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Neem Seed Oil: Encapsulation and Controlled Release - Search for a Greener Alternative for Pest Control

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1. Introduction

1.1 Challenge and need of environment-friendly pesticides

Plants constitute the world's primary food source and so there has been a tendency always to maximize agricultural yields which has been achieved through the development of modified high yield crops and the use of synthetic fertilizers. But this productivity is negatively hampered by plant diseases that contribute significantly to the total crop losses. One third of the world's potential food supplies are estimated to be lost to pre and post harvest pests and diseases. According to FAO [Food and Agricultural Organization] estimates, potential losses worldwide are 35% in the field and 14% in storage bringing the total loss to 50%. In Eastern and Southern Africa, these losses are estimated to be even higher. In order to mitigate these losses, pesticides are being used. The discovery of Bordeaux mixture is significant in the history of chemical control of plant diseases. The use of synthetic pesticides has undoubtedly resulted in increased crop production. However, synthetic pesticides that are used to control plant diseases are doing irreparable harm and damage to our fragile environment. Detrimental health effects, environmental issues, insect resistance or marketing opportunities for organically produced food are well-known arguments against the use of pesticides. The increasing awareness and concern about the impact of agricultural practices on the environment and in food and fiber production is promoting the concept of sustainable agriculture, thus, raising the thrust for bio-pesticides over synthetic pesticides. The increasing incidence of pesticide resistance is also fuelling the search for more environmentally and toxicologically safe and more selective and efficacious new bio-pesticides. Thus, in the context of all these challenges, the modern pesticides will be needed to meet, there arises the need of environment-friendly pesticides in order to boost agricultural production with the ever escalating world population the use of pesticide is absolute.

1.2 Background history of pesticides

Combating with the diseases of plant, destructive animals and weeds to protect crops started along with the starting of agriculture by human, which is almost a 10,000 year struggle now. Until the early 20th Century, cultural and mechanical methods augmented by

a diverse range of organic and inorganic substances derived from plants, animal and minerals dominated pest control. Effective and affordable synthetic pesticides gained ground by the mid-20th Century, due to the maturing chemical industry (Ujváry, 2001). Most of the insecticidal organophosphates were developed during the early 19th century, but their effects on insects, which are similar to their effects on humans, were discovered in 1932. Very little progress has been made with regard to treatment of diseases until about 1882 when the value of lime and copper sulphate as a fungicide was accidentally observed in France and very quickly resulted in the development of the Bordeaux mixture by Millardet. The epochal discovery of Bordeaux mixture is believed to be the first important landmark in the history of chemical control of plant diseases (Berger & Kaitisha, 1995). While reckoned back, the discovery of dithiocarbamates in 1934 and introduction of several other pesticides in the late 1960's are considered to be the two most important events in the one hundred year long history of chemical control of plant disease.

Plant extracts were likely the earliest agricultural biopesticides, as history records that nicotine was used to control plum beetles as early as the 17th century (biopesticideindustryalliance.org). Biological control related experiments for insect pests in agriculture date back as far as 1835, when Agostine Bassi demonstrated that White-muscadine fungus (*Beauveria bassiana*) could be used to cause an infectious disease in silkworm. Mineral oils as plant protectants were also reported in the 19thcentury. One of the most important discoveries of bio-pesticides was spores of the bacteria *Bacillus thuringiensis* (Bt), in 1901. Japanese biologist Shigetane Ishiwata isolated Bt from a diseased silkworm. It was rediscovered after ten years, by Ernst Berliner in Thuringen, Germany in a diseased caterpillar of flour moth. Bt was started to use as natural insecticide in 1920 while commercially became available in 1938, in France as Bt product, Sporeine. After this, till 1999 several bio-pesticides were discovered, developed commercially, appeared in world market, yet toxic synthetic chemical insecticides were continuously leading the market of pesticides in the 20th century as they were cheap.

1.3 Pesticides: Synthetic vs natural

Pests refer to the living organisms that occur unwanted or cause damage to the crops. Insects, mice and other animals, unwanted plants (weeds), fungi, microorganisms such as bacteria , viruses, and prions etc. are included in pests. By definition, according to Food and Agricultural Organization [FAO]) and the World Health Organization (WHO: UNO, 1963), a pesticide is a substance or mixture of substances intended to prevent, destroy, repel, or to mitigate any pest including unwelcome species of plants or animals; during production and/or storage, transportation, distribution and elaboration of food; agricultural products or food for animals; or that may be administered to animals to fight ectoparasites. The term also includes herbicides and compounds used as growth regulators, insecticides, fungicides, defoliants, desiccants, and inhibitors of fruit thinning and germination. Pesticides include a wide variety of components and display a broad spectrum of chemical properties (biopesticideindustryalliance.org). Pesticides are classified in different ways. According to the source of origin, they may be of synthetic (Chemical) or natural (bio-pesticide). Another way of naming of pesticides is directly by the type of pests they control, e.g.,

- Algicides to control algae,
- Antifouling agents to kill or repel organisms that attach to underwater surfaces,
- Antimicrobials to kill microorganisms (such as bacteria and viruses).

- Attractants to attract pests (for example, to lure an insect or rodent to a trap). (However, food is not considered a pesticide when used as an attractant.)
- Molluscicides to kill snails and slugs,
- Nematicides to kill nematodes (microscopic, worm-like organisms that feed on plant roots).
- Ovicides to kill eggs of insects and mites.
- Pheromones to disrupt the mating behavior of insects.
- Repellents to repel pests, including insects (such as mosquitoes) and birds.
- Rodenticides to control mice and other rodents etc.

Chemical pesticides are usually classified by their common source or production method. There are four basic types of chemical pesticides that are most commonly used- (i) Organophosphate pesticides (ii) Carbamate pesticides (iii) Organochlorine pesticides (iv) Pyrethroid pesticides . Both organophosphate and carbamate pesticides affect the nervous system by disrupting the enzyme that regulates acetylcholine, a neurotransmitter. DDT and chlordane are the example of organochlorines which have been removed from the market due to their health , environmental effects and their persistence. Pyrethrin, a natural pesticide, is obtained from chrysanthemums. Pyrethroid pesticides are developed synthetic products of pyrethrins.

Organochlorides(DDT, dieldrin and aldrin) have high persistence in the environment of up to about 15 years. Organophosphates(parathion, carbaryl and malathion) have an intermediate persistence of several months Carbamates (Tenik, Zectran and Zineb) have a low persistence of around two weeks. Synthetic pyrethroids are non-persistent, contact and residual acting insecticides (cypermethrin, permethrin) and are suitable for a wide range of crops and target insects. Most pesticides are broad-spectrum, that is they kill all insects in a certain area and may kill other animals like birds and small mammals.

A bio-pesticide, according to FAO definition is - *a compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison. Differ from biocontrol agents in being passive agents, whereas biocontrol agents actively seek the pest. The rationale behind replacing conventional pesticides with bio-pesticides is that the latter are more likely to be selective and biodegradable.* Bio-pesticides are derived from natural materials like animals, plants, bacteria, and certain minerals. For example, garlic, mint, neem, papaya, canola oil ,baking soda etc. all have pesticidal applications and are considered bio-pesticides. Almost all the bio-pesticides are categorized among the three major groups such as (i) microbial pesticides (ii) plant-incorporated-protectants (PIPs) (iii) biochemical pesticides. According to the U. S. Environmental Protection Agency (USEPA), at the end of 1998, there were approximately 175 registered biopesticide active ingredients and 700 products. At the end of 2001, there were approximately 195 registered bio-pesticide active ingredients and 780 products. The most commonly used bio-pesticides are living organisms (bacteria, viruses and fungi) which are pathogenic for the pest of interest. These include biofungicides (Trichoderma), bioherbicides (Phytopthora) and bioinsecticides (Bacillus thuringiensis).

1.4 Advantages and disadvantages of biopesticides and chemical pesticides

Botanical pesticides also offer various means of combating insects resistant to products currently available. Almost all synthetic pesticides rely on neurotoxic agents, meaning they attack the nervous system of insects. But tropical plants have over time developed literally

thousands of weapons that kill insects in other ways. For example, the makabuhay vine, which grows in the Philippines, burns insects using a chemical that absorbs sunlight.
Synthetic pesticides are rapidly losing their effectiveness. To date, hundreds of insect species have developed resistance to at least one pesticide formula and a dozen or so species are immune to them all. Some scientists fear that pesticide manufacturers will eventually be unable to outwit insects. Chemical pesticides do suffer from several disadvantages due to which the use of bio-pesticides is preferred. Some of the disadvantages associated with the chemical pesticides are-

i. Environmental pollution,
ii. Creating health hazards due to the presence of the pesticide residues in food, fiber and fodder
iii. Development of resistance by the insects.

According to World Health Organization estimates, up to 20,000 people die of pesticide poisoning in the Third World each year. Some synthetic pesticides are accumulating in soil and groundwater where they threaten the health of entire ecosystems.
In contrast, the bio-pesticides offer several advantages over synthetic pesticides which are

i. Bio-pesticides are less harmful than chemical pesticides because bio-pesticides do not leave harmful residues,
ii. Bio-pesticides generally target one specific pest or a small number of related pests in contrast to broad spectrum chemical pesticides which affect, apart from the pest, other beneficial insects, birds, mammals or nontarget species.
iii. Bio-pesticides are effective in smaller quantities, decompose quickly and do not cause environmental problems.
iv. When used in Integrated Pest Management programs, bio-pesticides can greatly reduce the use of conventional pesticides, while the crop yield remains high.
v. Bio-pesticides are often cheaper than chemical pesticides.

Few limitations of bio-pesticides -

i. They are highly specific, due to which exact identification of the pest/pathogen or use of multiple pesticides may be required.
ii. They often suffer variable efficacy due to the influences of various biotic and abiotic factors (since bio-pesticides are usually living organisms, which bring about pest/pathogen control by multiplying within the target insect pest/pathogen)

1.5 Modification of pesticides with polymers-controlled release pesticides

A significant improvement can be experienced within the agricultural and pharmaceutical sectors by using such environmentally friendly technologies that mainly target the production of healthy, nutritious, quality foodstuff as well as pharmaceutical products by taking environmental characteristics into account, and adapting to them. To achieve such goals mentioned above and to bring sustainable, environmentally human economical systems to the forefront, scientists today are more than ever before being challenged to provide environmentally benign, more economical, and more efficient products for the health and well being of mankind. “Controlled delivery” technologies have emerged as an approach with promise not only to utilize resources in the maximum efficient way but also for the prevention of pollution. Moreover, if the resource is natural or renewable polymer, then it will draw attention as more new, more economical and more eco-friendly source for use of mankind.

Controlled delivery may be defined as a technique or method in which active chemicals are made available to a specified target at a rate and duration designed to accomplish an intended effect (Kyodonieus, 1980). Controlled delivery occurs when a polymer, natural or synthetic, is judiciously combined with an active agent in such a way that the active agent is released from the material in a predesigned manner. The release of the active agent may be constant or cyclic over a long period. It may be triggered by the environment or other external events. In any case, the purpose behind controlling the drug delivery is to achieve more effective therapies while eliminating the potential for both under and overdosing (Peppas, 1997). During the last decade, controlled release technology has received increasing attention in the face of a growing awareness of those substances which are excessively toxic and sometimes ineffective when administered or applied by conventional means. Conventional application of agricultural chemicals provides an initial concentration far in excess to that required for immediate results in order to assure the presence of sufficient chemical for a practical time period. Such overdosing wastes much of the chemical's potential and all too often causes toxicity problems for non target organisms (Kyodonieus, 1980). To improve the efficiency of agricultural pesticides for longer time, the use of controlled release technology is effective. Many of the controlled release formulations are highly efficient in sustaining the release of the biologically active components. It should be recognized that the polymeric material to be used in controlled release must degrade to some fashion before there can be any environmental impact in the chemical, biochemical or biological sense. However, it is encouraging to use naturally occurring polymers or degradable synthetic polymers. To ensure adequate pest control within a suitable period, pesticides are applied in concentrations greatly exceeding those required for control of the target organism, thus increasing the likelihood of runoff or leaching and pollution of surface or groundwater. Controlled release formulations have the potential to reduce the environmental problems associated with the application of pesticides. There are many advantages of controlled release formulations including an increased safety to the user and non-target organisms, a reduction in the amount of pesticides applied, reduced leaching of pesticides and increased persistence of the active ingredient.

The selection of an appropriate coating agent depends on the nature of the agrochemical, environmental aspect of the agent as well as cost effectiveness of its incorporation (Karak, 1999). The different approaches for controlling the release of agrochemicals are-

i. Controlled release by applying active coating of wax (Herdrie, 1976), sulfur (Miller & Donahue, 1992), polymer (Hansen, 1966a & Hansen, 1966b), neem (Bains et al., 1971), Lac (Kenawy, 1998a). Other coatings include coal tar, mahua oil, metal salts etc.)
ii. Controlled release by polymeric adduct formation
iii. Controlled release by incorporation of Nitrification inhibitor
iv. Controlled release by other techniques.

Controlled release by polymeric adduct formation is one of the best methods so far commercialized. Here, monomer or prepolymer or oligomers are used for adduct formation with active agent by cross linking reactions. Selection of cross linker, time of cross linking and temperature of cross linking reactions are very much important for controlling the rate of release of the active agent.

Over the last few years, there have been plenty of polymeric agrochemical formulations including pesticide, herbicides, growth regulators and fertilizers. However, the major drawback with these techniques is the residual polymers that might accumulate in the soil

and become harmful to the soil and plants. Therefore, an attempt to produce system, which can help to utilize the whole agrochemical and also conserve the water, soil, and economy, is getting considerable interest in the field of modern research.

1.6 Advantages of controlled-release pesticides

The need of controlled delivery device can be understandable by their advantages. Controlled-release technology has many advantages irrespective of its preparation and applications. In this article, the concentration is given mainly on the agrochemicals though other applications are also equally important.

Controlled delivery technology offers several advantages (Kenawy,1998a; Akelah, 1996) over conventional formulations. These can be summarized as follows:

Maintain of constant level of active agent: In conventional formulation, the active agent tends to release first at an overdose then undergoes to the local environment. Controlled releases offer a solution for this problem by maintaining the concentration of active agent between the minimum effect and toxic level.

- Use of smaller dose: This includes more efficient utilization of active agents, resource saving, safety etc.
- Reduces the loss by limiting leaching, volatilization and degradation.
- Minimizes potential negative effect (if there any) associated with overdose.
- Economical because less active material is needed due to reduction of excessive amounts for a given time interval.
- Facilitation of handling and masking of any odor.
- Toxic material becomes chemically nontoxic when attached with polymers.
- Extention of the duration of the activity of less-persistent or non-persistent active agent unstable within the aquatic environment by protecting them from leaching and degradation, hence aiding the practical applications of these materials.
- Reduction of phytotoxicity by lowering the mobility of the active agent in soil, hence reducing its residue in the food chain.
- Convenience: it converts liquids to solids; hence it results in easily transported materials with the reduction of flammability.

1.7 Disadvantages of controlled release technology

Although the advantages of controlled release technology are impressive yet the merits of each application have to be examined individually, and the positive and negative effects weighed carefully before large expenditures for developmental work are committed. In other words, controlled release is not a panacea, and negative effects may, at times, more than offset advantages. Some of the disadvantages of controlled release or the areas that require a thorough appraisal include (McCormick, 1987; Cardarelli, 1980)

- The cost of controlled release preparation and processing is substantially higher than the cost of standard formulations, but this could be compensated by minimizing the repeated applications.
- The fate of using excessive amounts of polymers as matrix and its effect on the environment is very important. This could be eliminated by using biodegradable polymers and improving weight efficiency by using polymers that may be beneficial to the environment when degraded.

- The fate of polymer additives, such as plasticizers, stabilizers, antioxidants, fillers, etc. left behind, once application is over, may cause some impact on environment.
- The polymer degradation products generated by heat, hydrolysis, oxidation, solar radiation and biological degradation may damage the environment.

1.8 Mechanisms and types of controlled release systems

Polymer controlled release formulations are divided into two broad categories, physical and chemical combinations. In physical combination, the polymer acts as a rate-controlling device while in chemical combination, it acts as a carrier for the active agent (Akelah, 1996). The choice of the best system to release the active agent in sufficient quantity to achieve the desired effect with minimum biological or ecological side effects depends on many considerations. These include the properties of the active agent, its physicochemical interactions with the polymer; the polymer nature (cross-linking degree, thermal behavior, and compatibility with the active agent); stability of the combination during processing; desired release rate; shape and size of the final product; duration; seasonal conditions; cost and ease of formulation and application.

1.8.1 Physical combinations

Two different approaches have been reported in literature in the case of the physical combination of biologically active agent with polymeric materials. Firstly, the biologically active agent can be encapsulated in a polymeric material in which the release of the active agent is controlled by Fick's law of diffusion through the micro pores in the capsule walls.The equation is given below:

$$R_d = dM_t / dt = A / h\, D\, (C_s - KC_e)$$

Where M_t is the mass of the agent released, dM_t/dt is the steady state release rate at time t, A and h are the surface area and thickness through which diffusion occurs, D is the diffusion coefficient of the active agent in the polymer, Cs is the saturation solubility of the active agent in the polymeric membrane, K is the partition coefficient of the active agent and the medium which surrounds the device, Ce is the concentration of released active agent in the environment.

In the second approach, biologically active agent is heterogeneously dispersed or dissolved in a solid polymeric matrix, which can be either biodegradable or non-biodegradable. The release of the active agent is generally controlled by diffusion and erosion (Kenawy et al.,1992). Release by erosion is a surface area dependent phenomenon, and the general expression which describes the rate of release (R_r) by an erosion mechanism is:

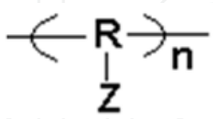
$$R_r = dM / dt = K_e C_o A$$

Where Ke is the erosion rate constant, A is the surface area exposed to the environment, Co is the loading of active agent in the erodible matrix. The design of such physical combinations is generally not influenced by the structure of the active agent molecule.

1.8.2 Chemical combinations

An active agent can be chemically attached to a polymer either as a pendant side groups, or as a part of the main backbone. Obviously only those biologically active agents, that contain

a structural moiety with at least one reactive functional group suitable for use as link to the functionalized polymer, can be used in this technique (Kenawy et al.,1992).
Polymeric chemically bonded active agents can be prepared by two synthetic methods. The first involves chemical modification of a preformed polymer with the desired active agent via a chemical bond, leading to a polymer having the active species linked to the main chain as a pendant group.



Z= active agent, R= monomer unit

Fig. 1(a). Active agent attached to polymer as side chain

The second method requires synthesis and polymerization of a biologically active monomer which leads either to polymer having the active group as repeat units in the main chain backbone.

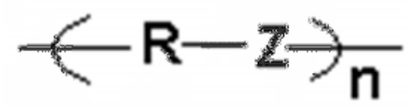


Fig. 1(b). Active agent in the polymer backbone

The chemically attached active agent is released from the polymer by the hydrolytic cleavage of the active agent-polymer linkage or via a slow degradation of the polymer itself induced by the water in the surrounding environment. The kinetic expressions which describe the release rate depend on the extent of branching and crosslinking of the macromolecules, i.e., on whether the cleavage reaction occurs on the surface of an insoluble particle or in the matrix.
Release of the active agent is usually dependent on surrounding environmental conditions that break the linkages via chemical attack (hydrolytic by moisture; thermal / photo by sunlight) or biological degradation (enzymatic by microorganisms), pH of the medium, ionic strength of the dissolution medium, competing ions, electrolyte concentration and temperature etc.

1.9 Release mechanisms

The release profile looked for a controlled delivery system is generally the steady state release of active agent or a zero-order release mechanism kinetically. The release rate in such systems is not affected by the amount of active agent released or not released at any moment (Paul, 1976). However, this type of release profile do not exhibit by many controlled delivery systems. The controlled delivery systems can be classified into several classes depending on the mechanism of release rate. According to Fan and Singh (Fan & Singh, 1989), the major release mechanisms involved in controlled delivery formulations are:
i) Diffusion ii) Swelling iii) Osmosis and iv) Erosion or chemical reaction controlled

i. Diffusion-controlled systems

Diffusion of active agent through the polymer is the rate-determining step in these systems. The polymer is hardly affected by the environmental factors. There are mainly two types of diffusion-controlled release systems.

(a) Reservoir systems

Here, the active agent is released out to the environment by diffusion, through the micropores of the capsule walls. The active agent is surrounded or encapsulated by a thin layer of polymeric membrane. Commonly used techniques in drug delivery systems are microencapsulation, nanoencapsulation, coacervation and spray encapsulation (Nagpal et al., 2001).

(b) Monolithic systems

In these systems, the active agent is dispersed or dissolved in the polymer. Release of the active agent may take place either by diffusion or leaching along with diffusion, if there is interaction between polymer and the environment. If a soluble additive is incorporated in the polymer matrix, the environmental fluid can easily penetrate the matrix by dissolving the additive and interconnected channels will be formed, through which the release would be easy. This technique has enormous applicability because, these types of physical combinations need not be influenced by structure of the active agent or polymer.

ii. Swelling controlled systems

Here, the dispersed or dissolved active agent in polymer matrix is unable to diffuse to any considerable extent. The active agent is released out slowly when the polymer system gets into contact with a compatible solvent or fluid in the environment and swelling takes place. Examples used in such systems are Poly(hydroxyl methyl methacrylate), polyacrylamide and poly(ethylene glycols) etc. (Omolo et al., 2004).

iii. Osmosis controlled system

Osmotic force is the driving force in osmosis-controlled systems. Such systems generally consist of a solid and water-soluble active agent, which is enclosed by a water-permeable, but active agent impermeable polymer membrane with a small opening. Water is transported into the core by permeation and hydrostatic pressure will be built up in the core and subsequently, the dissolved active agent comes out (Fan & Singh, 1989). In the field of controlled delivery, several newer techniques and methods like targeted delivery, viable cell immobilization, microspheres and nanoparticles/nanocomposites are finding great research interest (Hwang et al., 1985; Dua et al., 1996).

iv. Erosion or Chemical reaction controlled system

(a) Erosion-controlled system

The release of the active agent occurs here by erosion of the polymer. The active agent is physically immobilized in the polymer matrix. Active agent release rate is generally proportional to the erosion rate of the polymer matrix which undergoes surface erosion. A zero order release can be achieved in these systems if the erosion rate is constant and matrix dimension remains unchanged.

(b) Chemical reaction controlled system

Here, the active agent is released only when the polymer active agent bond is cleaved or the polymer is degraded. A zero order release profile may be obtained when the active agent is a co-monomeric unit in polymer backbone and release occur by polymer degradation.

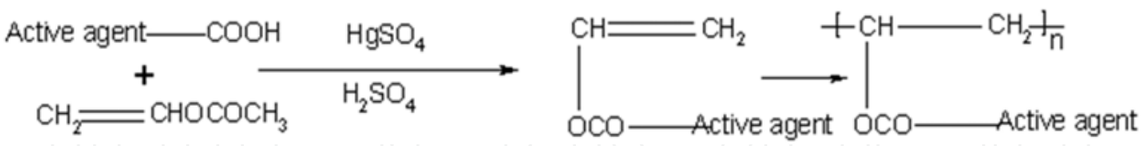
1.10 Manufacturing techniques of controlled release formulations

There are several techniques for the preparation of controlled release formulations. Among them, the most widely used techniques are discussed below:

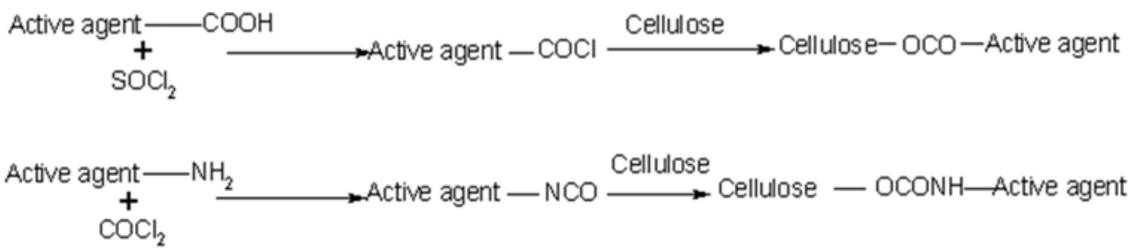
1.10.1 Chemically bound

Chemically bound active agents are of two types:

Those which are prepared by attaching a polymerizable site to the active ingredient, followed by polymerization of the new derivative. For example,



Those which are prepared by chemically binding derivatives of active ingredients to a suitable polymer. For example, active agents containing carboxylic functionalities have been reacted to form acid chlorides, which in turn are attached to natural polymers through its hydroxyl group . Similarly, active agent containing primary amino functionality were reacted with phosgene to form isocyanates, which in turn were attached through the hydroxyl group of natural polymers.



Such chemically bound combinations have found application in forestry and agronomic crops. The rate of release can be increased by lowering the molecular weight or increasing the hydrophilicity of the polymer carrier. The rate of release also depends upon the degree of substitution of the herbicide moiety within the polymer, the pH of the hydrolysis medium and the size of the particles.

1.10.2 Matrix encapsulation technique

The most common and widely used method for the encapsulation of active agents as controlled release product is the matrix encapsulation technique. Controlled release products obtained by this technique lack a distinctive wall surrounding each particle of the active ingredient. The active ingredient is dispersed within a polymer and becomes entrapped within many small cells of a continuous matrix. The active ingredient may be dissolved or suspended in various polymers to yield ribbons, sheets or granules. Often an excipient such as an inorganic filler is added to such formulations.

1.10.3 Microencapsulation

Microencapsulation is the coating of small solid particles, liquid droplets, or gas bubbles with a thin film of coating or shell materials. The product so obtained is termed as microcapsules. Microcapsules are small particles that contain an active agent or core material surrounded by a coating or shell. At present, there is no universally accepted size range that particles must have in order to be classified as microcapsules. However, many

workers classify capsules smaller than 1 μm as nanoparticles and greater than 1000μm as macrocapsules. Commercial microcapsules typically have a diameter between 3 and 800 μm and contain 10-90 wt% core. A wide range of core materials has been encapsulated, including adhesives, agrochemicals, live cells, active enzymes, flavors, fragnances, pharmaceuticals and inks. Most capsule shell materials are organic polymers, but fats and waxes are also used.

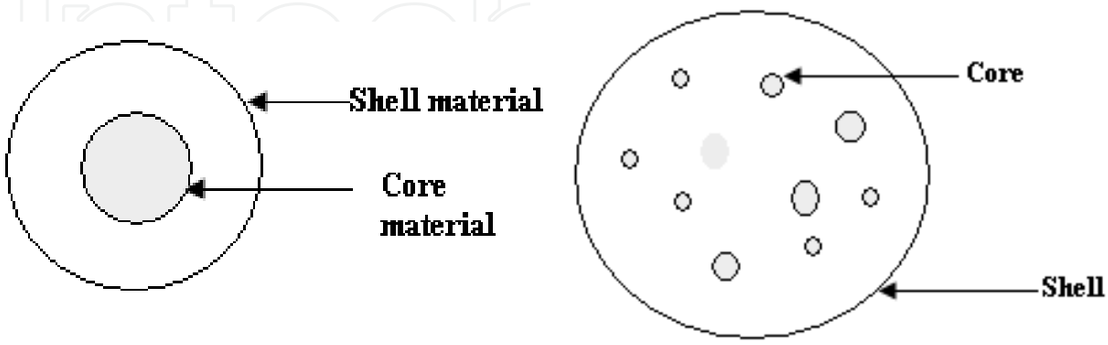


Fig. 2. Schematic diagrams of two representative types of microcapsules: (a) Continuous core/shell microcapsule; (b) Multinuclear microcapsules [Courtesy of C.Thies]

Microcapsules can have a variety of structures. Some have a spherical geometry with a continuous shell as shown in Fig.2(a). Others have an irregular geometry and contain a number of small droplets or particles of core material Fig.2(b).
Within the broad category of microparticles (Fig.3), 'microspheres' specifically refer to spherical microparticles and 'microcapsules' applies to microparticles which have a core surrounded by a material which is distinctly different from that of the core. The core may be solid, liquid or even gas. A microparticle usually refers to a homogeneous mixture of the polymer and active agent, whereas microcapsules have at least one discrete domain of active agent.

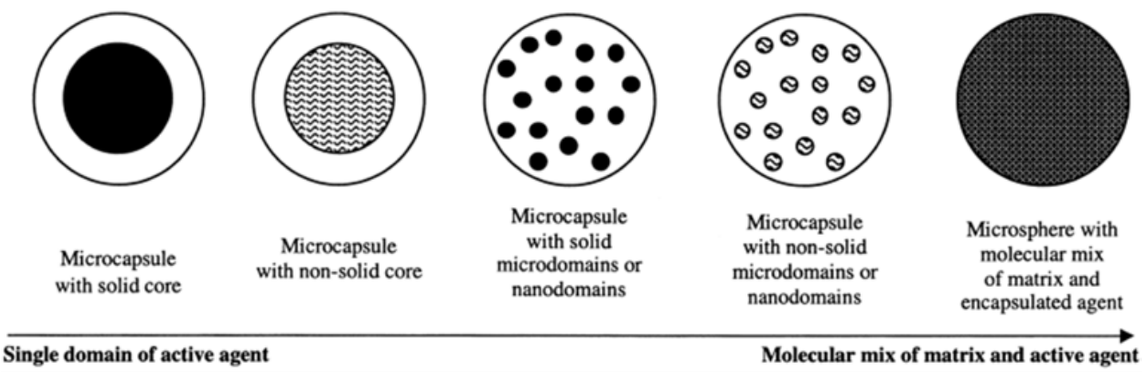


Fig. 3. Different categories of microparticles (Birnaum & Peppas 2004)

A steady stream of encapsulation technologies continues to appear in the patent literature. Some are simply modifications or improvements of the established technologies, where as others new technologies are confined to laboratory scale.

Thus, numerous preparation technologies available for the encapsulation of core material have been reported (Benita, 1996; Arshady, 1999; Sliwka, 1975; Ranny, 1969). Some of the important processes used for microencapsulation are summarized in Table 1.

Chemical processes	Physical processes	
	Physico-chemical	Physico-mechanical
• Suspension, dispersion and emulsion polymerization	• Coacervation	• Spray-drying
	• Layer-by-layer (L-B-L) assembly	• Multiple nozzle spraying
	• Sol-gel encapsulation	• Fluid-bed coating
• Polycondensation	• Supercritical CO_2-assisted microencapsulation	• Centrifugal techniques • Vacuum encapsulation • Electrostatic encapsulation

Table 1. Different techniques used for microencapsulation.

1.10.3.1 Solvent evaporation and extraction based process

1.10.3.1.1 Phase separation or coacervation

IUPAC defined coacervation as: "The separation into two liquid phases in colloidal systems. The phase more concentrated in colloid component is the coacervate, and the other phase is the equilibrium solution." The first systematic approach of phase separation, that is, partial desolvation of a homogeneous polymer solution into a polymer-rich phase (coacervate) and the poor polymer phase (coacervation medium) was realized by Bungenberg and colleagues (Bungenberg de Jong,1949; Bakkan &Anderson,1976). These authors termed such a phase separation phenomenon "coacervation".

Currently, two methods for coacervation are available, namely simple and complex processes. The mechanism of microcapsule formation for both processes is identical, except for the way in which the phase separation is carried out. In simple coacervation, a desolvation agent is added for phase separation, where as complex coacervation involves complexation between two oppositely charged polymers.

(a) Simple Coacervation

Aqueous solutions of water-soluble polymers are phase separated in aqueous media when sufficient salt is added to such solutions. This phenomenon is called simple coacervation. As long as phase separation produces a liquid polymer-rich phase, simple coacervation can be used to produce microcapsules (Bakkan&Anderson,1976). Microcapsules with gelatin, or poly(vinyl alcohol) or different natural polymers shell have been produced in this manner.

(b) Complex coacervation

Complex coacervation occurs in aqueous media and is used to encapsulate water-immiscible liquids or water-insoluble solids (Ghosh, 2006). Complex coacervation is carried out by mixing two oppositely charged polymers in a solvent (usually water); the process is shown schematically in Fig.4.

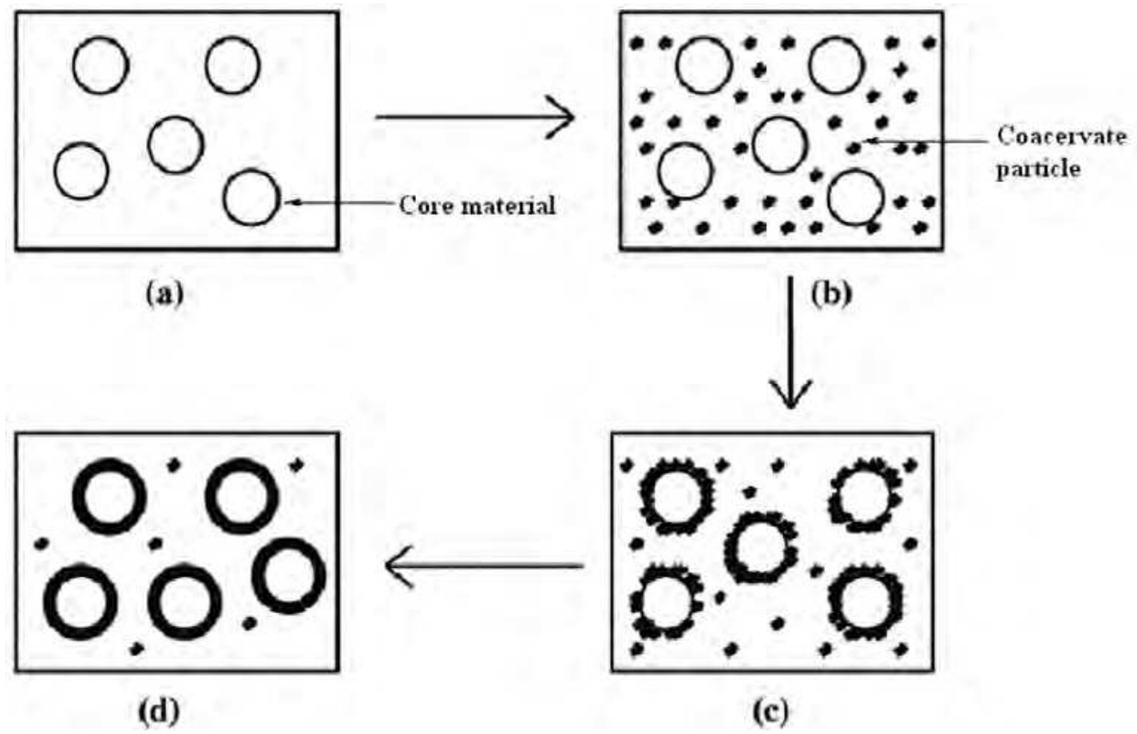


Fig. 4. Schematic representation of complex coacervation process. (a) Core material dispersion in shell polymer solution; (b) separation of coacervate from solution; (c) coating of core material by microdroplet of coacervate; (d) coalescence of coacervate to form continuous shell around core particles.

The three basic steps in complex coacervation are: (i) preparation of the dispersion or emulsion; (ii) encapsulation of the core; and (iii) stabilization of the encapsulated particle. The core material (usually an oil) is first dispersed into a polymer solution (e.g., a cationic aqueous polymer). The second polymer (anionic, water soluble) solution is then added to the prepared dispersion. Deposition of the shell material onto the core particles occurs when the two polymers form a complex. This process is triggered by the addition of salt or by changing the pH, temperature or by dilution of the medium. The shell thickness can be obtained as desired by controlled addition of the second polymer. Finally, the prepared microcapsules are stabilized by crosslinking, desolvation or thermal treatment. Complex coacervation is used to produce microcapsules containing fragrant oils, liquid crystals, flavors, dyes or inks as the core material. Porous microcapsules can also be prepared using this technique. When using this technique, certain conditions must be met to avoid agglomeration of the prepared capsules (Matiowitz, 1999).

1.10.3.2 Emulsion based process

1.10.3.2.1 Single emulsion process

This process involves oil-in-water (o/w) emulsification. The o/w emulsion system consists of an organic phase comprising of a volatile solvent with dissolved polymer and the active agent to be encapsulated, emulsified in an aqueous phase containing a dissolved surfactant. A surfactant is included in the aqueous phase to prevent the organic droplets from coalescing once they are formed. The polymer-solvent-active agent solution is emulsified

(with appropriate stirring and temperature conditions) to yield an o/w emulsion. The emulsion is created by using a propeller or magnetic bar for mixing the organic and aqueous phases.

Once the emulsion is formed, it is subjected to solvent removal by either evaporation or extraction process to solidify the polymer droplets. In the case of solvent removal by evaporation, the emulsion is maintained at a reduced pressure or at atmospheric pressure and the stir rate is reduced to enable the volatile solvent to evaporate. The organic solvent leaches out of the droplet into the external aqueous phase before evaporating at the water air interface. In the case of extraction, the emulsion is transferred to a large quantity of water or other quench medium, into which the solvent associated with the oil droplets is diffused out. Several reports are available that discusses on the formation of microspheres, for increase of encapsulation efficiency and release pattern of microspheres formed by this method (Jeyanthi, 1996; Arshady, 1991; Cavalier et al., 1986; Jalil, 1990; Tsai et al., 1986).

1.10.3.2.2 Double emulsion process

The problem with encapsulating hydrophilic active agent is the loss of the active agent to the external aqueous phase during the formation of the microparticle. Along with the loss of active agent to the external phase, the remaining active agent may migrate to the surface of the droplet before solidifying. To minimize these problems, the organic droplets should be solidified into microparticles as quickly as possible following their formation (Thies, 1992).This is achieved by using a viscous organic solution of polymer and active agent and a large secondary volume of water that attracts the organic solvent into the aqueous phase immediately, thus leaving the microparticle with the encapsulated active agent.The viscous dispersed phase minimizes the volume of organic solvent, facilitating its quick removal from the droplet and also makes it more difficult for the solid active agent particles/crystal to migrate to its surface, resulting in a more homogeneous distribution of the active agent within the particle. Another alternative to encapsulate hydrophilic active agent is to employ the water-in-oil-in water (w/o/w) emulsion process. An aqueous solution of the active agent is added to an organic phase consisting of the polymer and organic solvent with vigorous stirring to form the first w/o emulsion. This emulsion is then dispersed in another aqueous phase containing more surfactant to form the w/o/w emulsion. The problem with this type of emulsion occurs when the inner emulsion is not sufficiently stabilized, resulting in loss of aqueous droplets containing active agent to the external aqueous phase. The choice of surfactants that can be used to stabilize the inner emulsion is limited to materials that will dissolve in the organic solvent. Typically, the fatty acid esters of polyoxyethylene or sorbitan are used due to their high solubility in organic solvents and good biocompatibility.

1.11 Neem (Azadirachta Indica A. Juss.)

Neem is a fascinating tree. This plant may usher in a new era in pest control, provide millions with inexpensive medicines, cut down the rate of human population growth, and perhaps even reduce erosion, deforestation, and the excessive temperature of an overheated globe (National Research Council, 1992). Neem played an important role in the life of ancient Indian people. The efficacy of neem as a medicine has been documented in several different ancient treatises. Neem has achieved a relatively wide distribution in the tropical areas of Asia, Africa, South America, and Oceania.

More than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds (Koul,1990; Chatterjee & Pakrashi, 1994; Mitra & Patel, 1963; Taylor, 1984; Champagne et al. 1992; Kraus, 1995; Devakumar &Dev, 1996; Govindachari, 1992). The compounds have been divided into two major classes: isoprenoids and others (Devakumar &Dev,1996). The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and csecomeliacins such as nimbin, salanin and azadirachtin. The nonisoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalcone, coumarin and tannins, aliphatic compounds, etc. The details of the chemistry of various compounds falling under these groups have been reviewed (Kraus, 1995; Devakumar &Dev, 1996).

Azadirachtin was first isolated based on its exceptional antifeedant activity in the desert locust, and this substance remains the most potent locust antifeedant discovered to date. Some entomologists concluded that neem had remarkable powers for controlling insects that it would usher in a new era in safe, natural pesticides. Extracts from its extremely bitter seeds and leaves might be the ideal insecticides. They attack many pestiferous species; seem to leave people, animals, and beneficial insects unharmed; biodegradable; and they appear unlikely to quickly lose their potency to a build up of genetic resistance in the pests. Neem seems to provide nontoxic and long-lived replacements for some of today's most suspect synthetic pesticides.

Childs et al.(Childs et al., 2001) reported that there were at least 12 brands of neem pesticides registered in India. Neem-based pesticides refer to those formulated pesticides containing azadirachtin as the major active compound. The most popular product currently seems to be 0.3% azadirachtin EC (emulsifiable concentrate) although some companies are producing products with up to 5% azadirachtin content (Childs et al, 2001).

1.11.1 Neem seed oil components

The neem seed kernel is very rich in fatty acids often up to 50 percent of the kernel's weight. Neem seed oil is very bitter with a garlic/sulfur smell and contains vitamin E and other essential amino acids.Studies of the various components of the oil have been found the following fatty acids (i) oleic acid - 52.8% (ii) stearic acid - 21.4% (iii) palmitic acid - 12.6%
(iv)linoleic acid - 2.1% (v) various lower fatty acids - 2.3%. The percentages vary from sample to sample depending on place and time of collection of the seeds. Neem oil is an excellent moisturizing oil that contains compounds with historical and scientific validity as medicinals. Use of the oil for cosmetics and medicines has been limited by its strongly bitter taste and sulfur/garlic smell.

1.11.2 Biologically active compounds from neem seed oil

Two types of insecticides can be obtained from seeds of the Indian neem tree, Azadirachta indica (Maliaceae) (Isman, 2006). Neem oil, obtained by cold-pressing seeds, can be effective against soft-bodied insects and mites but is also useful in management of phytopathogens. Apart from the physical effects of neem oil on pests and fungi, disulfides in the oil may likely to contribute to the bioactivity of the material. Neem seeds actually contain more than

a dozen azadirachtin analogs, but the major form is azadirachtin. Seed extracts include considerable quantities of other triterpenoids, notably salanin, nimbin, and derivatives thereof. Besides azadirachtin, the role of all other biologically active compounds has also been in high esteem.

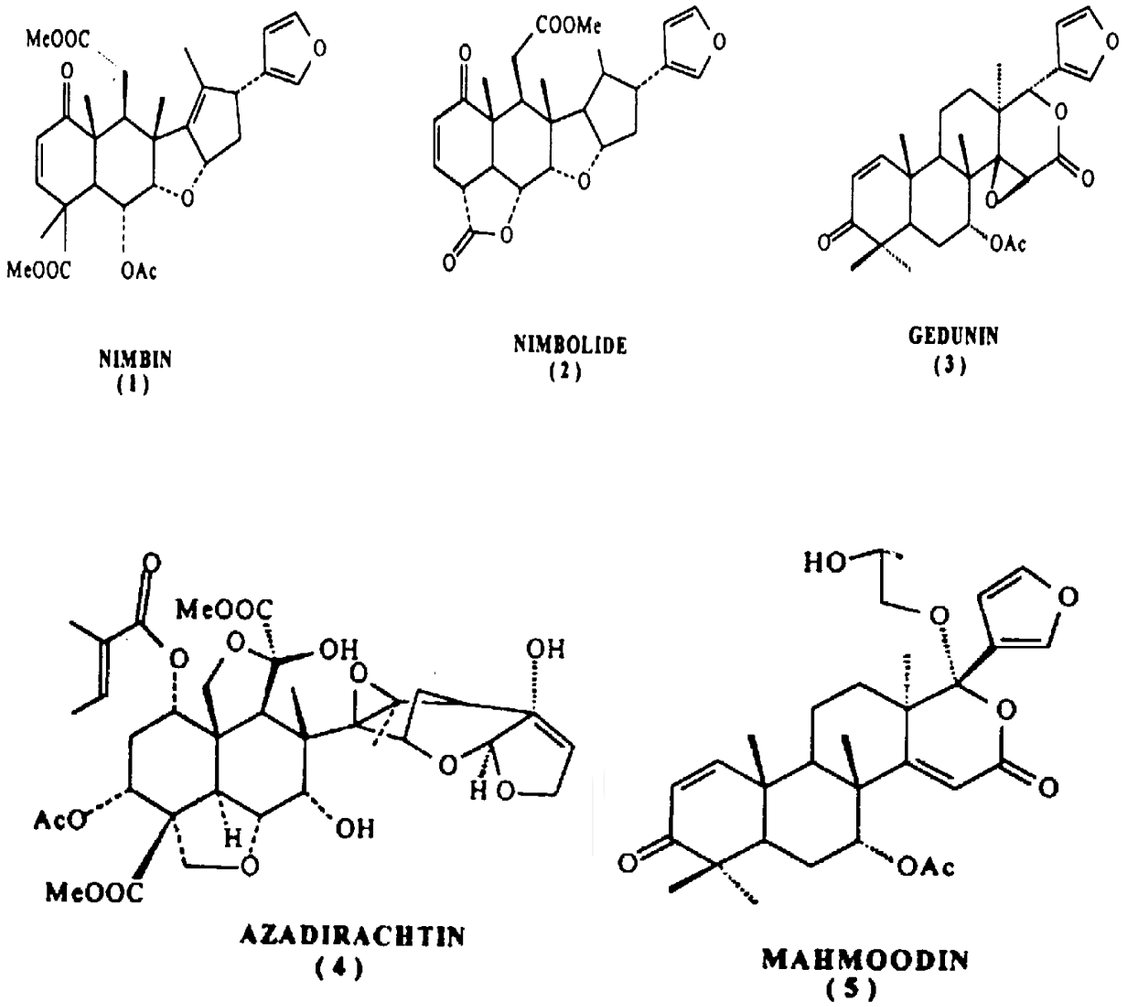


Fig. 5. Major biologically active compounds present in neem seed oil

Neem seeds typically contain 0.2 to 0.6% azadirachtin by weight. From the crude, some tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide and nimbidic acid have been isolated (Mitra et al., 1971). These entire compounds possess valuable pesticide, repellent as well as medicinal properties. Nimbidin shows antifungal activity by inhibiting the growth of *Tinea rubrum* (Bhargava et al.1970). In vitro, it can completely inhibit the growth of *Mycobacterium tuberculosis* and has been found to be bactericidal (Murthy, & Sirsi, 1958). The spermicidal activity of nimbidin and nimbin (1) is reported in rats. Nimbolide (2) is reported to inhibit the growth of *Plasmodium* falciparum (Rochanakij et al., 1985; Khalid et al., 1989). Nimbolide also shows antibacterial activity against *S. aureus* and *S.* coagulase Rojanapo et al., 1985). Gedunin (3), isolated from neem seed oil is reported to possess both antifungal (Rao et al. 1977) and antimalarial (Khalid et al., 1989) activities. Azadirachtin (4), highly oxygenated C-secomeliacins isolated from neem seed and have strong antifeedant activity (Butterworth & Morgan, 1968). It can inhibit to the development of malarial parasites (Jones et al., 1994). Mahmoodin (5), a deoxygedunin isolated from seed oil, is reported to possess moderate antibacterial action against some strains of human pathogenic bacteria (Devakumar &Dev, 1996).

1.11.3 Need of encapsulation of NSO pesticide

Search for environmentally sound pesticides received an impetus following the publication of 'Silent Spring' by Rachel Carson in 1962. It was around this period that Pradhan et al.(Pradhan et al., 1962) reported the feeding deterrent property of neem seed kernel suspension against desert locust, *Schistocerca gregaria*. Subsequently, several bioactive ingredients notably meliantriol and azadirachtin were isolated from various parts of the tree which aroused worldwide interest in the insecticidal bioactivity of the neem tree and thus a substantial work has been carried out globally to establish the insecticidal activity of neem. Isolation of several bioactive ingredients notably meliantriol and azadirachtin from various parts of the tree aroused worldwide interest in the insecticidal bioactivity of the neem. In fact neem has been recommended by most of the authors as a desirable method of pest control and repellent as it does not cause harm to the people and the plant but selectively affects the insects. Neem products affect different physiological processes in insects, e.g. metamorphosis including insect growth regulation, adult fertility, toxicity, and they also affect behaviour, having antifeedant and oviposition deterrent effects. This way the neem extract could influence over 200 species of insects many of which are resistant to modern pesticides. Several review papers have been published regarding the use of neem pesticides in cotton pest management, other vegetable pest management, mosquito repellent activity etc. and almost all the published papers have confirms its strong pesticidal activity.

Though Neem Seed Oil (NSO) has been in high esteem due to its potent pesticide properties, yet, application of it to the soil is limited due to its liquid nature. This limitation can be overcome by the encapsulation of NSO to give rise to a solid formulation. Moreover, azadirachtin, the key component of NSO is rapidly degraded by sunlight. Thus, encapsulation of NSO not only gives it a solid form but also protects it from sunlight. Another reason behind the encapsulation and controlled release of NSO is that, along with potent pesticide activity, it also shows toxicity in some cases. The toxic effect is observed to fish like tilapia and carp and also in human in several isolated cases. NSO intoxication by human produces nausea, vomiting, acidosis, encephalopathy, etc. Microencapsulation and controlled delivery technology seems to be the most useful technique to minimize this toxicity and make an appropriate as well as efficient use of this effective natural resource NSO.

2. Literature review

2.1 Controlled release agrochemicals

The different classes of polymers viz., elastomers, plastics and fibres were extensively used in agriculture for varied purposes. The major application fields included CR pesticides, herbicides and fertilizers, soil conditioning, plant protection, seed coating and gel planting (McCormick, 1984). Several pesticides like sevin, dimethoate, ethyl trithion, methyl trithion, diazinon, malathion, chloropyriphos and temephos could be incorporated in plasticized poly(vinyl chloride) to obtain CR products (Cardarelli,1980).

El-Refaie and coworkers (Kenawy, 1998b) prepared controlled release formulations based on crosslinked polyacrylamide derivatives. The release data of the herbicide 2,4-D in vitro from formulations were described. Micro-or macro encapsulation of active agents using polymers is one of the methods widely used for the preparation of CR products. Condensation polymerization reactions yielding polyamide, polyester, polyurea, polyurethane, polycarbonate and polysulphonamide could be well utilized to prepare CR formulations. Crosslinking of the polymer wall provided durable and storage stable capsules (Lowell et al., 1977; Koestler,1980). Several controlled release pheromone formulations were also synthesized by microencapsulation. The utilization of starch as a polymer matrix for CR agrochemical was reported (Shasha, 1980). Pfister, Bahadir and Korte (Pfister et al., 1986) claimed another system based on calcium alginate with a series of herbicides. Starch was used as an encapsulating material for S-ethyldipropylthiocarbamate (EPTC), atrazine and trifluralin (Shasha et al., 1981a;Trimnell et al. 1984; Shasha,1981b). Teft and Friend (Teft & Friend,1993). synthesized controlled-release polymeric microspheres of herbicides Dicamba(DA) based on ethylcellulose, polyarylsulfone or a combination of the two.

Kulkarni, Kumbar, Dave and Aminabhavi (Kumbar et al., 2001a) reported the release kinetics and encapsulation efficiency of urea-formaldehyde (UF) crosslinked matrices of starch, guar gum (GG) and starch+guar gum (St+GG) for controlled release of solid (chloropyrifos) and liquid (neem seed oil) pesticides. In another report, Kulkarni and his group (Kulkarni et al., 2002) claimed the synthesis of novel polymeric sodium alginate interpenetrating network (IPN) beads for the controlled release of chloropyrifos. They also synthesized IPN beads of poly(vinylalcohol)-g-poly(acrylamide) with sodium alginate for the controlled release of cypermethrin pesticide (Kumbar & Aminabhavi, 2002).

Marei et al. (Marei et al., 2000) compared carbofuran encapsulated controlled release formulation with the granular formulation in term of mobility of carbofuran and reported that leaching potential of alginate formulation decreased more than nine times compared with granular formulation. Chitosan gel beads and film were assessed for their ability to control the release of herbicide atrazine and fertilizer urea (Teixeira et al., 1990). Elabahni et al. (Elbahri &Taverder, 2005) developed a technique for encapsulation of herbicide inside ethyl cellulose microsphere and evaluated the shape and size of microspheres by scanning electron microscopy. Polysaccharides like cellulose, chitin, amylose and amylopectin were found to be useful natural polymers for the CR formulations of 2,4-dichlorophenoxyacetic acid and metribuzin (McCormick, 1984). CR formulation of kraft lignin and propachlor had been successfully prepared by Wilkins and Blackmore (Wilkins & Blackmore,1987). It was reported that rice husk lignin could be combined with 2,4- dichlorophenoxyacetic acid (Kenawy et al.,1992). The application of lignin in CR formulations was reviewed by Wilkins (Wilkins,1983). Zhu and Zhuo (Zhu & Zhuo, 2001) synthesized a new starch-g-poly(butylacrylate) for encapsulating carboxylic group containing herbicides. Polymerizable

derivatives of pesticides containing acid groups could be prepared by a reaction with alcohols having a vinyl group (Harris & Post, 1974; Harris, 1980). Copolymers of vinyl 2,4-dichlorophenoxyacetate and trimethl amine methacrylamide were reported to be used for CR application (Kenawy et al.,1992). Increased release of herbicide was obtained as the hydrophilic co-monomer content increased.

Kenawy and his group (Akelah et al., 1993) prepared controlled release systems based on polyureas and poly(Schiff`s bases). The effects of structure and temperature of the aqueous environment on the hydrolysis rate of the obtained polymer had been reported. Cheillini and Akelah (Solaro et al. 1991) synthesized polymeric herbicides containing 2,4-D and MCPA by modification of oligoethyleneoxylated styrene/divinylbenzene(DVB) resins. The release features for these systems were greatly affected by the pH.

Akelah et al. (Akelah & Rehab,1994) reported chemical modifications of a series of polyamides containing hydroxyl groups with 2,4-D in the presence of dicyclohexylcarbodiimide(DCC) as a condensating agent to yield a series of polymer. They reported that the rates of release of 2,4-D from the formulations were mainly dependent on hydrophilicity, the pH and the temperature of the release medium.

Pesticides containing acid groups were converted to more reactive acid chlorides, which could react with polymers containing pendant hydroxyl or amino groups. Acylation of synthetic and natural polymers were possible in this manner (Neogi,1970; Wilkins, 1976; Allan et al., 1971; Allan et al., 1977). Pentachlorophenol intercalated on mineral clay was reported by Akelah and Rehab (Akelah & Rehab,1996). The release of pentachlorophenol from the formulations was studied in different media at 30^0C and it was concluded that the release of pentachlorophenol from the formulations was dependent on the structure, swelling degree and the medium of release.

A series of preformed polymers modified with pesticides were reported (Kenawy et al., 1992). Chitin (Kemp & Wrightman, 1981; Trenkel, 1997) as a naturally occurring polymer was used as carrier for herbicide metribuzin and the system showed slow release when polymer was directly attached to metribuzin.

2.2 Nanocomposite for controlled release of pesticide

Nanocomposites technology with layered silicate as in situ reinforcement has been extensively investigated in recent years. The increasing interest for development of polymer clay nanocomposites is due to the improvement in properties of these composites resulting from the synergic effect of interaction of both the components at nanometer level (Pinnavaia & Beall, 2000).Among the different nanocomposites, the bio-hybrid nano structured materials have evoked considerable interest in recent years.The current trend involves in the development of nanocomposite based on natural polymers and inorganic solid particularly clay minerals. Nano clays and layered double hydroxides are being developed in this regard(Chaoi et al. 2007). Both materials show good biocompatibility, low toxicity, and the potential for controlled release.Chemicals can be loaded between layers of both materials (an arrangement that can beinfluenced by buffer conditions, in particular pH). In the case of hydrophobic chemicals, this arrangement prevents re-crystallisation, increases solubility, and therefore bioavailability. A number of research activities have been completed with regards to layered double hydroxides as pesticides, growth regulators, plant nutrients, and slow-release fertilizer(Olanrewaju et al., 2000; Lakraimi et al., 2000).

Imazapyr (IMP), a herbicide was bound to polydiallyldimethyl ammonium chloride-montmorillonite composites for reducing the leaching of IMP. IMP release in the soil from

the controlled release formulation was substantially slower than its release from the commercial formulation (Radian & Mishael, 2008). The inorganic Zn-Al layer double hydroxide was used as a matrix to encapsulate the herbicide 2,4 dichlorophenoxyacetate. The release of the herbicide was rapid initially followed by a sustained release. The release behavior was dependent on the on the type of anions(like chloride,carbonate etc.)and their concentration present in the release medium(Hussain et al., 2005). Mg/Al layered double hydroxide(LDH) was intercalated with herbicides 2,4-D MCPA and picloram. The complexes were assayed for the controlled release of herbicides. It was concluded that LDH had the potentiality for the preparation of slow release formulation of 2,4-D MCPA picloram (Cordoso et al., 2006). The release of alachor and altrazine into aqueous solution from the controlled formulation prepared from alginate and pectin,with and without the addition of clay minerals, was studied by Gerstl et al.(Gerstl et al., 1998). The addition of clay to the formulations was found to have a profound inhibitory effect on the release of alachor.

2.3 Natural pesticides

Four major types (pyrethrum, rotenone, neem and essential oils) along with three minor types (ryania, nicotine, and sabadilla) of botanical products used for pest control were reported in literature (Isman, 2006). Isman (Isman, 2005) reported that pyrethrum accounted 80% of the global botanical insecticide market. Several plant derived compounds with pesticidal potential were discussed and reviewed in the literatures (Duke, 1990, 1986a, 1986b; Duke & Lydon, 1987;Duke et al., 1988).

Shay et al. (Shay et al., 1998) studied the insecticidal and repellent properties of nine volatile constituents of essential oils against the American cockroach, *Periplaneta americana*. Alali et al. (Alali et al.; 1998) studied the six compounds, representing the mono-tetrahydrofuran (THF) (gigantetrocin A, annomontacin), adjacent bis-THF (asimicin, parviflorin), and nonadjacent bis-THF (sylvaticin, bullatalicin) classes of annonaceous acetogenins, and compared them with technical grades of synthetic amidinohydrazone (hydramethylnon), carbamate (propoxur, bendiocarb), organophosphate (chlorpyrifos), and pyrethroid (cypermethrin) insecticides to determine their dietary toxicities to insecticide-resistant and insecticide-susceptible strains of the German cockroach, *Blattella germanica*.

Mugisha-Kamatenesi et al. (Mugisha-Kamatenesi et al., 2008) demonstrated that usage of botanical pesticides in field pest management was normal around Lake Victoria basin for the subsistence farmers. Mahfuz and Khanam reported (Mahfuz & Khanam, 2007). on the efficacy of seven different plant extracts viz. Acorus calamus rhizome, leaves of Datura fastuosa, Datura stramonium and seeds of D. stramonium, Corchorus capsularis, Aphanamixis polystachea and Jatropha curcas on Tribolium confusum adult.

Mishra et al. (Mishra et al., 1989) isolated essential oils from leaves of Chenopodium ambrosioides, Cinnamomum zeylanicum, Citrus medica, Melaleuca lucadendron, Ocimum canum and O. grattissium. These oils demonstrated fungitoxicity against Aspergillus flavus at 200, 300, 400 and 500 ppm and most of them were shown to be more effective than synthetic fungicides viz; Agrosan G.N., Copperoxychloride, Ceresan, Thiovit and Dithane M45. Asthana et al. (Asthana et al., 1986) found the leaf extract of Ocimum adscendens to be fungitoxic against Aspergillus flavus. The volatile fungitoxic fraction was identified to be an essential oil, and was observed to be more active than some five synthetic fungicides tested.

Wang et al. (Wang et al., 1990) were able to isolate antifungal and larvicidal polyacetylenes for Artemisia borealis (B. campestris subsp. borealis). Dichloromethane extracts for the whole plant showed antifungal activity against Cladosporium cucumerinum.

Upadhyaya and Gupta (Upadhyaya & Gupta, 1990). demonstrated the inhibitory effect of some medicinal plants on the growth of Curvularia lunata (Cochliobolus lunatus). Ethanol extracts of garlic followed by those of Ocimum santum, Datura alba and hemp were found to be most inhibitory to growth of the fungus. Aqueous extracts were less effective. Garlic extracts were shown to be inhibitory on the growth of a number of fungi (Tansey & Appleton, 1975). From methanol extracts of twigs of Oxymitra velutina - a west african plant, 12 alkaloids; 5 aporphinoids including lysicamine, which is active against Bacillus subtilis, Botrytis cinerea, Saprolegnia asterophora and Rhizoctonia solani were isolated (Achenbach & Hemrich,1991).
Yegen et al. (Yegen et al., 1992) studied the fungitoxic effect of extracts of six selected plants from Turkey. Results indicated that aqueous and essential oils of Thymbra spicata, Satureja thymbra, Laura nobilis, Mentha spicata, Salvia futicasa and Inula viscosa were fungitoxic to Fusarium moniliforme, Rhizoctonia solani, Sclerotinia sclerotiorum and Phytophthora capsici. Kumar and Tripathi (Kumar &Tripathi,1991) screened leaf extracts of 18 plant species belonging to 11 families for their control of Pythium debaryanum, Fusarium oxysporum, R. solani and Sclerotium rolfsii.

2.4 Neem seed oil as pesticide

Pradhan et al. (Pradhan et al., 1962) reported the feeding deterrent property of neem seed kernel suspension against desert locust, *Schistocerca gregaria*. Subsequently, several bioactive ingredients were isolated from various parts of the tree, more notable being the isolation of meliantriol (Lavie & Jain, 1967) and azadirachtin (Butterworth & Morgan, 1968). Both meliantriol and azadirachtin inhibited feeding of locust. In fact neem was recommended by most of the authors as a desirable method of pest control and repellent as it did not cause harm to the people and the plant but selectively affected the insects (Dhawan & Patnaik, 1996). Neem products affected different physiological processes in insects which were reported in literature (National Research Council, 1992). Neem extract could influence over 200 species of insects. Many of which were resistant to modern pesticides. Active constituents like azadirachtin showed larvicidal and anti-feedant (Rao et al., 1988; Morgan & Thornton, 1973; Miller & Chamberlain, 1989) activities.
It was reported that a spray of 1-percent neem oil in water "stopped 95 to 100 percent of the powdery mildew on hydrangeas, lilacs, and phlox." Locke and Larew demonstrated that neem oil could reduce damage caused by various pests, including spider mites. It was also reported that a 2-percent spray of neem seed oil applied directly to spider mite eggs resulted in an 87-percent mortality (Becker, 1994). Neem oil provided effective control of rice plant hoppers like *Nilaparvatha lugens*, *Nephotettixs* spp. and *Sogatella furcifera*. A spray of 3% neem oil discouraged settling of hopper, *N. legens* on treated plants (Jayaraj, 1993).. Similarly, 3% neem oil and 5% neem seed kernel extract were reported to control *Helicoverpa armigera* in bengal gram. Isman (Isman,1997; Isman, 1996). reported a significantly higher growth inhibition on grubs of rice seedlings while using the crude oils extracted from the seeds of neem, custard apple and china berry. An oil based neem formulation containing 300 ppm of azadirachtin was used effectively against the desert locust, grasshoppers and other lepidopteran pest (Ramarethinam & Marimuthu, 1998). Application of neem oil was also reported to reduce the incidence of plant viral diseases like yellow vein mosaic of okra, yellow mosaic of grain legumes, leaf curl of chillies and ragged-stunt virus of rice. Neem oil was reported to inhibit or reduce the transmission of Tungro virus and Tobacco mosaic virus (Vijayalakshmi et al.,1995).

The use of neem pesticides in cotton pest management was thoroughly reviewed by R.T. Gahukar (Gahukar, 2000). Pesticides derived from neem (*Azadirachta indica A. Juss.*) controlled pests without having the nontarget toxicological effects associated with conventional pesticides (Gahukar, 2000). Both azadirachtin and azadirachtin analogues were studied for antifeedant activity against the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) and azadirachtin was found to be the most active (Simmonds et al., 1995).Neem oil (2%) inhibited normal growth and development of early third instar larvae of *S. litura* under laboratory conditions by affecting growth hormone systems (Koul, 1987). Azadirachtin reduced pupation and adult eclosion in *S. litura* by 47% and 42% respectively (Ayyangar & Rao, 1991; Mohan, 1990). Several reports were found on using neem oil against mosquito and as larvicide (Virendra et al., 1995; Sharma et al., 1993; Fredros et al.,2007; Nagpal et al.,1995; Govindachari et al., 2000).

Antifungal activity of neem oil for mycelia growth inhibition was studied and reported by Mohanty et al. (Mohanty et al., 2008). Wanyika et al. (Wanyika et al.2009) reported that pyrethrum-botanical oil (neem sedd oil, cotton seed oil and yellow oleander oils) blends against maize weevils was effective insecticide compared to pure pyrethrum based insecticide. Senthil-Nathan et al. (Senthil-Nathan et al., 2009) studied on the toxicity and physiological effects of neem pesticides applied to rice and found neem-based pesticides to be more effective to inhibit the growth and survival of N. lugens, the brown planthopper. When the synthetic insecticides chlorpyrifos (Termex®), cypermethrin+acetamiprid (Conquest®), and the natural insecticide neem (*Azadirachta indica* A. Juss) seed oil extract were used, the insecticides significantly reduced the populations of diamondback moth, *Plutella xylostella* L., and the cabbage head caterpillar, *Crocidolomia binotalis* Zeller (Umeh et al., 2009). Reports were also available on the advantages of neem oil based pesticides over other chemical pesticides (Gauri, 2007).

2.5 Encapsulation and controlled release of NSO

In the field of encapsulation and controlled release of NSO, a number of research works were carried out and reported by Aminabhavi and co-workers. Several controlled release formulations containing NSO were developed by them (Aminabhavi et al, 1998; Kulkarni et al.,1999; Kumbar et al., 2001b; Kulkarni et al., 2000). Preparation and characterization of granules encapsulating the natural liquid pesticide viz., *A. indica* A. Juss. (neem) seed oil (NSO) was reported by Kulkarni et al. (Kulkarni et al.,1999). The polymer matrices used for encapsulation were urea formaldehyde crosslinked starch (UF–St), guar gum (UF–GG) and UF–(St + GG). The release of the active ingredient was depended on the type of matrix and its swelling ability. The entrapment efficiency was more than 95% in majority of the cases indicating the efficient encapsulation. The percentage loading of NSO with different matrices and their density exerted an influence on the release data.

The study regarding the release kinetics and encapsulation efficiency of the urea formaldehyde (UF) crosslinked matrices of St, GG, and St + GG for the controlled release of the solid (chlorpyrifos) and liquid (neem seed oil) pesticides (Kumbar et al., 2001b) was carried out and reported by Kumbar et al. They indicated that variable release rates were related to the polymer type and especially the pesticide type. It was concluded that it was possible to slow the release rates of pesticides using these cheaply available polymers.

Kulkarni et al. studied and reported the encapsulation of NSO as liquid pesticide using sodium alginate (Na–Alg) as a controlled release (CR) polymer after crosslinking with

glutaraldehyde (GA) (Kulkarni et al., 2000). The FTIR spectral study confirmed the absence of chemical interaction between active ingredients and polymer as well as crosslinking agents. An increase in the crosslinking of the polymer resulted in a significant decrease of NSO release from the beads.

Development and invention of an improved granular formulation of neem seed extract containing neem Aza-A having enhanced storage stability (Sreenivasa et al., 2006) and the ability for gradual release of neem Aza-A was reported in a patent.The formulation consists of an inert particulate compound as a carrier, at least one lipophilic substance as a deactivator/binder, optional colorant and neem seed extract containing neem Aza-A (Sreenivasa et al., 2006).

Controlled release microcapsules containing neem (*Azadirachta Indica A. Juss.*) seed oil (NSO) were prepared by encapsulation of natural liquid pesticide NSO in polyelectrolyte complexes of naturally occurring polymers namely carrageenan-chitosan (Devi & Maji, 2009a, 2009b) gelatin-carrageenan (Devi & Maji, 2010) and gelatin-sodium carboxymethyl cellulose (Devi & Maji, 2011). Microencapsulation was carried out at the optimized ratio and pH for complexation between the biopolymers in order to get maximum yield and to form stable polyelectrolyte complex. The microencapsulation method for NSO loading was optimized. The release rates of NSO were studied by varying the percentage of oil loading, concentration of crosslinking agent and polymer concentration. Scanning electron microscopy study demonstrated free flowing to bursting look of the microcapsules for different formulations. Microcapsules formed from different biopolymer polyelectrolyte complex systems were compared. Fourier transform infrared spectroscopy (FTIR) study indicated the absence of any significant interaction between the polyelectrolyte complexes and NSO. The interaction between the complex forming polymers was indicated by theformation of new bond and shifting of peaks in FTIR spectra.

The effect of a natural rubber coating of a capsule obtained from sodium alginate on the release of neem Aza-A was studied by Riyajan et al.(Riyajan & Sakdapipanich, 2009).The effect of physically modifying the hydrophobicity of alginate beads and the optimum conditions for release of neem Aza-A from such capsules were investigated in this study. To enhance the stability of neem Aza-A which is not stable in the environment, a biodegradable novel semi-interpenetrating polymer network based on poly(vinyl alcohol) (PVA) and sodium alginate containing neem (*Azadirachta indica*) in the presence of azadirachtin-A (neem Aza-A) as well as glutaraldehyde as a crosslinking agent was prepared for use in the controlled release of neem Aza-A(Riyajan & Sakdapipanich, 2010).

3. Methods of synthesis of encapsulated controlled release NSO pesticides

3.1 Some significant methods used

The most common and frequently used method for preparing granules and beads of NSO is matrix encapsulation technique. This technique was used for preparing crosslinked polymeric granules containing 20, 35 and 50% (w/w) of the natural liquid pesticide viz., *A. indica* A. Juss. (neem) seed oil (NSO) and was reported (Kulkarni et al.,1999). The crosslinked granules were obtained bypreparing urea-formaldehyde (UF) prepolymer followed by gelatinizing starch (St), guargum (GG) , or St-GG matrices containing NSO by boiling St, GG, or St-GG combinations in distilled water to form a transparent muscilage. Three concentrations of NSO i.e., 20, 35 and 50% (w/w of the polymer) was added after cooling and then dispersed by mechanical mixing. The pH of the mixture was reduced to 3.0

for completion of crosslinking reaction by using urea-formaldehyde prepolymer as crosslinker. The crosslinked mass was sieved, dried in vacuum oven and furthr sieved in order to obtain uniform sized granules ranging between 0.5-2mm.

The amount of NSO in the granules, density of the matrices, extent of swelling of the granules and the cumulative release of NSO from the matrix were studied thoroughly and reported. It is concluded that starch matrix is more effective in low moisture containing soil, while in high moisture containing soil, GG matrix is preferred for effective release of NSO (Kulkarni et al.,1999). Urea formaldehyde (UF) crosslinked matrices of St, GG, and St + GG were used to study the release kinetics and encapsulation efficiency of two pesticides-chlorpyrifos, a solid and neem seed oil , a liquid pesticide (Kumbar et al., 2001b).

Sodium alginate beads containing NSO were prepared by mixing NSO in a sodium alginate solution followed by dropwise addition of this solution into methanol containing glutaraldehyde (1%) and HCl(1% of 1N) solution under constant stirring (Kulkarni et al., 2000). The beads were separated from methanol at different time intervals, washed with water and dried.

In our laboratory, microencapsulation of NSO was carried out in three natural polymer systems by the method of polyelectrolyte complexation and then crosslinking. For the carrageenan- gelatin A system (Devi & Maji,2010), the procedure was as follows- known amount of (100ml) 0.5-1.5% (w/v) of carrageenan solution in distilled water was taken in a beaker. This polymer solution was stirred by mechanical stirrer under high agitation at 70 ± 1^0C. This temperature was maintained throughout the experiment. To this, NSO (1-4g) was added under high agitation to form an emulsion. A known amount of (200ml) gelatin-A solution of 1-3% (w/v) was added to the beaker drop wise to attain complete phase separation. However, the weight ratio of carrageenan to gelatin was maintained at 1: 2 during all the experiments. At this ratio, interaction between gelatin and carrageenan was maximum as judged by the measurement of coacervate yield (%) and viscosity. The pH of the mixture was then brought down to 3.5 by adding 2.5% (v/v) glacial acetic acid solution. At this pH, maximum interaction took place as measured by yield (%) and turbidity. The beaker containing the microcapsules was left to rest at the same temperature for approximately 15 minutes. The system was then brought to $5\text{-}10^0$C to harden the microcapsules. The cross linking of the polymer capsule was achieved by slow addition of certain amount of genipin solution (a natural crosslinker) (0.5225% w/v). The temperature of the beaker was then raised to 45◦C and stirring was continued for another 3-4 h to complete the crosslinking reaction. The beaker was then cooled to room temperature slowly while stirring. The microcapsules were filtered through 300-mesh nylon cloth, washed with 0.1% (w/v) Tween 80 surfactant solution to remove oil, if any, adhered to the surface of microcapsules. This was further washed with distilled water, freeze dried and stored inside a refrigerator in a glass ampule.

3.2 Encapsulation and controlled release studies

UV-visible spectrophotometric methods were used to study the encapsulation efficiencies and controlled release behavior of encapsulated NSO. Encapsulation efficiencies were calculated by estimating the pesticides before (theoretical) and after encapsulation (actual loading). The encapsulation efficiency is dependent on both the nature of the matrix material and the pesticide type. A highly rigid polymer (shell) is obviously a good polymer for encapsulation. A calibration curve of NSO is required for the determination of

encapsulation efficiency, oil content and oil load and release rate of oil from the microcapsules.

3.2.1 Calibration curve of NSO

In our study (Devi & Maji, 2009, 2010), it was found that 0.1 gm of NSO could be easily dissolved in 100 ml of water containing 0.1 g Tween 80. A known concentration of NSO in DDI water containing 0.1% Tween 80 was scanned in the range of 200-400 nm by using UV visible spectrophotometer. For NSO having concentration in the range 0.001 to 0.08 g /100ml, a prominent peak at 254 nm was noticed. The absorbance values at 254nm obtained with the respective concentrations were recorded and plotted. From the calibration curve, the unknown concentration of NSO was obtained by knowing the absorbance value.

3.2.2 Encapsulation efficiency, oil content and oil load

For the calculation of encapsulation efficiency, oil content and oil load, a known amount of accurately weighed microcapsules are grounded in mortar, transferred with precaution to a volumetric flask containing a known amount of 0.1% (w/v) aqueous Tween 80 solution and kept for overnight with continuous stirring. The encapsulation efficiency (%), oil content (%) and oil loading (%) are generally calculated by using the calibration curve and the following formulae (Maji et al., 2007).

$$\text{Encapsulation efficiency } (\%) = (w_1 / w_2) \times 100$$

$$\text{Oil content } (\%) = (w_1 / w) \times 100$$

$$\text{Oil load } (\%) = (w_2 / w_3) \times 100$$

where
w = weight of microcapsules
w_1 = actual amount of oil encapsulated in a known amount of microcapsules
w_2= amount of oil introduced in the same amount of microcapsules
w_3 = total amount of polymer used including crosslinker
In all the studied complexes, the oil loading (%), oil content (%), encapsulation efficiency (%) and release rate of oil were dependent on various factors like amount of oil, concentration of polymer, type and concentration of crosslinker. In general, encapsulation efficiency increased as oil load increased. Similarly encapsulation efficiency was found to increase when concentration of polymer (κ-carrageenan-chitosan, gelatin A-κ-carrageenan, sodium carboxy methyl cellulose-gelatin A) or cross-linker (glutaraldehyde, genipin, tannic acid) increased. At higher polymer concentration, the availability of the polymer was more to encapsulate oil droplets and thereby efficiency increased. Higher crosslinker concentration (glutaraldehyde /tannic acid/ or genipin) increased the cross-linking which improved the oil retention capacity.

3.2.3 Oil release studies

Oil release studies of encapsulated oil can also be carried out by using UV–visible spectrophotometer. A known quantity of microcapsules is placed into a known volume of 0.1% Tween 80 surfactant solution. The microcapsule-Tween 80 mixture is shaken from time to time and the temperature throughout is maintained at 30◦C (room temperature). An aliquot sample of known volume (5 ml) is removed at appropriate time intervals, filtered

and assayed spectrophotometrically at 254 nm for the determination of cumulative amount of oil release up to a certain time t. Each determination is carried out in triplicate. To maintain a constant volume, 5 ml of 0.1% Tween 80 solution is returned to the container. A typical oil release study pattern is given as below.

The faster or slower release rate of oil by the microcapsules prepared by varying different conditions could be explained on the basis of either decrease or increase in the thickness and compactness of microcapsule wall formed due to addition of polymer and crosslinker. Higher oil loading decreased the thickness of microcapsule wall whereas higher concentration of polymer increased the thickness of the wall. This in turn would control the release rate. Microcapsules wall became more compact as the amount of cross-linker increased. This led to decrease in the release rate. Effects of various crosslinking agents (glutaraldehyde, genipin, tannic acid) on NSO encapsulated microcapsules of carrageenan-chitosan complex were compared and glutaraldehyde was found to be the most effective crosslinker followed by genipin and tannic acid.

3.3 Characterization

Characterization of encapsulated NSO microcapsules and granules/beads are mainly carried out by using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC) studies. FTIR spectrum of NSO shows the absorption bands at 1745.90, 1463.04 and 1163.85 cm^{-1} which are due to carbonyl stretching, CH_2 asymmetric deformation and C-C stretching vibration (Fig.6). The position of these bands in the physical mixture as well as in the NSO loaded microcapsules/beads/matrices remained unaltered (not shown in figure) indicating the absence of any significant interaction between NSO and the polymer complexes or polymer mixtures used for encapsulation.

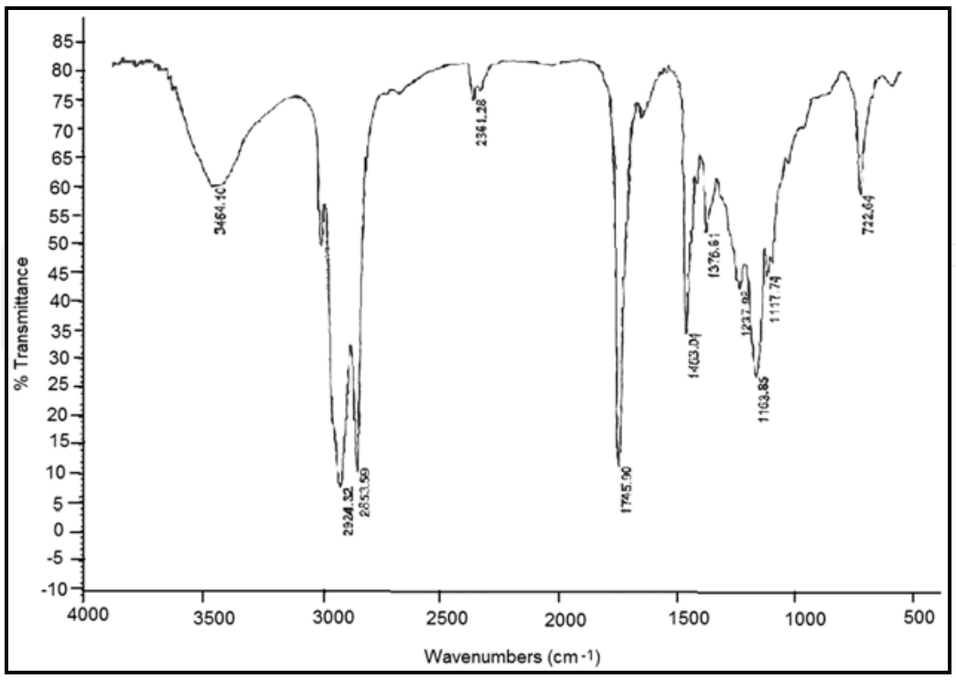


Fig. 6. Pattern of FTIR spectrum of NSO

The DSC thermogram of NSO shows an endothermic peak at around 220◦C (Fig.7). Hardly, any remarkable difference in the thermograms of the NSO loaded microcapsules and physical mixture of complex polymer and NSO was observed (not shown in figure). In the thermograms, the endothermic peak due to NSO appeared almost in the similar position. Thus both FTIR and DSC study reveal no interaction between polymers and NSO.

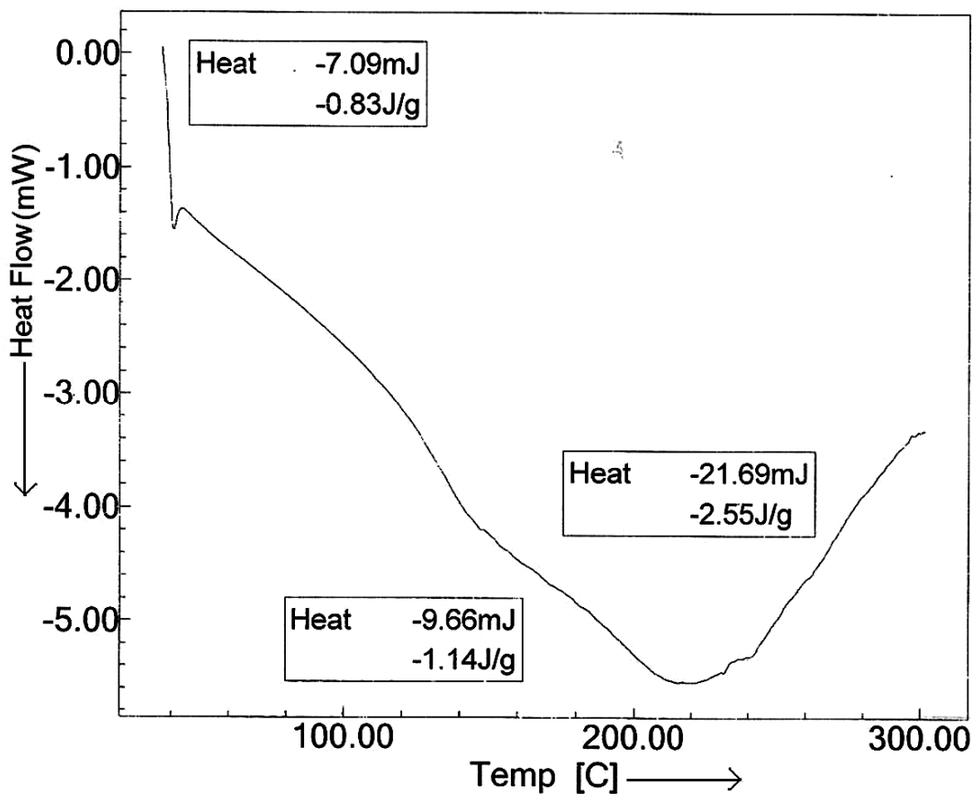


Fig. 7. Endothermic heat absorption pattern of NSO in DSC.

Extent of weight loss due to temperature and temperature of decomposition at different weight loss of NSO as observed in a thermogravimetric analyzer instrument are shown in the Fig.8 and Table 2 below-

Temperature of decomposition (T_D) (^{0}C) at different weight loss (%)							Residue (%) at 500 (^{0}C)
20	30	40	50	60	70	80	
315	340	358	375	395	415	430	7

Table 2. Temperature of decomposition at different weight loss (%) of NSO

Thermal stability of chitosan-carrageenan microcapsules (as suggested by thermogravimetric analysis) was found to improve with the increase in the concentration of crosslinker. In case of various crosslinkers, glutaraldehyde crosslinked samples showed highest thermal stability followed by genipin and tannic acid (Devi & Maji, 2009b).

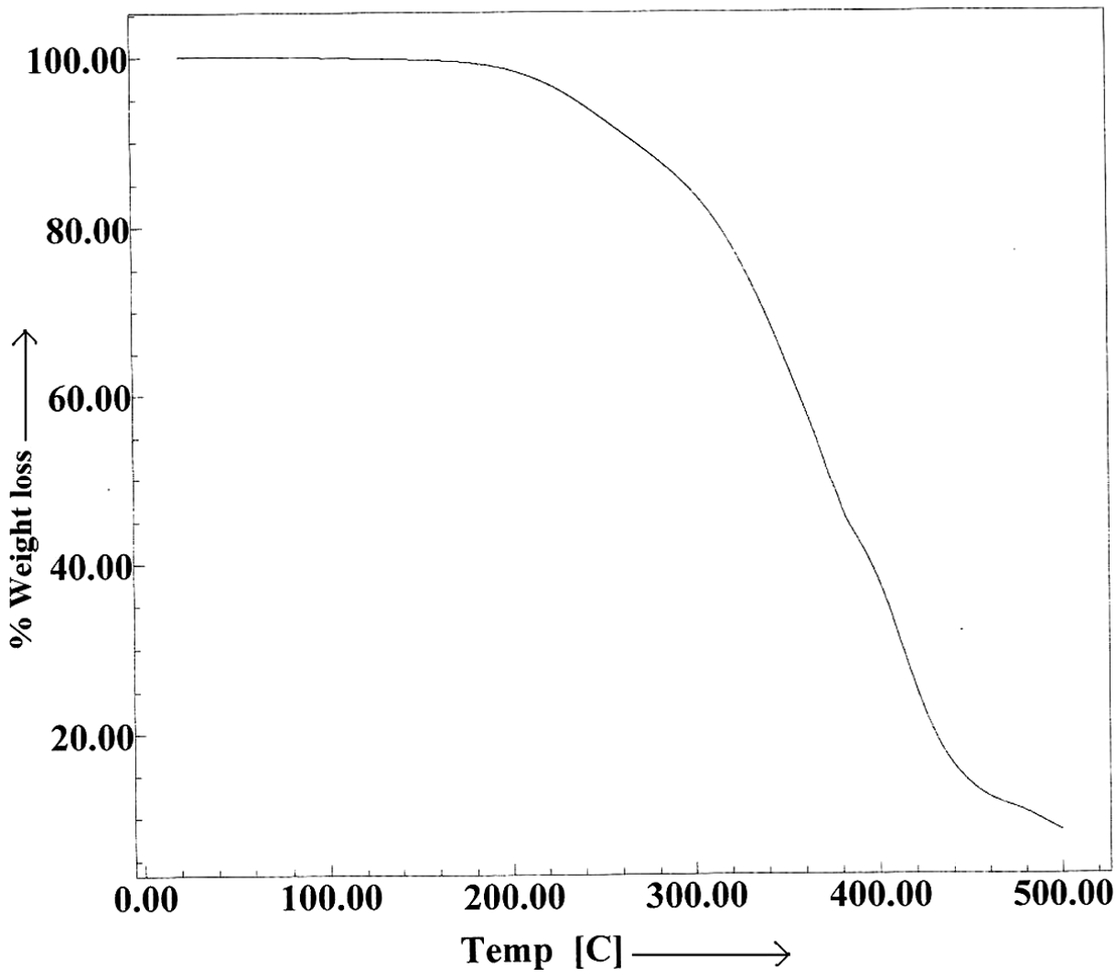


Fig. 8. Weight loss pattern of NSO in TGA

Formation of spherical beads with smooth surface and having particle sizes ranging from 1.21 to 1.40 mm in diameter was reported for sodium alginate beads (Kulkarni et al., 2000). The SEM photographs of capsules of sodium alginate matrix, crosslinked by glutaraldehyde and coated with natural rubber were shaped like an egg (Riyajan & Sakdapipanich, 2009). The mean particle size was 0.14 mm as measured by using both optical microscope and SEM. The diameter of capsules increased drastically from 0.14 mm to be 3 mm and had a smoother surface after coating with natural rubber.

SEM study showed the formation of spherical microcapsules having free flowing to bursting look depending on the extent of oil loading (Devi & Maji, 2009, 2010). SEM micrographs of microcapsules prepared at higher oil loading appeared oily, agglomerated and having a bursting look compared to those of microcapsules prepared at lower oil loading. The sizes of microcapsules increased with the increase of the concentration of the polymer. There was a clear distinction between the polymers with (lump of polymer) or without NSO encapsulation (free flowing spherical microcapsules). Fig.9 below shows the SEM photographs of formed NSO loaded microcapsules of carrageenan-gelatin complex and the neat complex.

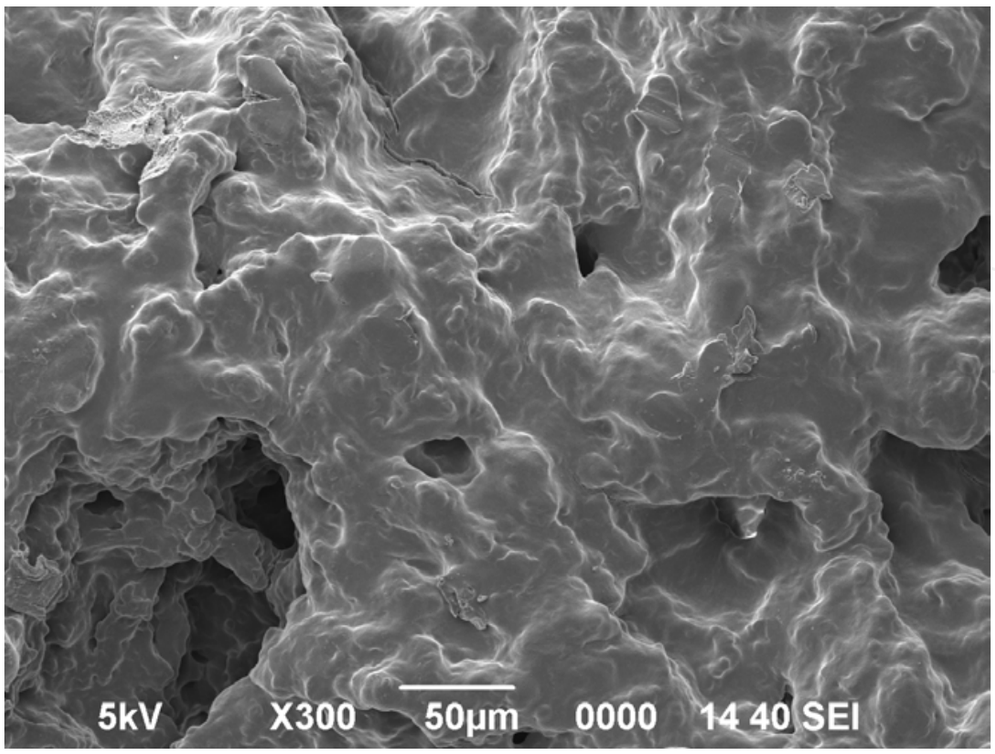


Fig. 9(a). Scanning electron micrograph of polymer complex without NSO encapsulation

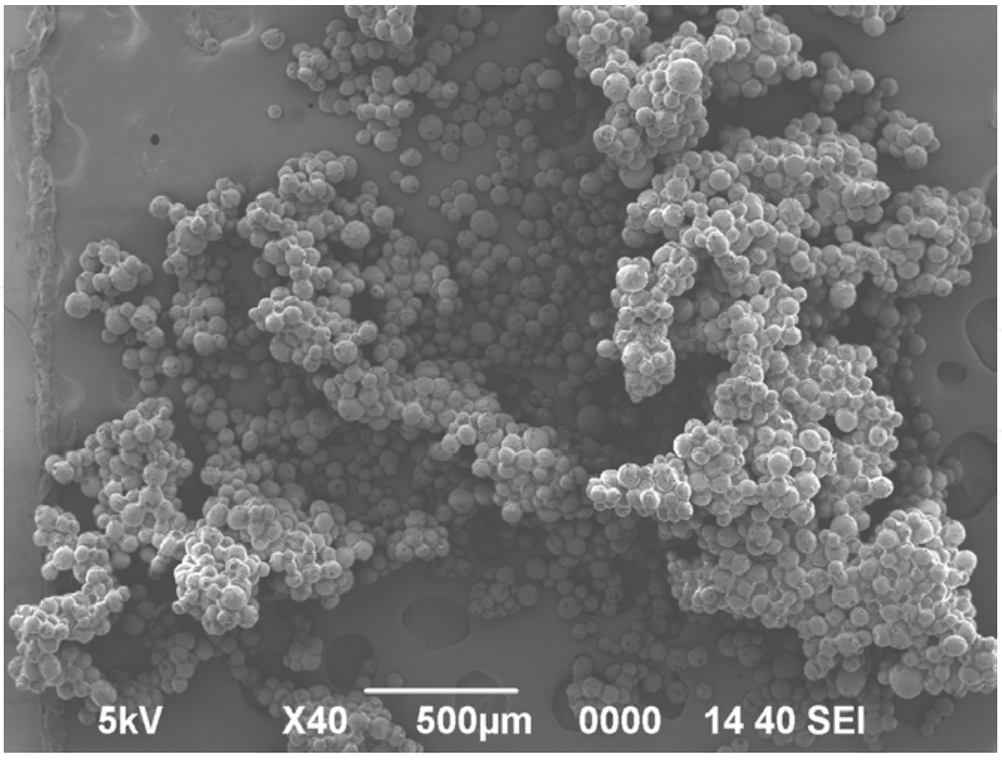


Fig. 9(b). Scanning electron micrograph of polymeric NSO controlled release microcapsules

In a study, the chemical structure of the controlled release neem Aza-A capsule wall (Riyajan & Sakdapipanich, 2010) was evaluated through X-ray diffraction. In addition, the swelling behaviour of the capsules and their thermal stability were investigated. The strength of the capsule wall depended on the polymer (PVA) in the matrix and the crosslinking density. Scanning electron microscopy, electron probe microanalysis and atomic force microscopy data indicated that the structure of the bead walls was rough and nonporous.

4. Commercialization, future direction and conclusion

4.1 Commercialization of neem and controlled release neem pesticides

Over 300 species of insects are stated to be controlled by neem all over the world. In India alone 106 species belonging to 10 orders of class Insecta, 12 species of nematodes and a fungus species are reportedly controlled by neem (Singh & Kafaria, 1991). The bio-efficacy results have attracted the attention of the pesticide industry in India and abroad. Nearly five dozen products are either marketed or are awaiting commercialization in India. Most of the products are either oil based or based on various extractives (Table 3) (Parmar & Ketkar, 1996).

4.2 Future direction of research

While the use of high yield-short duration hybrid varieties of seeds, fertilizers and irrigation increased agricultural productivity, pesticides complemented it by protecting the plants. The focus has now shifted towards sustainable agriculture in the light of harm caused by indiscriminate use of fertilizers and pesticides, laying stress on organic farming and use of biopesticides. Efforts in this direction have led the use of Bt- and plant-derived biopesticides. The later category has provided pyrethrum, nicotinoids, rotenoids for use and rediscovering the applications of neem (*Azadirachta indica*) based formulations. Alternative strategies for plant protection emerging for 21st century comprise of introduction of transgenic varieties of plants resistant to pesticides/pests. They appear to be members of biopesticide consortium, constituting an integral part of integrated pest management (IPM). Efforts are underway to understand the mechanism of action of biopesticides, so that light is thrown at molecular events for designing biopesticides appropriate for different pests. Concerted efforts are hoped to remove limitations in biopesticides raw material availability, potency variations, standardization of extraction methods, quality control, shelf life and improved bioefficacy. Biopesticides appear target specific, do not leave residues on food and feed by virtue of their biodegradability, economical and eco-friendly and hence are hoped to provide cleaner and safer eco-system (Mendki et al., 1994). Thus, the direction for future research of neem seed oil as a greener alternative for future generation technology as well as commercialization of neem products is the most important issue.

Jayaraj et al. (Jayaraj et al. 1996) reported that the future research should aim at the following-

i. Research on genetic divergence in neem and their phenotypic stability
ii. Research on maximizing the viability, vigour and storability of neem seed
iii. Selection of varieties with higher oil content and high yielding capacity
iv. Research on definitions of quality control and general biological properties
v. Methods to check the photodegrability of neem formulations
vi. Enhancement of efficacy of insect repellents and antifeedents of botanical origin by improved botanicals, better timing and improved application methods

Product	Active ingredient(s)	Activity claimed	Manufacturer
Amitul mosquito oil	Oil	Against mosquitoes	M/s Amitul Agrochem Pvt.Ltd., P.O.New Sheopuri Colony, Gorakhpur 273001, U.P.
Azadirachti EC	Oil (300ppm aza)	Pesticidal	M/s Sunida Exports, Mumbai 400 042
Bioneem	Oil (300ppm aza)	Pesticidal	M/s Ajay Bio-tech Ltd., Pune 411 040, Maharashtra State
Godrej Achook	Oil	Antifeedent, repellent,male sterility, molt/chitin inhibitor,ovicidal etc	M/s Bahar Agrochem and Feed Pvt. Ltd. E-24, Lote Parshuram, Firozshah Nagar, Mumbai 400 079
Jaineem	Oil	Pesticidal	M/s Jai Chemicals, Faridabad 121 001, Haryana
Limonool	Oil (300ppm aza)	Pesticidal	M/s Sri Bio-Multi-Tech (P) Ltd., Banglore, 560 032, Karnataka
Margosom	Oil (300ppm aza)	Pesticidal	M/s Som Phytopharma(India) Ltd., Hyderabad 500 016, A.P.
Moskit	Oil	Mosquito repellent	M/s Investment and commercialization enterprises, Bombay- 400 022.
Neemolin	Oil (300ppm aza)	Pesticidal	M/s Khatan Janker Ltd. Mumbai 400 001
Nimbitor (ZA-199)	Oil (300ppm aza)	Pesticidal	M/s Zandu Pharmaceutical Works Ltd., Mumbai 400 025
Neem oil emulsion	Oil	Pesticidal	M/s Sio Agro Research Laboratories, Dhiraj Apartments, Mulund (West), Mumbai 400 080
Nimbecidine	Oil (300ppm aza)	Antifeedent, repellent, metamorphosis disruptor, synergist	M/s T. Stanes and Co. Ltd., Coimbatore 641 018, Tamilnadu.
Neemrich	Oil (300ppm aza)	Pesticidal	M/s West Coast Herbochem Pvt. Ltd., Banglore 560 022
TRIC	Oil	Against household pests	M/s Amitul Agrochem Pvt.Ltd., P.O.New Sheopuri Colony, Gorakhpur 273001, U.P.

Table 3. Some of the commercial neem oil based pesticidal products in India including those in the pipelines

vii. Use of tissue culture and biotechnological approaches to obtain innumerable neem trees with high yield in shorter time.
viii. Making neem cultivation mandatory in all social forestry and other nutrition gardening programmes, and
ix. Further research on aspects of manufacturing ready-to-use and effective formulations can be thought of and conducted.

Nanotechnology has the potential to revolutionize the agricultural industry with new tools for the molecular treatment of diseases, rapid detection of deficiency of nutrients etc. Smart sensors and smart delivery systems will help the agricultural industry combat viruses and other crop pathogens. In the near future nanostructured catalysts will be available which will increase the efficiency of pesticides and herbicides, allowing lower doses to be used. Nanotechnology will also protect the environment indirectly through the use of alternative (renewable) energy supplies, and filters or catalysts to reduce pollution and clean-up existing pollutants. Researchers are engaged to develop technologies to make pesticide delivery systems which can respond to environmental changes. Their aim is to tailor these products in such a way that they will release their cargo in a controlled manner in response to different signals e.g. magnetic fields, heat, ultrasound, moisture etc.

5. Conclusion

Neem products are finding favour particularly because of their environment friendliness. In the field of pest control, they are revolutionizing the total concept by shifting emphasis from instant pest mortality to gradual, nearly invisible, pest extinction. The use of neem materials for medicinal, toiletries and other purposes has also a large consumer acceptability and almost no report regarding any undesirable effect could be traced (Parmar & Ketkar, 1996). In industrialized countries, it is hard to imagine botanicals playing a greater role in future than at present, except organic food production (Isman, 2006). Organic production is estimated to be growing by 8% to 15% per annum in Europe and in North America (National research council, 2000) and it is in those market places that botanical pesticides face the fewest competitors. In conventional agriculture, botanicals face tremendous competition from the newest generation of 'reduced risk' synthetic insecticides such as the neonicotinoids.

Once a decision is taken to produce and use such products on a large scale, requisite research and development effort will have to be put in to make the neem based technology durable and practically sustainable. Stabilization of neem products against photo, thermal and microbial degradation may be of interest where persistence under such conditions is desired. Easy handling and transportation of the products are also important for industrial scale production and commercialization. In this context the microencapsulation and controlled release technology posses great potential for further research and commercialization of neem oil as a suitable and superior pesticide of the next generation. Extensive and systematic research work is needed to marshal the various facts about it and to speed the realization of its potential for application to plants, soil and water as a greener alternative for future generation technology.

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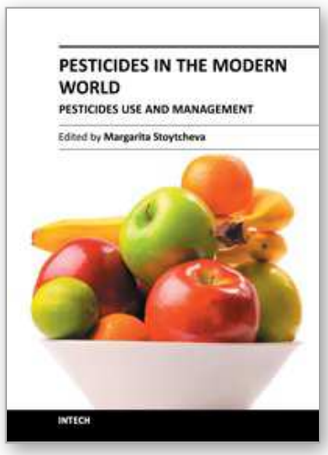

Pesticides in the Modern World - Pesticides Use and Management
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This book brings together issues on pesticides and biopesticides use with the related subjects of pesticides management and sustainable development. It contains 24 chapters organized in three sections. The first book section supplies an overview on the current use of pesticides, on the regulatory status, on the levels of contamination, on the pesticides management options, and on some techniques of pesticides application, reporting data collected from all over the world. Second section is devoted to the advances in the evolving field of biopesticides, providing actual information on the regulation of the plant protection products from natural origin in the European Union. It reports data associated with the application of neem pesticides, wood pyrolysis liquids and bacillus-based products. The third book section covers various aspects of pesticides management practices in concert with pesticides degradation and contaminated sites remediation technologies, supporting the environmental sustainability.

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