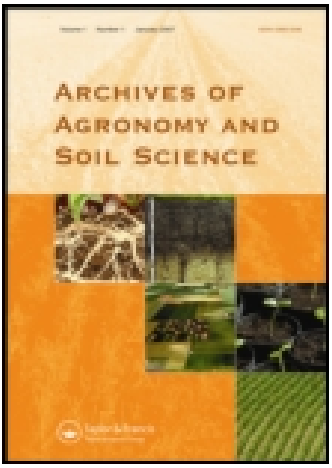


Archives of Agronomy and Soil Science
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gags20

# Effect of pesticide application on soil microorganisms

Anu Kalia [a] & S. K. Gosal [b]
[a] Electron Microscopy and Nanoscience Laboratory , Punjab Agricultural University , Ludhiana, India
[b] Department of Microbiology , Punjab Agricultural University , Ludhiana, India
Published online: 21 Jul 2011.

To cite this article: Anu Kalia & S. K. Gosal (2011) Effect of pesticide application on soil microorganisms, Archives of Agronomy and Soil Science, 57:6, 569-596, DOI: 10.1080/03650341003787582

To link to this article: http://dx.doi.org/10.1080/03650341003787582

*Archives of Agronomy and Soil Science*
Vol. 57, No. 6, September 2011, 569–596



# Effect of pesticide application on soil microorganisms

Anu Kalia[a]* and S.K. Gosal[b]

[a]*Electron Microscopy and Nanoscience Laboratory, Punjab Agricultural University, Ludhiana, India;* [b]*Department of Microbiology, Punjab Agricultural University, Ludhiana, India*

(*Received 9 March 2009; final version received 11 March 2010*)

Modern agriculture largely relies on the extensive application of agrochemicals, including inorganic fertilizers and pesticides. Indiscriminate, long-term and over-application of pesticides have severe effects on soil ecology that may lead to alterations in or the erosion of beneficial or plant probiotic soil microflora. Weathered soils lose their ability to sustain enhanced production of crops/grains on the same land. However, burgeoning concern about environmental pollution and the sustainable use of cropping land have emphasized inculcation of awareness and the wider application of tools, techniques and products that do not pollute the environment at all or have only meager ecological concerns. This review covers the types of, concerns about and current issues regarding the extensive application of agrochemicals, in particular pesticides, on a variety of microorganisms integrated in successive food chains in the soil food web.

**Keywords:** microbiota; pesticide; biological degradation; soil probiotic microbes



## Introduction

Life on earth is sustained by a series of intricately woven dependencies among organisms, with each individual having its own important role in the biosphere. These interdependencies may be described as the relationship between prey that acts as food and higher organisms, and involves even microscopic organisms, i.e. microorganisms.

The region below the surface of the earth has components that enact their respective roles in manner similar to those of the above-ground food web, i.e. the pioneer bacteria and fungi, the protozoans and microarthropods that feed/prey on the pioneer flora, and the somewhat larger worms and earthworms that devour those that eat the pioneers (Coleman 2001). If the above components beneath the soil are healthy and finely tuned then this is exhibited as an extravagant number of plants and a similar diversity in animal numbers on land.

The current era of 'instant production' demands the excessive use of chemicals, which are unhealthy for the environment. Disturbance-invoked alterations arise because of the excessive application of agrochemicals such as inorganic fertilizers and a range of pesticides (Wilson 2000). The thoughtless use of these agrochemicals has been suggested to have various negative impacts, such as the killing of beneficial nontarget organisms involved in nutrient retention and recycling, and

*Corresponding author. Email: kaliaanu@rediffmail.com

ISSN 0365-0340 print/ISSN 1476-3567 online

DOI: 10.1080/03650341003787582
http://www.informaworld.com

impoverishment of the nutrient pool, leading to decreased soil fertility (Schuster and Schroder 1990; Chowdhury et al. 2008). Moreover, these chemicals have a tendency to move in the environment by adhering to the organic matter in the soil or to the roots/aerial parts of the primary producers; they therefore enter the food web, finally biomagnifying in the lipid bodies or other tissues of higher organisms (Hafez and Thiemann 2003). Vaporization is another way by which pesticides may move into the atmosphere, and a substantial amount of applied pesticide may seep through the soil to contaminate fresh water aquifers or may reach bodies of surface water following heavy showers. In addition, a small amount of pesticide is mineralized and degraded by sunlight (photodegradation) and by microbes (microbial degradation) present in soil. With such a range of movement in the biosphere, curbing the use of agrochemicals and implementing alternative techniques would be a useful step towards conservation of the soil by maintaining microbial diversity.

The microbial population of 1 g of soil in a country is an index of the agricultural prosperity of that country. The total mass of the microflora and -fauna beneath the soil is 20 times that of the human population of the earth (Torsvik et al. 1990). One gram of healthy soil contains 1 million to 100 million bacteria involved in the breakdown of organic matter, 0.15–0.5 mg of fungal hyphae, 10,000–100,000 protozoa, few to several hundred microarthropods, 15–500 nematodes and a few earthworms (Coleman 1994). The bacteria and fungi break down organic matter and help retain soil particles as macroaggregates/clumps, while the remaining components of the soil food web maintain the correct number of bacteria and fungi by prey–predator interactions, and thus help in the recycling and retention of basic nutrients in the soil. This is necessary to maintain soil fertility and crop productivity.

These components of the soil food web exist in harmony and are intricately woven in interdependent relationships (Johansson et al. 2004). The structure and function, i.e. number, activity and community structure of the soil food web, may act as a prime indicator of ecosystem health. Monitoring the active and total biomass of each organism group directly may help in detecting the dynamics of change leading to a damaged ecosystem.

Once applied, agrochemicals persist in the soil for long periods and have negative impacts on soil microbial flora (Araujo et al. 2003), such as the killing certain specific group(s) of microorganisms. A consequent decrease in microbe number disturbs a specific process in the food web performed by an individual or group, and disrupts the components relying upon it. A series of modifications is triggered in almost all microbial flora/fauna groups, which leads to changed prey–predator modules that may cause changes in soil aggregation, soil chemistry, pH and structure as soil organic matter is depleted (Bossuyt et al. 2001). The degraded soil becomes more prone to erosion to surface water bodies following heavy rain and also responds less to ever high fertilizer input. Soil becomes barren over a period of few decades because only negligible organic matter is left to sustain microbial growth and development (Chowdhury et al. 2008).

There is a need for the advent and use of cheaper and eco-friendly alternatives that result in increased crop production, along with the judicious use of the known arsenal of agrochemicals, as suggested by the Integrated Pest and Nutrient Management protocols. Current trends are towards the use of organic farming, the application of biofertilizers, the popularization of biological control agents/biopesticides, conservation tillage, cautious use of inorganic fertilizers and skillful pesticide management (precision pesticide application or the use of nanopesticides).

## Pesticides

### *Indian scenario*

Pesticide application forms is at the forefront of increased agricultural food production and management practices. Approximately 80,000 t of pesticides are used annually in Indian agriculture (Srinivasan 1997), mostly in the production of cotton (45% of total pesticide use; Dhaliwal and Pathak 1993), rice (23% of total pesticide use), fruits and vegetables ($\sim$ 8% of total pesticide use), and cereals, pulses, oilseeds and millets ($\sim$6 to 7% of total pesticide use). The Indian states of Haryana, Punjab and Uttar Pradesh have the largest pesticide consumption, using $>$5000 t of (technical grade) pesticides in 2000–2001. This enhanced consumption has led to the depletion of soil fertility and a reduction in sustainable crop production (Sharma 2003). Because of an awareness of the negative impacts of extensive pesticide use on ecology and the environment, and Integrated Pest Management practices, the consumption of chemical pesticides has decreased by 27.69%, from 66,360 t during 1994–1995 to 43,590 t during 2001–2002 (Anonymous 2002).

### *What are pesticides?*

Pesticides include chemically synthesized compounds, devices or organisms that are routinely utilized in agriculture to manage, destroy, attack or repel pests, pathogens and parasites. Pesticides include both organic and inorganic moieties and may be classified into different groups based on their chemical composition. These include organochlorines, organophosphates, carbamates, formamidines, thiocyanates, organotins, denitrophenols, synthetic pyrethroids and antibiotics (Bohmont 1990). A pesticide may be categorized on the basis of the organism (pest/pathogen/parasite) curbed/controlled or killed by its application. Approximately 700 pesticides, including insecticides, herbicides and fungicides, act on $\sim$ 95 biochemical targets in pest insects, weeds and destructive fungi, and hence can be classified according to their mode of action. The most commonly applied pesticides, i.e. insecticides (organochlorines, organophosphates and carbamates), act primarily by disrupting nervous system function, in particular, four nerve targets, acetylcholinesterase, voltage-gated chloride channel, the acetylcholine receptor and the $\gamma$-aminobutyric acid receptor. By contrast, herbicides often kill or injure plants by targeting plant-specific pathways, for example, by blocking photosynthesis (Ecobichon 1991; DeLorenzo et al. 2001), carotenoid synthesis or aromatic and branched chain amino acid synthesis, which are essential in plants. Herbicides affect mechanisms associated with vital processes such as photosynthesis, respiration, growth, cell and nucleus division or the synthesis of proteins, carotenoids or lipids (Ecobichon 1991). Fungicides target various pathways that disrupt basic cellular functions, block fungal lipid biosynthesis (e.g., ergosterol), protein biosynthesis (e.g., tubulin biosynthesis), or essential enzymes (e.g. cytochrome *c* reductase) (Casida 2009).

The fate, on application, of pesticides in the soil and the transport processes involved depend on the cumulative effects of the pesticide's characteristics (e.g., adsorptivity, solubility, volatility and degradation rate), the soil's charateristics (e.g., texture and organic matter), the application methods used (e.g., aerial or ground) and the site conditions (e.g., topography, weather and irrigation) (Jeong and Forster 2003).

Pesticides have the ability to cling to soil particles and microbes, which prevents nontarget effects of applied pesticides. Soil microbes, and the large quantities of metabolites they produce, provide extra surfaces to which pesticides can bind, thereby reducing bioavailability. The greater the activity of microorganisms, the greater the amount of pesticide that could bind to the soil, and so microbe numbers give an indication of the amount of freely available pesticide in the soil. Moreover, soil type also affects pesticide binding to soil particles because each type of soil interacts differently with each type of pesticide (European Commission 2009). In addition to this, a substantial amount of applied pesticide is degraded by native soil microbes, in particular, soil bacteria and fungi. A review by Van Eerd et al. (2003) gives the various mechanisms of pesticide metabolism in plants and microbes.

Certain pesticides, which are more resistant to degradation by abiotic (physical, chemical and other factors) and biotic (living organisms i.e. the micro-, meso- and macroorganisms of the soil food web) agencies, leach into the lower strata of the soil, are absorbed by plant roots, accumulate in the food chain and are ultimately biomagnified in the food web. There have been several reports on the accumulation of pesticide residues in plant (Babu et al. 2003; Waliszewski et al. 2008) and animal tissues (Sofina et al. 1993; Hans and Farooq 2000; Nakata et al. 2002). Saenz-de-Cabezon et al. (2008) have reported the persistence of applied pesticide inside protistian and microarthropod internal tissues and unfertilized eggs. According to them, this enhances both the residual activity of the active ingredient and its movement in the soil, depending on predator and prey dispersal capacity. The applied pesticide can be transported from the sprayed area to nontarget areas away from the crop, which thus affects not only pest species, but potentially nontarget endangered species also. Mano et al. (1996) reported the bioaccumulation and enhanced persistence of the acaricide dicofol which is protected from hydrolysis by adsorption on *Azospirillum lipoferum* cells.

The biomagnification of pesticides in plant and animal tissues (particularly in lipid bodies) makes their use hazardous to health and may lead to several ailments. Over the decades, there has been a considerable increase in pesticide use and a simultaneous increase in the problem of biomagnification has been encountered in soil (Hans and Farooq 2000), in plant and animal products such as cereals (Babu et al. 2003), fruits and vegetables (Waliszewski et al. 2008), and in milk and milk products (Kannan et al. 1997). In addition, there is the emerging problem of the development of pesticide-resistant pests, which may resist even higher concentrations of pesticides.

A field study on the movement of isomers of the organchlorine compounds dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH) was performed by Waliszewski et al. (2008). This study revealed the diffusion of organochlorine pesticide from agricultural soils to growing carrot plants and the adsorbtion of organochlorine vapors by the leaves. Organochlorine pesticides accumulate within the carrot plant, especially in the root peel, which shows levels 3–7 times higher than those in the flesh of the root (Waliszewski et al. 2008). The principal source of these residues is considered to be their deposition in agricultural soils from where they are subsequently adsorbed by the roots, although they may also be adsorbed by the leaves of growing plants on volatilization (Bidleman and Leone 2004).

The negative effects of applied pesticides in higher organisms include direct impacts such as fish kills, reproductive failure in birds and acute illnesses in humans.

Human exposure to or ingestion of pesticides usually occurs as a result of the misapplication or careless disposal of unused pesticides and pesticide containers. Moreover, as the top consumers, humans are exposed to high levels of pesticides on ingesting contaminated plant and animal products. Sofina et al. (1993) found residues of DDT and HCH in the food of pregnant women and in human milk in rice-growing areas of Kransnodar region, Russia.

## Impact on soil microflora

Soil fertility is determined by the presence of sufficient nutrients and also a sufficient number and diversity of soil microflora. Microbial diversity is mostly due to the occurrence of various types of organic substrates in soil. The diverse groups of organisms that are mostly unicellular of prokaryotic or eukaryotic origin include bacteria (eubacteria and archaebacteria), cyanobacteria, actinomycetes, fungi and algae. These soil microbes perform a variety of activities required for the proper functioning of the soil as a dynamic system.

Although pesticides are important, their effects on nontarget organisms are of great concern because this poses a risk to the entire ecological system (Kalia and Gupta 2004). In general, the effects of pesticides on microorganisms will vary depending on the chemical dosage, the properties of the soil and various environmental factors (Ecobichon 1991). Because the application or extensive use of pesticides has led to a rapid decline in the quality of the organic matter in soil it also affects the diversity of the microbial flora and fauna. Because these microbes are involved in various element-recycling and -transformation processes, any change in their number or ratio could potentially prohibit/enhance one or other of the reaction chains important for soil fertility. Pesticides affect nontarget microbes by interfering with vital processes such as respiration, photosynthesis and biosynthetic reactions, as well as cell growth and division and molecular composition (DeLorenzo et al. 2001). Initially, the application of pesticide decreases microbe number and activity, but as the chemical persists microbes develop tolerance/resistance and recolonize. Ryan (1999) reported that the conventional practice of fertilizer and pesticide application may affect some groups of organisms in the soil, but the overall effect on the soil community would be small. Gupta et al. (2000) reported a negative impact of pesticide application on all soil microbes with a decrease in the average population of all groups studied in soil samples taken from fields under a rice–wheat cropping system.

### *Microbial activities*

Several soil microbial enzymes are hampered or affected by the application of pesticides to the soil. Soil microbial biomass is affected by an array of factors including the physical and chemical properties of the soil (soil factors), temperature, moisture and pH (soil environmental factors) and the use of chemical fertilizers, pesticides, heavy metals, the addition of organic matter, cultivation and crop rotation, seasonal variation, tillage, etc. (soil management factors). Engelen et al. (1998) observed that, on application to soil, Herbogil (dinoterb), a reference herbicide, resulted in the inhibition of biomass-related activities and the stimulation of nitrogen mineralization. They recorded the effects on metabolic parameters as determined by monitoring substrate-induced respiration (SIR) and dehydrogenase activity, as well as carbon and nitrogen mineralization. Variations in the complex metabolic fingerprints were recorded using the Biolog system which demonstrated the inhibition of many catabolic pathways after the application of Herbogil.

A laboratory microcosm study to investigate the impact of pestcide (insecticide, herbicide and fungicide) application on the health of paddy field soil showed a decrease in soil dehydrogenase activity with increased pesticide concentrations and toxicity increased in the order insecticide > fungicide > herbicide. However, pesticide application did not produce any significant change in soil protein content, although it did cause an increase in the soil phenol content (Subhani et al. 2002). Yao et al. (2006) reported that the application of a new pesticide acetamiprid at normal field concentrations (0.5 mg $kg^{-1}$ dried soil) and at high concentrations (5 and 50 mg $kg^{-1}$ dried soil) had a strong negative influence on soil respiration and phosphatase activity, however, it enhanced dehydrogenase activity after 2 weeks of application. Pampulha and Oliveira (2006) reported that the herbicide combination 60% bromoxynil + 3% prosulfuron induced significant changes in the microbial populations of the soil with a long-lasting negative impact on dehydrogenase activity. Adebavo et al. (2007) reported a reduction in fungal, actinomycete and protozoal populations in soil on application of Thiodan (4000 and 8000 mg $kg^{-1}$) and Karate (6000 and 12000 mg $kg^{-1}$), although there was a significant increase in the bacterial count.

### *Bacteria and actinomycetes*

The application of bactericides, in particular antibiotics in laboratory, glass house and field studies, decreases the number of soil bacteria. Application of bactericides like oxytetracycline led to growth suppression in soil bacteria (Bossuyt et al. 2001). Piotrowska-Seget et al. (2008) performed a laboratory study to assess the impact of applying successive doses of oxytetracycline (bactericide) or Captan (fungicide) on microbial biomass and activity. They reported that both oxytetracycline and Captan significantly decreased the numbers of culturable bacteria, although total bacterial biomass was not affected. The study indicated that oxytetracycline or Captan application may negatively affect nontarget soil microorganisms and their activities.

Soil bacterial and actinomycetal forms are involved in carbon transformation reactions, along with the archaebacterial methanogens. Franzluebbers et al. (1994) observed seasonal changes in soil microbial biomass and mineralizable C and N in continuous wheat, continuous wheat–soybean and wheat–soybean–sorghum systems. Endosulfan and butachlor also inhibit the total methanogenic bacterial population with degree/severity of inhibition being influenced by rate of endosulphan application. Butachlor treatment inhibits the methanogenic bacterial population (Kumaraswamy et al. 1998).

Ibekwe et al. (2001) reported variations among Biolog fingerprints showing the severe effect of methyl bromide on heterotrophic microbial activity in the first week of application, as well as a shift in all pesticide treatments to a microbial community dominated by Gram-positive bacterial biomass, as demonstrated by the phospholipid fatty acid profile. However, pesticide-metabolizing microbes may dominate and overpopulate on pesticide application and thus increase the viable cell count of microbial species. Zhang et al. (2008) reported an increase in the number of Gram-negative bacteria on application of the insecticide cypermethrin, which may have acted as a nutrient for the growth of these microbes in the cucumber phyllosphere.

Moreover, a phospholipid fatty acid (PLFA) assay indicated a significant increase in total and bacterial biomass and a decrease in fungal biomass following insecticide treatment. Digrak and Kazanici (2001) studied the application impacts of three different organophosphorus insecticides and reported higher total viable bacterial numbers in an isofenphos-treated soil sample compared with control groups on incubation.

Nitrogen mineralization processes, such as ammonification and nitrification, are also affected by the application of pesticides, with the former being inhibited less because it is carried out by a vast diversity of microflora. Odokuma and Osuagwu (2004) have shown that the organochlorine pesticides Lindane and dieldrin were more toxic than the organophosphate pesticides pirimphos methyl and malathion to *Nitrsomonas*, *Nitrobacter* and *Thiobacillus*. The carbamates benomyl and methomyl were as toxic as the organochlorines to these microorganisms. Tu (1995) reported severe suppression of nitrification for $\sim 1$ month following application of the fungicides Telone 17, Vortex, dazomet and fenemiphos in sampled soils. In addition to the above N mineralization processes, a major group of soil rhizobia are involved in the nitrogen fixation in soil. Pesticides may directly affect free-living populations of nodular bacteria in soil or indirectly influence the extent of infection and thus the number of nodules formed. The infection process may be changed either by the influence of a pesticide on the virulence of the attacking bacteria or by affecting the root fibers of the plants in which the infection occurs (Niewiadomska and Sawicka 2002; Niewiadomska 2004). Because few pesticides can mimic naturally occurring biochemicals, application of these pesticides result in interference with various biochemical signaling processes between rhizobia and appropriate host plants, leading to the disruption of early nodulation events (Mussarat and Haseeb 2000; Fox et al. 2007).

Bertholet and Clark (1985) highlighted the inhibition of nodulation by the application of trifluralin and metribuzim pesticides. Thiobencarb at 2 and 4 mg kg$^{-1}$ inhibited *Azospirillum* populations, anaerobic nitrogen fixers and *Azotobacter* in an alluvial soil (Jena et al.1990). Singh et al. (1995) reported severe impairment of legume rhizobia symbiosis following the application of fungicides as a seed dressing at recommended field rates. The rhizobial counts were drastically decreased even at low fungicide concentrations of 1 and 10 mg kg$^{-1}$.

The application of herbicides can affect *Rhizobium*–legume symbiosis in several ways because herbicides can: (1) affect the host plant directly; (2) negatively affect the growth and survival of the rhizobia, thus reducing the potential for nodule formation; (3) reduce the efficacy of the rhizobia in terms of the ability to nodulate or form an effective symbiosis (Anderson et al. 2004); and (4) reduce the nitrogen-fixing effectiveness of the symbiosis via enzymatic inhibition or interruption of biochemical pathways in the bacteroids (Drew et al. 2007).

Sawicka and Selwet (1998) reported that seed application of imazethapyr and linuron can cause a decrease in the nitrogenase activity of root-nodule bacteria and stimulate the development of resistant bacteria. The effect depends on the herbicide, its concentration and the weather conditions. Niewiadomska (2004) reported a reduction in nitrogenase activity on the active strain of *Rhizobium leguminosarum* bv. *trifolii* KGL under both pot and field experiment conditions in red clover following the application of selected pesticides [fungicide Funaben T (Carbendazim and thiram)] and herbicide [Pivot 100 SL (a.i. imazetapyr)]. Application of both of these pesticides also inhibited the multiplication of the microorganisms in soil under red clover plantations in the first days after application, and later stimulated their multiplication. Similarly, Khan et al. (2004) reported the negative impact of herbicide application on chickpea–*Mesorhizobium* symbiosis. A study by Dunfield et al. (2000) showed that seed application of the fungicides Captan and thiram in pea after 24 h of treatment not only significantly reduced the numbers of rhizobia recovered from seed, but also altered the fatty acyl methyl esters (FAME) and Biolog profiles of the recovered rhizobia. Dunfield et al. also observed that at high concentrations Captan affected nodulation and plant growth in pea.

In certain instances, the application of pesticides may not have drastic effects on rhizobial growth or reduce the number or activity of rhizobia, however, subsequent nodule formation by rhizobia may be reduced leading to a reduction in nodule size and total nitrogen fixation (Anderson et al. 2004). Fox et al. (2007) reported *in vivo* evidence of inhibition or delayed recruitment of *Sinorhizobium meliloti* bacteria to host alfalfa roots, the formation of fewer root nodules, lower rates of nitrogenase activity and a reduction in overall plant yield at the time of harvest due to application of the organochlorine pesticides methyl parathion and pentachlorophenol.

Similarly, heterotrophic nitrogen fixation in soil is greatly influenced by the application of different pesticides. Carbofuran application under flooded conditions stimulates nitrogen fixation with a concomitant increase in the populations of *Azospirillum* and anaerobic nitrogen fixers, and also increases Indole acetic acid (IAA) accumulation in a culture of *Azospirillum* isolated from rice roots and soils. Benomyl-amended flooded soil also showed a striking stimulation of *Azospirillum* populations (Charyulu and Rao 1978).

### *Fungi*

Soil fungi have a greater ability to resist the application of pesticides, but the application of fungicides drastically affects their population and hence a variety of mineralization/decomposition processes controlled by them. Moharram et al. (1994) performed a laboratory study to show the effect of applying pyrazofos (fungicide), bromoxynil (herbicide) and profenfos (insecticide) on the nitrogen fractions (amino-N, peptide-N, ammonia-N, total soluble-N, protein-N, total-N) of six soil fungi. They observed a significant increase in total-N in *Trichoderma harzianum* and *Fusarium solani*, whereas a significant decrease was observed with *Aspergillus niger* and *Penicillium chrysogenum* following the application of pyrazofos. In addition to fungicide, the herbicide bromoxynil caused a significant decrease in total-N in *A. niger, F. solani*, *T. harzianum* and *Mucor racemosus*, whereas it significantly increased total-N in *Stachybotrys chartarum*. Similarly, the insecticide profenfos caused a significant reduction in total-N in *P. chrysogenum*.

In addition to hyphal molds, soil yeasts are also affected by the application of pesticicdes. Slavikova and Vadkertiova (2003) reported that the fungicide prochloraz inhibited the growth of the majority of yeast strains, while the insecticide triazamate restricted or inhibited growth in all tested yeast strains. *Cryptococcus* strains were shown to be most sensitive to pesticides, while *Cystofilobasidium capitatum, Debaryomyces occidentalis* var. *occidentalis* and *Trichosporon cutaneum* strains were most resistant to pesticide application.

In general, the majority of herbicides do not have a drastic negative impact on the soil fungal population, at least at the recommended levels, however, a few insecticides have an initial inhibitory on soil fungi, followed by a stimulatory effect. The fungi belong to the group of microorganisms that, after an initial sensible response to the presence of pesticides in the soil, rapidly establish normal metabolism, even enabling them to increase in number, particularly in the case of fungicide and insecticide application (Mandic et al. 2005). In a field study performed by Iqbal et al. (2001), only application of endosulfan caused a decrease in fungal population. The majority of pesticides do not affect fungi, rather pesticide application (monocrotophos, methamidophos, endosulfan + dimethoate, fenpropathrin, bifenthrin + acetamide, profenophos, chlorpyriphos, carbosulfan, etc.) stimulated fungal growth/number in cotton agroecosystem.

The application of fungicide decreases both the number and type of soil fungi. Bossuyt et al. (2001) have reported the direct suppression of fungal growth in soil following application of the fungicide Captan, which further led to a reduction in the formation of macroaggregates in soil, thereby indicating the positive influence of fungal activity on macroaggregate formation.

The most affected group of fungi are the well-known glomealian arbuscular mycorrhizal fungi (Redecker et al. 2000), which are involved in increased plant nutrient (P, N and micronutrients) acquisition (Read 2002), impart protection against soil-borne pathogens in plants and improve soil structure by either the direct production of glomalin protein (Bever et al. 2001) or by enhancing rhizodeposition (required to glue soil particles) in the infected plant (Jones et al. 2004). Moreover, arbuscular mycorrhizal colonization may influence the composition of bacterial communities in the hyphosphere or the mycorrhizosphere, which may result in enrichment of the rhizospheric zone with beneficial bacteria. This would positively affect the growth and development of the inoculated plant (Toljander et al. 2006).

The application of herbicides may decrease arbuscular mycorrhizal fungal symbiosis by lowering viability and growth, or by decreasing host plant diversity (Bethlenfalvay and Schuepp 1994). However, fungicides have direct effects on soil arbuscular mycorrhizal fungal populations. Sukarno et al. (1993) reported a decrease in the active proportion of external hyphal lengths following fungicide treatments. Similarly, detrimental effects of the application of a large number of fungicides on arbuscular mycorrhizal population and hyphal growth have been catalogued and reported by Kurle and Pfleger (1997). A laboratory study by Chiocchio et al. (2000) revealed that the fungicide benomyl inhibits spore germination and hyphal length of the arbuscular mycorrhizal fungus *Glomus mosseae* when applied at the agronomic dose and doses below the recommended (21.25, 10.62 and 10 mg $kg^{-1}$) level. However, small spores of *Glomus mosseae* were seen to be more resistant to benomyl than larger spores.

Apart from the effects on hyphal length and spore germination, negative effects of fungicide application may also be assayed by using certain important enzyme activities as markers. Kuperman and Carreiro (1997) discussed the use of stains specific for fungal enzyme activities such as alkaline phosphatase as a marker of phosphorous metabolism in arbuscular mycorrhizal symbiosis to rapidly assess the negative effect of fungicide application on arbuscular mycorrhizal fungi. In a field study, Kjoller and Rosendahl (2000) assessed the effect of four fungicides, fenpropimorph, propiconazole, a commercial mixture of both, and benomyl, on alkaline phosphatase activity in internal and external hyphae of an arbuscular mycorrhizal fungus in 4-week-old plants. They reported that benomyl inhibited fungal alkaline phosphatase activity in both internal and external hyphae at the low application level of 1 $\mu$g g$^{-1}$ soil, whereas fenpropimorph had an intermediate effect at the high application level of 125 $\mu$g g$^{-1}$ soil, inhibiting both internal and external hyphae. Propiconazole decreased the activity of the external hyphae at low applications (0.21 $\mu$g g$^{-1}$ soil) but did not affect the internal activity at any level. Kjoller and Rosendahl also observed a decrease in arbuscular mycorrhizal colonization and decreased P uptake due to the application of fungicides.

Abd-Alla et al. (2000) reported the negative effect on application of various pesticides (Afugan, Brominal, Gramoxone, Selecron and Sumi Oil) on root colonization by arbuscular mycorrhizal fungi of the legumes cowpea (*Vigna sinensis* L.), common bean (*Phaseolus vulgaris* L.) and lupin (*Lupinus albus* L.). Pesticide application significantly inhibited arbuscular mycorrhizal root colonization and the number of spores in all legumes. However, Abd-Alla et al. reported stimulation of the spore formation in pesticide-treated cowpea 60 days after planting.

In a contradictory report by Schweiger et al. (2001), application of the commercial fungicides carbendazim and a mixture of propiconazole and fenpropimorph (as their active ingredients) stimulated hyphal P uptake from inside hyphal compartments at recommended rates. Stimulation of hyphal P uptake may be attributed to the negative effect of the fungicides on other components of the soil microbial community that interact with arbuscular mycorrhizal fungi. However, application of fungicides at high concentrations (100 $\times$ the recommended rate) completely inhibits hyphal phosphorus uptake and arbuscular mycorrhizal colonization (Schweiger et al. 2001). Similar results have been reported by Bary et al. (2004) who demonstrated that fungicides applied at low levels over a period do not affect arbuscular mycorrhizal fungal populations.



### *Cyanobacteria and soil algae*

Algae and cyanobacteria are located at the base of the food chain and are important organisms that maintain the ecological equilibrium in both aquatic and terrestrial environments. Among nitrogen fixers in soil and water environments, blue–green algae are probably the most important becasuse of their unique capacity to fix both atmospheric nitrogen and carbon dioxide (Pandher et al. 1994). Martensson (1993) reported the use of nitrogen fixation (deduced by acetylene reduction assay) as an indirect method to assess the impact of pesticide application on soil biological properties. Mallavarapu (2002) reported a decrease in the diversity of soil algal forms, as well as the replacement of cyanobacteria by more resistant green algae, in soils contaminated by the insecticide DDT. These shifts in genera may lead to a decrease in biodiversity leading to the loss of some ecological functions. Similarly, application of the insecticide trichlorfon inhibited the nitrate uptake rate in *Anabaena* PCL7119, and reduced the nitrate uptake rate and the chlorophyll and phycobiliprotein content in other cyanobacteria.

At low concentrations, herbicides influence morphology and photosynthesis by affecting electron transport in cyanobacteria (Venkateswarlu 1993). At higher concentrations, all herbicides reduce the total chlorophyll content, total sugar content and dry weight of cyanobacteria compared with untreated controls (Sreenivas and Rana 1994). Application of the herbicide atrazine severely decreases beneficial soil enzyme activities (dehydrogenase and arylsulfatase) and significantly reduces the microbial carbon biomass and soil algal population (Mallavarapu 2002).

Soil algae (edaphophytes) are quite abundant and highly diverse forms existing in soil sampled from both arable and virgin land. Being photoautotrophic, these microbes are concentrated in the top few inches of the soil profile and are organized in a community structure that varies depending on soil type, farming method and pesticide application (Berard et al. 2004). Because of their photosynthetic activity, these microbes could be used as early bioindicators of disturbances related to pesticide pollution or xenobiotic contamination occurring in the pesticide-applied soil (Kostikov et al. 2001). In a study by Megharaj et al. (2000), the viable soil algae count was seen to decline with increased DDT contamination of the soil. Megharaj et al. also reported alteration in the composition of algal and cyanobacterial species in contaminated soils and the elimination of sensitive algal species in medium and highly contaminated soils as negative impact of DDT application. Resistant algal species, including the nitrogen-fixing cyanobacteria, are the most potent DDT degraders and show catabolism of DDT to generate different metabolic intermediates. In a recent study by Caceres et al. (2008), a toxic xenobiotic organophosphorus insecticide fenamiphos was reported to be degraded by soil algae (*Chlorella, Scenedesmus* sp., *Chlamydomonas* sp., *Stichococcus* sp.) and cyanobacteria (*Nostoc* sp., *Nostoc muscorum, Anabaena* sp.)

### *Protozoans protists and others*

Protozoa are resplendent in diversity of their translucent adaptations to microcosmic environments, both in terms of numbers and biomass, and occur in a wide range of habitats, including soil (Cotterill et al. 2008). The soil food web has diverse types of microfaunal forms including the protozoan protists and the microarthropods that inhabit the water/air-filled porosity of the soil system, which in turn determines their access to resources, exposure to predators and inclusion in the micro-food webs (Lavelle et al. 2006). The major soil protozoan groups include the naked amoebae, ciliates and flagellates. Protozoan activities within the water-film communities are extensive, with an average of 10–12 turnovers per year. Protozoa, particularly amoebae and flagellates, probably have more impact on soil microbial C and N turnover on a per unit mass basis than any other fauna. Predator–prey interactions in the rhizosphere are important to stimulate plant growth according to the 'microbial loop in soil'; the mechanism that involves protozoa and nematodes (Bonkowski et al. 2009). An increase in microbial density in the rhizosphere stimulates bacterial grazers such as protozoa, which on grazing the bacteria excrete one third of the ingested N as ammonia (Lambers et al. 2009).

Being microbial grazers and predators of bacteria and fungal forms, protozoans have a great impact on the population of pioneer communities in soil food web. Bonkowski et al. (2000) reported that the presence of protozoa decreases the length of fungal hyphae by 18%, while the presence of ectomycorrhizas leads to a reduction in the numbers of bacterial and protozoan grazers. Moreover, certain protozoans, particularly ciliates, maintain their specific number in the soil system. Because the soil system exhibits a complexity several orders of magnitude higher than that of aquatic systems, the soil ciliate population remains in a constant ciliatostasis by maintaining a particular number of ciliates in encysted form. In a soil microcosm study, the dynamics of encysted to physiologically active forms of soil ciliates was determined by Ekelund et al. (2001). They reported that internal population regulation is the major factor governing ciliate encystment and the rate of encystment depends on ciliate density. This internally governed encystment may be an essential adaptation to an unpredictable environment in which it cannot be known when the soil will dry out and if individual protozoa will survive desiccation.

These eukaryotic microscopic soil faunal forms are quite sensitive to pesticide input. Ekelund et al. (1994) reported a negative impact of pesticide application (dimethoate, pirimicarb and fenpropimorph) on the colonization of a sterilized soil by a natural population of soil protozoa. A maximum decrease in the protozoan population, particularly soil flagellates, was reported in fenpropimorph-treated soil. A similar report by Ekelund (1999) further emphasized the negative impact of corbel pesticide on the number and diversity of soil protozoans as pesticide concentration increased. A decrease in naked amoebae and heterotrophic flagellates (bacterivorous protozoa) at concentrations lower than the recommended field application were observed and a significant decrease in hetertrophic flagellates was recorded at even lower concentrations. This may be attributed to a change in the microbial food web leading to increased competition from and/or predation by ciliates. Because protozoa play an important role in nitrogen mineralization, the beneficial effect of the fungicide may be counteracted by detrimental side effects on soil N-mineralization.

In a study carried out by Downing et al. (2004), application of pesticides (atrazine, chlorothalonil and endosulfan) decreased the number of protist taxa, with a decrease in the relative abundance of chlorophyte and chrysophyte taxa by atrazine, chrysophyte and heterotrophic protists by chlorothalonil, and chrysophyes, cryptophytes and dinoflagellate taxa by endosulfan application. Soil protozoa also exhibit a decrease in number and diversity with time of pesticide exposure. Trielli et al. (2006) recorded the dose-dependent effect of organophosphate pesticides on cell viability and mean fission rate in *Colpoda* protist. They also reported a significant decrease in the number of excysted cells in comparison with untreated controls. Moreover, at the cytology status, an interference with the ability of *Colpoda* to extrude macronuclear chromatin was also recorded to be due to pesticide exposure. Adebavo et al. (2007) reported a decrease in the protozoal population corresponding to an increase in the number of days after treatment, i.e. time of exposure of soil to Thiodan and Karate insecticides at high concentrations (8000 and 12,000 mg $kg^{-1}$, respectively). Similar alterations in the species communities of soil diatom populations have been reported following the application of atrazine herbicide in soil sampled from a corn field with a relative abundance of diatom communities which developed tolerance to atrazine (Berard et al. 2004). Ojo et al. (2007) observed a differential impact of the application of four fungicides, pentachloronitrobenzene (PCNB), benomyl, Captan and Cresan, and reported that PCNB was the most potent biocidal compound because it completely eliminated all soil protozoan and fungal propagules in treated soil samples and also reduced almost all bacterial and actinomycetal forms.

Soil protozoans also exhibit profound biochemical diversity by virtue of the occurrence of several enzymes, in particular the soil amoebae and some ciliates exhibit chitinase enzyme system (including enzymes viz., lysozymes, exochitinases and endochitinases) activities that help to lyse fungal cell walls by degrading the chitin polymer. Several amoebae have been shown to be significant control agents of *Gaeumannomyces graminis tritici*, the notorious ‘take-all’ fungus of South Australian wheatlands (Coleman 1994).

Microarthropods are a group of microflora that include the microscopic larval forms of the insect arthropods and have the prime function of maintaining the number or diversity of bacterial, fungal or protozoal forms on which these prey/graze. This group of soil micro-food web is quite sensitive to the application of xenobiotics, particularly pesticides. Krogh (1991) reported a significant decrease in the members of the major composite taxonomic groups including the Collembola, Acarina, Cryptostigmata, Brachychthonoidea, Prostigmata, Mesostigmata and Astigmata, with a stimulation in the Tarsonemidae, during the drought period in first few months of isofenphos pesticide application. However, they reported that benomyl application resulted in a more complicated response pattern, with an increase in the population of the whole microarthropod group and negative effects remaining for only the first five months. In a microcosm study, Parmelee et al. (1996) reported a lowering of the total microarthropod abundance and a specific decrease in the prostigmatid and oribatid mites in malathion (400 mg $kg^{-1}$) and Aroclor 1254 applied treatments. The application of pesticides not only decreases the number of microarthropods, but also results in a reduced reproduction rate. Joy et al. (2005) reported a reduction in the number of major taxonomic and trophic groups of Collembola as well as decreased rates of reproduction by application of heptachlor and endosulfan pesticides.



## Successful alternative strategies to pesticide use applied to date

### *Use of biopesticides*

Biopesticides are included among alternative techniques to amend and decrease the impact of chemical pesticide usage, and form one of the foremost strategies of the Integrated Pest Management (IPM) protocol. These include cultural methods of plant resistance to pests, conservation of natural enemies in the crop and the use of insect pest-control products such as 'microbial insecticides'. The IPM concept partly includes the integrated use of biocontrol agents (microbes, insects, extracts/compounds) along with the judicious, timely and lesser use of chemical pesticides, as well as wise management of agricultural tools and cropping practices/protocols.

Biopesticides, an important part of the biological control system, are products/processes derived from animals, plants and microorganisms such as bacteria and viruses. The most commonly used biopesticides include *Bacillus thuringiensis*-based products, baculoviruses and botanical extracts such as the use of rotenone, pyrethrin, nicotine and azadirachtin (alkaloid produced by neem). In addition to these a fungus, *Trichoderma*, has been used along with biocontrol agents (*Trichogramma* parasitizes and preys upon pest eggs) and *Bacillus thuringiensis* modification.

### *Bioremediation of pesticide-contaminated soils/land*

Bioremediation utilizes the already available arsenal of pesticide-degrading gene pools coding for a number of enzymes such as oxygenases, hydroxylases, hydrolases and isomerases in native pesticide-resistant vanguard microbial populations of contaminated soils/lands to reclaim the site for sustainable agriculture and crop production (Farrar et al. 2002). This spans phytoremediation, mycoremediation, mycorhizoremediation, etc., depending on the type of organism applied/used (Khan 2007). The choice of microbes, plants, mycorrhiza, mushrooms or their combinations in a remediation effort depends on the extent of the contamination, the nature of the chemicals present, and the amount of the source available for decontamination (Chowdhury et al. 2008). However, the hydrophobic nature of most pesticides is the major obstacle to their uptake by microbes or plants.

### *Utilization of plant-associated microbes*

Plant-associated microorganisms play essential roles in agricultural and food safety, and contribute to environmental equilibrium. Their study has classically been based on cultivation-dependent methods, which often recover only 0.01–10% of direct counts. However, studies based on molecular analysis have estimated $>$ 4000 species per gram of soil. Most of these microorganisms are probably noncultivatable, such as the plant symbiotic arbuscular mycorrhiza endomycorrhizae common in many angiosperms and gymnosperms, or are transiently in a viable but noncultivable state (Montesinos 2003). Hence, it could be suggested that certain plant growth-promoting microbes have fastidious nutritional interdependencies on their supporting microfloral forms, resulting in an inability to culture them in pure forms. These plant growth-promoting or disease-suppressive microbes not only exhibit nutritional interdependency, but also have evolved in a manner that, on co-inoculation, may impart extra benefit(s) on the plant. Dwivedi et al. (2009) have reported the positive effect of co-inoculation of various arbuscular mycorrhizal fungi (*Glomus* species) with phenazine and diacetylphloroglucinol positive *Pseudomonas fluorescens*, however, an unknown antifungal metabolite-producing *Alcaligenes faecalis* strain SLHRE425 negatively influenced arbuscular mycorrhiza root colonization.

Certain soil bacteria and several actinomycetes are well known to produce a few secondary metabolites having antimicrobial activities (Haas and Keel 2003). Production of these antimicrobial compounds by the producers in biologically active concentrations at localized sites in the environment has a substantial impact on the soil rhizospheric population (including soil probiotic and pathogenic microbes) lacking the potential to produce antibiotics (Thomashow et al. 2008). Moreover, a single microbe may have the potential to produce a variety of, perhaps five or six, different antimicrobial products. These antibiotics may contribute to microbial competitiveness and to the suppression of plant root pathogens.

Soil bacteria that exhibit antibiotic production include the genera *Pseudomonas, Bacillus, Rhizobium* and many more. Among the *Pseudomonas* species inhabiting the rhizosphere, certain strains of fluorescent pseudomonads have received particular attention (Vlami 2008) because of their potential to control seed- and soil-borne pathogenic fungi and oomycetes (Raaijmakers and Weller 2001). Important antimicrobial compounds for which a major contribution to biocontrol has been demonstrated are 2,4-diacetylphloroglucinol, pyoluteorin, phenazines, pyrrolnitrin, cyclic lipopeptides and the volatile hydrogen cyanide (Raaijmakers et al. 2006; Loper et al. 2007). Some strains, such as *Pseudomas fluorescens* CHA0 and Pf-5, produce multiple antibiotics with overlapping or different degrees of activity against plant pathogens (Bottiglieri and Keel 2006).

Karpunina et al. (2003) reported growth suppression in *Rhizobium leguminosarum* and *Bacillus subtilis* by lectins I and II (at a concentration of 1–10 $\mu$g ml$^{-1}$) isolated from the nitrogen-fixing soil bacterium *Paenibacillus polymyxa*. Moreover, lectin I inhibited *Azospirillum brasilense* and *Erwinia carotovora* subsp. *citrulis*, while lectin II exerted bactericidal activity against *Xanthomonas campestris* and

*Azospirillum brasilense*. Using a culture-independent approach, Robleto et al. (1998) have reported a significant effect on trifolin toxin-sensitive bacteria in the rhizosphere of beans after inoculation with trifolin toxin-producing *Rhizobium* strains. They recorded an alteration in the alpha-proteobacterial ribosomal intergenic spacer analysis (RISA) profile showing a considerable reduction in diversity by the trifolin toxin-producing strain, although very little impact on the total microorganism RISA profile was reported.

Apart from the fluorescent pseudomonads and alpha-proteobacteria, soil actinomycetes are the biggest group of antibiotic producers and thus have an enormous impact on other soil microbes in the rhizospheric region. A streptomycin producer, *Streptomyces bikiniensis*, has been reported to have a significant negative effect on the survival of *Salmonella enterica* serovar Dusseldorf in soil compared with a nonantibiotic-producing *Streptomyces* species (Turpin et al. 1992). However, it is not always the case that antibiotic-producing actinomycetes have a negative impact on the indigenous soil probiotic microbes. In certain instances, antibiotic producers may maintain similar population levels because of several possible reasons such as necrotrophic growth of resistant bacteria in the presence of killed sensitive groups, inhibition of only some susceptible soil bacteria, highly localized antibiotic production in soil and significant inhibition of bacteria in a restricted area, and the stimulatory effect of killing some members of the indigenous microflora due to enhanced nutrient availability. Hence, the overall impact on the population change may be too small to record for inoculation of a single antibiotic-producing microbe (Anukool et al. 2004).

The presence of these microbes in an intricate microbial community may be identified using culture-independent techniques (Kaur et al. 2005; Offre et al. 2007; Saito et al. 2007). Utilization of these plant-beneficial microbes, which may exist in symbiosis, loose associative or endophytic association with the plant would comprehensively enhance the benefits imparted to the plant (directly or indirectly) and decrease the incidences of plant pathogenic infections.

### *Effect of transgenic crop cultivation*

Transgenic plants carry genes and produce compounds foreign to their environment, which leads to concerns about their environmental use because of potential ecological ill effects. The issue of direct and indirect effects on nontarget organisms and the ecosystem is particularly important because many transgenic plants are being developed that have new or enhanced antimicrobial properties for protection against phytopathogens. These transgenic plants express antimicrobial compounds such as chitinases, glucanases, lysozymes, thionins, defensins and systemic acquired resistance (SAR) gene products, harbor antibiotic, herbicide/weedicide resistance genes or produce novel toxins for pest resistance.

Microcosm and field studies have shown that exposure to transgenic plants produced changes in the population levels and composition of some soil and plant microorganisms. Microcosm studies also revealed that *Bacillus thuringiensis* var. *kurstaki* (*B.t.k.*) endotoxin persists at low levels and would be of ecological concern on repeated use of *B.t.k.* endotoxin-producing plants because this would lead to accumulation of the endotoxin and potentially affect the soil microorganisms adversely. Genetically modified potatoes consistently altered the physiological profile of the rhizosphere microbial community at harvest, but the effect did not persist from one season to the next (O'Callaghan and Glare 2001). Transgenic crops alter both the population levels of bacteria and fungi, and the species composition of bacteria. Ahrenholtz et al. (2000) reported the bactericidal effect of transgenic potato plant roots expressing the phage T4 lysozyme gene on cells of *Bacillus subtilis* attached to root hair cells using appropriate staining and fluorescence microscopy techniques. They also reported that the transgenic plants always showed significantly (1.5–3.5-fold) higher killing which was independent of the age and growth of plant.

However, in certain instances, transgenic plants can also cause transient but significant increases in the levels of culturable aerobic bacteria and fungi. Transient changes may include stimulation of bacterial and fungal populations (Donegan et al. 1995) or a decrease in their diversity. There may be a higher incidence of the plant pathogen *Verticillium dahlia* in transgenic *Bacillus thuringiensis* var. *morrisoni* strain *tenebrionis* (*B.t.t.*)-producing plants owing to their enhanced longitivity. Transgenic plants that produce opines can influence the composition of rhizosphere bacteria by altering their populations due to the selective utilization potential of opines by rhizosphere bacteria. A delay in the onset and a decrease in the level of colonization by the arbuscular mycorrhizae *Glomus mosseae* was observed in transgenic tobacco plants with chitinase expression. Light microscopy revealed distinct differences in the fungal structures on transgenic plants compared with control plants. Pesticidal proteins produced in transgenic plants can persist in soil and binding of these proteins to soil particles can protect them from biotic degradation. Even the DNA in transgenic plants can persist in a field environment for several months. There is potential for the horizontal gene transfer of antibiotic resistance from transgenic plants to microorganisms (Dunfield and Germida 2004). However, Demaneche et al. (2008) reported no significant differences in bacterial antibiotic-resistance levels between transgenic and nontransgenic corn fields, although the bacterial populations were different.

Castaldini et al. (2005) have assessed the effects of *Bt* corn plant residues on soil respiration, *Glomus mosseae*, and rhizospheric and bulk soil eubacterial communities. They observed differences in rhizospheric eubacterial communities associated with the three corn lines by means of denaturing gradient gel electrophoresis (DGGE) analyses of 16*S* rRNA genes and also noted a significantly lower level of mycorrhizal colonization in Bt 176 corn roots. At four European locations that differed in their climatic conditions or soil properties, Cortet et al. (2007) reported a transient decrease in the soil microarthropod population with significant negative effects recorded in soil with a high clay content due to transgenic *Bt* maize expressing Cry1Ab protein under field conditions. Selim et al. (2007) reported a significant reduction in the number of bacterial colony-forming units after 2 and 4 days of treatment with paenimyxin (biopesticide produced by *Paenibacillus* sp. Strain B2). They also reported a significant modification to the genetic structure of the bacterial communities, as indicated by RISA fingerprinting. However, treatment did not affect the quantification of 16*S* rDNA or of the denitrifying bacterial community. Thus the genetic structure of soil bacterial communities was recorded as transient, because no effect was reported after 7 days in comparison with the untreated control. However, the negative impact on soil microarthropods was more evident for pesticide usage than for the use of *Bt* maize. Icoz et al. (2008), however, reported inconsistent yet statistically significant differences in the numbers of different groups of microorganisms, the activities of the enzymes, and the pH between soils planted with *Bt* and non-*Bt* corn over a period of four years of continuous corn cultivation. All these points advocate for thorough discussions on the facts and fallacies surrounding the utilization of transgenics.

## Future perspectives

### *New generation pesticides*

New generation pesticides include the reformulation of known pesticides and the development of new types, particularly organic and nanopesticides. Futuristic pesticides such as propesticides, natural base pesticides, chitin inhibitors, pheromones, metamorphosis disruptor sulfonyl ureas, dinitroanillines and triazoles are more active against pests (Technology Information 2009). Advanced molecular biology techniques have been instrumental in identifying several targets for new pesticides. Particularly in the case of fungicides, complex III containing a cytochrome *b* with two ubiquinone-binding sites (electron transport system of fungal mitochondria) has been identified as a potential target for methoxyacrylate fungicides. Similarly, the osmotic signal transduction pathway consisting of histidine kinase and mitogen-activated protien (MAP) kinase cascades has been a target for dicarboximide fungicides (Isamu and Makoto 2005).

Formulation technology is now seen as enabling, and can add significant value and attractive presentation to pesticide products, while at the same time improving operator safety and reducing the dose rate and wastage of pesticides applied to crops, thereby reducing environmental impact and enhancing food safety (Knowles 2009). Volgas et al. (2005) reported the reformulation of the well-known herbicide 2,4-D as iso-octyl ester emulsifiable concentrate in solvents like hydrocarbons and methyl esters of fatty acids, which gives the compound excellent weed-control activity. Improving the types of formulations from the simple solutions in water, emulsifiable concentrates in a petroleum-based solvent, or dusts and wettable powders to water-based liquid formulations such as suspension concentrates, oil-in-water emulsions, microcapsules and water-dispersible or -soluble granules not only increases the activity of the pesticide, but also the environmental safety. Heylings et al. (2007) have discussed the development of new environmentally safer formulation of the herbicide Paraquat which contains an increased concentration of the active ingredient together with the addition of purgative-like magnesium sulfate and gel-forming substances such as alginate which delay absorption of the ingested product. Bateman (2008) also discussed the development of a new formulation of Paraquat which is commercially available from Syngenta.

Formulations that contain a mixture of various types of active ingredients impart augmentive effects. Application of a mixture of pesticides can bring about an increased level of performance; however, interactions among the active ingredients can also have adverse effects on performance and possibly on the safety of the product to users, consumers and the environment. Because of this, a product containing a mixture of active substances must be considered by the registration authorities (Godson et al. 1999).

The development of organic pesticides might be a substitute for synthetic pesticides, particularly when two or more active components are combined to provide novel modes of action against a wide variety of pests, particularly insects. Organic pesticides containing various bioactive ingredients will also exhibit reduced risk of cross-resistance because pests would have difficulty in adapting to a group of bioactive compounds. Antonious et al. (2007) reported the use of capsaicinoids (pungent compound from Chinese capsicum) to control a variety of insects and spider mites in vegetables.

Nanopesticides are one of a number of new strategies being used to address the declining efficacy of older-style chemical pesticides, combined with the inevitable rising price of petrochemical-based inputs. Nanopesticides primarily include nano-encapsulated pesticides (nano-insecticides and nano-herbicides are currently available) or the reformulation of a previously approved pesticide through nano-sizing of the active ingredients. The latter could be formulated by creating nano-sized versions of pesticide molecules leading to the development of nanopesticide emulsions that are more stable, more toxic to pests and better absorbed into plants (Norwegian Pollution Control Authority 2008).

The formulation of a pesticide must be designed to meet the demands of efficacy and suitability to the mode of application while minimizing damage to the environment. Nanoencapsulation of pesticides meets these demands because it enables smaller quantities to be used effectively over a given period and the pesticide design enables them to resist the severe environmental processes that act to eliminate conventionally applied pesticides, i.e., leaching, evaporation and photolytic, hydrolytic and microbial degradation. Nanocapsules are solid hollow particles ranging from 10 to 1000 nm in diameter, which can be filled with pesticides in small dosages, resulting in sufficient effects on pests yet a reduction in side effects. Moreover, their small size makes their deposition on the sprayed sites easy, helping to reduce wastage (Le Roy Boehm et al. 2000). Several nanoproducts such as carbon nanotubes, carbon nanofibers and fullerenes could be used to encapsulate known/formulated pesticides.

The most well-known encapsulated nano-insecticides are the aluminosilicate nanotubes containing active ingredient/pesticide, which could easily be picked up by insect hairs while crawling on the sprayed aerial parts of plants. Once the nanotubes reach the surface of the insect, they are actively imbibed inside the tissues. Consumption of pesticide-filled nanotubes leads to release of the active ingredient in the body and finally the death of the insect. Guan et al. (2008) reported the formulation of photodegradable insecticide imidacloprid microcrystals (directly encapsulated with chitosan and sodium alginate through layer-by-layer self-assembly), which have high toxicity against the adult stage of *Martianus dermestoides*.

Nanoemulsions are another type of nanopesticide that are biologically more active and relatively environmentally safe. Moreover, nanoemulsions are useful in the delivery of water-insoluble pesticides in aqueous systems. Zhu et al. (2009) reported the use of an oil-in-water microemulsion as a template to prepare nanocomposites with diameters $< 100$ nm by complex coacervation of acacia and protists containing water-insoluble Lepidopteran insecticide, beta-cypermethrin.

Research on the molecular mode of action of nanopesticide formulations in insects, biosafety and molecular interaction with plants, soils and the environment is scanty. Similar to conventional pesticides, on application (e.g., foliar spray or soil application), nanoformulations interact with the soil, insect, plant and atmosphere, but here both the active ingredient of the pesticide and the encapsulating material show their individual and cumulative effect on the interaction. Nanopesticides, in conjunction with a number of other physiochemical parameters including shape, particle size, crystalline structure and surface chemistry, possess potentially greater toxicity (regarding human exposure) and eco-toxicity risks than conventional chemical pesticides (Nel et al. 2006).

However, there are very contradicting reports on the impact of nanopesticides or their constituting components, i.e., encapsulating materials/emulsions. In a study on *Escherichia coli* and *Bacillus subtilis* (Fortner et al. 2005), bacterial tests of the toxicity of C60 fullerenes have shown reduced growth and respiration at low concentrations. Similar changes have been observed at a higher resolution (community level) in both terrestrial bacteria and protozoa *in vivo* (Johansen et al. 2008). In the latter experiment, bacteria and protozoa were exposed to nC60 at 5–50 mg $kg^{-1}$ in soil for 7 and 14 days, and subsequent changes in the community structure were measured using both culturing and growth-independent techniques (polymerase chain reaction [PCR] amplification of total genomic DNA and separation by [DGGE]). Changes in the community structure indicated a growth impediment for certain species of the indigenous microbial community and demonstrated the ecotoxicological effects of nC60 even in the presence of a highly complexing medium like soil. A report by Tong et al. (2007) provided contradicting conclusions regarding fullerene toxicity. They reported that fullerene had the least detremental impact (1 or 1000 mg $kg^{-1}$ of C60) on the structure and function of the soil microbial community and microbial processes. The impact on community structure was evaluated by fatty acid profile analysis and DGGE–PCR of total genomic DNA using bacterial variable V3 region targeted primers, while the functional analysis was performed by studying several soil enzymatic activities for $\beta$-glucosidase, acid-phosphatase, dehydrogenase and urease (Tong et al. 2007).

Similar to nanocomposites or nanoemulsions, engineered nanoparticles of Ag, CuO and ZnO have microbicidal activity, particularly towards soil bacteria. These nanoparticles are now being prepared and sold as common antimicrobials in various household products and their application is currently restricted to nanomedicine or nanohygiene. However, these engineered nanoparticles contaminate soil and water through the sewage system or by the inefficient disposal of the nanoproducts. On release into the environment, these engineered nanoparticles exhibit toxic effects on beneficial soil microbes leading to both cidal and static effects on soil microbes. Nanoparticles show a varying degree of antimicrobial activity, which is photo- and dose/concentration sensitive. Usually nanoparticles attack the bacterial cell wall forming pits (Sondi and Salopek-Sondi 2004; Gogoi et al. 2006) or attack bacterial membranes, i.e., destabilization of the outer membrane by treatment with nano-silver particles leading to collapse of the plasma membrane potential and decreased ATP production (Lok et al. 2006) as well as loss of membrane integrity (Reddy et al. 2007). The ecotoxicity of $TiO_2$ (APS 330 nm), $SiO_2$ (APS 205 nm) and ZnO (APS (air plasma spraying) 480 nm)-engineered nanoparticles to Gram-positive (*Bacillus subtilis*) and Gram-negative (*Escherichia coli*) bacteria in water suspensions containing citrate and low phosphate concentrations has also been reported (Adams et al. 2006). Gajjar et al. (2009) reported the antimicrobial toxicity of engineered nanoparticles of Ag, CuO and ZnO in soil probiotic bacteria *Pseudomonas putida* strain KT2440 as detected by a plasmid construct bearing *luxAB* reporter genes.

Once released into the environment, engineered nanoparticles may aggregate to some degree, be associated with suspended solids, or sediment to be accumulated by organisms and enter sources of drinking water and food materials. Because soils, sediments and water bodies contain solid and dissolved matter that can be a powerful geosorbent, nanoparticles could be easily adsorbed. These fate processes are dependent on the characteristics of the particle and of the environmental system (Boxall et al. 2007). However, the aggregation of nanoparticles into larger particles, possibly by factors present in the environment may reduce their nontarget antimicrobial activity. Shah and Belozerova (2009) reported a statistically insignificant influence of nanoparticles in the soil on the number of colony-forming units, peak areas of methyl ester of fatty acids in the FAME profile or on the total soil community metabolic fingerprint.

### *New approaches for pesticide application/sensing*

Currently, methods of pesticide application are being revised and upgraded from the traditional bulk or split application techniques. The novel method of using an irrigation system to apply fertilizers and pesticides, i.e. chemigation, is quite revolutionary regarding the precision application of pesticide. Moreover chemigation via drip irrigation has advantages compatible with environmental stewardship. Felsot et al. (2000) reported the suitability of drip and furrow irrigation techniques for insecticides of the neonicotinoid class, including imidacloprid, thiamethoxam and acetamiprid because there was less loss of the applied pesticide by leaching or volatilization.

Several new methods have been developed to sense the presence of the applied pesticide using rapid and cost-effective on-field tests because applied pesticides have the ability to move away from the site of application or produce recalcitrant breakdown intermediates/products. Nanosensors have superior properties over existing techniques such as high-performance liquid chromatography or gas chromatography, because they can provide rapid, sensitive, simple and low-cost on-field detection (Liu et al. 2008). Measurement protocols based on nanoparticles and nanotubes are also suitable for the mass fabrication of miniaturized devices. M Wang and Li (2008) reported the fabrication of $ZrO_2$/Au nanocomposite films that can detect (voltammetric detection) the organophosphate pesticide parathion. In addition to nanocomposites, the use of nanoparticles joined to substrate-integrated enzyme activation and carbon nanotubes or carbon nanofibers are other common types of nanobiosensors. The majority of nanobiosensors developed to date produce electrochemical, amperometric or voltametric signals. Kim et al. (2008) used an approach for substrate-integrating enzyme activation (substrate-bound tyrosinase electrode using gold nanoparticles anchored to pyrroloquinoline quinone) and corresponding current response at the substrate-bound enzyme electrode identified with cyclic and differential pulse voltammetry. Du et al. (2007) have developed a sensitive, fast and stable amperometric sensor for the quantitative determination of the organophosphorous insecticide triazophos by immobilization of acetylcholinesterase enzyme on a multiwall carbon nanotube–chitosan composite. Electrochemical biosensors based on carbon nanotubes and carbon nanofibers have also been used for sensing environmental pollutants such as pesticides (J Wang and Lin 2008).

## Conclusions

Extensive agrochemical applications, particularly of pesticides, affect the soil microbial flora, thereby indirectly affecting soil aggregation and fertility. Among all the groups of soil microbes discussed above, a primary decrease in the number and diversity of microbes occurs on pesticide application. In particular, a decrease in bacteria is more evident in terms of diversity followed by an increase in the population of certain resistant bacterial that dominate, as indicated by altered RISA profiles for pesticide-treated soils. However, certain microbes such as fungi and actinomycetes are better able to metabolize xenobiotics like pesticides and thus have the ability to flourish and multiply following an initial transient decrease in number. The application of insecticide has a positive impact on a few soil cyanobacteria, in particular because arthropod larvae (which are predatory cyanobacterial grazers) are killed, although they are are negatively affected by other types of pesticides. Although conventional pesticides have been useful in the green revolution in Asia, sensible use of these products is now required to avoid environmental pollution. Novel protocols for a lower but better application of pesticides, i.e. precision application, would be useful in decreasing the ecotoxic effects and human health hazards. Moreover, further developments to popularize alternative methods, such as biopesticides, organic pesticides, novel biocontrol agents and nanopesticides should be emphasized to avoid careless pesticide application. The use of nanopesticides (the latest entrants in the pesticide world) is debatable because of their unknown ecological concerns and negative environmental impacts.

**Acknowledgements**

Authors are thankful to the state government of Punjab, India for providing the necessary funding for carrying out of the research work.

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