Effect of Antibiotics on the Growth of Entomopathogenic and Antagonistic Fungi

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Abstract
The effect of antibiotics, Chloramphenicol, Tetracycline, Streptomycin, and Bacteriomycin on the growth of fungus was studied. Bacteriomycin inhibits the growth of *Verticillium lecanii* but accelerate the growth of *Trichoderma viride.* The presence of tetracycline and chloramphenicol does not inhibit or influence the growth of *Verticillium lecanii.* The presence of antibiotics influences the growth of *T.viride* at optimum concentration and inhibits the growth at high concentration. Presence of chloramphenicol in *Trichoderma harzianum* produces more growth compared with other antibiotics. Above the optimum concentration, the growth depletes. Hence chloramphenicol can be used at minimum concentration of less than 20 ppm to get the maximum yield of *T.harzianum*. Streptomycin inhibits the growth of *V.lecanii* and accelerates the growth of *T.viride* and *T.harzianum.*

Introduction:
The yield of agricultural products depends on the destructive activities of numerous pests like fungi, weeds and insects from time immemorial and it requires the use of pesticides and fertilizers. Violative pesticide residues raise food safety concerns among domestic consumers and pose trade impediments for export crops (Gupta *et al.,* 2010). Chemical pesticides causes serious threats like genetic variations in plant populations, reduction of beneficial species, damage to the environment or water bodies, poisoning of food and health problems such as cancer which makes biopesticides to represent the need (Waage, J. K., *et al*., 1988). The environmental concerns with the dependence on chemical pesticides necessitated for alternatives (Vincent *et al., 2003*). Pesticides based on pathogenic microorganisms specific to a target pest offer an ecologically sound and effective solution to pest problems and meet the demands of biopesticides in comparison with the beshram, neem, garlic, triphala, pinus kesia (Lianfang *et al., 1998).* These ecofriendly alternatives include the use of biofungicides (Trichoderma), bioherbicides (Phytopthora) and bioinsecticides (Bacillus thuringiensis). The usage of biopesticides reduces risk of exposure to chemicals, reduces water pollution through fertilizer runoff, reduces number of applications, causes less harm to beneficial pests, biodegradable and provides better nutritional quality (Vincent *et al., 2003*).

Verticillium lecanii is a cosmopolitan entomopathogenic fungus, the conidia (spores) of which are slimy and attach to the cuticle of insects. The fungus infects insects by producing hyphae from germinating spores that penetrate the insect's integument; the fungus then destroys the internal contents and the insect dies (Ray Cloyd). T*richoderma viride* is an antagonistic fungus used for seed and soil treatment for suppression of various diseases caused by fungal pathogens (Dennis *et al., 1971*). The mycelium of *Trichoderma* produces a wide variety of enzymes, including cellulases and chitinases. Because of its chitinases,

Trichoderma acts as a parasite to other fungi. *Trichoderma harzianum* is also an antagonistic fungus that occurs widely in nature in soil substrate and is being commercialized owing to its ability to compete with phytopathogenic fungi and produce toxins *(*Wirth *et al., 1992*).

The fungus is grown in open space during large scale production in the industries. To avoid contamination by bacteria, the medium is formulated with the antibiotics (Butt *et al., 2001)*. Chloramphenicol is a bacteriostatic that inhibits peptidyl transferase activity of the bacterial ribosome, binding to A2451 and A2452 residues in the 23S rRNA of the 50S ribosomal subunit, preventing peptide bond formation. Tetracycline is a polyketide antibiotic that inhibits the prokaryotic 30S ribosome, by binding aminoacyl-tRNA. Streptomycin binds to the 16S rRNA of the bacterial ribosome, interfering with the binding of formyl-methionyl-tRNA to the 30S subunit Streptomycin. A broad-spectrum Immunomodulator cum plant defence activator based on *Dibromo dinitro propane 1-3-diol*, with prophylactic, preventive and curative properties in controlling the bacterial diseases among crop (Stretton, R. J, *et al., 1973*). Bacterimycin exhibits a unique mode of action, which mimics the natural systemic activated resistance (SAR) response found in most plant species (Mallikarjunaswamy *et al., 2016)*.

The present research focuses on the effect of antibiotics on the growth of *Verticillium lecanii*, *Tricoderma viride* and *T.harzianum.*

Materials and Methodology:

Antibiotics:

The antibiotics Chloramphenicol (Acichem Laboratories, 250mg capsule), Tetracycline (Lupin Pharmaeuticals, 500mg capsule), Streptomycin (Macleods, 750mg), Bacteriomycin were purchased from medical shop.

Microorganisms:

The biopesticides were purchased from the local market and the spores are allowed to regenerate on the native media. After 5 days, *Verticillium lecanii* was inoculated on the Potato carrot agar medium and *Tricoderma* spp was inoculated on Potato dextrose agar medium.

Media preparation

Potato carrot medium

30g Potato and 30g carrot were sliced and boiled well. After boiling, the extract is filtered and made upto one litre using reverse osmosis water. 5g Glucose is added to the final volume and sterilised. For solid medium, 25g agar per litre is added to the liquid preparation and boiled well for complete mixing and then sterilized (Sørensen *et al., 2009*).

Potato dextrose medium

250g Potatoes were sliced and boiled well. After boiling, the extract is filtered and made upto one litre using reverse osmosis water. 5g Glucose is added to the final volume and sterilised. For solid medium, 25g agar per litre is added to the liquid preparation and boiled well for complete mixing and then sterilized (Arriagada, A. *et al*.,1948).

Methodology

A loop of culture, *Verticillium lecanii, Tricoderma viridi and Tricoderma harzianum* were inoculated into the respective broth medium and incubated for three days at room

temperature. After incubation a loop of culture is taken from the broth and inoculated on the agar plate and incubated for three day at room temperature. 2 litre of agar medium was prepared, sterilised and cooled (Harry K *et al*., 1996). Antibiotics were measured in the concentration of 10, 20, 30, 40 ppm and each antibiotic at specific concentration is added to the cooled medium and mixed well. The medium containing th**e** antibiotic is poured on the petriplate. A small disk of culture about 0.6 cm is placed on the cooled solidified medium and kept for incubation at room temperature. The radial growth of the culture was measured daily and compared with the growth on the control plate (Chang *et al., 2010*).

Results and discussions

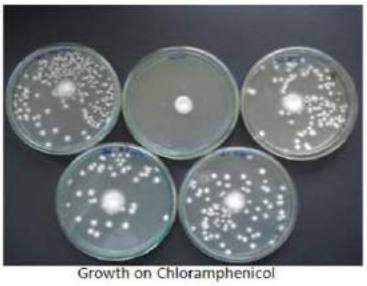
The effects of antibiotics on the growth of the fungus on second and third day after inoculation were observed and the results were tabulated.

Growth of *Verticillium lecanii*

The growth of the culture on the plate is influenced and inhibited by the presence of the antibiotic and its concentration. The addition of bacteriomycin at large concentration inhibits the growth of *Verticillium lecanii.* The presence of streptomycin at low concentration influences the growth of the culture whereas at high concentration inhibits the growth of *V.lecanii*. The presence of tetracycline and chloramphenicol does not inhibit or influence the growth of *V.lecanii.*

Antibiotic	Concentration (ppm)	10		20		30		40	
		day 3	day 4	day 3	day 4	day 3	day 4	day 3	day 4
Chloramphenicol	Influence of growth (cm)	0.1	-0.3	0.1	0.1	-0.1	-0.3	0	-0.3
	Percentage of growth	6%	-14%	6%	5%	-6%	-14%	0%	-14%
Tetracycline	Influence of growth (cm)	0	-0.2	0.1	-0.1	0	-0.1	0	0.1
	Percentage of growth	0%	-9%	6%	-5%	0%	-5%	0%	5%
Streptomycin	Influence of growth (cm)	0.1	0.1	0	0.1	-0.1	0	0	-0.1
	Percentage of growth	6%	5%	0%	5%	-6%	0%	0%	-5%
Bacteriomycin	Influence of growth (cm)	-0.1	-0.1	-0.2	-0.3	-0.3	-0.6	-0.4	-0.7
	Percentage of growth	-6%	-5%	-13%	-14%	-19%	-27%	-25%	-32%


Mother plate of *Verticillium lecanii*


Growth on Chloramphenicol


Growth on tetracycline

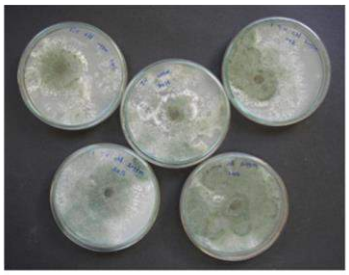

Growth on streptomycin

Growth of *Tricoderma viride*

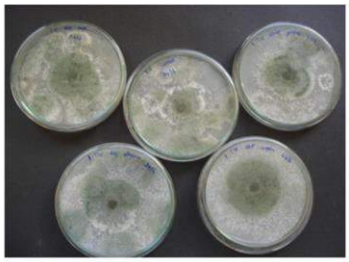
The growth of the culture on the plate is influenced and inhibited by the presence of the antibiotic and its concentration. The antibiotics used in the study influences the growth of *T.viride* at optimum concentration and inhibit the growth at high concentration. Presence of bacteriomycin at very low concentration influences the growth and produces more colonies when compared with other antibiotics.

Antibiotic	Concentration (ppm)	10		20		30		40	
		day 3	day 4	day 3	day 4	day 3	day 4	day 3	day 4
Chloramphenicol	Influence of growth (cm)	0.45	0.85	0.95	3.25	0.7	4.4	-0.05	1.6
	Percentage of growth	23%	21%	49%	81%	36%	110%	-3%	40%
Tetracycline	Influence of growth (cm)	0.7	1.25	0.25	1.35	0.05	1.5	-0.1	5
	Percentage of growth	36%	31%	13%	34%	3%	38%	-5%	125%
Streptomycin	Influence of growth	0.45	1.8	0.8	3.05	1	4.1	0.45	1.75
	Percentage of growth (cm)	23%	45%	41%	76%	51%	103%	23%	44%
Bacteriomycin	Influence of growth	2.05	1.25	2.00	1.50	1.92	1.35	1.67	0.98
	Percentage of growth (cm)	105%	31%	103%	38%	99%	34%	85%	24%

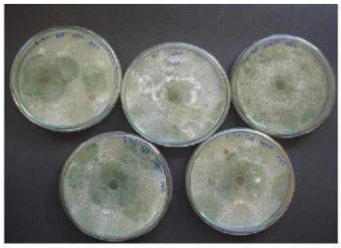
Mother plate



Growth on Chloramphenicol



Growth on Streptomycin



Growth on tetracycline

Growth of *Tricoderma harzianum*

Antibiotic	Concentration (ppm)	10		20		30		40	
		day 3	day 4	day 3	day 4	day 3	day 4	day 3	day 4
Chloramphenicol	Influence of growth (cm)	-0.3	4.1	-0.05	4.8	-0.3	4.1	-0.2	4.1
	Percentage of growth	-10%	103%	-2%	120%	-10%	103%	-7%	103%
Tetracycline	Influence of growth (cm)	-0.2	3.8	-0.1	4.1	-0.4	3.8	-0.6	3.5
	Percentage of growth	-7%	95%	-3%	103%	-14%	95%	-21%	88%
Streptomycin	Influence of growth (cm)	-0.3	4	-0.3	3.6	-0.4	3.9	-0.3	3.4
	Percentage of growth	-10%	100%	-10%	90%	-14%	98%	-10%	85%
Bacteriomycin	Influence of growth (cm)	-0.8	2.9	-0.8	2.3	-1.6	1.4	-1.7	1.3
	Percentage of growth	-28%	73%	-28%	58%	-55%	35%	-59%	33%

The growth of the *T.harzianum* is influenced by the presence of the antibiotic and its concentration. Presence of chloramphenicol produces more growth compared with other antibiotics. Above the optimum concentration, the growth depletes. Hence chloramphenicol can be used at minimum concentration of less than 20 ppm to get the maximum yield of *T.harzianum*.

Conclusion

The usage of antibiotics at low concentration influences the growth of the microorganism. The effect of antibiotic depends upon the microbial community and the incubation period. Among the antibiotics used, bacteriomycin depletes the growth of the micro organisms and effectively controls the contamination of bacteria. But at very low concentration, it influences the growth of *Tricoderma viride.* Chloramphenicol at very low concentration influences the growth of *Verticillium lecanii and Tricoderma harzianum.*

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