International Archive of Applied Sciences and Technology
Int. Arch. App. Sci. Technol; Vol 9 [4] December 2018 : 62-69

[ISO9001: 2008 Certified Organization]
www.soeagra.com/iaast.html

IAAST
ONLINE ISSN 2277- 1565
PRINT ISSN 0976 - 4828

CODEN: IAASCA ORIGINAL ARTICLE

DOI: .10.15515/iaast.0976-4828.9.4.6269

Isolation and Screening of Pesticide Resistant Rhizobacteria from Wheat (*Triticum aestivum*) Rhizosphere Soil

S. B. Waghmare[1], S. S. Khandare[2*], M. G. Ingale[3]
[1] P.G. Student, Post graduate Dept. of Microbiology, J. B. College of Science, Wardha.
[2] Asso. Professor and Head, Dept. of Microbiology, J. B. College of science, Wardha.
[3]Asstt. Professor, Post graduate Dept. of Microbiology, J. B. College of Science, Wardha
*Corresponding author e-mail: ksuhas21@gmail.com

ABSTRACT
Pesticides are chemical substances that are used to prevent, kill or repell any pest. During application, if not handled properly these pesticides accumulates in soil and water and causes lethal effects on living beings of ecosystem. This problem can be overcome by elimination of pollutants from contaminated sites by using microorganisms. This process of bioremediation is the cheaper alternative to chemical technology. Therefore it is a need to isolate, identify and characterize such microorganisms that are resistant to such pesticides. The present study aims to isolate pesticide tolerating microbes from wheat rhizosphere soil of Waigaon (Nipani) region of Wardha district, Maharashtra. In this study total 10 pesticide resistant bacterial isolates capable of tolerating two pesticides such as Mancozeb 75 % WP (Indofil M-45) is a dithiocarbamate non-systemic agricultural bactericide as well as fungicide and Carbendazim 50 % WP (Camry 767) is broad spectrum benzimidazole systemic fungicide and a metabolite of benomyl were obtained by spot assay enrichment technique. Among them Pseudomonas sp. and Arthrobacter sp. showed highest resistance and were used for further studies. In our study, data revealed that Pseudomonas sp. showed the highest level of tolerance viz. 1900 ppm to pesticide Mancozeb 75 % WP. Highest level of tolerance exhibited by all the strains to Carbendazim 50 % WP and were found to be 1800 ppm. Thus such findings may be useful in designing the multi resistant bacterial consortium that can be used efficiently for bioremediation of pesticides.
Key Words: *Pesticides Tolerance, Mancozeb 75 % WP, Carbendazim 50 % WP, Wheat rhizosphere Rhizobacteria.*

Received 28/07/2018 Revised 25/08/2018 Accepted 24/10/2018

Citation of this article
S. B. Waghmare, S. S. Khandare, M. G. Ingale. Isolation and Screening of Pesticide Resistant Rhizobacteria from Wheat (*Triticum aestivum*) Rhizosphere Soil. Int. Arch. App. Sci. Technol; Vol 9 [4] December 2018. 62-69.

INTRODUCTION

The term pesticide is a composite term that includes all the chemicals that are used to kill or control pests. In agriculture this includes herbcides, insecticides, fungicides, nematocides and rodenticides (1). Millions of tons of pesticides are applied every year in agricultural fields to increase crop production and pest control. Now a day's use of pesticides is increased. Excessive and indiscriminate use can lead to microbial imbalance, environmental pollution and health hazards [2]. Pesticides can reach water through runoff from treated plants and soil. The pesticides leach down to soil and contaminate the ground water or if immobile, they would keep on the top soil where it could accumulate to toxic level in the soil and become harmful to microorganisms (3). The ecosystem is polluted due to widespread use of pesticides in controlling the agricultural pests and accumulation of such pesticides adversely affects the environment [4]. The world health organization data show that only 2-3 % of applied chemical pesticides are effectively used for preventing, controlling and killing pests, while the rest remains in the soil [5]. Pesticides possess

serious threat to ecosystem. Deaths and chronic diseases occurring due to pesticide poisoning are about 1 million per year globally [6]. The microbial degradation of hazardous waste offers promising strategy by which such chemicals may safely be detoxified. For that reason there is need to isolate, identify and distinguish the microorganisms that interact in contaminated environment and to isolate genetic determinants of resistance [7].

Bioremediation constitutes an attractive alternative to physico-chemical methods of remediation, as it is less expensive and can selectively achieve complete destruction of organic pollutants [8]. For bioremediation to be effective, the pollutant must be subject to microbial attack, the metabolite product must be safe and the process must not cause adverse ecological side effects. [9]. Therefore, these toxic compounds have been implicated in various disorders and diseases including cancer, adverse reproductive outcomes, peripheral neuropathies, neurobehavioral disorders, impaired immune functions and allergic sensitization reactions, particularly of the skin, cumulative inhibition of cholinesterase activity because of long-term low doses of exposure [10]. Bacteria have several mechanisms that allow them to be tolerant or resistant to toxic compounds. Bacteria may utilize efflux pumps, which remove toxic compounds from the cell (11). Bacteria may alter surface receptor sites to block entry into the cell or alter the chemical by mechanisms such as methylation. Bacteria may also produce degrading enzymes that metabolize the compound (12).

The present study involves the use of two most widely used pesticides in Waigaon (Nipani) region of Wardha district, Maharashtra, namely Mancozeb and Carbendazim. Mancozeb (Indofil M-45) is a dithiocarbamate non-systemic agricultural fungicide. Carbendazim (Camry 767) is broad spectrum Benzimidazole systemic fungicide and a metabolite of Benomyl. Both of these pesticides can persist in the environment and biomagnifies in the food chain. To cope with elevated concentrations of toxic pesticides present in environment, effective and ecofriendly bioremediation process is required for removing such pesticides from environment. For this reason this investigation was undertaken to study pesticide tolerance capacity of bacteria from wheat rhizospheric soil.

MATERIALS AND METHODS

Soil sample collection

The rhizosphere soil samples from wheat (*Triticum aestivum*) plant were collected from agricultural field of Waigaon (Nipani) region of Wardha district, Maharashtra. These fields had been already sprayed with the pesticides for the past few years. The soil sample was collected from the 2-5 mm deep from surface in sterile sample bag and transported to the laboratory aseptically and processed. The remainder of each sample was preserved at 4 ^{O}C for further analysis.

Isolation of pesticide tolerant bacteria from wheat rhizosphere

Nutrient broth supplemented with 10 mg, 20 mg, 30 mg and 40 mg/L of Mancozeb75 % WP and Carbendazim 50% WP, used for enrichment of microbial populations. The respective flasks were inoculated with 1% (w/v) rhizospheric soil samples collected from wheat (*Triticum aestivum*) and incubated at 28±2°C on an orbital rotary shaker (Remi CIS-24 Plus) at 150 rpm. A series of 10-fold dilutions were prepared down to 10^{-9}. 100 µl from each dilution was plated on Congo red yeast extract mannitol agar plate (CRYEMA). CRYEMA Medium: Yeast extract (1.0 gm/lit), Mannitol (10.0 gm/lit), Dipotassium Phosphate (0.50 gm/lit,) Magnesium Sulphate (0.20 gm/lit), Sodium Chloride (0.10 gm/lit), Congo Red (0.025 gm/lit), Agar (20 gm/lit), pH 6.8 – 7, Distilled water (1L) and autoclaved at 121^{0}C for 20 min. After incubation, plates were observed for different isolates based on morphological traits. Morphologically variable colonies picked up. Pure cultures of the soil isolates were made and preserved on the nutrient agar slants (10).

In vitro analysis of isolates against pesticide tolerance

Nutrient agar medium was used for screening of rhizobacterial isolates for pesticide tolerances. The one litre medium contained beef extract (3.0 gm), peptone (5.0 gm), agar (20.0 gm) pH 7.0 ± 0.2. For screening against Mancozeb 75% WP and Carbendazim 50% WP, the nutrient agar medium was supplemented with variable concentrations of 10, 20, 40, 60, 80 and 100 ppm of both the pesticides and poured in petri plates. Inoculums of Rhizobacterial culture isolates was spotted on the medium plate and incubated for at 37℃ for 24 to 48 hrs. (13).

Identification of efficient pesticide tolerant bacterial isolates.

Isolates showing efficiency to tolerate pesticides were identified firstly on the basis of macroscopic (colony morphology, pigmentation, etc.) and microscopic (Grams reaction, motility, cell shape, etc.) observations. This identification was followed by several biochemical and enzymatic tests. The various biochemical tests performed are, production of enzyme oxidase, catalase, urease, indole, voges-proskaur test, methyl red test, citrate utilization test, H_2S producton test, nitrate reduction test, fermentation of glucose, lactose, mannitol and sucrose (8).

RESULTS AND DISCUSSION

Isolation of pesticide resistant Rhizobacteria

These isolates were initially screened for tolerance to Mancozeeb 75 % WP and Carbendazim 50 % WP with 10 to 40 ppm concentration. In the present study total ten isolates, IS1, IS2 IS3, IS4, IS5, IS6, IS7, IS8, IS9 and IS10 were obtained from rhizospheric soil sample. These isolates were further examined for their efficiency of pesticide tolerance (Table1).

In vitro analysis of isolates against pesticide tolerance

Among ten isolates IS1, IS2, IS3, and IS8 were able to tolerate Mancozeb 75 % WP and Carbendazim 50 % WP with 100 ppm concentration. IS5 shows tolerance only up to 40 ppm for Mancozeb 75 % WP and 60 ppm for Carbendazim 50 % WP as well as IS6 was able to tolerate Carbendazim 50 % WP up to 60 ppm. IS4, IS7, IS9 and IS10 strains demonstrated poor tolerance toward both the pesticides. Therefore only efficient pesticide tolerant isolates (IS1, IS2, IS3 & IS8) were subjected for further study (Table 1). All the four efficient isolates were further tested for tolerance towards Mancozeb75 % WP and Carbendazim 50 % WP at higher concentrations from 200 to 2000 ppm . All the four isolates showed tolerance up to 1800 ppm for Carbendazim 50 % WP, while for for Mancozeb75 % WP IS1, IS2, IS3 and IS8 showed tolerance up to 2000 ppm, 1500 ppm, 1500 ppm and 900 ppm respectively (Table 3 and 4).

Table 1: Screening of isolates for pesticide tolerance.

Pesticides	Concentration (in ppm)	Bacterial isolates									
		IS1	IS2	IS3	IS4	IS5	IS6	IS7	IS8	IS9	IS10
Mancozeb 75 % WP	10	+	+	+	+	+	+	+	+	+	+
	20	+	+	+	-	+	-	-	+	-	-
	40	+	+	+	-	+	-	-	+	-	-
	60	+	+	+	-	-	-	-	+	-	-
	80	+	+	+	-	-	-	-	+	-	-
	100	+	+	+	-	-	-	-	+	-	-
Carbendazim 50 % WP	10	+	+	+	+	+	+	-	+	+	+
	20	+	+	+	-	+	+	-	+	-	-
	40	+	+	+	-	+	+	-	+	-	-
	60	+	+	+	-	+	+	-	+	-	-
	80	+	+	+	-	-	-	-	+	-	-
	100	+	+	+	-	-	-	-	+	-	-

[+ Positive reaction, - Negative reaction]

Biochemical characterization and identification of Rhizobacteria

These isolates were characterized morphologically and biochemically on the basis of cultural, morphological and biochemical characteristics. The isolates IS1, IS2 & IS3 were Gram negative, motile bacilli, forms yellowish creamy colonies on nutrient agar while IS8 is Gram Possitive, non motile also forms forms yellowish creamy colonies on nutrient agar. All four isolates use citrate as a source of carbon ,produce the enzymes catalase, urease and oxidase, produce hydrogen sulphide and able to reduce nitrate. The isolates IS1, IS2 & IS3 demonstrated fluroscence when exposed to UV (Table 2). As described in Bergey's Manual of Determinative Bacteriology. The isolates IS1, IS2 & IS3 were identified as *Pseudomonas* sp. and isolate IS8 as *Arthrobacter* sp.

Table 2: Mancozeb tolerance efficiency of four bacterial isolates.

Isolates	Mancozeb 75 % WP concentration (ppm)																		
	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000
IS1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IS2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
IS3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-
IS8	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-

Table 3: Carbendazim tolerance efficiency of bacterial isolates.

Isolates	Carbendazim 50 % WP concentration (ppm)																		
	200	300	400	500	600	700	800	900	1000	1100	1200	1300	1400	1500	1600	1700	1800	1900	2000
IS1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
IS2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
IS3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
IS8	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-

Table 4: Cultural and Biochemical characteristics of pesticide tolerant bacterial isolates

Tests	Pesticide tolerant bacterial isolates			
	IS1	IS2	IS3	IS4
Grams Reaction	Gram Negative bacilli	Gram Negative bacilli	Gram Negative bacilli	Gram Possitive bacilli
Motility	Motile	Motile	Motile	Non motile
Colony on Nutrient agar	Circular	Circular	Circular	Circular
Color	Yellowish	Cream	Cream	Yellowish
Glucose	-	+	+	+
Sucrose	-	-	-	-
Lactose	-	+	+	+
Maltose	+	-	-	-
Mannitol	-	-	-	-
H_2S Production	+	+	+	+
Nitrate reductase	+	+	+	+
Indole test	-	-	-	-
Methyl Red test	-	-	-	-
Vogues- Proskauer test	-	-	-	-
Citrate utilization test	+	+	+	+
Urease	+	+	+	+
Oxidase	+	+	+	+
Catalase	+	+	+	+
Fluorescence	+	+	+	-

In the present study we have isolated four isolates that could tolerate pesticide concentration as high as 1800 ppm of Carbendazim 50% WP and tolerate 900 to 2000 ppm of Mancozeb 75 % WP. The isolates demonstrated varied efficiency for two different types of pesticides. Tolerant bacterial isolates showed some degree of diversity with regards to their nutrient utilization, biochemical phenotypes and tolerance toward both pesticides, despite somewhat similar colony and cell morphologies. This gives support to the hypothesis that diverse indigenous microbial communities will increase the likelihood that these pesticides are metabolized (14). Several other studies showed that bacterial isolates have the ability to use specific pesticides as a sole source of carbon, nitrogen or phosphorus. These results strongly suggested that genes responsible for the ability to metabolize various pesticides might be plasmid mediated (2). Data revealed that bacteria closely associated with roots of wheat (*Triticum aestivum*) plants and the immediate surrounding soil significantly tolerates two commonly used pesticides in wardha region viz. Carbendazim 50 % WP and Mancozeb 75 % WP. Growth of *Pseudomonas* was confirmed on King's B medium on which all the strains showed fluorescence under UV light.

In one of the study of Singh *et al.* [15] total eight isolates belonging to *Bacillus* sp., *S. aureaus* and *Aspergillus* sp. isolated from various soil samples found to degrade pesticide Dimethonate. In another study conducted by Naphade *et al* [8] five soil isolates namely *Pseudomonas psychrophila, Devoshia yakushimensis, Paracoccus chinensis, Planococcus rifietoensis, Pseudomonas aeruginosa* were found to tolerate two pesticides (Endosulfan, Cypermethrin and Chlorpyrifos) efficiently. These isolates could tolerate endosulfan up to 19000 ppm, cypermethrin up to 15000 ppm and chlorpyrifos up to 15000 ppm .

Hussaini *et al.* [16] during his study isolated six bacterial strains which were found to show significant ability to carry out the degradation of selected pesticides (Chlorpyrifons and malathion), these cultures were identified as *Acinetobacter radioresistens, Pseudomonas frederiksbergensis, Bacillus pumilis, Serratia liquefaciens, Serratia marcescens, Burkholderia gladioli.* Pesticide resistant cyanobacterial cultures were also isolated from selected soil samples from Baramati region of Maharashtra by Pawar *et al.* [17].These cyanobacterial cultures were found to effectively tolerate Monocrotophos (900 mg/L) and Endosulfan (500 mg/L).Tyagi *et al.* [18] demonstratd that ,*Serratia nematodifila,* a cypermethrin degrading bacteria isolated from rhizospheric soil of cauliflower found to use cypermethrin as sole source of carbon and degraded 97.5% of the pesticide added at an initial concentration of 100 ppm in FTW minimal salt medium in 7 days.

In the present study of tolerance to pesticides by Rhizobacteria, showed that *Pseudomonas* sp. and *Arthrobacter* sp. could be a promising candidate for bioremediation of pesticides in regions with high levels of pesticide, Mancozeb 75 % WP and Carbendazim 50 % WP.

Fig.1: Tolerance efficiency of isoalates to Manconezeb 75 % WP & Carbendazim 50 % WP.

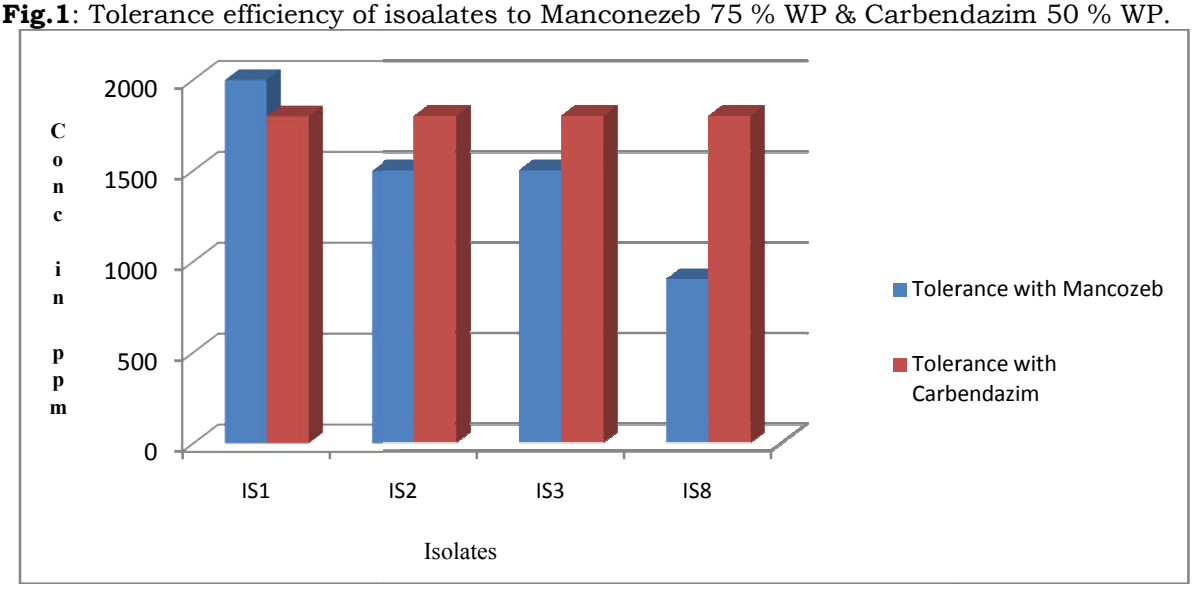

Fig 2: IS1, IS2, IS3 & IS8 tolerate 900 ppm with Mancozeb 75 % WP.

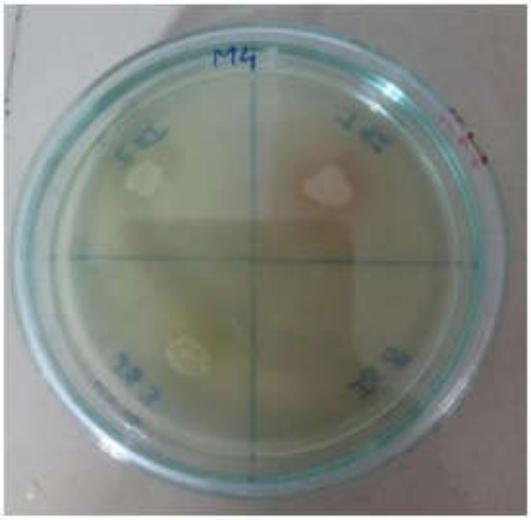

Fig 3: IS1, IS2 & IS3 tolerate 1500 ppm with Mancozeb 75% WP.

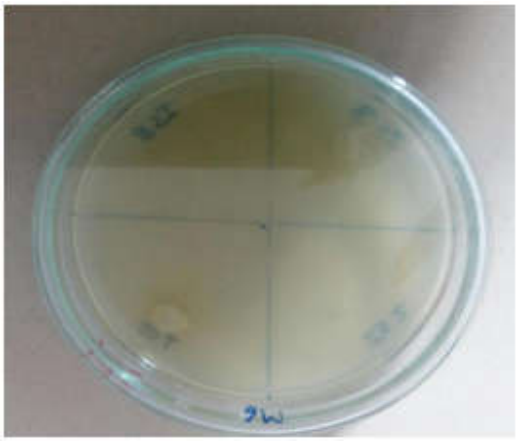

Fig 4:IS1 tolerate 2000 ppm with Mancozeb 75% WP.

Fig 5:IS1, IS2, IS3 & IS8 tolerate 1800 ppm with Carbendazim 50 %WP.

Fig 6: Colonies of *Pseudomonas* sp. on King's B plates observed under UV.

Fig 7: Colonies of *Arthrobacter* sp. on on nutrient agar.

Fig 8: Mancozeb 75% WP

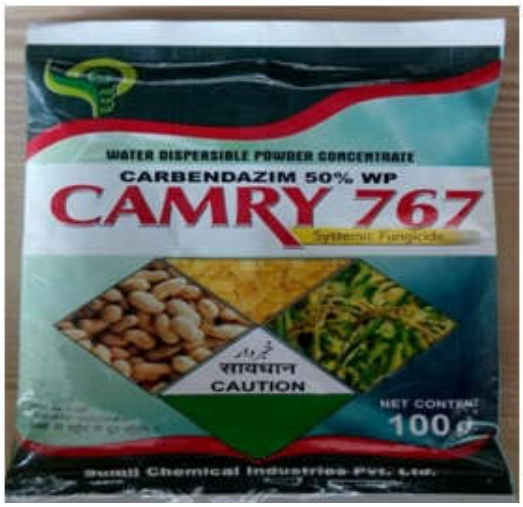

Fig 9: Carbendazim 50% WP.

CONCLUSION

The results of the present study are quite encouraging and shows that the bacteria isolated from wheat rhizosphere are able to tolerate Mancozeb 75 % WP and Carbendazim 50 % WP pesticides efficiently and may therefore be used for bioremediation of pesticide contaminated soil. These isolates could be used as a microbial consortium for efficient removal of pesticides from environment.

REFERENCES

1. Rehmat H. Enzyme based removal of pesticides from soil and water: A review. http:// www.ejmanager.com/mnstemps.
2. Rashmi, P. & Joseph D. (2015). Isolation of Pesticide Tolerating Bacteria from Cultivated Soil in Kerala and the Study of the Role of Plasmid in Pesticide Tolerance. Int. J. Of Pure App. Biosci.3 (1): 109-114.
3. Amakiri, M.A. (1982). Microbial degradation of soil applied herbicides. J.Microbiol. 2:17-21.
4. John, P. J. (2007). Alteration of certain blood parameters of freshwater teleost Mystus vittatus after chronic exposure to Metasystox and Sevin, Fish Physiol. And Biochem.33:15-20.
5. World health organization (1990). Public health impacts of pesticides used in agriculture. http:// apps.who.int./iris/handle/10665/39772.
6. Environews Forum (1999). Killer environment. Environ health perspect.107 (2): A62-A63.
7. Akhter, M. & Laz R. (2013). Isolation and molecular characterization of pesticide (Fenitrothion) resistant bacteria from agricultural field. IOSR Journal of Pharmacy. 3 (5): 31-38.
8. Naphade, S.R., Durve, A.A., Bhot, M., Varghese, J. & Chandara, N. (2012). Isolation, characterization and identification of pesticide tolerating bacteria from garden soil. Euro J Exp Bio.2(5):1943-1951.
9. Tewari, L.,Saini, J. & Arti. Bioremediation of Pesticides by Microorganisms: General Aspects and Recent Advances. http://www.semanticscholar.org/paper. 25-48.
10. Warsi, M., Bee. N., Hashim, M., Ahmad, A.,Kumar, U. & Khan, S. (2017). Isolation and molecular identification of pesticide tolerant bacteria in agriculture soil of rampur (U.P). International Journal of Bio-Technology and Research. 7 (1): 25-40**.**
11. Silver, S. & Phung L. (1996). Bacterial heavy metal resistance:New surprises. Ann.Rev.Microbiol. 50:753-789.
12. Bentley,R. & Chasteen T. (2002). Microbial methylation of metalloids: Arsenic, antimony and bismuth. Microbiol.Mol.Biol.Rev.,66:250-271.
13. Shadab,A.,Kumar, A.,Kumar,A.,Prasad, S.,Tiwari, A.,Shrivastava, D., Shrivastava,S.,Tiwari, P., Singh, J. & Mathur, B. (2018). Isolation and Characterization of Pesticide Tolerant Bacteria from Brinjal Rhizosphere. Int.J.Curr.Microbiol.App.Sci.,7: 4849-4859.
14. Aguirre, S. & Lowe, K.(2010). Pesticide-Tolerant bacteria isolated from agricultural canals in the lower rio grande valley of south texas. Online J. Biol.Sci.,10 (3): 126-135.
15. Singh, S & Iyer P.R. (2017). Isolation and characterization of microorganisms that degrade dimethonate. J Bioremediat Biodegrad. 8:410.
16. Hussaini, S.Z., Shaker, M. & Iqbal M.S. (2013). Isolation of Bacteria for Degradation of Selected Pesticides. *Adv. Biores.,*4(3), 82-85.

17. Pawar, S.T. (2015). Isolation and screening of pesticide resistant cyanobacteria from pesticide contaminated soil. Phycos. 45(1):85-84.
18. Tyagi, H. & Prashar P.(2015). Isolation and identification of cypermethrin degrading Serratia nematodiphila from cauliflower rhizosphere. Int. J. Pharmtech Res.,7.(1): 64-71.