ridomil and sodium metabisulphite (table 6).

3.2: Effect of pesticides on mycelia growth of fungal isolates

Mycelia growth of *A. niger* was effectively inhibited at 50, 100, 150, 200 and 250 ppm of copper sulphate, benlate, brestan and kocide; the organism was inhibited at 100 and 150 ppm using sodium chloride, formalin and ridomil. There was no inhibition at these concentrations by sodium metabisulphite, demosan and tecto-60, while the mycelia growth of *A. flavus* was effectively inhibited at 50, 100, 150, 200 and 250 ppm of benlate and brestan only, also at 100 ppm, kocide and copper sulphate inhibited the mycelia growth of *A. flavus*. Similarly, 150 and 250 ppm of sodium metabisulphite, formalin and demosan inhibited the mycelia growth of the organism. However, inhibition of *A. flavus* by sodium chloride and tecto-60 was not observed.

The effect of pesticides on four fungi isolated from *P. tuber-regium* is shown in table 1, the effect of CuSO₄ on *A. niger* is significantly different from benlate, nacl, ridomil, kocide, NA₂S₂O₄, formalin, tecto-dust and demosan while brestan shows the

highest significant effect on *A. niger* and significantly different from CuSO₄, NaCl, tecto-dust and demosan produced high significant effect on *A. flavus* while the effects were non-significantly different from each other. Na₂S₂O₄ and brestan were non-significantly different from each other, the effect of formalin is significantly different from kocide and CuSO₄, the effect of formalin is significantly different from kocide and CuSO₄. The tecto-dust effect on *Trichoderma* sp is significantly different from formalin on *Trichoderma* sp, there is no significant difference between Ridomil and kocide, also NaCl and brestan are non significantly different from each other but different from Na₂S₂O₄, CuSO₄ and benlate effect are non significantly different from each other. The effect of tecto-dust is higher on *Mucor* sp and significantly different from all other pesticides used. Ridomil and brestan effect are not significantly different from each other but significantly different from benlate. Table 2 shows the effect of concentration of the pesticides on the fungi isolated from *Pleurotus tuber-regium*, there is no significant difference between 0,100 and 250 concentration on *A. niger* but the effect of 50 and 150 concentration are non significantly different from each other while there is a high significant effect on *A. niger* at the concentration of 200. The effect of the concentration at 100,200,250 is non-significantly different for *A. flavus* but significant different from 0 concentration while there is no significant difference between the concentration at 50 and 150 on the *A. flavus* The effect of the concentration at 50,100,150,200 and 250 are non significantly different on *Trichoderma* sp but significantly different from concentration at 0, also the same effect was recorded for *Mucor* sp. The result from table 3 shows the mean square of pesticides and concentration on the four fungi isolates from *P. tuber-regium*. The effect of pesticides is highly ($p < 0.01$) significant with *A. flavus*, *Trichoderma* sp and *Mucor* sp but non significant with *A. niger*. The effect of concentration on the *A. niger* is significant ($p < 0.05$) but highly significant for *A. flavus*, *Trichoderma* sp and *Mucor* sp. Table 4 shows the correlation matrix among the fungi isolated from *P. tuber-regium*, The pesticides is negatively correlated and non significant with *A. niger*, *A. flavus* and *Trichoderma* sp but positive and non-significantly correlated with *Mucor* sp and concentration. *A. niger* shows significant relation to *A. flavus* and *Trichoderma* sp but non significantly correlate with *Mucor* sp and negatively related to concentration. *A. flavus* is positive and highly correlated with *Trichoderma* sp and *Mucor* sp but negatively correlated but significant with concentration. *Trichoderma* sp is positive highly correlated with *Mucor* sp but negatively correlated

with concentration while *Mucor* sp is also negatively correlated but significant with concentration. However, the pathogenic fungi isolated included; *A. niger*, *A. flavus*, *Trichoderma* sp., *Rhizopus* sp. This work is in accordance with Hollingsworth (2012) who reported that mushroom green moulds were caused by *Aspergillus* sp., *Penicillium* sp., *Spicaria* sp. and *Cladosporium* sp. constitutes a serious damage in mushroom cultivation on the field. Fasidi *et al.*, (2008) reported that *Cladobotryum ladobotryum* and *Cladobotryum dendroides* affected *Pleurotus* sp. and *Agaricus* sp. has caused cobweb mould parasites on mycelium. It grows rapidly on the surface of the substrate, causing the mushrooms spoilage. *Gliocladium* sp. affected *Pleurotus* sp. and causes discoloration and rotting of fruiting body. *Penicillium* sp. affected *Pleurotus* on pasteurized substrate, all mushroom growth in sterilized plastic bags causes competition for nutrient, enters during spawning under unhygienic condition or is still present in the substrate. *Paecilomyces variotii* affected all wood inhibiting mushroom growth.

The organisms isolated in this study have been reported as soft rot pathogens on other crops by various workers. *A. niger* has been reported to cause soft rot of yam tubers and while *Rhizopus* sp causes soft rot of green pepper and pear (Amusa, 2003, Zhao 2012). *A. flavus* is a phyto-pathogenic fungus associated with internal mouldiness of cocoa beans and other economically important crops in Nigeria (Fagbohun, 2011). This work agrees with the observation made by Ogiehor (2010) who reported that *A. niger*, *T. harziarium* and *A. flavus* contamination can usually be recognized by the typical colours of their mycelium. At times, a distinctive zone can be recognized between inoculated mushroom mycelium, and contamination very common is *Penicillium* sp. and *Aspergillus* sp.

From this study, it is advised that care be taken during cultivation and spawning of mushroom, not to allow the spores of the fungal isolates be dispersed all over the field.

This work also confirms the observation made by Andras Geosel (2011). He stated that the most commonly known means of controlling fungi diseases on the field and green house is through the use of chemical compounds that are toxic to the pathogen. He also mentioned that chemical either inhibit germination, mycelia growth and multiplication of the pathogen or an outright lethal to the pathogen. The general morphological effects on mycelia after application of these chemicals include mitochondrial swelling, distortion and disintegration.

Formalin inhibited the mycelia growth of *P. tuber-regium* but favoured the growth of weed fungi on the substrate. Therefore, the growth of the

mushroom was hindered and could not produce fruit bodies. This is similar to the result obtained (Shakma and Suman 2006) *Sclerotium rolfsii* can be completely controlled at 800 ppm formaldehyde and 100 ppm liquor NH₂ in vitro, but these concentrations did not favour the growth of the mushroom

For a successful control of fungal contaminants by chemicals during the mushroom cultivation, it is necessary that the chemicals should not be toxic to the mushroom growth, hence the pesticides used in this study were considered safe regarding fruit injury and human consumption and the bio-assay has revealed that the pesticides were effective against the fungi. Also, (Fletcher JT and Gaze RH 2008) have suggested the use of dithiocarbamate fungicides for the selective control of *Verticillium* sp., *Dactylium* sp; *Mycogone* sp; *Trichoderma* sp. and *Penicillium* sp. during mushroom cultivation. Also, Fasidi *et al.*, (2005) reported that dry and wet bubbles can be controlled by *carbendazim*, *benomyl*, *chlorothanil*, *prochloraz* and *thiophanate-methyl*. Fungi competing for nutrient in the substrate can be controlled by panmush, benomyl (benlate) or thiophanate methyl, vinclozolin. Fungi affecting the fruiting bodies can be controlled by *panmush*, *benomyl*, *chlorothanil*, *prochloraz* and *thiophanate-methyl*. Fungi competing for nutrients in the substrate can be controlled by *panmush*, benomyl (benlate), or *thiophanate methyl*, *vinclozolin* using 20 ppm to 50 ppm during or after moistening the substrate. Fungi affecting the fruiting bodies may be controlled by spraying a mist of pulverized fungicides (*benomyl*, *prochloraz*, *prodione*). A much used fungicide in Japan and Taiwan is panmush. The active ingredient is 2 – (4-thiazolyl) – benzimidazole. Other names include: Mertect – 340, Penmush Y 101, Tecto – 60. Dosage per bag of 1kg of sawdust is 0.2g if a 60% w/w active ingredient is used. The fungicide should be mixed with small amount of the substrate.

4.0: Conclusion

From the findings of this study, the pesticides inhibited the germination of *A. niger*, *A. flavus*, *Trichoderma* sp. and *Rhizopus* sp. between 50 ppm and 150 ppm. At 200 ppm, there was inhibition of the spores of *A. niger* using ridomil, formalin, copper sulphate, tecto-60 and sodium metabisulphite and also there was inhibition of the spores of *A. flavus* using benlate, kocide and sodium metabisulphite respectively.

Mycelia growth of the isolated fungi were highly inhibited between 50 ppm and 150 ppm using copper sulphate, benlate, brestan, kocide, ridomil and formalin. The mycelia of *A. flavus*, *Trichoderma* sp. and *Rhizopus* sp were not inhibited at these concentrations using sodium chloride and tecto-60.

Also, there was no inhibition of mycelia of *A. niger* at these concentrations using sodium metabisulphite, demosan and tecto-60. Hence, the effect of benlate, bestan, kocide, formalin and copper sulphate was determined on mycelia growth of *P. tuber-regium* between 50 ppm and 250 ppm as stated above.

Brestan, kocide and copper sulphate are toxic to the mushroom, hence, inhibited the mycelia growth of *P. tuber-regium*. It was determined that benlate and formalin favour the growth of *P. tuber-regium* at 50 ppm and 100 ppm effectively.

From these results and present study, benlate can be regarded as a broad spectrum pesticide in cultivation of mushroom. However, benlate at 50 ppm and 100 ppm exerted greater growth inhibitory effect on the weed fungi but favoured the growth of *P. tuber-regium* in the laboratory and on the substrate in the field. Hence, it could be recommended that benlate when applied either at 50 ppm or 100 ppm inhibited the mycelia growth of the pathogens without affecting the mycelia growth of the mushroom.

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