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Effect of inhibitory substances and pesticide on *Streptomyces sp.* isolated from soil under cultivation of *Curcuma Longa* L.

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ABSTRACT

In the present day soil pollution is major problem because different types of pesticides and non biodegradable substance which are used to control plant pest. The residual component of pesticide diffuses into the soil and causes soil pollution. Some microorganisms like actinomycetes have ability to degrade these components and control the soil pollution and water pollution. In Ayurved system of medicine the rhizome portion of *Curcuma longa* L. was used as an anti-inflammatory and antiseptic. Antimicrobial substances released from these plants diffuse into the surrounding soil area of the plant. Actinomycetes which grow in these areas were resistant to these substances. Hence considerable attention had been given in studying the effect of pesticides and inhibitory substances on actinomycetes especially *Streptomyces sp.* isolated from soil under cultivation of *Curcuma longa* L. The present study deals with effect of pesticides and inhibitory substances like Confidor, Carbaryl, Follidol and Diethane M- 45, dettol, phenol, sodium azide, and crystal violet. The results were recorded on the basis of presence or absence of growth. Total seven *Streptomyces* isolates were found resistant against Confidor and Carbaryl each, five were resistant against Diethane M- 45 and four were resistant against follidol. Total nine *Streptomyces sp.* were found sensitive to dettol, two were resistant against phenol and five were resistant against Sodium azide and Crystal violet. From the study performed we conclude that *Streptomyces sp.* which is resistant to pesticide and other inhibitory substances can be used to degrade the residual components of pesticide which are released into the soil. It may have better option to prevent soil pollution.

Keywords: *Streptomyces sp., Curcuma longa* L., Pesticides, inhibitory substances, soil pollution.

INTRODUCTION

Actinomycetes were originally considered to be an intermediate group between bacteria and fungi but now are recognized as prokaryotic organisms. They show common characteristics of both bacteria and fungi. So possess sufficient distinctive features to classify them into a separate category. Actinomycetes have been considered as biotechnologically and industrially valuable prokaryotes since they have produced a large number of compounds of pharmaceutical and agricultural importance (Ripa *et al.,* 2009 and George *et al.,* 2012).

Streptomycetes are most significant component of the microbial population in the soils. *Streptomyces* is the largest antibiotic producing genus of actinomycetes in the microbial world discovered so far. *Streptomycetes,* the Gram Positive filamentous, strict aerobes, lack cross wall and their growth is at the tip of filament, and are widely distributed in a variety of natural and manmade environments (Watve *et al.,* 2001).

Now a day's various types of pesticides used for agriculture purpose are the main cause of soil pollution. Residual quantity of pesticide has great effect on flora and fauna of soil. Actinomycetes play major role to abate such soil pollution and sustain the flora and fauna of soil ecosystem. Actinomycetes decompose organic matter which acts as a storehouse for energy and nutrients for plants and other soil organisms to use. Plant−bacterial associations can improve the degradation of organic pollutants in soil. The rhizome underground portion of *Curcuma longa* L. used as antibacterial substances. These substances diffuse into the surrounding soil area of the plant. The release of antibacterial substances inhibits growth of other microorganism. Actinomycetes which are resistant to these substances only grow in this area. Little attention has been given in studying the effect of other inhibitory substance and pesticide on actinomycetes especially *Streptomyces sp.* isolated from soil under cultivation of *Curcuma longa* L.

MATERIALS AND METHODS

Materials -

1. Soil-Soil under cultivation of *Curcuma longa* L. from the villages around Barshi, Dist. Solapur, M.S, India
2. Glycerol aspargine agar
3. Pesticide – conidifor, carbaryl, Follidol and Diethane M- 45
4. Inhibitory substances- Dettol, Phenol, Sodium azide, Crystal violet.

Methods-

Isolation of actinomycetes: For the present study 4 soil samples collected from the villages around Barshi, Dist. Solapur M.S, India were used for isolation of actinomycetes. Total 15 actinomycetes were isolated by streak inoculation technique on glycerol aspargine agar (L-aspargine- 0.1g, K_2HPO_4-0.1g, glycerol- 1g, trace salt solution- 0.1mL, agar- 2.5g, distilled water- 100 mL pH-7.4) after incubation at an ambient temperature for 5-7 days.

Identification of actinomycetes: These actinomycetes were identified by performing morphological, cultural and biochemical studies. Morphological characters were studied by cover slip culture technique (Mycelium pattern e.g., Arial, submerged and surface mycelium and structure of spore chain). Cultural characters were studied by observing growth on different media e.g., Bennet's agar, Dextrose agar. Biochemical studies include enzymatic and sugar utilization test.

Identification of *Streptomyces* by Probabilistic Identification of bacteria for windows, version: 2.0 - These isolates were also identified by using Bergey's Manual of Systematic Bacteriology Vol-4 and Micro IS software. Based on microscopic, cultural characteristics actinomycete isolates were primarily identified to genus level. Biochemical, resistance pattern and other characters were used to identify isolates to species level. The PUBWin software version 2.0 (Bryant 2004) were downloaded from the web site: http://www.som.soton.ac.uk/staff/tnb/pib.htm

Probabilistic identification matrices used were *Streptomyces* species major cluster (Williams *et al*., 1983), *Streptomyces* species minor cluster (Langham *et al*., 1989 and Kampfer and Kroppenstedt, 1991).

Effect of pesticides and inhibitory substances on *Streptomyces sp.* :

The effect of inhibitory substances like Dettol, Phenol, Lysol, Sodium azide, Crystal violet were carried out by using Glycerol aspargine agar. These inhibitory substances were added in glycerol aspargine agar having concentration Dettol (0.1), Phenol (0.1), Lysol (0.1), Sodium azide (0.01), Crystal violet (0.0001) % w/v separately. Pesticides such as Confidor 0.3ml/ L, Carbaryl 3g/ L, Follidol 2g/L and Diethane M- 45 0.2g/L were added in Glycerol aspargine agar separately. The pesticides and inhibitory effect were studied by spot inoculating on glycerol aspargine agar containing these pesticides and inhibitory substances and incubated at an ambient temperature for 5-7 days. Results were recorded on the basis of presence or absence of growth

RESULTS AND DISCUSSION

A. Identification of organism:

Among all 15 actinomycetes isolates total ten actinomycetes were identified as *Streptomyces alboflavus, Streptomyces antibiotius, Streptomyces cellulosae, Streptomyces chromofuscus Streptomyces chromogenus, Streptomyces cyaneus, Streptomyces exfoliates, Streptomyces filipinensis, Streptomyces flaveolus* and *Streptomyces flavoviridis* on the basis of morphological and cultural characteristics. Morphological studies were carried out by cover slip culture technique and slide culture technique (Mycelium Pattern e.g. Aerial, Submerged and Surface mycelia and Structure of spore chain Photo plate 3 and 4) and MICRO-IS software and PUBWin software version 2.0.

B. Effect of Pesticides and inhibitory substances on *Streptomyces sp.*

Out of 10 *Streptomyces* isolates, total 70% *Streptomyces* were resistant to confidor and carbaryl and then followed by diethane i.e. 50%. Total 40% *Streptomyces* were resistant to Follidol. Total 50% *Streptomyces* were resistant to Sodium azide and Crystal violet. Least resistant activities was shown against dettol i.e. 10% and then followed by Phenol i.e. 20%

Effect of Pesticides and inhibitory substances on *Streptomyces sp.* was shown in Table 1. Fig.1 shows Percentage of resistant *Streptomyces sp.* to pesticides and Fig.2 shows Percentage of resistant *Streptomyces sp.* to inhibitory substances.

Photo plate1 Sample Collection sites *Curcuma longa* L. Photo plate 2 *Streptomyces* isolates


Photo plate 3 & 4 Light Microscope and Scanning electronic photograph of *Streptomyces* spore chain

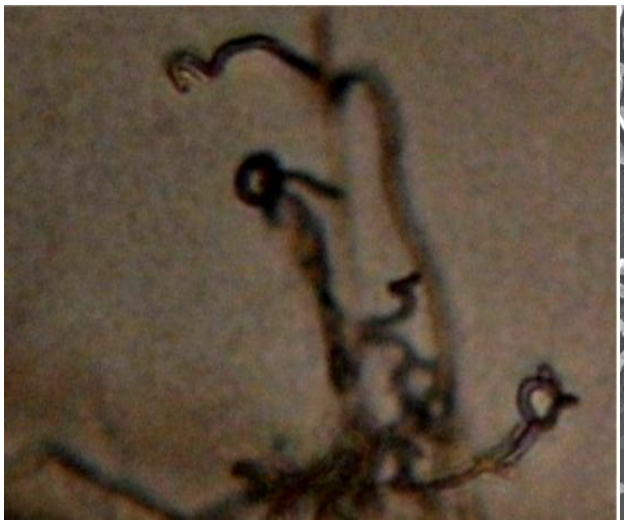
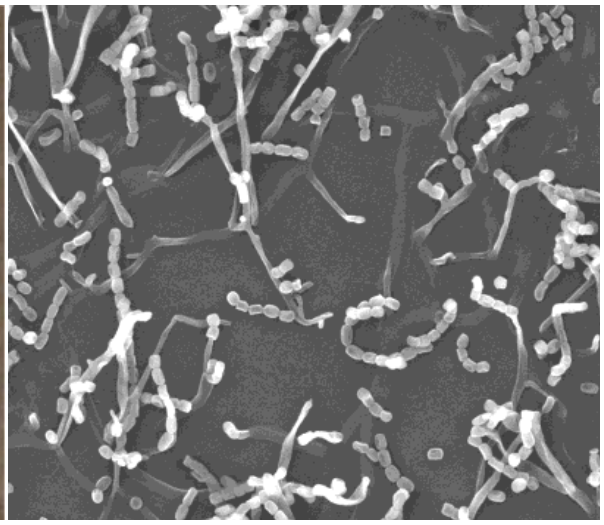



Table 1. Effect of Pesticides and inhibitory substances on *Streptomyces sp.*

Sr. No	Name of Isolate	Pesticides				Inhibitory Substances			
		Confidor	Carbaryl	Follidol	Diethane M- 45	Dettol	Phenol	Sodium azide	Crystal violet
1	*Streptomyces alboflavus*	+	+	-	+	-	+	-	-
2	*Streptomyces antibiotius*	+	+	-	-	-	-	+	-
3	*Streptomyces cellulosae*	-	+	-	+	-	-	-	+
4	*Streptomyces chromofuscus*	+	+	-	-	-	-	+	-
5	*Streptomyces chromogenus*	+	-	+	-	-	-	-	-
6	*Streptomyces cyaneus*	+	-	+	+	-	-	+	+
7	*Streptomyces exfoliates*	+	-	-	-	-	+	-	-
8	*Streptomyces filipinensis*	-	+	+		-	-	+	+
9	*Streptomyces flaveolus*	+	+	-	+	-	-	-	+
10	*Streptomyces flavoviridis*	-	+	+	+	+	-	+	+
		7	**7**	**4**	**5**	**1**	**2**	**5**	**5**

* **Where + = resistant, - = sensitive**

Fig.1 Percentage of resistant *Streptomyces sp.* to pesticides.

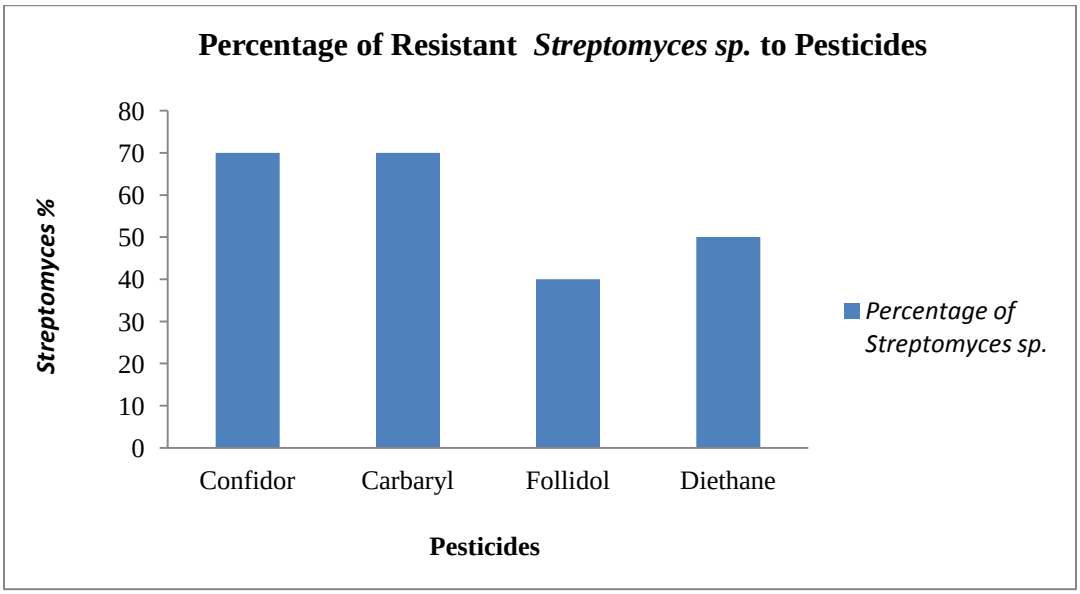
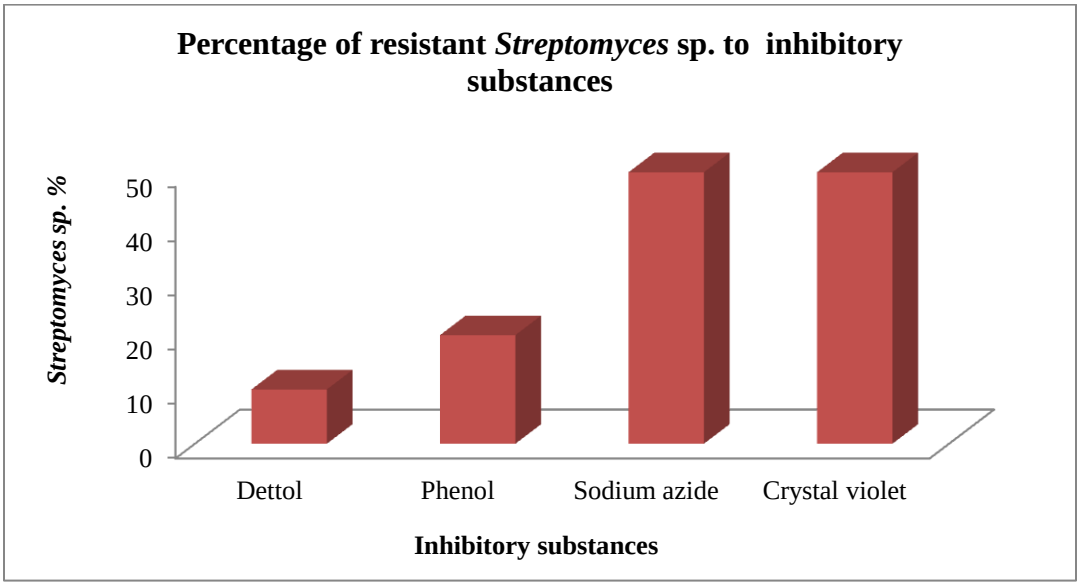


Fig.2 Percentage of resistant *Streptomyces sp.* to inhibitory substances.



Analia (2013) investigated *Streptomyces spp.* which degrades highly toxic organic compounds like the organochlorine pesticide (OP) γ-HCH (hexachlorocyclohexane). Koli *et al.*, (2014) reported pesticide resistant *Streptoverticillium olivoverticillatum* isolated from marine sand sample of Alibagbeach. India. They found that *Streptoverticillium olivoverticillatum* was resistant to all tested pesticide *Viz.* Carbaryl, Diethane M-45, Folidol powder, Quinalphos 25%, Chloropyrofos 20% and Confidor.

Mustafa *et al.*, (2004) reported actinomycetes isolated from farming soils of Turkey tolerance to NaCl (7%), NaCl (10%), Sodium Azide (0.01), (0.02), Phenol (0.1), Potassium Tellurite (0.001) and Crystal violet (0.0001) (growth with % w/v). Oliveira *et al.*, (2009) reported intrinsically arsenic tolerant actinobacteria, in a long-term heavy metal contaminated soil. Koli *et al.*, (2014) reported resistance pattern of *Streptoverticillium olivoverticillatum* against inhibitory substances. They found that *Streptoverticillium olivoverticillatum* resistant to dettol (0.1), crystal violet (0.0001), Lysol (0.1), sodium azide (0.01) % w/v while sensitive to phenol, mercuric chloride, arsenic trioxide and sodium azide (0.02% w/v).

These results support our study.

CONCLUSION

From the result it is concluded that strain of *Streptomyces sp.* resistant to pesticides and inhibitory substances can be used for degradation of residual components of pesticide which are released into the soil and control soil pollution to some extent.

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