Increase in food production is the prime-most objective of all countries, as world population is expected to grow to nearly 10 billion by 2050. Based on evidence, world population is increasing by an estimated 97 million per year (Saravi and Shokrzadeh, 2011). The Food and Agricultural Organization (FAO) of the United Nations has in fact issued a sobering forecast that world food production needs to increase by 70%, in order to keep pace with the demand of the growing population. However, increase in food production is faced with the ever-growing challenges especially as the new area that can be increased for cultivation purposes is very limited (Saravi and Shokrzadeh, 2011). The increasing world population has, therefore, put a tremendous amount of pressure on the existing agricultural system so that food needs can be met from the same current resources like land, water, etc.

In the process of increasing crop production, herbicides, insecticides, fungicides, nematicides, fertilizers and soil amendments are now being used in higher quantities than in the past. These chemicals have mainly come into the picture since the introduction of synthetic insecticides in 1940, when organochlorine (OC) insecticides were first used for pest management. Before this introduction, most weeds, pests, insects and diseases were controlled using sustainable practices such as cultural, mechanical, and physical control strategies (Gill and Garg, 2014).

Pesticides have now become an integral part of our modern life and are used to protect agricultural land, stored grain, flower gardens as well as to eradicate the pests transmitting dangerous infectious diseases. It has been estimated that, globally, nearly $38 billion is spent on pesticides every year (Pan-Germany, 2012). Manufacturers and researchers are designing new formulations of pesticides to meet the global demand. Ideally, the applied pesticides should only be toxic to the target organisms, should be biodegradable and eco-friendly to some extent (Rosell et al.,
Unfortunately, this is rarely the case as most of the pesticides are non-specific and may kill organisms that are harmless or useful to the ecosystem. In general, it has been estimated that only about 0.1% of the pesticides reach the target organisms and the remaining bulk contaminates the surrounding environment (Carriger et al., 2006). The repeated use of persistent and non-biodegradable pesticides has polluted various components of water, air and soil ecosystem. Pesticides have also entered into the food chain and have bioaccumulated in the higher tropic level. More recently, several human acute and chronic illnesses have been associated with pesticides exposure (Mostafalou and Abdollahi, 2012; Gill and Garg, 2014).

Increasing use of pesticides is creating substantial health problems to humankind all over the world. Although not all pesticides are equally risky and not all people are equally at risk, the effects can be acute or chronic. Acute toxicity effects are better understood. Depending upon the type of pesticide used, exposure time and the dose absorbed by the body, they can produce symptoms within minutes or hours; most of which diminish in time. These poisoning or intoxication effects result in mild to severe headache, nausea, flu, skin rashes, blurred vision and certain neurological disorders.

Toxic reactions may be worse for those suffering from poor nutrition or dehydration and warmer temperatures may also increase the toxic effects. This implies that field labourers working in the heat may be more susceptible to poisoning. Of all the possible health impacts from pesticide exposure, cancer has been the most frequent focus of attention, as there are numerous reports of carcinogenic potential of pesticides in animals (Wahid Abdul, 2004).
In contrast to acute effects, much more uncertainty surrounds the chronic effects, which are believed to arise from low-level exposures to pesticide residues in food and water. Some of these agrochemicals persist in the soil for long time and show cumulative residual effect. They not only affect the soil microflora but change the physicochemical properties of soil, too. The residual effects are likely to prevail more in the developing countries due to the use of supra-optimal doses together with unplanned crop treatment. They are taken up by the roots from the soil and transported to aerial parts. When absorbed directly by the leaves, they follow the apoplastic/symplastic pathways of phloem transport for storage into the seed and consequently enter the food chain. Many reports witness that seed consumed as a whole or as its product contain the amount of pesticides that can cause significant hazardous effects on the consumer health (Wahid Abdul, 2004).

Major environmental consequences of pesticides are their accumulation in the body of organism, and movement through the components of environment due to their higher persistence. Another problem associated with the use of pesticides is the development of resistance in pests with time. The resistant population of pests grows faster and damages the crop with more vigour.

The undue persistence, high mammalian toxicity and developing resistance of the organochlorine, organophosphate and carbamate insecticides led to a ban or restriction on their use in many developed and developing countries. It has become imperative for the humans to synthesize new types of molecules that would prove to be more effective killers of pests to reduce crop and stored grain losses. These newly synthesized chemicals pose fewer hazards to the environment and their long term effects on the non-target organisms in the environment.
Many people believe that there is no alternative to pesticide use to raise crops that is required to feed the ever growing human population. Besides helping in the production of greater quantities of food, pesticides also help reduce loss of food in storage and vector borne diseases. Every year, above 3 billion pounds of pesticides are used for these and other purposes. Agriculture accounts for the greatest percentage, followed by the industry, forestry and government, and home gardening and lawn usage (EPA, 1982).

Pesticides also have some major drawbacks. With their persistant nature, they move up through the food chain from plankton or insects to animals and humans, making dietary exposure unavoidable in many situations. They also move downward through soil to contaminate groundwater used for drinking. Through these exposures, pesticides pose a threat to human health by causing cancer or birth defects or other health and environmental problems (Veeraiah et al., 2002).

Pesticides, after applications, eventually reach the aquatic ecosystem in considerable amounts via agricultural run off from land, contaminated ground water, bottom sediments, urban run of and atmospheric fall out through rain, etc. These pesticides affect the target as well as non-target species (Singh et al., 1998).

The four main groups of pesticides, namely, organochlorine, organophosphate, carbamate, and pyrethroid insecticides (Smith and Gangolli, 2002; Ahmed et al., 2000), are of particular concern because of their toxicity and persistence in the environment. However, several of the banned pesticides are still used on a large scale in developing countries, and they continue to pose severe health and environmental problems (Biswa and Kshirsagar, 2004). Pesticide use raises a number of environmental concerns, and human and animal health hazards (Ahmed et al., 2000). Over 98% of sprayed insecticides and 95% of herbicides reach a destination other
than their target species, including non-target species, air, water and soil (Miller, 2004). Pesticides are one of the causes of water pollution, and some pesticides are persistent organic pollutants and contribute to soil contamination (Smith and Gangolli, 2002). As a result, these chemicals are non biodegradable, persistent and get accumulated in the environment and thus into the human food chain. Despite regulatory measures, these compounds continue to be detected in measurable amounts in the ecosystem, including marine life (Smith and Gangolli, 2002).

Many species of fish, some birds and small mammals, and a number of plants, including phytoplankton, as well as beneficial insect species, are extremely sensitive to chemical pesticides, and they die immediately after coming into contact with such substances (Anne Nadakavukaran, 1990).

The pesticides are being carried to ground water. The Centre for Science and Environment (CSE), a non-governmental organization, has revealed the presence of pesticide residue in bottled water and cool-drinks which rattled not only the industry but also millions of consumers across the country (Dhar Aarti, 2004). The test results showed the presence of organochlorines like gamma-hexachlorocyclohexane, chlordane, orlindane and DDT. Of the organophosphorous pesticides, malathion and chlorpyrifos were most common. The samples had enough poison to cause the chronic cancer, liver, and kidney damage, disorders of nervous system, birth defects and cause irreparable health disorders as they accumulate in the body fat (Dhar Aarti, 2004).

Since the chlorinated hydrocarbons are not water-soluble, and lipophilic in nature, they are not excreted from the body when ingested but, instead, are stored in fatty tissues such as liver, kidneys, and fat around the intestine. Toxic substances present in minute amounts in the general environment can thus become quite
concentrated as they move along a food chain, sometimes reaching lethal doses in organisms at the highest trophic levels. This process (biomagnification) is responsible for reproductive failure in the various birds of prey. Eagles, pelicans and falcons are all top carnivores, fish-eaters, at the end of relatively long food chains. As such, they have accumulated amounts of DDT, sufficiently high to interfere with important bodily processes leading to depletion of species to the state of being endangered or extinction (Anne Nadakavukaran, 1990).

In addition, pesticide use reduces biodiversity, reduces nitrogen fixation (Rockets, 2007), contributes to pollinator decline (Hackenberg, 2007; Haefeker, 2000; Wells, 2007; Zeissloff, 2001), destroys habitat (especially for birds) (Palmer et al., 2007), and threatens endangered species (Miller, 2004). It also happens that some of the pest adapt to the pesticide and develop resistance. To eliminate the offspring of this pest, a new pesticide or an increase the dose of pesticide will be needed. This will cause a worsening of the ambient pollution problem.

What is even more disastrous is that these pesticides make the life of non-target species miserable, and, amongst them, fishes are the worst victims. Hence, fish can be used as indicator species or test species to measure pesticide pollution.

One of the reasons for large-scale fish mortality and low-fish landing has been attributed to the increasing concentration of pesticides in natural water bodies (Holden, 1964; Nicholson, 1967; Saunders, 1969; Skea, 1970). In fact, interacting physical, chemical and biological factors of the aquatic ecosystem complicate the process and the response of a fish to a particular pesticide exposure. A minor difference in physical and chemical characteristics of environment may result in different response of a species.
With the accumulation in aquatic ecosystem, a good amount of pesticides undergo breakdown and transformation depending upon the physico-chemical and biological factors of the water ecosystem. Simultaneously, a considerable amount of pesticides and their byproducts enter the fish body where they are distributed and metabolized depending upon the detoxifying ability of the fish, and elicit some responses in fish. It depends on the nature and concentrations of pesticides as well as the duration of their persistence in fish. Hydrophilic pesticides are more readily transported to fish than hydrophobic ones. Water soluble pesticides enter the fish either through the body surface or gill or mouth. Pesticides in food stuff get ingested and absorbed through the gastrointestinal tract. Pesticides may enter singly or through multiple routes. The route of entry of pesticides and the duration of exposure have substantial impact on their absorption, distribution, biotransformation, and consequently their effects on fish (Singh et al., 1998).

For half a century, scientists and the public have been well aware of the risk posed by pesticides to humans and the environment. World-wide concern about pesticide residues in food and drinking water has led to legislative efforts to restrict the user of traditional, broad-spectrum pesticides. In many countries, a severe reduction in the use of many such pesticides for a wide range of agricultural uses has been imposed. The principal rationale for restricting the use of many of these chemicals is to protect consumers, especially children, who are more susceptible to the effect of pesticides (NRC 1993, Goldman 1998). The situation has compelled us to search for some other alternatives and eco-friendly technologies, ultimately leading to the birth of “bio-pesticides”. So pesticide producers have developed a suite of new biorational pesticides designed to target only selected organisms (NRC, 1993).
Many modern pesticides do not persist for long in the environment. They act quickly and are then degraded to non-toxic substances by chemical or microbial processes. This helps to prevent their build-up in crops or other organisms. The persistence of pesticides depends on its chemical properties, as well as environmental factors such as temperature, moisture, soil pH and the availability of micro-organisms.

One of the promising alternatives has been the use of biopesticides. They can replace, at least in part, some hazardous chemical pesticides with their incorporation into integrated crop management technology (Kumar and singh, 2014). Although potential and scope of biopesticides and biofertilizers for promoting sustainable agriculture has been known for years, organic farming has emerged now in view of the growing demands for safe and healthy food, and concerns about environmental pollution (Kumar and singh, 2014).

Food is the basic necessity of life, and food contaminated with toxic pesticides is associated with severe effects on human health. Hence, it is pertinent to explore strategies that address this situation of food safety, especially for the developing countries where pesticide contamination is widespread due to indiscriminate use as a major part of population lives below poverty line (Margarita Stoytcheva., 2011).

I.1. Biopesticide

Biorational pesticides/biopesticides are considered as third-generation pesticides that are rapidly gaining popularity. The “word biorational” is derived from two words, “biological” and “rational”, which means pesticides of natural origin that have limited or no adverse effects on the environment or beneficial organisms (Gill and Garg, 2014).

Biopesticide is a formulation made from naturally occurring substances that controls pests by nontoxic mechanisms and in an ecofriendly manner. It is, therefore,
gaining importance all over the world. Biopesticides may be derived from animals (e.g. nematodes), plants (Chrysanthemum, Azadirachta) and micro-organisms (e.g. Bacillus thuringiensis, Trichoderma, nucleopolyhedrosis virus), and include living organisms (natural enemies), their products (phytochemicals, microbial products) or byproducts (semichemicals) which can be used for the management of pests control (Mazid et al., 2011).

Biopesticides pose less threat to the environment and human health. They are generally less toxic than chemical pesticides, often target specific, have little or no residual effects and have acceptability for use in organic farming. They are often effective in very small quantities and often decompose quickly, thereby resulting in lower exposures and largely avoiding the pollution problems caused by conventional pesticides (Kawanishi and Held, 1990).

Biopesticides differ from conventional chemical pesticides in many ways. They are distinguished from the latter by their unique modes of action, low use volume, target specificity, or natural occurrence. They are, additionally, living entities capable of survival, growth, reproduction, and infection (Kawanishi and Held, 1990).

More generally, manufacturers are examining botanical pesticides, biological pesticides, and bioengineered products as potential new pesticides. Pyrethrum, purified from chrysanthemums, is an example of a botanical pesticide. Pyrethrum itself has limited applications, and more effective synthetic pyrethrins have been developed. Four insecticides now available in the United States are derived from the neem tree, grown in India (Hill, 1997). Biopesticides have the low toxicity to nontarget species, do not migrate because they bind tightly to the soil and are not bioaccumulate.
A microbial pesticide is a microorganism that has pesticidal properties and is, in fact, applied as a chemical pesticide. A well-known microbial is the bacterium, *Bacillus thuringiensis* (Bt), used to control caterpillars, beetles, and flies. Microbial insecticides such as Bt and baculoviruses are more pest-specific than chemical pesticides, and have lessening threat to nontarget species. But, like other pesticides, a microbial must be shown not to threaten humans, animals or the environment (Hill, 1997).

Table I.1. Alternatives to conventional pesticides (Hill, 1997):

<table>
<thead>
<tr>
<th>ALTERNATIVE</th>
<th>DEFINITION</th>
<th>EXAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>An extract prepared from living organism</td>
<td>An extract of chrysanthemum, tobacco, or the neem tree</td>
</tr>
<tr>
<td>Botanical</td>
<td>An ingredient purified from a natural extract</td>
<td>Pyrethrum purified from chrysanthemum extract or nicotine from tobacco extract</td>
</tr>
<tr>
<td>Biological a</td>
<td>A living agent such as bacterium, insect, or fish that acts as a pesticide</td>
<td>After rabbits were introduced into Australia, their population explode; they were long controlled by a microorganism that infected and killed them</td>
</tr>
<tr>
<td>Microbial a</td>
<td>A biological that is a microorganism bacterium, virus, fungus, protozoan</td>
<td>The Bt toxin or baculovirus, either of which can act as a pesticide</td>
</tr>
<tr>
<td>Bioengineered organism b</td>
<td>A living organism with genes from another organism inserted into its DNA, which allow it to make a pesticide that it was previously unable to produce</td>
<td>A plant with the Bt gene inserted into its DNA, allowing it to produce a product that repels several insects, or a plant given genes allowing it to make a product, making it resistant to herbicides</td>
</tr>
</tbody>
</table>

a These are not extracts or chemicals, but living organisms.
b In a *biological*, a living organism is the pesticide. In a *bioengineered organism*, the living organism produces the pesticide after it has been given the necessary genetic material to do so.
Biotechnology companies are heavily investing in research aimed at developing bioengineered organisms. In a bioengineered plant, for example, specific genes are inserted into the genetic material to give it properties it did not previously have. Plants already modified by bioengineering include cotton and tomatoes that can resist an herbicide. When the crop is sprayed with that herbicide, only the weeds growing with the crop are killed, not the crop itself (Hill, 1997).

In a different application of bioengineering, genes are taken from the Bt. bacteria mentioned above. After manipulation these are transplanted into such plants as cotton, tomato, and potato (Hill, 1997). The plants receiving the Bt. genes use the new generic information to produce a protein that kills specific insects.

### I.2. Spinosad

Spinosad insecticide is based on a compound found in the bacterial species, *Saccharopolyspora spinosa*. The genus of *Saccharopolyspora* was discovered in 1975 by Lacey and Goodfellow, who described isolates from crushed sugar cane which produce yellowish-pink aerial hyphae, with bead-like chains of spores enclosed in a characteristic hairy sheath (Mertz *et al*., 1990).

This genus is defined as aerobic, gram-positive, non-acid-fast actinomycetes with fragmenting substrate mycelium. *S. spinosa* was isolated from soil collected inside a non-operational sugar mill rum still in the Virgin Islands. Spinosad represents a new class of insecticides acting by a novel mode of action (Salgado, 1998; Salgado *et al*., 1998; Thompson *et al*., 2000). Spinosad is currently sold in various formulations and concentrations as the basis for products such as Tracer, Conserve, Success, SpinTor and Justice. Spinosad is currently sold in over 30 countries for control of a broad range of foliar-feeding insect pests (Dow, 2003).
While spinosad provides highly efficacious control of many lepidopteran insects on a variety of crops such as cotton, apples, sweet corn, potatoes, and fruiting/leafing vegetables, it is relatively nontoxic to most beneficial insects and mites (De Amicis et al., 1997; Kirst et al., 1992).

Spinosyns occur in over 20 natural forms, and over 200 synthetic forms (spinosoids) have been produced in the lab (Watson and Gerald, 2001). Spinosyns are novel macrolides produced by *Saccharopolyspora spinosa* (Kirst et al., 1991; Mertz and Yao, 1990). Spinosyns are comprised of a tetra cyclic macrolide containing forosamine and tri-O-methylrhamnose, with different degrees of methylation on the polyketide or deoxysugars. The spinosad insecticide contains a mix of two spinosyns, Spinosyn A, the major component, and Spinosyn D (the minor component), in an approximately 17:3 ratio (Mertz et al., 1990).

The two major factors in *S. spinosa* fermentation, spinosyn A and spinosyn D, differ from each other by a single methyl substituent at position 6 of the polyketide (Fig.1). Spinosad, a combination of spinosyn A and spinosyn D, is used for control of agricultural insect pests, and is highly effective against target insects and has an excellent environmental and mammalian toxicological profile (Sparks et al., 1998). Incorporation studies with $^{13}$C-labelled acetate, propionate, and methionine established that spinosyns are assembled by a polyketide pathway, and that the two N-methyl groups of forosamine, and the three O-methyl groups of tri-O-methylrhamnose, are derived from S-adenosyl-methionine (Waldron et al., 2001a; 2001b). The polyketide portion of spinosyns differs from the more common type I polyketides (e.g. erythromycin, rapamycin, or tylosin) in that it contains three intramolecular carbon-carbon bonds (Fig.1). Rhamnose biosynthesis is the limiting step in spinosad biosynthesis (Madduri et al., 2001a; 2001b).
1.2.1. Properties of spinosad:

Technical spinosad is composed of tan or white low melting crystals (Spinosyn A, m.p. 84-99.5°C; Spinosyn D, m.p. 161-170°C), which have low volatility and an earthy odour. Crystals are soluble in a number of organic solvents. Solubility is higher in polar solvents such as acetone, dichloromethane, acetonitrile, and methanol than in non-polar solvents such as hexane. Spinosad is a relatively non-polar molecule that is not easily dissolved in water (Crouse et al., 2000)

Figure 1.1. Structure of spinosyn A and D

a. Spinosyn A, Empirical Formula C41H65NO10; MW 731.98

b. Spinosyn D, Empirical Formula C42H67NO10, MW 745.99
<table>
<thead>
<tr>
<th>Common name (ISO)</th>
<th>SPINOSAD a mixture of 50-95% spinosyn A and 5-50% spinosyn D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade Name</td>
<td>XDE-105, Tracer</td>
</tr>
<tr>
<td>Development Code (for new actives only)</td>
<td>Code</td>
</tr>
<tr>
<td></td>
<td>232105</td>
</tr>
<tr>
<td></td>
<td>275043</td>
</tr>
<tr>
<td></td>
<td>XR-105</td>
</tr>
<tr>
<td></td>
<td>XDE-105</td>
</tr>
<tr>
<td></td>
<td>DE-105</td>
</tr>
<tr>
<td>Chemical family</td>
<td>Spinosyns (Macrylic lactone isolated from soil organism <em>Saccharopolyspora spinosa</em>).</td>
</tr>
<tr>
<td>Chemical name (IUPAC)</td>
<td>Spinosyn A:</td>
</tr>
<tr>
<td></td>
<td>Spinosyn D:</td>
</tr>
<tr>
<td>Chemical name (CA)</td>
<td>Spinosyn A:</td>
</tr>
<tr>
<td></td>
<td>Spinosyn D:</td>
</tr>
<tr>
<td>CIPAC No</td>
<td>636</td>
</tr>
<tr>
<td>CAS No</td>
<td>131929-60-7 Spinosyn A:</td>
</tr>
<tr>
<td></td>
<td>131929-63-0 Spinosyn D:</td>
</tr>
<tr>
<td>EEC No</td>
<td>434-300-1 (mixture of spinosyn A and D)</td>
</tr>
<tr>
<td>FAO SPECIFICATION</td>
<td>not established</td>
</tr>
<tr>
<td>Minimum purity</td>
<td>850 g/kg, with 50-95% spinosyn A and 5-50% spinosyn D</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>Spinosyn A: C_{41}H_{65}NO_{10}</td>
</tr>
<tr>
<td></td>
<td>Spinosyn D: C_{42}H_{67}NO_{10}</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>Spinosyn A: 731.98 Spinosyn D: 746.00</td>
</tr>
<tr>
<td>Year of Initial Registration</td>
<td>1997</td>
</tr>
<tr>
<td>Color</td>
<td>Light gray to white</td>
</tr>
</tbody>
</table>

I.2.2. Environmental Fate:

The routes of spinosad dissipation and transformation in the environment include photodegradation and biotransformation on plant surfaces, abiotic hydrolysis, aqueous photolysis, photodegradation on soil, and biotransformation via soil microorganisms. Volatilization from plant or soil is not a mechanism of transport of spinosad in the environment.

Spinosad persistence in the environment is variable in terrestrial and aquatic systems (USEPA, 1998). Photolysis is the primary route of dissipation from plant surfaces. After initial photodegradation, residues are available for metabolism by plant biochemical processes.

Spinosad is broken down rapidly by sunlight. In the presence of sunlight, half-lives on leaves are 2 to 16 days and less than one day in water. It does not readily spread from leaves to the rest of the plant. In the absence of sunlight, spinosad breaks down very slowly in water. Half-lives of more than 30 days to 259 days have been reported. However, it binds rapidly to sediment. The half-life in sediment, where no oxygen is available, ranges from 161 to 250 days (NPIC, USEPA fact sheet, 2014). Abiotic hydrolysis is relatively unimportant compared to other dissipation routes. Spinosa does not degrade at a significant rate of hydrolysis under neutral conditions and slowly hydrolyzes under basic conditions. Aqueous photolysis is rapid in natural sunlight, and is the primary route of degradation in aquatic systems exposed to sunlight.

The rapid photolytic breakdown of spinosad in laboratory studies has also been confirmed in microcosm studies (Cleveland et al., 2002). Solubility of spinosad in water is pH dependent and is dependent on the structurally similar active ingredients. Solubility for Spinosyn A ranges from 290 to 16 mg/L with increasing
pH, while the solubility for Spinosyn D is much less but still pH-dependent with values ranging from 28.7 to 0.05 mg/L for pH values between five and nine (Cleveland et al., 2002).

Table I.3. Half-life of spinosad.

<table>
<thead>
<tr>
<th>Reported Half-lives for Spinosad in Soil and Water. Environmental Fate Parameter</th>
<th>Reported Half-life</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis (Spinosyn A/D)</td>
<td>No degradation @ pH 5 and 7, pH 9 (200/259 days)</td>
</tr>
<tr>
<td>Aqueous Photolysis (Spinosyn A/D)</td>
<td>0.93/0.82 days @ pH 7</td>
</tr>
<tr>
<td>Soil Photolysis (Spinosyn A/D)</td>
<td>82/44 days</td>
</tr>
<tr>
<td>Aerobic Soil Metabolism (Spinosyn A/D)</td>
<td>9.0–17.3/14.5 days</td>
</tr>
<tr>
<td>Anaerobic Aquatic Metabolism (Spinosyn A/D)</td>
<td>161/205 days</td>
</tr>
<tr>
<td>Terrestrial Field Dissipation</td>
<td>0.3 to 0.5 days for Spinosyn A</td>
</tr>
</tbody>
</table>

In the soil environment, Spinosad also sticks to soil and has a very low potential to move through soil towards ground water. In the top layers of soil, spinosad is rapidly broken down by microbes (NPIC, USEPA fact sheet, 2014). Spinosad is photodegraded quickly on soil exposed to sunlight, but the degradation rate is decreased at longer exposure times. It is quickly metabolized by soil microorganisms under aerobic condition. Under anaerobic conditions, the degradation rate is slower (WHO, 2011).

Table I.4. Half life of spinosad metabolites, A and D.

<table>
<thead>
<tr>
<th>Spinosyn A half life</th>
<th>Spinosyn D half life</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-17 days</td>
<td>14 days</td>
</tr>
<tr>
<td>128-240</td>
<td>177</td>
</tr>
<tr>
<td>(pre-sterilized soils)</td>
<td>(pre-sterilized soils)</td>
</tr>
</tbody>
</table>

Sources: Hale and Portwood, 1996.
Plant based pesticides contain active principles with low half-life period and their effects on the environment are not too detrimental (Ahmad et al., 2012).

I.2.3. Hazard Identification

Spinosad has low acute toxicity and is classified by USEPA as Toxicity Category III (slightly toxic with “caution” warning on label) for acute oral and dermal toxicity and Toxicity Category IV (not acutely toxic with no warning on label) for acute inhalation toxicity, primary eye irritation, and primary skin irritation (USEPA, 2005). The rat oral median lethal dose ($LD_{50}$) is 3,738 mg/kg for males and >5,000 mg/kg for females, whereas the mouse oral $LD_{50}$ is >5,000 mg/kg. The rabbit dermal $LD_{50}$ is >2,000 mg/kg and the rat inhalation median lethal concentration ($LC_{50}$) is >5.18 mg/L air (USEPA, 1998).

USEPA (2005) reported the sub-chronic toxicity effects of spinosad in mouse. There were increased vacuolation of cells in the lymphoid organs, liver, kidney, stomach, female reproductive tract, and epididymis, and less severely in the heart, lung, pancreas, adrenal cortex, bone marrow, tongue, pituitary gland, and anemia. They also observed thyroid follicle epithelial cell vacuolation, anemia, multifocal hepatocellular granuloma, cardiomyopathy and splenic histiocytosis in rats. USEPA (1998) observed no dermal irritation or systemic toxicity of spinosad occurred in a 21-day repeated dose dermal toxicity study in rabbits at 1,000 mg/kg/day.

USEPA (2005) reported spinosad is not a neurotoxic agent in acute, sub-chronic, or chronic toxicity studies. No neurotoxic effects were observed at the limit dose in an acute neurotoxicity study in rats and at doses up to 42.7 mg/kg/day in a subchronic neurotoxicity study. They also reported chronic feeding study in dogs increasing in serum alanine aminotransferase, aspartate aminotransferase, and
triglycerides levels. The presence of tissue abnormalities, including vacuolated cell aggregations, arteritis, and glandular cell vacuolation (parathyroid) were observed.

Salgado (1997) and Salgado (1998a) observed that spinosyn A is more water soluble than the other component of spinosad, spinosyn D. Spinosyn A and its soil metabolites bind to soil and have low soil mobility. Spinosad kills insects through action on their nervous systems. Due to its low toxicity and perceived low impact on the environment, EPA registered spinosad as a reduced-risk material (EPA, 1997; DOW, 2001; Jachetta, 2001). However, adverse impacts against beneficial organisms are a potential concern. Fresh sprays could kill honeybees, trichograma and other parasitoids (Suh et al., 2000; Tillman and Mullrooney, 2000; Bret et al., 1997).

I.2.4. Aquatic Toxicity

Spinosad shows slight toxicity to birds, moderate toxicity to fish, and slight to moderate toxicity to aquatic invertebrates. It is highly toxic to bees in laboratory tests and is highly toxic to oysters.

Spinosad was less toxic to many non-target organisms than some natural insecticides such as pyrethrum that are currently on the National Organic Program Final Rules Listings (Farm Chemicals Handbook, 2002). Federal Register (2001) stated that spinosad’s reduced toxicity to many organisms has the potential to increase ecological balance and conserve biodiversity. It is also toxic to oysters and other marine mollusks (Dow, 2001). Salgado (1997) and Salgado (1998a) observed that spinosad has moderate toxicity to fish. Muthukumaravel et al., (2013) described pesticides wherever applied; they found their way into water bodies ultimately affecting aquatic fauna in general and fish in particular.
I.2.5. Mode of action

Spinosad is highly active, by both contact and ingestion, to numerous insect species (Hertlein, et al., 2011). Spinosad’s overall protective effect varies with insect species and life stage. Spinosad affects certain species only in the adult stage, but can affect other species at more than one life stage. The species that are subject to very high rates of mortality as larvae, but not as adults, may gradually be controlled through sustained larval mortality (Hertlein, et al., 2011).

Spinosad is primarily a stomach poison with some contact activity and is particularly active against Lepidoptera, Diptera, some Coleoptera, termites, ants and thrips (Bret et al., 1997). The mode of action of spinosoid insecticides is via a neural mechanism (Orr et al., 2009). The spinosyns and spinosoids have a novel mode of action, primarily targeting binding sites on nicotinic acetylcholine receptors (nAChRs) of the insect nervous system that are distinct from those at which other insecticides have their activity. Spinosoid binding leads to disruption of acetylcholine neurotransmission (Qiao Meihua, et al., 2007). It causes loss of muscle control. Immediately after application, insect pests exhibit irreversible tremors, prostrate trembling, paralysis and death. Continuous activation of motor neurons causes insects to die of exhaustion within 1-2 days. Spinosad also has secondary effects as a α-amino-butyric acid (GABA) neurotransmitter agonist (Qiao Meihua, et al., 2007). Spinosad kills insects via hyperexcitation of the insect nervous system (Qiao Meihua, et al., 2007). Spinosad so far has proven non cross-resistant to any other known insecticide (Sparks Thomas et al., 2001).
Figure 1.2. The metabolic pathway for spinosyn A and D (Domoradzki et al., 1995).

1.2.6. Usage of Spinosad

Spinosad has been used around the world for the control of a variety of insect pests, including Lepidoptera, Diptera, Thysanoptera, Coleoptera, Orthoptera and Hymenoptera, and many others (Sparks et al., 2011 and 2012). Spinosad was first registered as a pesticide in the United States for use on crops in 1997 (Sparks et al., 2011 and 2012). Its labelled use rate is set at 1 ppm (1 mg a.i./kg of grain), and its Maximum Residue Limit (MRL) or tolerance is set at 1.5 ppm. Spinosad is considered a natural product, and thus is approved for use in organic agriculture by numerous nations (Hertlein et al., 2011). Spinosad has recently been used in oral
preparations to treat *Ctenocephalides felis*, the cat flea, in canines and felines; the optimal dose set for canines is reported to be 30 mg/kg (Qiao Meihua *et al.*, 2007).

Spinosad has been applied to over 200 different crops. It is used on apples, citrus, cole crops, vegetables, cereal grains, almonds and cotton. It has been used to control caterpillars in cotton, loopers in cabbage, leafminers in various crops, leafrollers on apples, thrips in citrus, etc. (Dow, 1997; Thompson *et al.*, 2000; Bret *et al.*, 1997).

### 1.3. Significance of the present study

In India, fish production has been recorded 7.6 million tonnes comprising 4.1 Mt capture fisheries and 3.5 Mt aquaculture productions (FAO, 2008). The freshwater aquaculture in India is carp-oriented. The joint share of three Indian major carps and three exotic carps in freshwater aquaculture production is over 90 per cent (Katiha and Bhatta, 2002).

Andhra Pradesh ranks first in freshwater prawn and brackish water shrimp production and second in freshwater fish production (DoF, 2009). Three major carp species, viz. *Catla catla, Labeo rohita* and *Cirrhinus mrigala*, are farmed in the polyculture systems in this state (Veerina *et al.*, 1993). The farmers in India have taken up two or three major carps composite culture *Labeo rohita, Catla catla* and *Cirrhinus mrigala* (Jhingran, 1991).

Guntur district, Andhra Pradesh, India, is predominantly an agricultural district, located on the western bank of the lower reaches of river Krishna (lat. 15° 18’-16° 50’, north, long. 70° 10’ – 80° 55’ east). Traditionally, commercial crops such as, tobacco, cotton, chillies, greengram and blackgram are grown in dry land regions, whereas, paddy and sugarcane are cultivated in wetland regions. The dry land commercial crops entail heavy investments and they are promising good profits. It is
also well known that all these are heavily sprayed crops. It is interesting to note that the total annual sale of pesticides is high in 23 districts of the Andhra Pradesh. Nearly 30-40% of the total quantity is sold through various outlets in Guntur district alone (personal communication from the Joint Director of Agriculture, Government of Andhra Pradesh, and Guntur District) (Veeraiah et al., 2004).

The commercial outlook of farmers in Guntur has mainly aimed at the maximization of the profit margin, which in turn has resulted in heavy consumption of pesticides. Although many investigators are working on the effects of different pesticides on fish especially on commercially important carp species, the effect of biopesticide, spinosad 45% SC (Tracer) on fish, *Labeo rohita* is not so far studied. In the present study, an attempt is made to investigate the impact of spinosad on the fish, *Labeo rohita*.

**I.4. Biology of the Test Fish *Labeo rohita* (Hamilton, 1822)**

Freshwater aquaculture in India is dominated by carp (*Labeo rohita, Catla catla* and *Cirrhinus mrigala* - *Cyprinidae*), which contribute about 87% of the total freshwater production (ICLARM, 2001). *Labeo rohita* is a major carp, widely cultured throughout India owing to its high commercial value. Growth rate is one of the most important parameters determining the economic efficiency of commercial fish culture, which is influenced by several biotic and abiotic factors (Brett and Groves, 1979). Rohu (*Labeo rohita*) is the most important among the three Indian major carp species used in carp polyculture systems.

Information on its culture is available only from the early part of the 20th century. The compatibility of rohu (*Labeo rohita*) with other carps like catla (*Catla catla*) and mrigel (*Cirrhinus mrigala*) made it an ideal species for carp polyculture systems. While riverine collection of seed was solely meeting the requirement for
culture of the species until the first half of the 20th century, the success in induced breeding in 1957 and the assured seed supply thereafter was the major factor for the development of its culture in freshwater ponds and tanks. Its high growth potential, coupled with high consumer preference, have established rohu as the most important freshwater species cultured in India, Bangladesh and other adjacent countries in the region. Considering its importance in the culture system, emphasis has also been given to its genetic improvement through selective breeding in India.

1.4.1. Characters

Body is laterally compressed and fusiform, attaining maximum length of one meter. Colour is blackish grey on the back and silvery white below. Body is covered with overlapping cycloid scales. Head is prominent with blunt snout. Eyes are large without eyelids. Mouth is subterminal, directed downwards and surrounded by thick lips. Upper lip is with a pair of short barbells and lower lip is fringed. But in other species of \textit{Labeo} barbells are absent. Jaws are without teeth. Dorsal fin is large, present at about the middle of the body. Pectoral fins are without spinous rays. Tail fin is small and homoceral. Air bladder is physostonous type and divided into an anterior and a posterior chamber. Weberian apparatus joins the air bladder with the ear. Lateral line canal passes through the scales.

1.4.2. Special Features

Both upper and lower lips have an inferior transverse fold, which is fringed on the lower lip.

1.4.3. Economic importance

It is the most popular food fish. Its flesh is delicious. It is relished very much in food and rich in protein content. Carps are cultivated in specially constructed water ponds, having adjacent breeding areas. It is mostly cultivated with catla (\textit{Catla catla})
in fresh water ponds and lakes in the absence of carnivorous fish. It is also used as a
game fish where it is specially introduced into water reservoirs for the purpose of
sport fishing.

**Classification**

- Phylum: Chordata
- Sub phylum: Vertebrata
- Class: Osteichthyes
- Subclass: Actinopterygii
- Order: Cypriniformes
- Family: Cyprinidae
- Genus: *Labeo*
- Species: *rohita*

**Figure 1.3.** Freshwater fish, *Labeo rohita*

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1. **5. Objectives of the present study**

1) to determine the sensitivity of the test fish, *Labeo rohita* through static and
   continuous flow through methods and assessing the LC\(_{50}\) values and observing the
   behavioral changes,

2) to evaluate the impact of the test toxicant spinosed (45% SC, Tracer) on the
   oxidative metabolism of the test fish by measuring the changes in oxygen uptake,

3) to estimate the test toxicant induced changes in the biochemical constituents such
   as glycogen, proteins, and nucleic acids i.e. DNA and RNA,

4) to study the changes in the activity levels of enzymes involved in protein and
   carbohydrate metabolisms,
5) Since the test toxicant is bio-pesticide and its mode of action is through nervous system, an attempt has also been made to elucidate the activity changes in the neurotransmitter enzyme, Acetyl Cholinesterase in the test fish, *Labeo rohita*.

6) to study the changes in protein banding patterns and DNA abrasions in the test organs of the test fish,

7) to observe the histopathological changes in the vital organs viz, Gill, Liver, and Kidney after exposure of the fish for 24 h, 96 h and 8 days under sub-lethal and lethal exposures.

8) to study the residue levels in the vital organs viz, Gill, Liver, muscle, brain and Kidney after exposure of the fish, *Labeo rohita* for 8 days under sub-lethal concentrations.