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Research Article

ISOLATION AND SCREENING OF ACTINOBACTERIAL ISOLATES FROM CHEMICAL PESTICIDES USAGE FIELDS OFTHE UTTARAKHAND REGION

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ABSTRACT
The excessive use of pesticides causesmaximum soil stress and infertile conditions. The present study was performed in order to explore and identify the novel microbial source for the biodegradation of monocrotophos pesticides. Soil samples (approx. 500 g) were collected using some clean,dry, and sterile polythene bags along with a sterile spatula, marking pen rubber band, and other accessories. These samples were air-dried for 1 week, crushed, and sieved. The sieved soils were then used for Actinomycetes isolation. A totalof 120 Soil samples were aseptically collected from different field regions of Uttarakhand viz. Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi, and Haridwar havedominant usage of monocrotophos pesticides. Amongst these samples, A total of 280 microbes were isolated; out of which 24 isolates of Actinobacteria (8.57 %) were isolated. The results revealed the strains of the genera viz. Micromonospora (65%), Actinomycetes (25%,) and Streptomyces (10%)are meant to be responsible for the biodegradation of monocrotophos pesticides.

Keywords: Monocrotophos pesticides, Organophosphate, Biodegradation, Bioremediation, Actinobacterial isolates, Pesticides degradation.

1. INTRODUCTION
Chemicals and pesticides toxicity has been a meager issue in agriculture and farming practices. These chemicals not only leave toxic residues in the soil but also enters the food chain and ecosystem. WHO (2009) reported monocrotophos pesticides are the major reasons for accidental poisonings. The state-wise report on monocrotophos pesticides published in 2001-2006 revealed the highest usage by Andhra Pradesh (2,779 metric tons) followed by Punjab (1,274 metric tons), Gujrat (865 metric tons), Haryana (823 metric tons), Karnataka (624 metric tons), Madhya Pradesh (597 metric tons), Tamil Nadu (522 metric tons), Rajasthan (512 metric tons), West Bengal (169 metric tons), Kerala and Bihar (103 megatons).Due to the high consumption of pesticides 1531 death cases were reported in year 2000, Out of which 609 were due to organophosphorus pesticides whereas 86 cases were reported due to consumption of monocrotophos which was the largest number of insecticide poisonings. Monocrotophos is poisonous organophosphates observed all cross the country and are widely used for agriculture. It is a direct-acting cholinesterase inhibitor capable of penetration through the skin. Symptoms are similar to those of other organophosphate compounds but the effect can be observed within minutes or in a day. Its cholinesterase inhibiting activity causes nervous system effects. Cases of human poisoning are characterized by muscular weakness, blurred vision, profuse perspiration, confusion, vomiting, pain, and small pupils. This may involve vomiting, diarrhea, nausea, headache, abdominal cramps etc. Severe poisoning due to monocrotophos causes cardiac arrest or respiratory failure which leads to death of person in the severe cases [1-5]. The two main organizations related to health and agriculture, FAO and WHO encouraged countries to list out pesticides having highly hazardous components. Many countries involved Australia, China, the European Union, Cambodia, Laos, Indonesia, Philippines, Vietnam Sri Lanka, Thailand; the United States of America banned the use of monocrotophos. To take off this from market urgent

steps should be taken. Many developing countries of Asia also have banned the use of monocrotophos as it causes high health risks. India is very much familiar with the threats of pesticides. But in the fields of rural India, pesticides like monocrotophos is continuously produced, used and exported in India. The reason behind this is that it is cheap and necessary for agricultural productivity [6-10]. The image of chemical structure of monocrotophos pesticide is shown in **Fig. 1.**

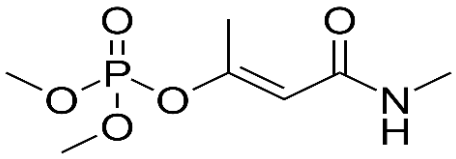

Fig. 1: Chemical structure of Monocrotophos ($C_7H_{14}NO_5P$)

2. MATERIAL AND METHODS

2.1. Collection and preparation of soil sample

About 120 Soil samples were aseptically collected from different field regions of Uttarakhand viz. Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi and Haridwar having dominant usage of monocrotophos pesticides. Soil sample (approx. 500 g) were collected using some clean, dry and sterile polythene bags along with sterile spatula, marking pen rubber band and other accessories. These samples were air-dried for 1 week, crushed and sieved. The sieved soils were then used for actinomycetes isolation as per the series wise methods as described in later sections.

2.2. Isolation of Actinomycetes

From collected samples, 5g of the soil was suspended in 50 ml of Normal saline (NaCl-0·85g/L). The soil suspension wasincubated in an orbital shaker incubator at 28°C with shaking at 200rpm for 3 minutes. Actinomycetes were isolated by spread plate techniques following the serial dilution of soil in YIM6 Starch-casein medium.

Different salt mixtures viz. NaCl- 100-150 g; KCl- 20 g; $MgCl_2$- 30 g; $MgSO_4$- 5 g; K_2HPO_4- 1g; Starch- 20 g; Casein/milk powder- 10 g.

The pH of each of the above medium was maintained from 10-12. In each of the medium, nalidixic acid (25-50 g/liter) was added. Isolated plates were incubated at 28°C for 25-35 days for the observation of growth of Actinomycetes [11-15].

2.2.1. Aerial Mass Color

The colour of the mature sporulating aerial mycelium is recorded in an exceedingly straightforward method (White, grey, red, green, blue and violet). Once the aerial mass color falls between two colors series, both the colors are recorded. If the aerial mass color of a strain to be studied shows intermediate tints, then also, both the colors series are noted [16].

2.2.2. Melanoid Pigments

The grouping is formed on the assembly of melanoid pigments (*i.e.* light-green brown, brown black or distinct brown, pigment changed by alternative colors) on the medium. The strains are grouped as melanoid pigment created (+) and not created (-) [17].

2.2.3. Reverse Side Pigments

The strains were divided into two groups, consistent with their ability to provide characteristic pigments on the reverse aspect of the colony, namely, distinctive (+) and not distinctive or none (-). In case, a color with low saturation like yellowness, olive or yellowish brown occurs, it is included in the latter group (-) [18-20].

2.2.4. Soluble Pigments

The strains are divided into two groups by their ability to provide soluble pigments apart from melanin: particularly, produced (+) and not produced (-). The color is recorded (orange, red, green, violet, blue and yellow) [21-22].

2.2.5. Spore Chain Morphology

With relevancy to spore chains, the strains are sorted into "sections". The species belonging to the genus Streptomyces are divided into three sections, particularly recti-flexibiles (RF), retina-culiaperti (RA) and spirales (S). Once a strain forms two types of spore chains, both are noted (*e.g.* SRA) [23-25].

2.2.6. Reproductive Structure Surface

Spore morphology and its surface options ought to be determined under the scanning electron microscope. The cross hatched cultures arranged for observation under the light microscope can be used for this purpose. The electron grid ought to be cleaned and adhesive tape should be placed on the surface of the grid. The mature spores of the strain ought to be rigorously placed on the surface of the adhesive tape and gold coating should be applied for half an hour and also the specimen is

examined under the electron microscope at completely different magnifications. The reproductive structure silhouettes are characterized as spiny, smooth, warty and hairy [26].

3. RESULTS AND DISCUSSION

With reference to the studies, total of 120 soil samples were collected from different field areas of Uttarakhand region (Tehri-Garhwal, Chamoli, Srinagar, Uttarkashi and Haridwar) having dominant usage of mono-crotophos pesticides. Amongst these samples, total of 280 microbes were isolated; out of which 24 isolates of Actinobacteria (8.57 %) were isolated. The results are shown in Table 1 and Fig.2. The actinobacteria isolates were screened on specific agar media and characterized by morphological colonies appearance and staining procedures. The actinobacteria isolates were categorized on the basis of a) type of pigment production (Table 2) and colony and color (Table 3) and Fig.3. These actinobacterial isolates were further screened for their identification by molecular. The results revealed the strains of the genera viz. Micromonospora (65%), Actinomycetes (25%) and Streptomyces (10%).

The results of the study suggest that, degraders of monocrotophos pesticides are available at the site where, monocrotophos pesticides are accumulated. The actinobacterial isolates were found in high density in the soil enriched with monocrotophos pesticides [27-28].

Table 1: Percent diversity of Actinomycetes isolates on YIM6 starch- casein agar medium

Soil sample	Total no. of microbes isolated	Actinobacteria isolates	Percent diversity of microbes isolated	Percent diversity of actinobacteria isolated
120	**256**	**24**	**91.42**	**8.57**

Table 2: Screening of isolated actinobacterial strains on the basis of pigment production

S. No.	Strain code	Pigment production		
		Melanoid pigment	Reverse sidepigment	Soluble pigment
1	ASUK03	+	+	+
2	ASUK07	-	+	+
3	ASUK254	-	+	+
4	ASUK145	+	+	+
5	ASUK67	+	+	+
6	ASUK86	+	+	+
7	ASUK46	+	+	+
8	ASUK34	+	+	+
9	ASUK23	+	+	+
10	ASUK60	+	+	+
11	ASUK79	+	+	+
12	ASUK224	-	+	+
13	ASUK185	-	+	+
14	ASUK145	-	+	+
15	ASUK76	-	+	+
16	ASUK216	-	+	+
17	ASUK237	-	+	+
18	ASUK259	-	+	+
19	ASUK263	-	+	+
20	ASUK283	+	+	+
21	ASUK292	+	+	+
22	ASUK308	-	+	+
23	ASUK315	+	+	+
24	ASUK423	-	+	+

*+, Presence -, Absence

Table 3: Screening of isolated actinobacterial strains on the basis of color of pigment, mycelium and appearance of colony and identified genera

S. No.	Strain code	Pigment color/mycelium/appearance of colony		
		Color of pigment	Mycelium	Appearance of colony
1	ASUK03	Yellow	Rough	Dirty based
2	ASUK07	Whitish yellow	Smooth	Round
3	ASUK254	Whitish green	Rough	Thick
4	ASUK145	White	Hairy	Thread like
5	ASUK67	Whitish pink	Branched	Wrinkled
6	ASUK86	Yellowish pink	Branched	Wrinkled
7	ASUK46	Whitish creamy	Branched	Wrinkled
8	ASUK34	Yellowish creamy	Branched	Smooth
9	ASUK23	Whitish concave	Spherical	Smooth
10	ASUK60	White cotton	Spherical	Smooth
11	ASUK79	Whitish	Spreader	Flattened
12	ASUK224	Whitish thread	Branched	Flattened
13	ASUK185	Whitish point	Aerial	Smooth
14	ASUK145	Whitish cotton like	Branched	Smooth
15	ASUK76	Purple spreader	Granular	Wrinkled
16	ASUK216	Whitish yellow cotton like growth	Rough	Flattened
17	ASUK237	Whitish cotton	Spherical	Smooth
18	ASUK259	Whitish scanty	Smooth	Smooth
19	ASUK263	Pinkish white	Flattened and spherical	Wrinkled
20	ASUK283	Whitish spreader	flattened	Wrinkled
21	ASUK292	Whitish yellow spreader	flattened	Wrinkled
22	ASUK308	Yellowish white spreader	flattened	Wrinkled
23	ASUK315	Whitish spreader	flattened	Wrinkled
24	ASUK423	Whitish brown spreader	flattened	Wrinkled

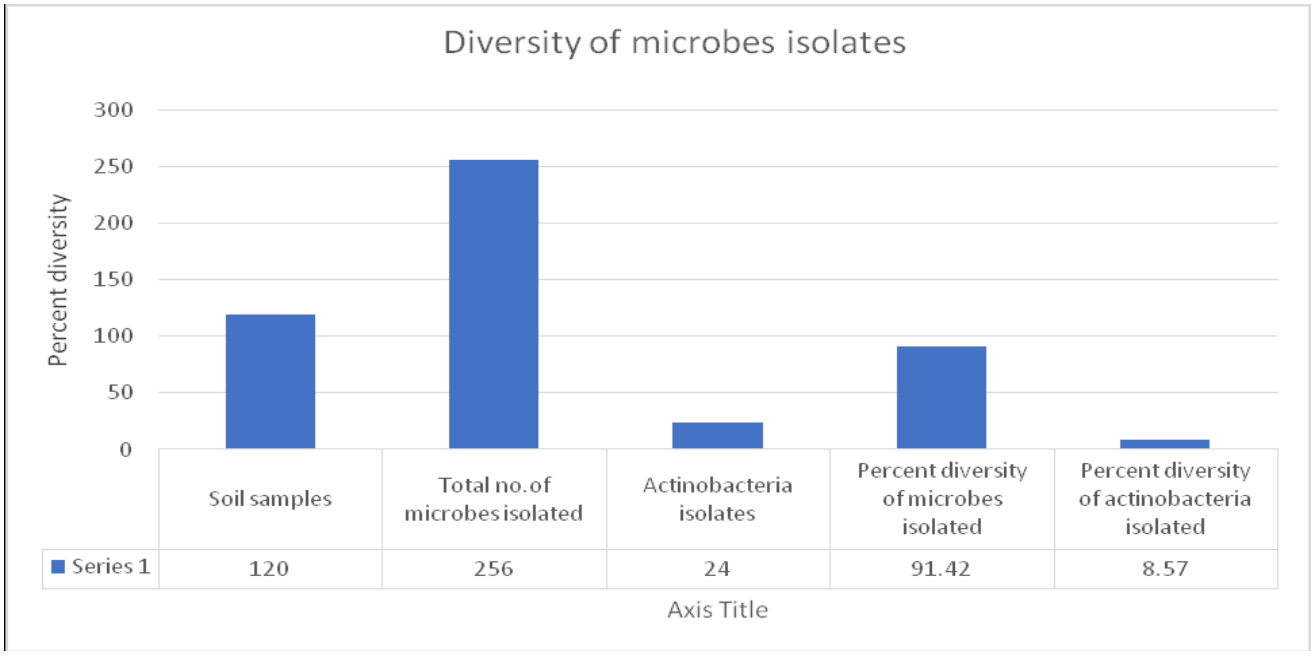

Fig. 2: Percent diversity of microbes and actinomycetes isolates on YIM6 starch casein medium

4. CONCLUSION

The study emphasizes the importance of isolated areas through which specific microflora community; thus, the sample sites are meant to be the repositories of the isolates of actinobacteria. The study concludes that, the microbial community like actinobacterial isolates increases naturally as per the availability and accumulation of such pesticides in the soil. The study thus concludes that, actinobacteria can be isolated from pesticides enriched areas and the same can be utilized in biodegradation of pesticides like these. More studies are however required to isolate and explore such microbial strains responsible for degradation of monocrotophos and other categories of pesticides.

Conflict of interest

None declared

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