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Prevalence of plasmid mediated pesticide resistant bacterial assemblages in crop fields

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Abstract: *Three crop fields namely, paddy, sugarcane and tomato exposed to bavistin [Methyl (1H-benzimidazol-2-yl) carbomate], monocrotophos [Dimethyl (E)-1-methyl-2-(methyl-carbamoyl) vinyl phosphate] and kinado plus [(EZ)-2-chloro-3-dimethoxyphosphinoyloxy-X1, X1-diethylbut-2-enamide], respectively were chosen for the present investigation to know the bacterial population and degradation of pesticides. The chemical nature of the soil and water samples from the pesticide contaminated fields was analysed along with counting of the total heterotrophic bacteria (THB), Staphylococci and Enterococcci population. Mean calcium, phosphate and biological oxygen demand were maximum in tomato field water. Field water recorded maximum phophate and silicate content, whereas, sugarcane field water elicited maximum dissolved oxygen content. On the other hand, available phosphate and exchangeable potassium were maximum is sugarcane field soil. Significant variations in the bacterial population were evident between the treatments in sugarcane field soil and tomato field water exposed to monocrotophos and kinado plus, respectively. In addition, significant variations between THB, Staphlyococci and Enterococci population were also evinced in both the sugarcane and tomato fields. The dominant pesticide resistant bacteria, Staphylococcus aureus, Enterococcus faecalis and Pseudomonas aeuroginosa harboured plasmids and the resistant trait observed were found to be plasmid borne.*

Key words: *Crop fields, Pesticide resistant bacteria, Kinadoplus, Bavistin, Moncrotophos, Plasmid*
PDF of full length paper is available online

Introduction

The indiscriminate and unplanned use of agrochemicals (El- Bestway *et al*., 2000) influence microbial processes that are an essential component of carbon, nitrogen and sulphur cycles. Monocrotophos is an organo-phosphorus insecticide, which finds its main use for foliar application to cotton (over 80% of monocrotophos applied). It is also recommended for application against foliage pests of maize, sugar cane, sugar beet, vegetables, potatoes and certain fruits. It is particularly effective against Lepidoptera, Homoptera and certain Coleoptera, acting by both systemic and residual contact properties. Bavistin is a broad spectrum, systemic fungicide for control of fungal diseases in various crops like paddy, wheat, chick pea (Sunita Gaind *et al.*, 2007) and kinado plus is used as an insecticide in tomato crop. These pesticides are known to induce changes in the microbial processes in agroecosystems. For instance, monocrotophos and bifenthrin and acetamiprid enhanced the bacterial population in cotton agroecosystem (Zafar Iqbal *et al.*, 2001). Monocrotophos affect the entomopathogenic microorganisms like *Bacillus thuringiensis*, *B. bassiana*, *M. anisopilae and S. insectorum* (Batista filho Antonio *et al.*, 2001). Bavistin induce transfection inhibition in *Mycobacterium smegmatis* (Pandita, 1988). The stimulation/ inhibition of nitrogen fixation of *Azospirillum* depended upon the nature, level and mode of pesticide application to the field soil. Furthermore, certain pesticides even at close to field application rates, may affect distinct changes in the microbial functions of a flooded soil. Bavistin when applied to chick pea (*Cicer arietinum*) and wheat (*Triticum aestivum*) seeds, declined the viable population of phosphate solubilising bacteria, *Pseudomonas striata* and *Bacillus polymyxa* (Sunita Gaind *et al.*, 2007). Bacteria like *Pseudomonas*, *Arthrobacter*, *Ralstonia* and *Rhodococcus* (Noordman and Janssen, 2002; Arnett *et al.*, 2000; Uragami *et al*., 2001; Widada *et al*., 2002 a,b), *Acinetobacter* (Margesin *et al.*, 2003); *Agrobacterium* (Horne *et al.*, 2002), *Methylobacterium* (Aken *et al.*, 2004) and *Alcaligenes* (Padmanaban *et al*., 2003) have amazing property to degrade xenobiotics, through evolution of new genes, which encodes enzymes that can use these compounds as their primary substrate (Suenaga *et al*., 2001).The survival of these bacteria under pesticide stress can provide an efficient, cheaper and eco-friendly solution for bioremediation of these xenobiotics contaminated soil (Hirano *et al*., 2004). The objective behind the present study was to assess the physico-chemical nature of the crop field soil and water. The bacteriological parameters like total heterotrophic bacteria, *Staphylococci*, *Enterococci* population were elucidated. The dominant bacteria screened were subjected to plasmid analysis. In addition, plasmid curing experiments were performed to precisely detect whether the pesticide resistant prevalent in these bacteria were plasmid or chromosomal DNA mediated.

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Materials and Methods

Sampling site: Soil and water samples in triplicate were collected in sterile autoclaved glass bottles from different agriculture fields namely, tomato, paddy, sugarcane exposed to kinadoplus, bavistin and monocrotophos respectively. Chemical analysis of the water samples were performed according to standard procedures (APHA, 1998). Selective physico-chemical properties of soil were explored by the following methods on air-dried samples: total nitrogen by Kjeldahl method (Bremner, 1965), available phosphate (Olsen and Sommers, 1982), and exchangeable potassium (Rowell, 1996).

Inoculation of bavistin, monocrotophos and kinado plus in field water and soil: The soil (S_A, S_B and S_C) and crop field water samples (W_A,W_B and W_C) from pesticide contaminated fields of paddy, sugarcane and tomato were randomly collected up to the depth of 10 cm and transported to the laboratory. 10 g of soil and 10 ml of water were aerobically incubated in 50 ml sterile salt media into duplicate 100 ml cotton plugged flasks containing 0.0, 0.02,0.04 and 0.08 g each of bavistin, monocrotophos and kinadoplus, with continuous shaking at room temperature for one week and observed for viability. Minimal salt media composed of (per liter of distilled water): $CaCl_2$:0.02 g, MgCl:0.2 g, K_2HPO_4:1.0 g, KH_2PO_4:1.0 g, NH_4NO_3:1.0 g and $FeCl_3$:trace (Sigma) in DH_2O, with pH=7.2-7.4 up to 1 liter were used for the incubation of soil and water samples.

Selection of pesticide resistant bacteria: The total heterotrophic bacteria (THB), *Staphylococcus* spp. and *Enterococcus* spp. were cultured and the bacterial isolates were identified according to the procedures described in Bergeys manual of determinative bacteriology (Sneath *et al*., 1994).

Plasmid DNA analysis: The bavistin contaminated paddy field was found to exhibit highest bacterial count when compared to other fields. Hence, the dominant bacteria, *Escherichia coli*, *Pseudomonas aeuroginosa* and *Staphylococcus* sp. were isolated from the paddy field and subjected to plasmid analysis. Plasmid DNA was extracted by alkaline lysis method (Maniatis *et al*., 1982) and analysed by gel electrophoresis on 1.2% (w/v) agarose gels stained with ethidium bromide and visualized under UV light.

Plasmid curing: Plasmid curing was attempted by adding acridine orange at sub-inhibitory concentrations (Hahn and Chiak, 1976) to 5 ml Luria broth, to which 0.1 ml of an 18 or 6 hr inoculums was then added. After incubation for 18 hr at 37°C, pour plates were made on nutrient agar and nutrient agar with bavistin. In addition 5ml luria broth containing no curing agent but inoculated and treated as described above was used for comparison.

Statistical analysis: The mean and standard error for the chemical parameters of the field and water samples were calculated. Bacterial populations and treatments were compared using analysis of variance (Two way-ANOVA) and Duncan new multiple range test (DMRT) was applied to test the significance of means by SPSS version 16.0.

Results and Discussion

Chemical parameters of the paddy, sugarcane and tomato field water and soil: The chemical parameters of the water samples of the paddy, sugarcane and tomato field contaminated with bavistin, monocrotophos and kinadoplus respectively, are presented in Table1. Among the crop fields, maximum mean calcium content was registered in tomato field water (0.116 ± 0.00047 mg l^{-1}), followed by paddy field water (0.0755 ± 0.00006 mg l^{-1}). The mean nitrite content of the paddy field water was maximum (6.22 ± 0.04041 mg l^{-1}) when compared to sugarcane field water (3.77 ± 0.03215 mg l^{-1}) and tomato field water (3.3 ± 0.05774 mg l^{-1}). It was interesting to note that there was no drastic fluctuation observed in the mean phosphate content between the crop field water (Paddy field:4.5 ± 0.05925 mg l^{-1}; sugarcane field:4 ± 0.05774 mg l^{-1}; tomato field :4.5). Paddy field registered maximum mean silicate content (0.950 ± 0.00333 mg l^{-1}), followed by tomato field (0.6 ± 0.01667 mg l^{-1}). Least mean silicate content was registered by sugarcane field water (0.4 ± 0.00577 mg l^{-1}). It was noticed that there was not much variation in the mean dissolved oxygen content of the sugarcane (1.40 ± 0.04410 mg l^{-1}) and tomato (1.12 ± 0.00577 mg l^{-1}) field water. On the other hand, paddy field water recorded minimum mean dissolved oxygen of 0.845 ± 0.00577 mg l^{-1}. The data pertaining to mean BOD content reveals that tomato field water elicited maximum of 1.654 ±0.01784 mg l^{-1} when compared to the paddy field water (0.563 ± 0.00780 mg l^{-1}) and sugarcane field water (0.113 ± 0.00100 mg l^{-1}).

The mean total nitrogen content was found to be high in paddy field soil (95.2 ± 0.0917 K/A).Tomato and sugarcane field soil registered mean total nitrogen content of 89.6 ± 0.18559 K/A and 82.6 ± 0.20008 K/A, respectively (Table 1). Similar trend was observed with mean available phosphate content of paddy and tomato field soil (7.0±0.14530 K/A and 7.0±0.16667 K/A, respectively). Maximum mean available phosphate was exhibited by sugarcane field soil (8.5±0.08819 K/A). The mean exchangeable potassium of the paddy and tomato field soil were almost similar (209±0.33333 K/A and 208.2±0.50000 K/A, respectively). Sugarcane field soil registered highest mean exchangeable potassium content of 218±0.50000 K/A.

Bacteriological profile of the paddy, sugarcane and tomato field water and soil: Total heterotrophic bacteria (THB), *Staphylococci* and *Enterococci* populations were enumerated and presented in Table 2. Higher bacterial counts (Too Numerable To Count-TNTC) were evinced in the paddy field soil. On the other hand, THB and *Staphylococcus* were found to exhibit higher count (TNTC) in paddy field water. Moreover, there was no *Enterococci* in the paddy field water. Hence the data displayed in Table 2 could not be subjected to statistical analysis.

Total heterotrophic bacteria (THB), *Staphylococci* and *Enterococci* populations were elucidated (Table 3b).There existed a significant variation between THB, *Staphylococci* and *Enterococci* bacterial population in sugarcane field water at p<0.05 level (Two

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Table- 1: Chemical analysis of the water and soil samples of crop fields contaminated with pesticides

Parameter	Crop field water		
	Paddy	Sugarcane	Tomato
Calcium (mg l^{-1})	0.0755 ±0.00006	0.0714 ±0.00214	0.116±0.00047
Nitrite (mg l^{-1})	6.22±0.04041	3.77±0.03215	3.3 ± 0.05774
Phosphate (mg l^{-1})	4.5 ± 0.05925	4.0±0.05774	4.5± 0.03333
Silicate (mg l^{-1})	0.95 ± 0.00333	0.4 ± 0.00577	0.6 ± 0.01667
Disolved oxygen (DO) (mg l^{-1})	0.845 ± 0.00577	1.40± 0.04410	1.12 ±0.00577
Biological oxygen demand (BOD) (mg l^{-1})	0.563± 0.00780	0.113 ± 0.00100	1.654 ±0.01784
		Crop field soil	
Total nitrogen (K/A)	95.2 ± 0.09171	82.6 ± 0.20008	89.6 ± 0.18559
Available phosphate (K/A)	7.0 ± 0.14530	8.5 ± 0.08819	7.0 ± 0.16667
Exchangeable potassium (K/A)	209 ± 0.33333	218 ± 0.50000	208 ± 0.50000

K/A-kilo/acre, Values are mean ± Standard error

Table - 2: Bacteriological profile of soil and water samples from paddy field exposed to Bavistin

Bacteria	Soil				Water			
	Control (cfu g^{-1})	0.02 g (cfu g^{-1})	0.04 g (cfu g^{-1})	0.08 g (cfu g^{-1})	Control (cfu ml^{-1})	0.02 g (cfu ml^{-1})	0.04 g (cfu ml^{-1})	0.08 g (cfu ml^{-1})
THB	TNTC	TNTC	TNTC	TNTC	TNTCX10^6	TNTCX10^6	TNTCX10^6	TNTCX10^6
Staphylococcus	TNTC	TNTC	TNTC	TNTC	TNTCX10^6	TNTCX10^6	TNTCX10^6	TNTCX10^6
Enterococcus	TNTC	TNTC	TNTC	TNTC	Nil	Nil	Nil	Nil

Cfu = Colony forming units, THB = Total heterotrophic bacteria, TNTC = Too numerable to count

way ANOVA, bacteriological parameters, F=45.118; between treatments, F=0.477; interaction, F=136.390). *Staphylococci* registered highest mean count of 446677 cfu ml^{-1}, followed by THB (1600000 cfu ml^{-1}) and *Enterococci* (32333.33 cfu ml^{-1}). On the other hand, significant variations were not detected among treatments in field water (Table 3a).

Statistically significant variation were evident between bacteriological parameters with regard to sugarcane field soil exposed to monocrotophos at p<0.001 level (Two way ANOVA, bacteriological parameters, F=15.642; between treatments, F=4.010; interaction, F=30.036). As in the case of sugarcane field water, soil also registered significant rise in the mean *Staphylococci* population (3E+007 cfu ml^{-1}), when compared to THB (4333333 cfu ml^{-1}) and mean *Enterococci* population (1501667 cfu ml^{-1}) (Table 3b). Control and 0.02 g dosage of monocrotophos registered maximum mean bacterial count 2E+007 cfu ml^{-1} (Table 3a).

The impact of kinadoplus on the tomato field soil bacteria reflected significant variation between the bacteriological populations at p<0.001 level (Two way ANOVA, bacteriological parameters, F=166.325; between treatments, F=0.533; interaction, F=171.602). *Staphylococci* bacteria registered highest count of 4E+011 cfu ml^{-1} when compared to *Enterococci* (5E+009) and THB (3E+007) (Table 4b). No significant variation was evident between the treatments in tomato field soil (Table 4a).

Significant variation between the bacteriological parameters and treatments were observed with regard to tomato field water. (Two way ANOVA, bacteriological parameters, F=33.971; between treatments, F=5.084; interaction, F=57.953). Except 0.04 g (3E+007), all the other treatments exhibited significantly (p<0.01) highest mean bacterial count (2E+008 cfu ml^{-1}) (Table 4a). Mean *Staphylococcus* bacteria were found to be significantly higher (4E+008 cfu ml^{-1}) than THB (7E+007 cfu ml^{-1}) and *Enterococci* (2500000 cfu ml^{-1}) at p<0.001% level of significance (Table 4b).

The Bavistin resistant bacteria were found to be *Enterobacter* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Achromobacter* sp. and *Enterococcus faecalis* in soil samples of paddy field. Water samples were composed of *Eschericha coli*; *Enterobacter* sp., *Pseudomonas aeruginosa*, *Acinetobacter* sp., S*taphylococcus aureus* and *Bacillus* sp., monocrotophos resistant bacteria were *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa Staphylococcus aureus* and *Bacillus* species in the soil of sugarcane field. On the otherhand, water samples constituted *Escherichia coli*, *Enterobacter* spp., *Klebsiella pneumoniae, Enterococcus faecalis* and *Bacillus* spp (Table 5).

Kinado plus resistant bacteria were *Escherichia coli, Klebsiella pneumoniae., Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus* species in tomato field soil, whereas water contained, *Escherichia coli, Enterobacter* species, *Klebsiella pneumoniae, Enterococcus facealis* and *Bacillus* sp.

Plasmid analysis and curing: Since higher counts of bacteria were registered in paddy field soil exposed to bavistin, the dominant bacteria namely, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphlococcus sp.* were selected and subjected to plasmid isolation and analysis. The plasmid profile as observed in the agarose gel

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Table - 3a: Impact of different doses of monocrotophos on the sugarcane field water and soil bacteria

Treatments	Sugarcane field soil bacteria (cfu ml^{-1})	Sugarcane field water bacteria (cfu ml^{-1})
Control	2E± 007a	2380000a
0.02g	2E± 007a	2153333a
0.04g	4142222b	2205556a
0.08g	349444b	178111a
F-test	*	N S

Table - 3b: Impact of monocrotophos on the bacteriological profile of sugarcane field water and soil

Bacteria	Sugarcane field soil	Sugarcane field water
THB (cfu ml^{-1})	4333333b	1600000b
Staphylococci (cfu ml^{-1})	3E+007a	446677a
Enterococci (cfu ml^{-1})	1501667b	32333.33c
F- test	***	***

cfu = Colony forming units, E = Exponent, NS = Non significant, * = Significant at p<0.05, *** = Significant at p<0.001.
In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

Table - 4a: Impact of different doses of kinado plus on the tomato field water and soil bacteria

Treatments	Field soil bacteria (cfu ml^{-1})	Field water bacteria (cfu ml^{-1})
Control	2E± 011a	2E± 008a
0.02g	1E± 011a	2E± 008a
0.04g	1E± 011a	3E± 007b
0.08g	2E± 011a	2E± 008a
F-test	N S	**

Table- 4b: Impact of kinado plus on the bacteriological profile of tomato field water and soil

Bacteria	Field soil	Field water
THB (cfu ml^{-1})	3E± 007b	7E± 007a
Staphylococci (cfu/ ml)	4E± 011a	4E± 008a
Enterococci (cfu/ ml)	5E± 009b	2500000b
F- test	***	***

Cfu = Colony forming units, E- Exponent, NS = Not Significant, ** = Significant at p<0.01, ***Significant at p<0.001
In a column, figures having dissimilar letters differ significantly according to Duncan New Multiple Range Test (DMRT)

electrophoresis revealed that the bavistin resistant *Escherichia coli* (L2-lane2), *Pseudomnas aeruginosa* (L3-lane3) and *Staphylococcus sp.* (L1-lane1) in soil indicates plasmid size of 240 kb (Fig. 1). Further after confirmation of the presence of plasmids in these bacterial isolates, they were subsequently subjected to curing experiments. After curing the plasmids from *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus sp.* they were exposed to bavistin and cultured and analysed for their pesticide resistant trait. Moreover, it was found that all three isolates were found to be susceptible to bavistin and lost their pesticide resistant potential (Table 6). Thus the above demonstration permits us to conclude that bavistin resistance prevalent in *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus sp*. were plasmid borne.

Relatively, paddy field water recorded maximum mean nitrite, phosphate and silicate content and tomato field water elicited maximum mean calcium, phosphorus, dissolved oxygen and biological oxygen demand. Among the crop field soil, maximum mean nitrogen was observed in paddy field, whereas, maximum mean phosphate and potassium content was registered in sugarcane field.

The yield and uptake of nutrients by crops is largely governed by the nutrient supply system of the soil through native and applied sources and their losses through leaching, weed infestation *etc.* (Kannaiyan, 1999). However, the native soil fertility of most tropical soils is low owing to the increased frequency of cultivation of land as demand for food increases, unsustainable nature of the bush fallow practices of naturally restoring the fertility status of the soil due to reduced fallow period occasioned by high population pressure and other human activities (Steiner, 1991), coupled with erosion, volatilization and immobilization (Law-Ogbomo and Remison, 2008; Molindo, 2009). N, P and K are primary nutrients in the soil (Chude *et al.*, 2004) because of their acute deficiencies in most light-textured soil and their application in the soil form the basis of applying the secondary and trace nutrients in the soil (Law-Ogbomo and Remison, 2007). Law-Ogbomo and Remison, (2009) have registered total nitrogen of 0.08 and 0.23% during the year 2005 and 2006, respectively in Benin City, Nigeria. They also recorded available phosphorus (8%:2005; 4%:2006) and exchangeable potassium (0.06 cmol Kg^{-1} :2005; 0.05 cmol Kg^{-1}: 2006).

The total nitrogen, extractable phosphorus and extractable potassium content of National Sugar Crops Research Institute (NSCRI), farm Thatta, Pakistan were 0.021%, 6.80 mg Kg^{-1} and 265 mg Kg^{-1}, respectively (Panhwar *et al.*, 2003). The soil at Adeyemi College of Education Research farm registered total nitrogen, phosphorus and potassium content of 0.12%, 7.23 cmol Kg^{-1} and 0.39 cmol Kg^{-1} (Ayeni, 2008).

Nutrient availability in the soil- plant system is dictated by complex interactions (or competition) between plant roots, soil microorganisms, chemical reactions and pathway of losses. The concentration dependents of most of the processes that nutrients undergo in soil include transformations induced by microbes (N_2 fixation, nitrification, denitrification, immobilization *etc.*), chemical processes (exchange, fixation, precipitation, hydrolysis, *etc.*) and physical processes (leaching, run off, volatilization *etc.*) (Jagadeeswaran *et al.*, 2005). Our results coincides with findings of Suett (1994) who have reported that insecticide exposure can have a significant impact on the soil microbial populations. Moreover, the activities of the soil microbial biomass are of paramount importance in determining the rate of degradation of pesticides applied. Similarly, Siddique *et al.* (2003) have isolated endosulfan degrading bacteria

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Table - 5: Prevalence of pesticide resistant bacteria in various fields

	Samples	
	Soil	**Water**
Paddy+bavistin	*Enterobacter* sp., *Pseudomonas aeruginosa, Bacillus* sp., *Achromobacter* sp., *Enterococcus faecalis*	*Escherichia coli, Enterobacter* sp., *Pseudomonas aeruginosa,* Acinetobacter sp., *Staphylococcus aureus, Bacillus sp.*
Sugarcane+ monocrotophos	*Escherichia coli, Klebsiella sp., Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp.	*Escherichia coli, Enterobacter* sp., *Pseudomonas aeruginosa, Enterococcus faecalis, Bacillus* sp.
Tomato+ kinado plus	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp.	*Escherichia coli, Enterobacter* sp., *Klebsiella pneumoniae, Bacillus* sp., *Enterococcus faecalis*

Table 6: Plasmid curing of bavistin resistant bacteria in paddy field soil

Bacteria	**Pre-curing**	**Post-curing**
Eschericha coli	+	- - -
Pseudomonas	+	- - -
Staphylococcus	+	- - -

+ = Presence of pesticide resistant, - = Loss of pesticide resistant

and fungi in media provided with endosulfan as sole source of carbon. Flooded rice soils harbour a myriad of microorganisms (aerobic and anaerobic) capable of mineralizing many of these pesticides and their degradation products to harmless end products in pure cultures and soils. *Arthrobacter* accelerated the degradation of carbofuran. Tipton *et al*. (2003) and Ohshiro *et al*. (1996) have reported that the soil that had been exposed to various xenobiotics previously, had a greater capacity to degrade such compounds and harbour a greater number of microorganisms than the soil that had not been exposed to similar compounds. Furthermore, soil microorganisms also play an important role in the dissipation of xenobiotic pesticides. Biodegradation of xenobiotics can be influenced by soil properties including pH, organic matter content, seasonal climatic factors, such as soil moisture contents and temperature (Caux *et al*., 1993). Organic fertilizer treatments (cow manure, composts or green manure) simultaneously increase insecticide adsorption onto soil and the insecticide soil persistence, indicating a mechanism of slow release of insecticide into soil by the organic matter (Rouchaud *et al.,* 1996). The variation in bacterial population observed in the crop fields could be attributed to variation in microbial adaptation in the presence of insecticides. The present findings is in accord with Sutherland *et al*. (2002) who have isolated bacteria from the sample of fertile grey clay (pH 7.5) top soil, obtained from a cotton field near Wee Wad, NSW, Australia, at the end of the growing season. The field had generally received several applications of pesticides. At higher doses of bavistin, reduction in the bacterial population was evinced in paddy field soil.

There is much evidence that, in soil, larger doses of insecticides degraded proportionately more slowly than smaller doses in the presence of adapted microorganisms (Suett, 1994). The present result gains support from the findings of Shakoori *et al.* (2000) who have isolated sixteen bacterial strains capable of tolerating carbosulfan and quinalphos upto the concentration of 0.4%.

The persistence of *Bacillus* spp. in the pesticide contaminated paddy, tomato, sugarcane field is in agreement with many researchers. *Bacillus* spp., *Arthrobacter* spp., *Rhodococcus* spp., bacteria capable of utilizing toxic xenobiotics including chlorinated insecticides have been reported from other laboratories (Annweiler *et al*., 2000; Shakoori *et al*., 1999; 2002; Awasthi *et al*., 2003; Kazunga and Aitken, 2000; Ohshiro *et al*., 1997; Cullington and Walker, 1999; Turnbull *et al*., 2001). Our findings also coincide with that of Padmanaban *et al.* (2003) who have reported that Alcaligenes are capable of degrading xenobiotic compounds. Furthermore, our findings also gains support from the observations of Shakoori *et al*. (2000, 1999) who have reported gram-positive and gram negative bacterial strains (rods and cocci) from industrial effluents and insecticide contaminated soil. The present observation is in good accord with Kaempfer *et al*. (1994) who had reported that gram positive bacteria *Coryneforms* and *Bacilli* from upper layer and gram negative bacteria such as *Pseudomonas* and *Aeromonas* sp. from aquifers. Further, the pesticide resistant *Pseudomonas* spp. and *Bacilli* spp. evinced in this study are well supported by Spain, 1995; Rangaswamy and Venkateswarlu (1992) and Edwards *et al.* (1992) who have isolated insecticide degrading *Pseudomonas* spp., *Bacillus* spp., *Blastobacter* spp. and *Cyanobacter* spp from insecticide contaminated samples. The dominant bacterial isolate resistant to bavistin namely, *E.coli, Pseudomonas aeruginosa* and *Staphylococcus sp.* possessed a single plasmid size of 240 kb. In addition, plasmid borne pesticide resistant bacteria was evinced in this study. This observation is well supported by Floodgale (1991) who had isolated such degrading strain which carry a number of plasmids. The present finding coincides with that of Fujita *et al.* (1993a;1993b) who have emphasized that plasmid harbouring bacteria are responsible for waste water clean-up. Furthermore, the plasmid mediated pesticide resistance evinced in this study has been proved by Head *et al.* (1992) and Furukawa *et al.* (1998) who have demonstrated the plasmid (199kb) mediated carbamate and biphenyl degrading potential of bacteria. Similarly,

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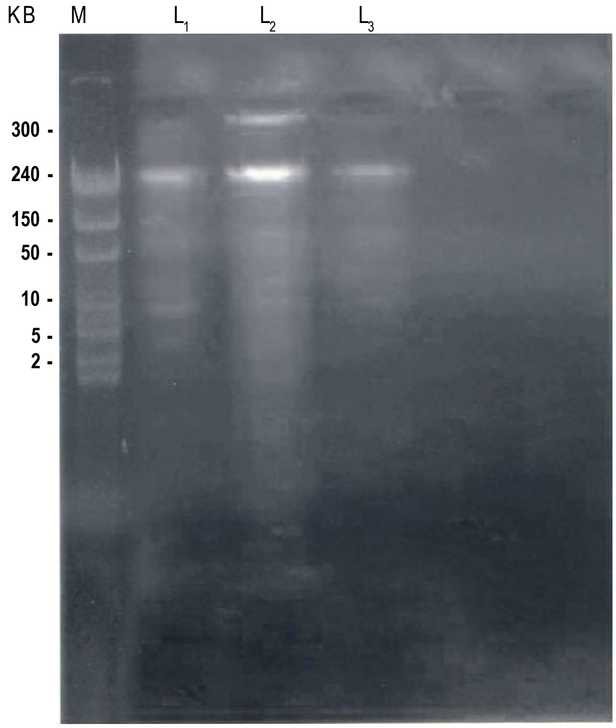

M = Marker plasmid, L_1 - Plasmid of staphylococcus sp.,
L_2 = Plasmid of E. coli, L_3 = Plasmid of pseudomonas

Fig. 1: Plasmid profile of pesticide resistant bacteria

Sphingomonas harbouring plasmids size in the range of 5.5 kb to over 200 kb have been reported by Orgam (1998).The pesticide degrading potential of *Pseudomonas aeuroginosa* has been well supported by Desouza *et al.* (1995) who have demonstrated plasmid mediated atrazine degrading potential of *Pseudomonas*. In addition, Gonzalez- Lopes *et al.* (1993) reported the versatility and plasticity 2,4-D degrading strain. *Ralstonia eutropha* using pathways carried out plasmid pJP4 (Perez-Pantoja *et al*., 2000). The degradative pathway for numerous chemical compounds has been found to be controlled and expressed by genes located on extrachromosomal replicons. Plasmid mediated pesticide resistance as observed in the present study is well supported by Thomas *et al.* (1996) who have reported seven cryptic plasmids ranging in size from 20-180 Mda in *Sphingomonas paucimobilis*.

The results of the present study is in agreement with Turnbull *et al*. (2001) who have reported that *Arthobacter globiformis* possessed 47 kb and 34 kb plasmid capable of degrading phenylurea. In contrast to the present finding, Ohshiro *et al*. (1997) have concluded that hydrolase-encoding gene(s) appears not to be plasmid-borne but chromosomal in origin.

All 240 kb plasmids could have genes for insecticide degradation as evidenced by curing experiments. In a similar study, 75 kb plasmid was recovered and gene for biodegradation of chlorobenzoate was located on it. The present demonstration that the plasmid cured bacterial isolates failed to survive in the presence of bavistin gains support from the study Brenner *et al*. (1993) who proved that cured bacterial strains were unable to utilize chlorobenzoates for growth. The results in present study also indicated that gene for biodegradation of bavistin, monocrotophos and Kinadoplus pesticides were located on 240 kb plasmid. The results of this investigation permits us to conclude that *E.coli, Pseudomonas aeruginosa, Enterococcus faecalis* and *Staphylococcus aureus* could be exploited for the degradation of pesticides.

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