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Role of Halonil in Reducing Contamination in Culture Media

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Abstract
An experiment was conducted to determine the role of halonil (chlorothalonil) on reduction of contamination on artificial culture media. Potato dextrose agar plates were amended with various concentrations of halonil which included 0.5%, 1% and 1.5% g/lit. Plates were initially placed on lab benches with lids removed and assessed for fungal growth. Individual spores of *Alternaria alternate* was picked with a pastier pipe and then placed in the center of PDA plates variously amended with halonil. Like wise, mycelial plugs excised from the periphery of an actively growth colony of the test fungus were also placed on PDA plates and checked for colony growth. No growth was observed when spores acted as inoculum growth however of observed when mycelial plugs were used as inoculum. Halonil can therefore be used affectively in checking lab contamination without the use of laminar flow unit.
Keywords: Halonil, Culture media

INTRODUCTION

Fungi are small, microscopic (except: mushrooms) eukaryotic, filamentous, non vascular, spore bearing branched organisms with no chlorophyll. Fungal cell walls contain chitin and glucans (the wall strengthening) but no cellulose as the skeletal components. These are embedded in the matrix of polysaccharide and glycoprotein. Some fungi known as obligate parasites or biotrophs, can grow and multiply only on or in their living host while others known as non obligate parasites require a living host plant for part of their life cycles.

Fungi cause a number of plant and animal diseases. In human's ringworm, athletes foot, and several other serious diseases are caused by fungi. Plant diseases caused by fungi include rusts, smut and leaf, root and stem rot, and may cause severe damage to crops. However a number of fungi, in particular the yeast, are important model organisms for studying problems in genetic and molecular biology (Tweeddell et *al.* 2002).

There are many methods of controlling fungi. Proper sanitation is necessary in controlling a fungal disease. Several fungal pathogens's overwinter or over summer on remnants of previous year's crop in the soil and these serve as a source of primary inoculums. These must be properly disposed (Qureshi, 2003). Crop rotation with non host is also important. Many weeds particularly biennial and perennial ones are potential sources of infection and harbor the pathogens which later infect the cultivated crops seeded in the same soil. Elimination of these wild and alternate hosts is a very effective measure of disease control. Normally the seeds, tubers, rhizomes bulbs etc. carry heavy loads of disease causing organisms or other spores (Vashishta et al., 2007). Under favorable conditions, these cause disease. In such cases the disinfection of these infected plant organs by using sterilizing agents before these are sown is quite effective. Many chemicals are also used to control fungal diseases e.g. halonil , Bordeaux mixture etc (Rodriguez *et al.* 1975 and Acuff 1988).

Contamination either in one way or the other in our labs foods and daily usage equipments is a nuisance. There are some of the basic reasons for fungal contamination. For example fungi may be in abundance in the environment where there is no proper ventilation for the exchange of air (Schipper, 1984). The working areas i.e. labs are not in a condition which stops the multiplication of fungi. In laboratories, generally a laminar flow unit is used to keep away contamination and must be in proper working condition. If there is no proper aeration and the equipments used inside the laminar flow unit are not well sterilized then these factors activate the growth of fungi and hence cause contamination (Agrios, 2000). Plant material and soil sometimes brought into the isolation chamber may also be a frequent source of contamination. Efforts should be made to keep them out of the area specifically used for isolation purpose. The present study focused on use halonil at various concentrations to control contamination of media in the lab.

MATERIALS AND METHODS

Potato dextrose agar (PDA) was used as a basal medium. PDA was prepared by boiling pieces of 200g of potato for 15minutes and then decanting the extract through a muslin cloth. To the decanted extract 20g each of dextrose and agar was added and the final volume was adjusted to 1liter (Beever and Bollard 1970). The medium was then autoclaved for 30 min at 121^0C. In order to determine the efficacy of halonil in reducing contamination, various concentrations of halonil including 0.5%, 1%, 1.5% were prepare and added to the PDA medium following autoclaving by vigorous shaking and allowed them to cool at 45^0C. PDA plates not amended with

halonil served as control. Halonil containing medium was then poured into Petri dishes and stored at 4^0C for subsequent use.

EXPERIMENT 1:
In order to check the growth of the contaminants, plates with and without halonil were placed with the lids removed on the lab benches for 72 hours. Plates were then assessed for any fungal/bacterial growth, under a microscope at 40 xs and number of colonies was enumerated. The treatments consisted of halonil amended media at various concentrations whereas plates without halonil served as control. Treatments were arranged in a completely randomized design with four replications.

EXPERIMENT 2:
The fungus used for this experiment was *Alternaria alternata* which was obtained from the culture bank of the Department of Plant Pathology, The University of Agriculture, Peshawar Khyber Pakhtunkhwa. The cultures were renewed by sub culturing on PDA under aseptic condition. Plates were sealed with Para film and incubated at 25^0C for one week. The culture thus obtained was used for subsequent studies.
The cultures were flooded with 10ml of SDW and scrapped with a rubber spatula to make a suspension which was then decanted through a muslin cloth to remove mycelial fragments. Using a hose pipe attached to the end of a fine Pasteur pipette, spores were individually picked and placed on media in each treatment. Plates were sealed and incubated at 25^0C for one week. Following incubation plates were examined for fungal growth. The experiment was arranged as completely randomized design with four replicates

EXPERIMENT 3:
A 5 mm mycelial plug was excised from the periphery of two day old cultures of *Alternaria alternata* and placed in the center of the plates containing PDA with and without halonil. Plates were sealed and incubated as described previously. Data were recorded after one week of incubation.

STATISTICAL ANALYSIS
Data were analyzed using an F test and analysis of variance with various concentration of halonil as independent variable. Treatments showing significant variations were then separated by least significant difference test.

RESULTS AND DISCUSSION
Table 1 show that the results were significance at 5% level of profitability. Maximum number of colonies 22.5 of *Alternaria alternate* were observed on PDA along (control) while that amended with various concentrations of halonil did not show any colony growth when plates were kept in open air. This effectively show that contamination which chiefly results from growth of fungal spores in the environment can be effecting checked by various doses of halonil. This is chiefly due to the fact that chlorothalonils do not allow fungal growth incited by growth of spores.

Statistically significantly results were obtained when effect of halonil was analyzed radical growth of *Alternaria alternate* by using conidia as inoculum. Radical growth (2.62 cm) was observed only on the PDA media while date amended with various concentration of halonil did not show only growth as showing in table 2. It appears that fungal growth started by using conidia on PDA along progressed to a full colony while those amended by various concentration of halonil effectively checked fungal growth.

There were significantly differences among the treatments at 5% level of probability ($P \leq 0.05$) on radical growth when mycelial plugs were used as source of primary inoculum. The maximum radial growth of the fungus (9 cm) was recorded on PDA not amended with halonil. This was followed by PDA supplemented with 0.5 g/lit of halonil (5) which were not statistically significantly from 1.0 and 1.5 g/lit. The lowest radial growth (4.42 cm) was recorded on PDA +1.5g/lit of halonil as shown in table 3.

There were significantly differences among the treatment at 5% level of probability ($P \leq 0.05$) on number of spores' ml^{-1} when mycelium was used as primary source of inoculum. The maximum number of spores of the fungus 17.31 $x10^4$ ml was recorded on PDA not amended with halonil. This was followed by PDA supplemented with 0.5g/lit of halonil ($10.5x10^4$) which were not statistically significant that 1.0 g/lit & 1.5 g/lit. The least number of spores ($7.81x10^4$) were recorded on PDA + 1.5 g/lit of halonil as shown in table 4.

There were significant differences among the treatments at 5% level of probability ($P \leq 0.05$) with respect to biomass of the fungus. The maximum biomass of the fungus of (13.15 g) was recorded on PDA not amended with halonil. This was followed by PDA supplement with 0.5 g/lit of halonil (4.19mg) which were not statistically significant than 1.0 g/lit and 1.5 g/lit/. The lowest biomass of 3.9 mg was recorded on PDA + 1.5 g/lit of halonil as show in table 5.

Table 1. Number of colonies of various contaminations on PDA media with and without halonil

Concentration	Number of Colonies				
	R1	R2	R3	R4	Mean
PDA	20	30	25	15	22.5a
0.5%	0	0	0	0	0b
1.0%	0	0	0	0	0b
1.5 %	0	0	0	0	0b

LSD Value: 4.973
Mean followed by same letters are non-significant different from each other at 5% level of probability.

Table 2. Radical growth (cm) of *Alternaria alternata* placed on PDA at various concentration of halonil with conidia as the source of inoculum

Concentration	Radical Growth (cm)				
	R1	R2	R3	R4	Mean
PDA	3.5	2.5	2.4	2.1	2.62a
0.5%	0	0	0	0	0b
1.0%	0	0	0	0	0b
1.5 %	0	0	0	0	0b

LSD Value: 0.3547
Mean followed by same letters are non-significant different from each other at 5% level of probability.

Table 3. Radical growth (cm) of A*lternaria alternata* on PDA amended with various concentration of halonil with mycelium as primary source of inoculum

Concentration	Radical Growth (cm)				
	R1	R2	R3	R4	Mean
PDA	9	9	9	9	9a
0.5%	4.35	4.4	5.65	5.6	5b
1.0%	4.45	5	5.25	5.5	5.05b
1.5 %	3.9	4.65	4.15	5	4.42b

LSD Value: 0.7579
Mean followed by same letters are non-significant different from each other at 5% level of probability.

Table 4. Number of spores of *Alternaria alternata* initiated by mycelial plug on media amended with various concentration of halonil

Concentration	Number of Conidia/ml ($x10^4$)				
	R1	R2	R3	R4	Mean
Control	13.75	22	23.25	10.25	$17.31x10^4$a
0.5g.litre	10.75	11	10	10.25	$10.5x10^4$b
1.0g/litre	11	7.75	7.5	9.75	$9.0x10^4$b
1.0g/litre	9.25	7.5	5.75	8.75	$7.8x10^4$b

LSD value 5.188
Mean followed by same letters are non-significant different from each other at 5% level of probability.

Table 5. Biomass of *Alternaria alternata* obtained on various concentration of halonil with mycelium as the primary source of inoculums

Concentration	Biomass (mg)				
	R1	R2	R3	R4	Mean
Control	13.92	17.97	11.44	10.73	13.51a
0.5g.litre	3.12	5.40	5.19	03.08	4.19b
1.0g/litre	5.82	3.04	4.30	03.56	4.18b
1.0g/litre	5.30	3.00	4.20	3.31	3.9b

LSD value 4.625
Mean followed by same letters are non-significant different from each other at 5% level of probability.

CONCLUSIONS

- Halonil has been reported not to allow fungal growth initiated by spores
- Thus halonil use can be effective in controlling fungal contamination without the use of laminar flow

unit.

- Halonil at 0.5% can used to check lab contamination

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