Research Article

Aphidicidal Potential of Ethyl Acetate Extract from *Pleurotus ostreatus*

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Abstract | This study was conducted to evaluate the insecticidal activity of the ethyl acetate extracts from fruit bodies, mycelia and fermentation filtrate obtained from *Pleurotus ostreatus* also known as the oyster mushroom. The percent mortality of *Macrosiphum rosae* (rose aphids) and the LC_{50} values were calculated and used as an indicative of insecticidal potential. The maximum mortality of aphids was achieved at 80 µg mL^{-1} and the LC_{50} for the fruit body extract after 24 HRS was calculated as 12.83µg mL^{-1} followed by the extract from the fermentation filtrate with LC_{50} value 25.03 µg mL^{-1}. The mycelial extract with LC_{50} (29.96 µg mL^{-1}) exhibited least insecticidal activity. The results obtained from this study revealed that these extracts possess metabolites with insecticidal attributes that could be used as a potential source for developing new and novel bio-pesticide(s).

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Introduction

Mushrooms are macro-fungi with a distinctive structure of fruit bodies. These can be seen with naked eyes and can be epigeous or hypogeous (Chang and Miles, 1992). *Pleurotus* species are spread around the world in forest environments (Bononi et al., 1999). *Pleurotus ostreatus*, is commonly known as the oyster mushroom. Although it is cultivated commercially for edible purpose, it also possesses medicinal properties (Jedinak et al., 2011) and can be utilized as therapeutic diet. It has been reported for antimicrobial (Wolff et al., 2008), anti-cancer (Jose et al., 2000) and antioxidant (Vamanu, 2012) activities. It has known nematicidal properties (Palizi et al., 2009), which make them worthy to screen for bioactive metabolites of agricultural importance. Certain fungi are used against the pathogenic insects which suggest that fungi contain toxic compounds that are active against noxious insects. The use of spores from the *Lycoperdon* to anesthetize bees is a traditional practice similarly *Amanita muscaria* can kill the houseflies when mixed with sugar solution and the powder of *Trametes odorata* keeps the cloths safe from insects (Riahi et al., 2009). Norman et al., (1996) worked on numerous fungal species against *Drosophila melanogaster* and *Spodoptera littarail* and showed that nearly half of 175 tested fungi possessed the toxicity against these insects. Similarly the mycelial extract of *Aspergillus flavus* is reported to possess the mosquito larvicidal activity with 34.34 µg mL^{-1} LC_{50} value (Govindarajan et al., 2005). Keeping in view the importance of microbes in medicine and agriculture, the present study was designed to assess the organic extract of edible fungi i.e. *P. ostreatus* extract for its insecticidal activity.

Materials and Methods

The reported procedure of Isman et al. (1987) with a few modifications was followed to evaluate the insecticidal activity. The fruiting bodies and mycelium of *P. ostreatus* were obtained from the department of Plant Pathology, The University of Agricultural Peshawar. The fruit body or the edible portion (1500 g) was carefully picked from the growth media i.e. wheat straw (Figure 1; a and b), whereas the mycelia (roots) were picked from the inside of growth media (Figure 1; c, 1000 g). Distilled water (100 mL each) was added to both samples and crushed separately to obtain the slurry. Ethyl acetate (500 mL) was added to the mixture and left for stirring on magnetic stirrer for 24 hours. The solid particles were removed from liquid portion by filtration and from liquid portion the organic and aqueous phase was separated. The organic portion was dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure in rotary evaporator to obtain the dense brown oil as a crude extract.

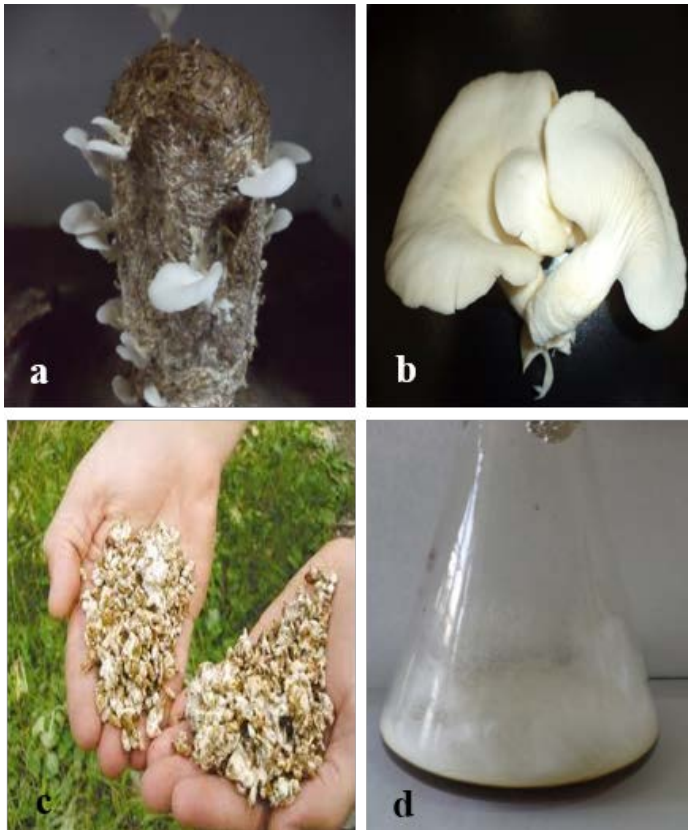

Figure 1: (a) *Growth of P. ostreatus on wheat straw,* **(b)** *Edible Fruit bodies of P. ostreatus,* **(c)** *Mycelia of P. ostreatus,* **(d)** *Fermentation filtrate of P. ostreatus*

Pure culture of *P. ostreatus* in Petri Plates was obtained by inoculating the Potato Dextrose Agar (PDA) media with a slice of the *P. ostreatus* in laminar flow unit (LFU) and kept in an incubator for 12 days at 25 °C. The fermentation filtrate (Figure 1; d) was then obtained by inoculating the Potato Dextrose Broth (PDB) media using a slant of pure PDA culture of *P. ostreatus*. The PDB was then cultivated in an incubator at 30°C for 12 days and the biomass obtained was crushed in electric grinder to obtain the slurry. Ethyl acetate (500 mL) was added to the slurry and the mixture was stirred overnight on magnetic stirrer. The extract was filtered and distilled water (250 mL) was added to it this was further stirred for 1 hour. Both organic and aqueous layers were separated and the organic phase was dried over anhydrous $MgSO_4$, filtered and evaporated *in vacou* to obtain the light brown crude oil.

Aphidicidal assay

The Aphids mortality was determined using a modified procedure of Isman et al. (1987). The stock solution (1000 µg mL^{-1}) was made by taking 1 mg sample from each extract in 10 mL of DMSO. The stock solution was further diluted to 20, 40, 60 and 80 µg mL^{-1} concentration. Each concentration was then adsorbed on to a filter paper; the filter paper was dried and was placed in a petri dish. To each plate thirty aphids were transferred along with their natural feed. The mortality count was done at the interval of 4, 12 and 24 hours. Percent mortality was calculated using equation 1 and the LC_{50} was measured using probit analysis (Finney and Stevens, 1948).

$$\%Mortality = (Nt - Nb) / Ni \ldots\ldots (1)$$

Where
N_t= Aphids killed by test solutions
N_b= Aphids killed in blank solution
N_i= Total number of aphids

Results and Discussion

Extraction of organic extract

All the samples were extracted with EtOAc and oily crude extract was obtained (Table 1). The fruit bodies (1 kg) produced 3.1 g (0.31%) of crude extract as dark brown oil. The mycelial extract 2.5 g Kg^{-1} (0.32%) was obtained as brown oil. The fermentation filtrate provided 2.4 g L^{-1} extract as clear brown oil. It is observed that the organic extract obtained from fruit bodies, mycelia and fermentation filtrate were comparable.

Aphidicidal assay

The ethyl acetate extract from all three samples of *Pleurotus ostreatus* were checked for aphidicidal potential. The petridishes containing filter paper pre-adsorbed

with different concentrations were prepared and the test insects were transferred to it. The dead aphids were counted after 4, 12 and 24 hours and the mortality was noted in terms of death of insects compared with positive control (Permithrine) and negative control (DMSO only). In case of positive control all the test insects were killed within 4 hours of application while in case of negative control no lethal effect was observed.

Table 1: *Details of extract obtained from different samples of P. ostreatus*

S. no	Parts used	Organic extract	Yield %
1	Fruit bodies	3.1 g Kg^{-1}	0.31%
2	Mycelium	2.5 g Kg^{-1}	0.25%
3	Fermentation filtrate	2.4 g L^{-1}	0.24%

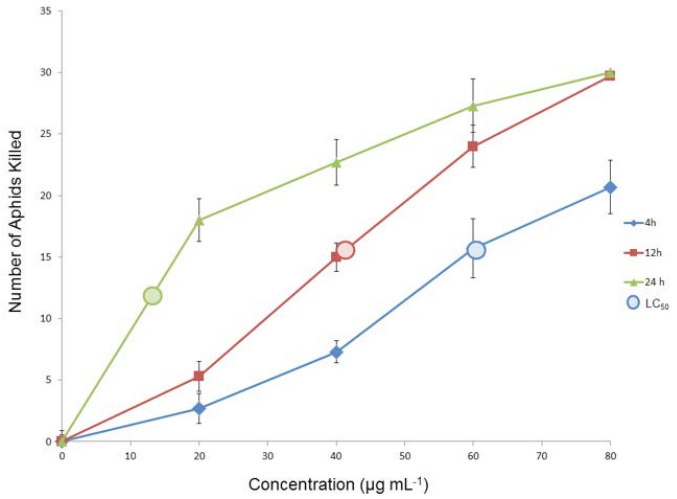

Figure 2: *Graph showing the effect of fruit bodies extract of P. ostreatus on aphids mortality at different interval of time along with LC_{50}*

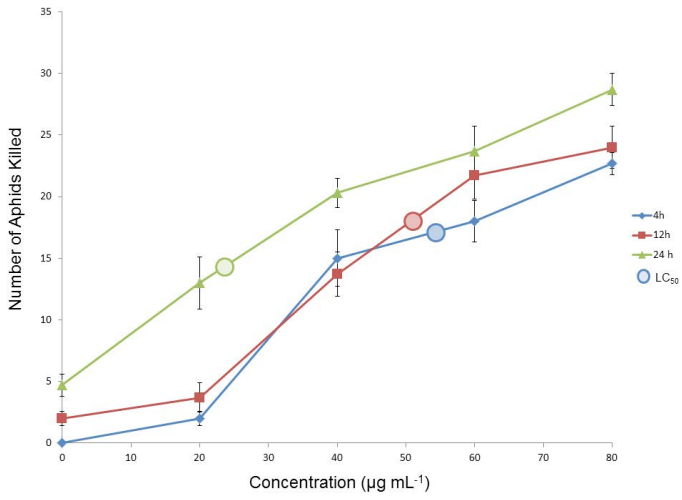

Figure 3: *Graph showing the effect of mycelial extract of P. ostreatus on aphids mortality at different interval of time along with LC_{50}*

Figure 2 demonstrates the killing of aphids by the fruit bodies extract of *P. ostreatus* at different time interval where after 4 hours, at 80 µg mL^{-1} concentration the mortality of 21 (70%) aphids was observed while all the test population was killed after 12 hours of application. The percent mortality was calculated and found 93%. The LC_{50} was calculated by probit analysis, for the fruit bodies of *Pleurotus ostreatus* it was found 12.80 µg mL^{-1} after 4 hrs, while after 12 h it was calculated as 12.80 µg mL^{-1}. The LC_{50} value increased to 61.95 µg mL^{-1} after 24 hours. Figure 3 illustrates the toxic effect of mycelial extract on insects. The mortality of insects at maximum concentration (80 µgmL^{-1}) was calculated as 80% after 24 hours whereas after 4 hours it was 77 %, interestingly the mortality rate slowed down to 73% after 12 hrs. For mycelial extract of *Pleurotus ostreatus* LC_{50} value after 4 hrs was 25.03 µg mL^{-1} while it increases with the passage of time to 49.03µg mL^{-1} after 12 hours and 53.48 µg mL^{-1} after 24 hours.

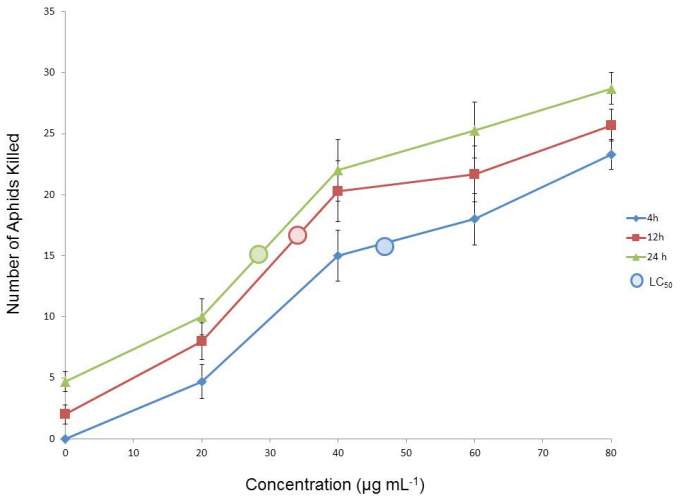

Figure 4: *Graph showing the effect of fermentation filtrate extract of P. ostreatus on aphids mortality at different interval of time along with LC_{50}*

Figure 4 illustrates the mortality of aphids by the extract obtained from fermentation filtrate of *P. ostreatus* at different intervals of time. After 4 hours of application of 20µg mL^{-1} difference in percent mortality was non-significant while significant difference in percent mortality was calculated by 40 µg mL^{-1} between 4 and 12 hours. The graph also highlight the mortality increase by increasing concentration i.e. 80 µg mL^{-1} the increased mortality of aphids was observed where twenty three insects were found dead that increased to 27 after 12 hours and 29 hours after 24 hours. The fermentation filtrate extract of *P. ostreatus* showed concentration dependent curve, i.e. after 4 hours at a

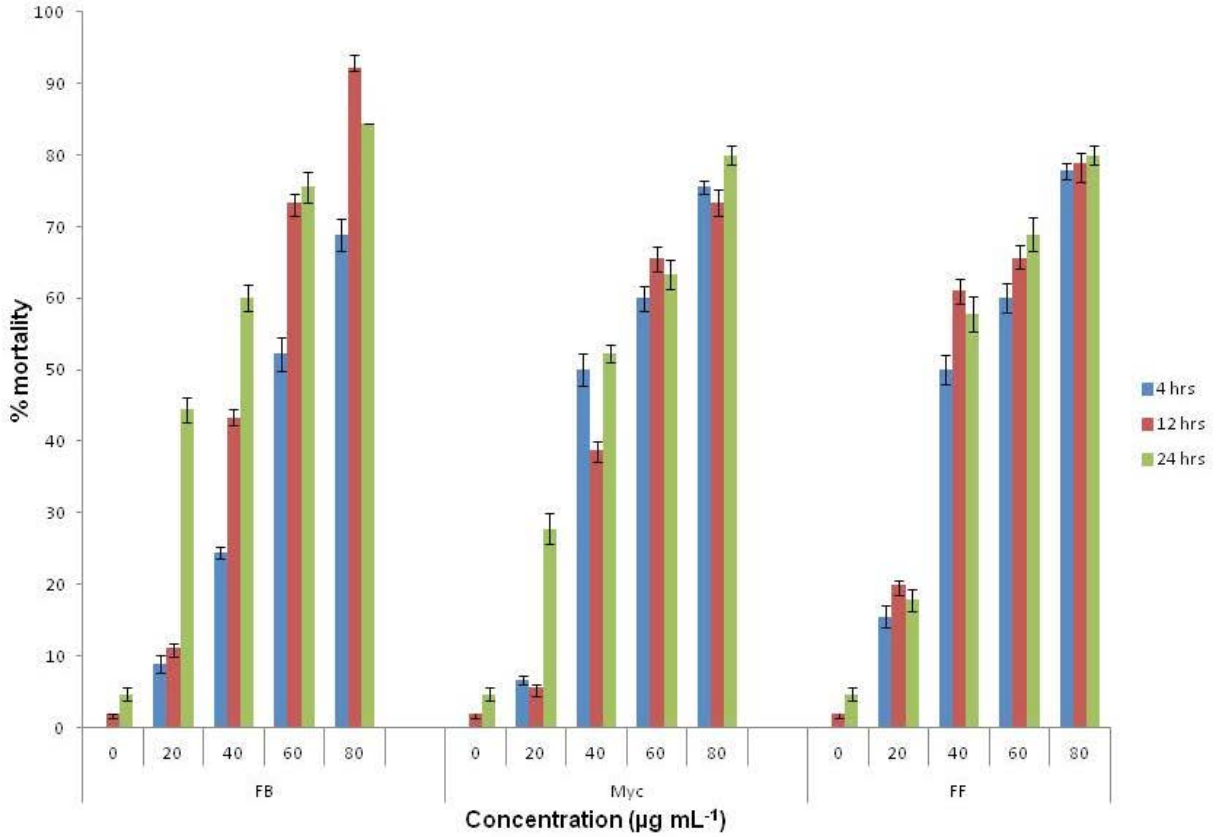

Figure 5: *Graph showing % mortality with standard error bar, DMSO + H_2O was used as a negative control that gave 0% aphids mortality. For positive control experiment Permethrin (Copex) at 20 µg mL^{-1} was used that showed 100% mortality, FB= fruit bodies, Myc= mycelial extract, FF= fermentation filtrate*

concentration of 80 µg mL^{-1} the LC_{50} was 49.13 µg mL^{-1} while it increases to 36.46 and 29.13 µg mL^{-1} after 12 and 24 hours respectively. It is reported that crude extracts having LC_{50} less than 250 µg mL^{-1} are potentially active (Rieser et al., 1996).

Overall the extract obtained from fruit bodies was found more potent even after 12 hours then mycelial and fermentation filtrate extract (Figure 5). The results also shows comparable mortality of aphids caused by mycelial and fermentation filtrate after 24 hours.

The study carried out by Dowd (1988) on the caterpillars suggested that fungal extracts such as kojic acid and fusaric acid possessed toxic effects on *Hliothis zea* and *Spodoptera frugiperda* as that of aflatoxin B1. They also reported that pyrethrin toxicity was synergized by kojic acid in both insects.

Conclusions

The results obtained from this study can be considered a step towards in the development of more potent insecticide against sap sucking aphids. The findings from this study shows that extracts obtained from different parts of *Pleurotus ostreatus* possess significant insecticidal (by killing Aphids) potential. It is concluded that the number of insects killed were time and concentration dependent while present mortality was only concentration dependent.

Acknowledgments

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