DISCUSSION
DISCUSSION

Pesticides constitute the key control strategy for pest control in agriculture and are becoming an important contributor in improvement of crop yields. Currently, organophosphates form a predominant and accounting for about 36 per cent of the total market of world because this group of insecticide is most frequently used, among various groups of pesticides that are being used world over (Kanekar et al., 2004).

This wide spread use of these OP pesticides over the years have resulted in hazardous effects occurred due to their interaction with biological systems of environment (Serrano et al., 1995). Quinalphos use and its Impact on biomass of microbial cells and their metabolic activities in clay loam soil were observed by Sengupta et. al (2009).

Thus, considering toxic effect of these pesticides, this is kindly necessary to reduce the level of these chemical pollutants from the applied environmental region. In this removal process involving biological removal of chemo-pollutants becomes a method of choice, because bacteria from natural environments can utilize different types of xenobiotic chemical compounds including insecticides for their growth and mineralize and degrade them.

Many scientists have isolated microorganisms from natural systems like soil which have the capacity to degrade chlorpyriphos and obtained good degradation yields (Singh et. al., 2003; Mukherjee and Gopal, 1996; Mallick et. al., 1999).

Hence, this study involves isolation of organophosphorus insecticides as Quinalphos and Dichlorvas degrading microorganisms after enrichment of soils and to select the most efficient strains. This study describes the enrichment of soil samples, isolation, characterization as well as identification of bacterial strains resistant to selected OP insecticides. Isolation of efficient bacterial strains was done from agricultural regions like
grape wine yard soil samples. The degradation of these chemical pesticides by soil-borne microorganisms was first demonstrated by Audus (1960).

5.1: Enrichment and isolation of bacterial strains resistant to organophosphorus insecticides Quinalphos and Dichlorvas

Soil samples were collected from areas where organophosphorus insecticides as Quinalphos and Dichlorvas were in constant use and used for further enrichment of soil samples by adding concentrations in mg/L as 5, 10, 15 and 20, for a period of 14 days. Because chances of isolating microbial strains from polluted soils, with high ability to metabolize a particular xenobiotic are brighter (Feng et al., 1997).

During enrichment with a specific xenobiotic compound, the natural selection of microorganisms which have been adapted to the presence of that xenobiotic and its rapid utilization and biodegradation are known to take place (Cullington and Walker, 1999).

Several previous studies from other laboratories have also reported isolation of microorganisms (bacteria, actinomycetes and fungi) able to utilize pollutants as their growth sources (Nagata et. al., 1993; Mueller et. al., 1990; Parske et. al., 1995; Thomas et. al., 1996; Sutherland et. al., 2002; Siddiqui et. al., 2002; Singh et. al., 2003)

Tipton et. al. (2003) reported, the soil which had been exposed to various xenobiotic constantly, had a higher capacity to degrade such compounds and had a higher number and diverse groups of microorganisms than soils that had not been exposed to similar compounds.

5.2: Morphological and biochemical characteristics of isolates and tolerance of bacterial strains towards insecticides

As many as twenty five bacterial isolates were obtained and isolated from enriched soil samples and it was interesting to note that all the isolates have ability to tolerate and grow
on higher concentrations of insecticides. Twenty five bacterial strains were isolated, of which 12 isolates were Quinalphos resistant (WL DumpQ10, WL Q5, PL Q10, PL Q5, VT Q15, VT Q20, MJ Q10, TK Q10, TK Q20, TS Q20 and TS Q15) and 13 isolates were Dichlorvas resistant (WL DumpD20, WL DumpD10, BS D10, BS D5, BS D15, PL D10, PL D15, VT D10, MJ D15, MJ D20, TK D10, TS D20 and TS D10) grown on Mineral Agar Medium consisting of insecticide as growth source.

Similarly, various scientists identified bacteria following enrichment culture technique and which having capacity of degrading organophosphorus insecticides. Different types of organophosphorus insecticide detoxifying bacteria have been enriched and isolated (Chapalamadugu and Chaudhry, 1989) and various hydrolyzing enzymes also have been discovered from these microbial cultures for remediation (Mulbry and Karns, 1989).

Sixteen bacterial strains have been isolated capable of tolerating quinalphos (an organophosphate) and carbosulfan (a carbamate) upto the concentration of 0.4%. Also six strains of yeasts were reported, having ability to utilize hepatochlor (a cyclodiene) and Quinalphos (an OP) (Shakoori et. al., 2000).

5.3: Biodegradation of Quinalphos and Dichlorvas insecticides

The efficiency of the isolated bacterial strains to degrade Quinalphos and Dichlorvas was analyzed by UV-Visible spectra of insecticides before and after degradation, and GCMS analysis of metabolites obtained after degradation of insecticides. Out of 25 isolates, six bacterial strains (WL DumpQ10, VT Q15, TK Q20, WL DumpD10, PL D15 and WL DumpD20) were selected for biodegradation study.

UV-Vis wavelength scans at 190-300nm showed absorption maxima of 220nm for Quinalphos and Dichlorvas. Decrease in absorption maxima in presence of bacterial culture was observed (Figure No.1, 6, 11, 16, 21 and 26) respectively for above isolates.
GCMS analysis of degradation metabolites showed that all the products formed after degradation of Quinalphos and Dichlorvas were simple and non-toxic. GCMS analysis also revealed the formation of new metabolites after degradation of Quinalphos and Dichlorvas. Table No.6, 7, 8, 9, 10, 11 show retention times of newly formed metabolites with its respective mass spectra for above selected isolates respectively.

Conversion of benzyl alcohol to benzaldehyde and finally to benzoic acid by *Pseudomonas putida* CSV86 has already been reported by Basu et. al., (2003), during benzyl benzoate degradation.

Pawar and Mali (2014), studied the biodegradation of Quinalphos by *Pseudomonas* species isolated from grape wine yard soils.

The detoxification and degradation of manmade chemicals like pesticides and role of microorganisms and their enzyme that degrade organophosphates have been represented by Karns et. al., (1987), Nannipieri and Bollag (1991).

Orgam (1998), reported insecticide degradation in a bacterium belonging to genus *Sphingomonas*, which had five plasmids.

Megharaj et. al.(1987), studied that the algae isolated from soil can metabolize monocrotophos and quinalphos.

Several studies have reported the comparative utilization and degradation of 32P-labelled Butonate, Trichlorphone as well as Dichlorvas on crop (Dedek et. al., 1979).

5.4: Effect of parameters as temperature and pH on degradation of Quinalphos and Dichlorvas by selected isolates

All selected isolates (WL DumpQ10, VT Q15, TK Q20, WL DumpD10, PL D15 and WL DumpD20) showed highest degradation rate at their optimum pH and temperature. Also degradation of insecticides was enhanced in glucose ammended mineral salts medium as
compare to insecticide alone. As glucose in the medium may be acting as growth activator for the bacteria.

Karpouzas and Walker (2000) detected capacity of *Pseudomonas putida* ep I for ethoprophos degradation and also additional carbon sources as glucose and succinate only slightly affects the degradation process. Also previous report found that supplementation of glucose as carbon source to mineral salts medium was not improved degradation of carbofuran (Behki *et al.* 1994).

Biotransformation of these chemicals in the active cells of microbes is connected with the structural features of the products and reagents and also properties related with the microbial metabolism (Critter and Airoldi, 2001).

Previous studies found that, biodegradation of insecticide by using different carbon sources and reported insecticide degradation can be increased by supplementing microorganisms with different carbon sources like dextrose, fructose, lactose and galactose in contaminated soil (KaviKarunya and Reetha, 2012).

Sarkar *et al.* (2008), reported that presence of glucose in the medium enhanced the dicofol degradation by *Pseudomonas* strain which is obtained from rhizosphere region of tea plant.

Effect of physicochemical parameters as pH on xenobiotic compound degradation was discovered by Moosvi *et al.* (2005) and resulted that pH influences rapid degradation of xenobiotic compounds. Also it was reported earlier that different soil environmental parameters as temperature, pH, moisture content, nutritional features are predominantly affected by the application of insecticides exerting hazardous effects on microbial activities of soil (Subhani *et al.*, 2001).

**5.5: Identification of a Microbial Culture using 16S rDNA based Molecular Technique**

A fragment of 16s rRNA was amplified for the analysis of phylogenetic relationship of selected organophosphorus insecticide degrading bacterial strains from the genomic DNA of respective bacterial strains and sequenced with their Gene bank Accession numbers.
Therefore, according to morphological characteristics, cultural properties, colony morphology and physiological and biochemical characteristics and phylogenetic analysis, the isolated Quinalphos and Dichlorvas degrading bacterial strains WL DumpQ10, WL DumpD10, WL DumpDQ20, PL D15 and VT Q15 were *Pseudomonas sp. FY1* (GenBank Accession Number: FJ534637.1), *Bacillus cereus* strain NMRL *PED1* (GenBank Accession Number: HQ596560.1), *Sphingobacterium mizutaii strain DSM 11724* (GenBank Accession Number: NR_042134.1), *Pseudochrobactrum asaccharolyticum strain ALK635* (GenBank Accession Number: KC456600.1), *Acinetobacter sp. 7-13* (GenBank Accession Number: KC170354.1) respectively.

Hussaini et. al.(2013) have isolated six bacterial strains as *Acinetobacter radioresistens, Pseudomonas frederiksbergensis, Bacillus pumilis, Serratia liquefaciens, Serratia marcescens, Burkholderia gladioli* and found to show significant ability for degradation of selected pesticides.

Kampfer et. al. (2006) described *Pseudochrobactrum* genus, with its *Pseudochrobactrum asaccharolyticum* species and *Pseudochrobactrum asaccharolyticum* species.
Yoo et al. (2007), reported a member of genus *Sphingobacterium* analysed as the strain 4M24<sup>T</sup> sequence of 16 rDNA gene and found similar to *Sphingobacterium daejeonense* TR6-04<sup>T</sup> and *Sphingobacterium mizutaii* ATCC 33299<sup>T</sup>

Several reports revealed that Acinetobacter strain from aerobic granules degrade phenol (Adav et al., 2007).