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SCREENING OF PESTICIDE RESISTANT CYANOBACTERIA AND THEIR ROLE IN SOIL FERTILITY.

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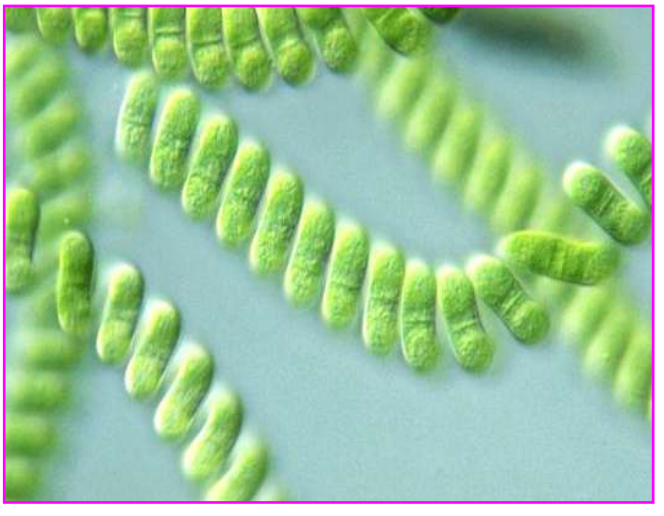
ABSTRACT



Pesticide contaminated soil tests (Grapes and Vegetables) were gathered and broke down from various areas like Katri, Kamtha, Bijanwadi, Khudawadi, Kakramba, Mangrul of Tuljapur taluka (District Osmanabad). Cyanobacterial confines were acquired by enhancement and segregation strategies by utilizing BG-11, Gerloff's medium. Secluded societies were saved in the research facility in both fluids and in addition on strong media containing Monocrotophos or endosulfan 100 mg/lit. In every one of the twenty-two disconnects acquired from ten soil tests were screened for its MCP and Endosulfan resilience limit in fluid media. Ten cyanobacterial societies were observed to endure MCP. Among these Synechocystis confined from the Grapes field (Khudawadi) was seen to be most raised MCP tolerant (900mg/L). However among twelve endosulfan persevering social orders disengaged, Anabaena from vegetable field was the most hoisted endosulfan tolerant (500 mg/L). Impact of pH and temperature on development of societies in nearness of pesticides demonstrated ideal development at pH 7 and pH 6.4 for Synechocystis and Oscillatoria individually. Be that as it may, both the way of life show ideal development at 30o Celsius. .Both these societies reestablish pesticide resilience subsequent to subculturing however others didn't.

KEYWORDS: *Cyanobacteria, Pesticide, Screening.*

INTRODUCTION :-

Cyanobacteria are excellent organisms to serve as models for the examination of a wide assortment of organic issues, for example, going about as natural contamination markers. In the most recent decade cyanobacteria were contemplated with various angles particularly regarding their utility in bioremediation, human nourishment, as biofertilizer (Venkataraman, 1975), wellspring of valuable chemicals and some novel applications. It has been watched that the number of inhabitants in soil microflora in charge of debasement of pesticides in soil does not demonstrate a noteworthy ascent in number upon rehashed use of the pesticide to soil. Utilizing a most likely number method the that bigger populace of EPTC (S-ethyl, N,N – depropyl carbamothioate) corrupting microorganisms did not exist in soils showing quickened debasement of EPTC and that expanded rates of digestion were in charge of quickened corruption as opposed to expanded number of

creatures. In India, utilization of pesticides is expanding @ 2-5 % pa. To date pesticide utilization extends between 480 – 520 g/ha and this records for around 3% of the aggregate pesticides utilized as a part of the world. Cyanobacteria have the capacity to make due in extraordinary conditions and are additionally answered to corrupt xenobiotics has screening of various strains of cyanobacteria for their development and resistance to pesticides like monocrotophos and Malathion

MATERIALS AND METHODS

Collection of soil samples

An overview of utilization of pesticides [endosulfan and monocrotophos (MCP)] in various rural fields from agriculturists in the Tuljapur taluka , (MS) was first completed. In light of preparatory review the exploration work was focused on two pesticides viz. monocrotophos (MCP) and endosulfan and 10 distinct locales of pesticide polluted soil. Soil tests were gathered according to standard convention before one week of pesticide application. All dirt examples were gathered in clean plastic sacks and conveyed to the lab for further reviews (Nayak, 2007).

Physicochemical analysis of soil

Soil examination was done at Krishi Vidnyan Kendra,Tuljapur. Soil pH was measured in a 1:2.5 soil-water suspension utilizing a glass terminal (Systronics, Digital pH meter, India). Soil electrical conductivity (EC) was controlled by measuring the electrical conductance of soil-immersion segregate with a conductivity meter (Equiptronics, India) (Pawar, 2008). State shaping unit was (CFU) figured by utilizing standard plate check system.

Detection of Pesticide from soil samples

Soil tests were extricated with equivalent volumes of ethyl acetic acid derivation for both sorts of pesticides endosulfan and monocrotophos (MCP). The natural stage was gone through MgSO4 for endosulfan extraction, the both kind of natural stages delicately vanished by vacuum evaporator and redissolved in CH3)2CO and ethyl acetic acid derivation separately. TLC plates were produced in either oil ether and CH3)2CO (8.5:1.5) or chloroform and ethyl acetic acid derivation (3:1). For endosulfan, spots were pictured by showering silver nitrate and introduction at UV light for 10 min (Bhalerao and Puranik 2007). If there should be an occurrence of monocrotophos redissolved natural stage in ethyl acetic acid derivation was created in n-hexane and CH3)2CO (7.5:3) and pictured der UV (Sharma 2005). Stock arrangements (1000 mg/l) were set up from

Enrichment, Isolation and purification of cyanobacterial cultures

The visual development of cyanobacteria in individual field was gathered and utilized for advancement in fluid media (BG-11 medium) in the research facility. The containers were brooded at 24± 2°C at 1800-2000 lux light force discontinuously for 30 days. The development of cyanobacteria was judged on the premise of visual perception following 30 days of brooding (Kaushik, 1987). For seclusion of unicellular cyanobacteria advanced specimen were streaked on separate agar plates and brooded at legitimate natural conditions. Noticeably unmistakable, very much segregated cyanobacterial settlements were chosen. In the event of filamentous societies, particular immunization of single fibers under aseptic conditions was the strategies for seclusion utilized. Filtration of the unicellular detaches was done utilizing fluid media (serial weakening technique) trailed by strong media. Filamentous societies were filtered on strong medium by strong agar plate technique.

Identification of Cyanobacteria

The way of life were additionally kept up in the soup medium at low power of light and at 20°C temperature. On the premise of morphological qualities cyanobacterial societies were distinguished at the family level. The morphology of secluded cyanobacteria was seen under high power magnifying lens (Labomed, demonstrate LX 300).

Screening of pesticide resistant Cyanobacterial cultures

The way of life gotten from soil movement were screened for MCP and endosulfan quality limit by taking after the point plate technique. Societies demonstrating the most noteworthy length of development along the slope were chosen for further trials. Keeping in mind the end goal to check the base inhibitory focus (MIC) of MCP and endosulfan a progression of 250 ml Erlenmeyer carafes containing 100 ml of BG-11 Medium was corrected with expanding pesticide fixations running from 100–1000 mg/l and 300 - 500 mg/l, of MCP and endosulfan, separately. Following 8 days of brooding the flagons were watched for Cyanobacterial development. The MIC was noted as the convergence of MCP and endosulfan bringing about entire hindrance of Cyanobacterial development in carafe

Effect of pesticides on growth of cyanobacteria

To concentrate the impact of pesticide on development of cyanobacteria, the BG-11 medium with Minimal inhibitory groupings of pesticides were utilized. The jars were immunized and brooded at 24± 2°C under 1800-2000 lux light force for 15 day.

RESULTS AND DISCUSSION

Sample collection and sampling sites

From the oral information of agriculturists, it was construed that up to 2014 –2015 there was an enormous usage of monocrotophos and endosulfan. At this moment, the usage of monocrotophos and endosulfan has been reduced by and large. Predominantly monocrotophos controls a sweeping scope of annoyances including sucking, gnawing and depleting frightening little creatures and 8-legged creature vermin on cotton, citrus, rice, maize, sorghum, sugarcane, groundnut, soybeans and vegetables. Endosulfan controls sucking, gnawing and depleting frightening little creatures and parasites on a broad assortment of harvests, including natural item vines, vegetables, cucurbits cotton, sugarcane, etc.The formulators and trade names of monocrotophos and endosulfan move with diff. regions.

As per our review report of pesticide spoiled cultivating soils 10 unmistakable goals were picked on the preface of the commitment of pesticides specifically handle (Table 1). The cyanobacteria are particularly observed among the photosynthetic prokaryotes for their capacity to make in an expansive grouping of conditions (Mahendra, 1997)

Table 1. Sampling sites and crops under cultivation

Sr. No.	Name of Sampling Place	Crops Under Cultivation
1.	Katgaon	Grams
2.	Kakramba	Soyabean
3.	Kamtha	Grapes
4.	Khudawadi	Grapes
5.	Chincholi	Lady's finger
6.	Bijanwadi	Vegitable.
7.	Tamalwadi	Grapes
8.	Sindphal	Brinjal
9.	Mangrul	Grapes

Physicochemical analysis of soil

The mean estimations of various physicochemical parameters are given in Table 2. pH was found amidst 7.0 to 9. Electrical conductivity of soil scopes from 0.10 to 0.95 dS/m. The most lifted SPC was seen at 0.11 EC and 7.8, pH

Table 2. Physicological analysis of soil

Sr. No.	Name of Sampling place	pH	Electrical conductivity (dS/m)	SPC (CFUX10^8/gm)
1.	Katgaon	7.8	0.11	48
2.	Kakramba	8	0.85	40
3.	Kamtha	9	0.9	30
4.	Apsinga	7.2	0.6	26
5.	Chincholi	7.9	0.7	29
6.	Bijanwadi	7.0	0.5	32
7.	Tamalwadi	7.2	0.55	24
8.	Sindphal	7.2	0.8	36
9.	Mangrul	7.5	0.2	15

Pesticide residues can adversely affect the soil biota and biological processes, contingent upon the dirt and climatic conditions, and administration rehearses. Different microbial gatherings, for example, microscopic organisms, parasites, blue green growth, and microflora (small scale arthropods) are altogether impacted to an alternate degree by various sorts of pesticides. Soil organisms have an essential catabolic part in the earth and add to the worldwide cycling of carbon, nitrogen, sulfur, phosphorus and different components through corruption of plants and creatures buildups. Among the dirt microorganisms, microscopic organisms are most inexhaustible in soil changing from 106 to 1014 for each g soil. They broadly take an interest in all the key natural exchanges to bolster the higher types of life and possess a critical position in the worldwide cycling of supplements. The quantities of microorganisms happening in soils are normally higher than those of alternate gatherings, notwithstanding, on account of their little size in connection to the huge cell estimate and broad fibers of alternate gatherings, microscopic organisms represent not as much as half of the aggregate microbial biomass in soil (Alexander, 1977).

Isolation and identification of cyanobacterial cultures

However more current methodologies over the most recent four decades have underlined vital basic and sub-atomic qualities of these life forms with plantlike life forms (Stanier and Cohen-Bazire, 1977; Ripika et al. 1979). Information of the full differing qualities and transformative progressive system of cyanobacterial ordered elements is as yet indistinct (Tripathi, 2006).

Table 3. Identification of cyanobacteria

Name of sampling place		Isolate identified
1.	Katgaon	*Ananaena spp.*
2.	Kakramba	*Syneachocystis spp.*
3.	Kamtha	*Gloeothece spp.*
4.	Apsinga	*Synechococcus spp.*
5.	Chincholi	*Synechocytis spp.*
6.	Bijanwadi	Oscillatoria spp.
7.	Tamalwadi	*Chroococcus spp.*
8.	Sindphal	Synechocystis spp.
9.	Mangrul	Synechococcus spp.

Detection of Pesticide from soil samples

All gathered soil samples from Tuljapur taluka were essentially broke down by thin layer chromatography to realize that whether there is a need of this review or not. The huge number of soil tests showed nearness of both pesticides even following six days after application. No recognition of pesticides in few examples for both pesticides may be expected the change of pesticides other middle of the road metabolites, e.g. endosulfan gets changed over to endosulfan-sulfate. In writing various reviews have detailed the natural

destiny of endosulfan in various sorts of tainted soils (Antonious et al., 1998;

Screening of pesticide resistant Cyanobacterial cultures

Pesticide angle plate test was connected to screen the secludes for most astounding resilience to monocrotophos and endosulfan autonomously. Development execution was recorded as length of development (in cm) over the pesticide inclination (Table 4). Among the disengages enduring endosulfan, recognized as Anabaena spp. had displayed most astounding resilience to endosulfan. From the seven monocrotophos enduring separates, recognized as Synechocystis spp., had the most elevated length of development (8.8 cm) along the angle displaying the most elevated resilience. Societies indicating development of >5 cm on angle plate were additionally surveyed utilizing stock examine. Aftereffects of the slope plate measure were affirmed by becoming these segregates in soup societies with shifting pesticide fixations, 100-1000 mg/l MCP and 100-500 mg/l endosulfan. The Synechocystis spp. could endure 900 mg/l of MCP and develop spring up to 500 mg/l of Endosulfan. The seclude, Anabaena spp. demonstrated lush development up to 500 mg/l of endosulfan. In light of these aftereffects of Anabaena spp. also, Synechocystis spp. was chosen for further biodegradation thinks about.

Table 4. Screening of pesticide resistant Cyanobacterial cultures

Isolate	Identification	Growth on gradient Plate (cm)		MIC* (mg/l)	
		Monocrotophos	Endosulphan	Monocrotophos	Endosulphan
1.	Oscillatoria spp.	3.2	5.6	500	300
2.	*Syneachocystis spp.*	*8.8*	*7.1*	*900*	*400*
3.	*Ananaena spp.*	*7.5*	*7.9*	*800*	*500*
4.	*Gloeothece spp.*	*6.2*	*4.7*	*700*	*300*
5.	*Chroococcus spp.*	*5.9*	*6.9*	*800*	*200*
6.	Synechococcus spp.	7.2	5.1	600	300

Microorganisms assume a vital part in the digestion of organochlorine bug sprays. Notwithstanding, the perseverance of various organochlorine bug sprays in soil and water for long stretches has been accounted for. This might be either because of the resistance of the bug spray to microbial debasement or to the development of a complex with some part of the earth which is to a great extent impervious to microbial assault (Aleder, 1965). Numerous commentators have shown the impacts of pesticides on soil microorganisms. There are expanding confirmations to propose that even at typical field rates, bug sprays have some effect on soil microorganisms.

Effect of pesticides on growth of cyanobacteria

Prolonged persistence of pesticides in soil can have an adverse impact on the soil health and its ability to sustain productivity. Rather than most amphibian biological systems, soil pH can be exceedingly factor, running from 2.5 in mine crown jewels to 11.0 in antacid deserts. Most heterotrophic microscopic organisms and parasites support a pH close impartiality, with growths being more tolerant of acidic conditions (Atlas, 1988). Extremes in pH, as can be seen in a few soils, would hence be required to impact the capacity of microbial populaces to corrupt pesticides. Ecological destiny of natural contaminations in soils is affected fundamentally by the pH and surface of the dirt, and furthermore the nearness of natural matter and co-toxins (Awasthi et al., 2000). Perseverance of pesticides, seen in present review could be ascribed to physicochemical properties of soil. The normal pH of soils under scrutiny was around lack of bias. Awasthi et al. (2000) revealed expanded debasement of endosulfan under un-immunized conditions when pH of soil was in soluble range

The impact of monocrotophos and endosulfan on development of Synechocystis spp. what's more, Anabaena sp. were considered separately. The detach Synechocystis spp. could endure 900 mg/l of monocrotophos.. The detach, Anabaena spp. indicated rich development up to 425 mg/l of endosulfan. The

observation shows that impact of monocrotophos and endosulfan on pH of soil within the sight of Synechocystis spp. what's more, Anabaena sp. individually. The pH of soil was diminished after fifth day of brooding. Then pH of soil was diminished after tenth day of brooding. As there is decline in pH, it demonstrates that cyanobacteria could debase the pesticides show in soil.

Broad and escalated utilization of pesticides in agro-biological community has brought about a worldwide pollution of the earth. Other than battling creepy crawly bothers, bug sprays likewise influence the populace and movement of useful microbial groups in soil (Singh and Parsad, 1991 and Bhuyan et al., 1992). In soil, it might change the microbial exercises (Bhuyan et al., 1992) and have affect on microbial populace (Ambrogioni et al., 1987). This may prompt incitement, diminishment or adjustment of soil organic process, which are basic for soil richness and product yield (Heinonen-Tanski et al., 1985). Plausibility that bug spray buildups in soil may effectsly affect soil microorganisms and their exercises has gotten significant consideration (Iqbal et al., 2001). Bug spray focuses surpassing the ordinary suggested field rates have impact on the dirt microbial populace and their exercises (Tu and Miles, 1976).

CONCLUSION

This review in view of the present overview led, monocrotophos and endosulfan developed as the significant pesticides utilized as a part of the Tuljapur taluka. The physicochemical examination uncovered the impact of pesticides on soil properties. In spite of the fact that, the impact of these pesticides is by all accounts impermanent, the outcomes have gone for malicious impacts. Thin layer chromatographic location technique demonstrated the tirelessness of monocrotophos and endosulfan in the dirt in any event for seven days. These perceptions had provided guidance to the review. In view of these perceptions additionally studies were confined to pesticides, monocrotophos and endosulfan. Pesticide safe cyanobacterial societies were disconnected from chosen soil tests. Strength of non-heterocystous genera over heterocystous cyanobacterial genera was affirmed with before reports which may assume an extraordinary part in bioremediation of pesticide polluted soil.

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