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REVIEWS

Contribution of Nanobiotechnology in Indian Agriculture: Future Prospects

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Abstract | Agriculture and livestock industries constitute the backbone of Indian economy. Nanobiotechnology is expected to make a seminal contribution in these areas in coming years. Two major constraints in these areas of research are as follows- (a) interventions should be low cost, preferably cheaper than the currently used products or technologies, so that farmers can afford and (b) they should be bio- and ecologically safe. Till date, significant research has not been done in India on nanobiotechnology with emphasis on Indian Agriculture. Our laboratory is working with the aforementioned objectives over the past few years. Results of our initiative towards this direction are presented. This review includes a short note on the future prospects of nanobiotechnology and its implications on various emerging sectors of Indian Agriculture.

Keywords: *Agriculture, Veterinary Science, Nanobiotechnology, Livestock, Poultry, Sericulture, Insecticide, Fungicide.*

1 Introduction

As per Food and Agriculture Organization (FAO) statistics, India was the fifth largest producer of over 80% agricultural food items in 2010[1] and livestock & poultry meat in 2011.[2] During the green revolution era, introduction of high yielding cereal crops served as a magic bullet.[3,4] Similar results were obtained in the white revolution era with introduction of high milk producing livestocks.[5] Nanotechnology in combination with Biotechnology offers a number of such exciting possibilities in the area of agricultural production, storage of foodgrains and designing better health conditions of livestock & poultry animals. For example, 25–30% of the total food grains produced in India is damaged due to pest and disease attacks.[6] Similarly, mortality and morbidity of live animals cause huge loss in productivity of livestocks.[7]

In contrast to products and technology generated for medicine industry, process of generation of products for Agriculture and Veterinary industry has to overcome certain serious limitations. For example, Indian consumers are often ready to pay exorbitant amount of money when it comes to saving the lives of their own as well as their kith and kin. This is not true for Agricultural crops and Veterinary animals. Farmers will never pay such a high price if their crops are destroyed due to natural calamities, insects and plant diseases or their livestock die due to diseases. In the times of epidemics (e.g. bird flu in recent times), livestock farmers are ready to pay some amount of money if the disease is curable, but often they resort to culling of their entire livestock if the disease is not curable. This is simply because high priced interventions are often more costly than replacement of entire livestock. In some cases of animals where emotional attachments (e.g. pets) are associated economically better off section of Indian society are ready to pay higher amount of money, but the size of such industry is very small in India to be commercially viable. Second, serious limitation in the growth of veterinary medicine and agrochemical industry is the consumers' concern about the biological and ecological safety. A glaring example in recent years is the introduction of genetically modified (GM) crops and genetically modified organisms (GMOs) in several countries around the globe. As agricultural and livestock products are directly consumed by the people, therefore the concerns often assume serious socio-political dimensions. Interestingly enough, introduction of novel

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ingredients like enzymes produced in recombinant living organisms (e.g. bacteria, fungi etc.) in life saving drugs do not cause such stir in the society.

Section 2.1, 2.2 and 2.3 would discuss about results from our laboratory pertaining to Indian sericulture, poultry, and agrochemical sectors respectively.[8–32] Section 3 will deal with my opinion about the future prospects of agro-nanobiotechno logy research.

Sericulture: Sericulture or silk farming is both an art and science of rearing silkworm for producing raw silk. Even today silk plays an important role for fashion industry worldwide. Being a rural industry, silk production and weaving (mostly manually) are done by relatively poorer section of India. India is the second largest producer of silk after China. In India, silk farming is not only a tradition, but also an integral part living of involving huge labor and creative ingenuity of rural artisans. Currently Indian domestic demand for silk is 25,000 metric tons, whereas the current production figures are 18,475 metric tons, rest being imported from China.

Poultry: Poultry refers to rearing domesticated birds for eggs and meats like that of chicken, quails, turkeys, fowl, domestic ducks and domestic geese, pigeons, doves or birds considered to be game pheasants. Poultry industries produce second largest amount of meat after pork worldwide.

Agrochemical: Agro-chemical is the hybrid term of agricultural chemical. This generic term includes various chemical products used in commercial agriculture worldwide. For example, broad range pesticides including insecticides (kills harmful insects), herbicides (kills weeds) and fungicides (kills fungus) fall under the category of agro-chemicals. Synthetic fertilizers, hormones, chemical plant growth agents and concentrated forms of raw animal manure also are classified as agrochemicals.

2.1 *Sericulture sector*

Silkworm farmers loose 15–20% of their larvae due to diseases.[33] In some seasons, the losses are so high that the farmers have to resort to complete overhaul of their larvae rearing set up. Different commercial strains of *Bombyx mori* silkworm (e.g. nistari) are usually fed on cultivated mulberry (*Morus alba*) leaves and maintained at a temperature of 22–25°C with proper ventilation up to cocoon stage. *B. mori* nuclear polyhedrosis virus (BmNPV) usually affects full grown early fifth instar larvae and the infection occurs through oral route. This 100% deadly viral disease kills larvae within 24–30 hours of infection. This disease is popularly known as 'Grasserie'. There are no currently available antidotes against this disease in the market. Intact BmNPV virus particles can be collected from artificially infected silkworm larvae. The disease develops when few microliters of the virus solution are injected in healthy silkworm by microinjection and thus could serve as an ideal model system.

Seven microliters of ethanolic solution containing 10^5 BmNPV particles were injected in fifth instar of nistari variety of silkworm larvae. In control experiments, only ethanol solution was found to have no deleterious effect. After six hours, one of the eight following types of amorphous nanosilica (ANS; 7 μls of ethanolic solutions of ANS; 7 μg/μl) was injected. AL60101, AL60102, AL60103, AL60104, AL60106, AL60108, AL60109, and AL60110 were tested during the study. The details of the AL-series nanosilica are as follows: (1) AL60101: 20–50 nm average particle size (APS), hydrophilic; (2) AL60102: 20–50 nm APS, lipophilic (Fig. 1); (3) AL60103: 20–50 nm APS, hydrophobic; (4) AL60104: 10–20 nm APS, hydrophilic; (5) AL60106: 10–20 nm APS, lipophilic; (6) AL60108: 10–20 nm APS, hydrophobic; (7) AL60109: 20–40 nm APS; lipophilic; and (8) AL60110: 20–40 nm APS; hydrophobic.

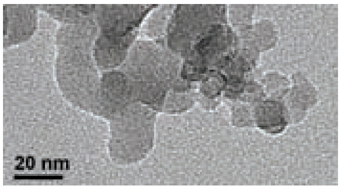

Figure 1: Transmission electron micrograph of AL60102 (A. Rahman and A. Goswami, unpublished data).

In BmNPV-infected larvae, AL60102 had the maximal effect followed by AL60106 and others. 81 ± 3% (n = 140) of the total number of larvae survived after 24 h following a single dose of AL60102, while all untreated virus-infected larvae (n = 40) died within 24–30 h. In control experiment, no mortality was observed in healthy larvae (n = 35) following a single dose of 7 μl (70% ethanol) injection. A larger number (n = 140) of larvae injected with AL60102 led to 94.13 ± 5.24% survival after 30 h. By 48 h, the survival percentage reduced to 68.33 ± 7.63%, and by 96 h (time for cocoon formation); it went down further to 9.16 ± 1.44%. In the next experiment, BmNPV infected larvae (n = 225) were injected with 7 μl solution containing 10^5 BmNPV (100°C heat inactivated) plus AL60102. 98.3 ± 0.88% of the control larvae (n = 100) completed normal life cycle. The rate of survival of live BmNPV infected larvae and the larvae injected with 100°C heat inactivated BmNPV prior to challenge by live virus was 0%. 21.6 ± 2.08% of the larvae injected with heat inactivated BmNPV plus AL60102 survived up to the cocoon stage and they laid normal eggs and produced adult moths like that of control. Although this was not a complete protection by any chance, but to the best of our knowledge this was the first report of its kind which could possibly lead to a field level interventions in future.[27]

In subsequent experiments, we studied the mode of action of the lipophilic ANS particles on the viral structure. We assumed that AL60102 acts first physically with supramolecular forces on the viral polyhedral surface which come into play due to excessive free surface energy of the AL60102. Capping layer like lipophilic moieties present on the surface try to stabilize the inherent instability of AL60102. Therefore, lipophilic capping layer interacts with environment and redistribute the high amount of free energy either as high energy in low entropy locations and thereby AL60102 surface then serves as activation site. We found that AL60102 disrupts the 3-D structure of the viral polyhedron into a much thinner and narrower one resembling 2-D structure of the virus (Fig. 2). If we consider that viral polyhedral surface as an organic polymer matrix, then we found following three types of physical interactions are important behind the viral neutralizing power of the AL60102- (i) AL60102-polymer matrix (A-PM), (ii) Polymer matrix-Polymer matrix (PM-PM)

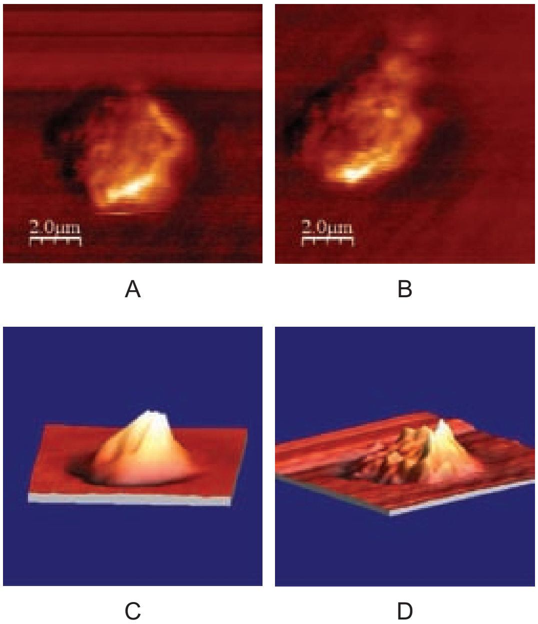

Figure 2: Confocal micrographs and reconstruction of the 3-D images from the images obtained from the microscope. Panel A: Pristine BmNPV polyhedron; Panel B: BmNPV polyhedron treated with AL60102; Panel C: Pristine BmNPV surface; Panel D: BmNPV polyhedron surface treated with AL60102 (Rahman A. and Goswami, A. unpublished data).

and (iii) AL60102- AL60102 interactions. We found competition amongst these three kinds of forces for stabilizing excessive surface free energy of AL60102. It can also be concluded that due to high surface free energy of AL60102, AL60102 acts as activation site for severe distortion of the contour of the viral polyhedral surface resulting into the death of the viruses inside the silkworm haemolymph.[20]

Lipophilic AL60102 due to its inherent affinity for lipids might absorb body lipids of silkworm larvae. Biochemical studies show that AL60102 could nullify the host lipid increase following invasion of the BmNPV.[27] At present it is far from clear which class of lipids or which lipid binds to AL60102. Investigations on the molecular events underlying these observations are in progress.

2.2 *Poultry sector*

Indian poultry industry is the fastest growing sector in the Agriculture market. The growth of agricultural crop production is 1.5–2.0% per annum whereas that of chicken eggs and broilers is 8–10% per annum. As a result, India now is the 5th largest producer of eggs and broilers worldwide. A significant paradigm shift in Indian poultry industry occurred in the last four decades due to introduction of sizeable investments by the private companies (with minimal government interventions), improvement in indigenous breeding of high value breeds, hatching, rearing and farm produce processing methodologies with complementary health support from veterinary sectors. The per capita consumption of eggs and poultry meat has increased enormously throughout India in the last two decades. For example, the per capita consumption of Eastern and Central Indian regions (20% of egg production) is 18 eggs and 0.13 kg of broiler meat and that of Southern states (45% of egg production) is 57 eggs and 0.5 kg of broiler meat. At present, total number of eggs and broilers produced per year is estimated to be higher than 37 billion and 895 million respectively. The country produces more than 735,000 tons of poultry meat.[6]

Protozoan parasite, e.g., *Plasmodium gallinaceum* is the major cause of malaria in poultry chickens. Young birds are more susceptible than the older birds. Virulent strains of *P. gallinaceum* cause mortality in birds. Non-virulent strains of the parasite cause morbidity in animals. Following are the major modes of transmission of the disease in poultry farms- mosquito bites, blood transfer during mass scale vaccination of chickens, caponization and injection. Signs of morbidity due to chicken malaria include depression, somnolence, off feed, muscular incoordination, anemia, increased thirst, reduced mating and respiratory distress. Vomiting, decreased egg production, egg weight & hatchability, and green diarrhea may also be seen. In order to combat chicken malaria in poultry farms administration of anti malarial drops containing plasmochin, quinine hydrochloride and pyrimethamine and their combinations are regularly practiced.[7] Farmers usually go for early sale of malaria affected birds and eggs produced by them, often, at low prices. Consumers cannot read symptoms due to lack of awareness about this disease. Indian livestock industry suffers from a number of other parasitic diseases.[19,21] Nanobiotechnology can offer a set of drug based interventions against a large number of common diseases which affect poultry birds. We demonstrated *in vivo* amorphous mesoporous silica (AMS) can be utilized as effective drug against virulent strains of chicken malaria (*P. gallinaceum*).[27]

The reasons for choosing AMS as therapeutic molecule are- (i) International Agency for Research on Cancer (IARC), has not rated amorphous silica dusts as carcinogen contrary to crystalline silica. Detail studies under IARC's supervision, scientists did not find any association of mesothelioma with biogenic amorphous silica fibers.[34]

(ii) Traditionally, naturally occurring mesoporous silica diatomaceous earth (DE) has been used in animal feed and cosmetics for centuries. Therefore, no side effects are expected from the use of mesoporous silica as drug against malaria either on eggs or poultry meat.

Frozen stocks of virulent strain of *P. gallinaceum* obtained from NIMR (National Institute of Malaria Research, New Delhi, India) were injected into 30 day old pure Ven Cobb breed broilers as described in ref. 27. At day 5, when the parasitemia was found to be 4.5 ± 0.2%, physiologically relevant dose of AMS dispersed in absolute alcohol were fed to the chicken twice a day (Fig. 3). In control experiment, no mortality was observed in healthy birds (n = 15) following ethanol administration. We observed 100% untreated malaria infected chicken (n = 15) died within 7–10 days of inoculation whereas AMS-treated chicken were found to be normal. On day 7, in malaria infected untreated chicken (n = 15), the parasitemia were found to be 5.5 ± 1.1%. In all AMS treated malaria infected chicken (n = 15), total parasite burden was found to be reduced by 78 ± 2% (n = 15). Several biochemical components of chicken blood were analyzed. Marked changes in different components level of serum cholesterol in uninfected, malaria infected and AMS treated but malaria treated chicken gave an important clue for studying the mode of action of AMS against the blood stages of the parasite. In uninfected chicken, the amount of total serum cholesterol, serum HDL, serum LDL, serum VLDL and serum triglycerides were 130 ± 4.46 mg/dl, 78 ± 3.01 mg/dl, 43 ± 2.93 mg/dl, 9 ± 1.12 and 31 ± 2.47 mg/dl respectively. In untreated malaria infected chicken there was a marked increase in all the components and the corresponding values were found to be 194 ± 3.12 mg/dl, 98 ± 1.1 mg/dl, 86 ± 2.03 mg/dl, 40 ± 2.13 mg/dl and 125 ± 3.07 mg/dl respectively. Significantly, in AMS treated malaria infected chickens, the values were 131 ± 1.21 mg/dl, 80 ± 1.86 mg/dl, 45 ± 2.08 mg/dl, 10 ± 1.72 mg/dl and 32 ± 5.47 mg/dl respectively. AMS treatment could thus bring back the increased levels of different serum component to near normal level. Therefore, AMS could be used as efficient drug against chicken malaria in future. We are interested to investigate whether nanopores inside the AMS absorb different kinds of serum cholesterol and release them when needed in appropriate environment. If so, AMS could be used as lipophilic drug delivery agents in other model systems.[27]

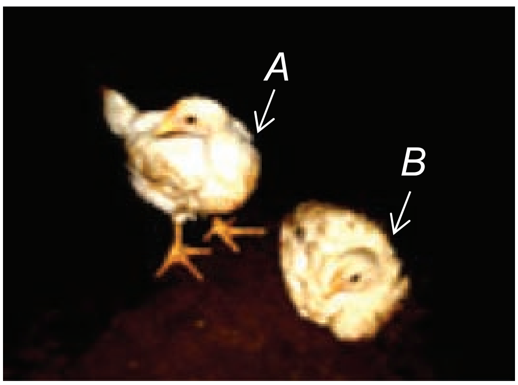

Figure 3: AMS treated malaria infected chicken (A) at day 7 and malaria infected chicken at day 5 after infection (B) (D. Seth and A. Goswami, unpublished data).

2.3 *Agrochemical sector*

Approximately 20–30% of the agricultural produce in India worth of 17 billion US$ is lost every year due to insect infestation and diseases. Indian agrochemical market grew at the rate of 11% per annum in the last decade. Worldwide, India ranks fourth in the agrochemical production after USA, Japan and China. During rice cultivation in India highest amount of agrochemicals (28–30%) is used. Cotton cultivation uses up 20% of the total pesticide produced in India. However, introduction of Bt cotton in the cotton belt has reduced the usage from 63% to 50% of the total volume use. There are 125 technical grade pesticide manufacturers who operate in India and they produce high purity chemical in bulk (generally 200–250 kg per drum) for formulator companies. Today, only 60 technical grade agrochemicals are produced in India and the rest are imported from abroad. Top ten multinational corporations like Bayer Cropscience Ltd, Rallis India Ltd, Syngenta India Ltd, BASF India Ltd etc. control 80% of the total market share. These top ten companies maintain their leadership positions due to good portfolio and introduction of new chemicals at regular intervals through intense R&D done abroad. There are 800 formulator companies in India who convert technical grade agrochemicals, to formulations by adding inert carriers, solvents, surface active agents, deodorants etc for direct field use. These formulations are packed for retail sale and bought by the farmers. There are approximately 1,45,000 distributors who sell these products to farmers. In 2009, India had the capacity to produce 146,000 tons of agrochemicals but could only produce 85,000 tons leading to a low capacity utilization of fifty percent. As the production cost of agrochemical is among the lowest in the world and quality manpower is available, India exports to the tune of 79 billion US$ to countries like USA, EU and African companies. In the year 2008, exports formed 50% of the total industry turnover and had achieved 29% CAGR during the period 2004–2008. Therefore, in order to save the presence of dominance of agrochemical sector in India strong R&D efforts towards development of new highly potent ecologically safe molecules which can act at low dosage are urgently warranted. Nanotechnology derived nanomaterials which could be used as agrochemicals offer new possibilities.[17,35]

2.3.1 Fungicide sector: Fungicides are commonly used to control fungal diseases affecting crops. The market share of different brands of fungicides has almost doubled due to Indian government's sustained support to horticulture industry. Elemental sulfur, most abundant multivalent non-metal on earth exists in different isotopic and allotropic forms. Sulfur is an important component of protein synthesis and at a very high concentration is toxic to microorganism like fungus and bacteria. Elemental sulfur at a very high dose was found to be effective against a large number of plant diseases like brown rot in peaches, powdery mildew diseases in apples, gooseberries, grapes, strawberries, sugar beets, etc., scab in roses as well as against certain smut and rust like fungal diseases of crop plants.[36] Researchers have proposed a number of explanations regarding the mode of action of S^0. After uptake by the pathogens, S^0 gets oxidized to form pentathionic acid or reduced to form H_2S, which are fungitoxic in nature. H_2S promotes oxidation of free –SH attached forms of essential enzymes involved in the mitochondrial metabolic pathways. Excess S^0 also induce non-specific cross-linking of proteins or lipids with free radicals of sulfur.[10]

However, due to its high-volume requirement during application in agricultural fields and decelerated efficacy due to acquired resistance in target pathogens, use of S^0 as a fungicide is no more popular among farmers worldwide. *Aspergillus niger* is an opportunistic, ubiquitous fungus and is a mycotoxigenic food and feed contaminant, especially of certain vegetables, fruits, nuts, beans, and cereals. The fungus is also responsible for invasive aspergillosis in immunocompromised patients and significantly contributes to the increase of morbidity and mortality among neonates. On the other hand, *Fusarium oxysporum* is a soil-borne plant pathogen, responsible for vascular wilts in a wide range of woods, crops, vegetables and ornamentals. Despite the use of a number of commercially available fungicides like Thiram, Zineb, Captan, Benomyl, Thiabendazole, Carbendazim, Thiovit, Thiophanate-methyl and Benomyl, which are commonly, used antifungal agents against *F. oxysporum* effective control over these fungi remains unsatisfactory. Repeated use of synthetic pesticides also induces toxicity in the crop fields.[11] Hence, developments of alternative eco-safe antifungal agents like sulfur nanoparticles (SuNPs) is the need of the hour. We synthesized, characterized two different-sized SuNPs and tested their antifungal efficacy against *A. niger* and *F. oxysporum*. Antifungal property of SuNPs was evaluated in terms of their effect on radial growth, frequency of spore formation, and phospholipid content of the fungal isolates.[23]

During the course of investigation we found that treatment with SuNPs considerably reduced fungal growth and rates of spore germination, which led to the logical demand for quantification of phosphorus in fungal bodies. Phospholipids in germinating spores serve as reservoirs of phosphorus during synthesis of nucleotides, sugars, and other morphogens involved in a number of developmental processes. Change in phospholipid content as mentioned above, amongst *A. niger* and *F. oxysporum* strains insinuates that reduction in content of phospholipids is a highly species specific phenomenon. However, it can be concluded that contact fungitoxicity of sulfur cannot be always correlated to its direct effect on phospholipid content of fungal body.[14] Moreover, the effect of size specific SuNPs on fungal morphogenesis warrants further study in this aspect. Fungicidal effects of nano-hexaconazole (I. Roy and A. Goswami, unpublished data; Fig. 4), and nano-ZnO (P. Patra and A. Goswami, unpublished data; Fig. 5) have shown excellent results recently in our laboratory. DNA microarray data on the effect of nano-sulfur, nano-hexaconazole and nano-ZnO in *A. niger* have been generated[11,15] and currently we are experimentally confirming the results by RT-PCR (S. Roy Choudhury, P. Patra, I. Roy and A. Goswami, unpublished data).

Therefore, all data taken together indicate that nano-sulfur, nano-hexaconazole and nano-ZnO would be preferred alternatives in agrochemical sectors over existing chemical fungicides.[11,14]

2.3.2 Insecticide sector: Insecticides are commonly used to drive away harmful insects from the crop fields or kill insect even in indoor settings. Global generic pesticide market is Rs. 45000 crores. Out of this, the opportunities for Indian Companies are immense; for instance 10% of US$ 20 billion would equal US$ 2 billion market in India, given a favorable climate. India already achieved US$ 500 million milestone.[37] Pesticide use in India has increased 50-fold since 1950.

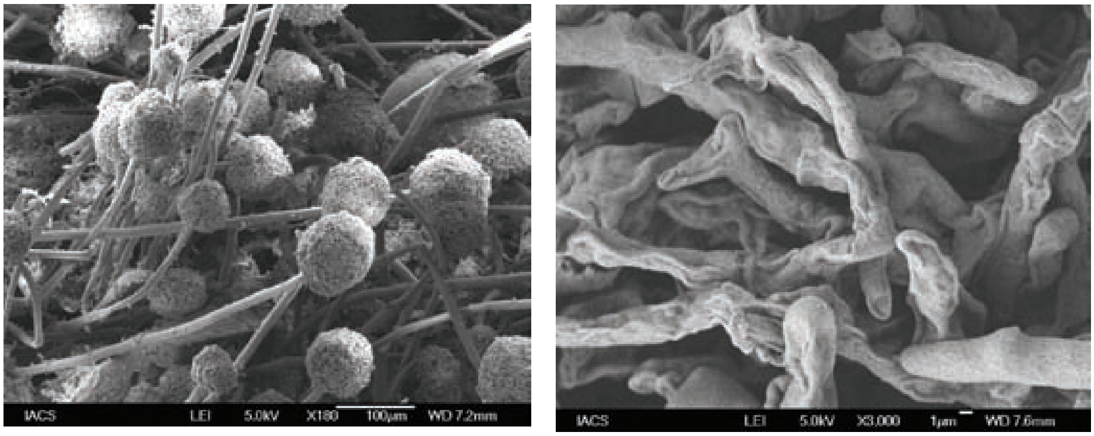

Figure 4: Left panel shows normal *A. niger* conidiophores. Right panel shows the nano-hexaconazole treated *A. niger* condiophore. Nano-hexaconazole kills the fungus by severely inhibiting spore production and altering normal hyphal morphology.

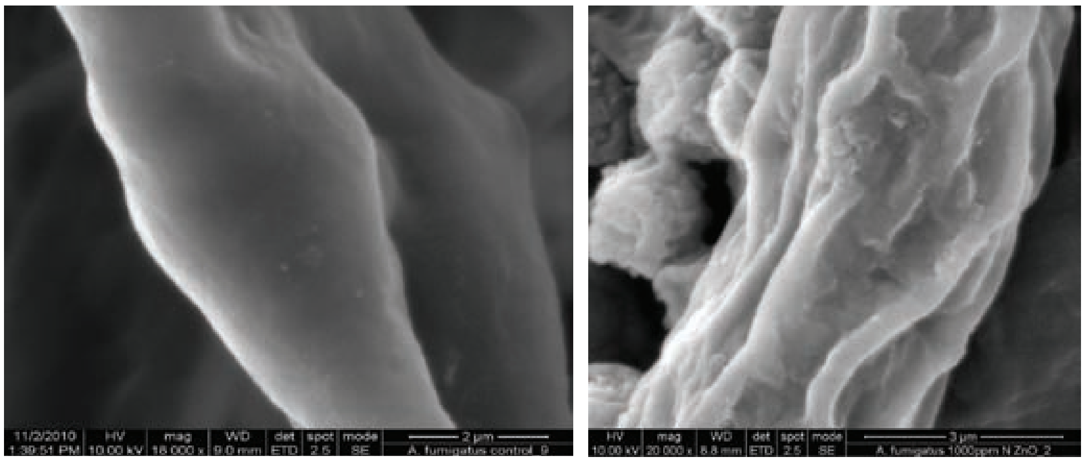

Figure 5: Left panel shows normal *A. niger* hyphae. Right panel shows the nano-ZnO treated *A. niger* hyphae. Due to application of nano-ZnO, the surface of the hyphal structure is distorted leading to death of fungi.

India uses about 90,000 tons of pesticides every year, more than 60% of it on food crops. In India pesticide use is bound to increase in coming years, because we cannot afford 10–20% loss in our production due to insect pests. The losses are higher (30–40%) if post harvest losses are taken into account. Total losses due to insect pests in India exceed Rs. 900 billion US$. Value of Indian Pesticide Industry is estimated at 74 billion US$ for 2015. This excludes exports of Rs. 29 billion US$. Among all the factors which contribute to the loss of grains produced, loss due to store grain pests is the maximum. On average, out of a total 6% loss of food grain in storage structures, about half is due to rodents, and half due to insects and fungi.[38]

Fumigants and residual insecticides are commonly used for protection of stored grains against pest infestation. Unfortunately, this leads to contamination of food with toxic pesticide residues. Moreover, many stored-product insects have become resistant to a variety of grain protectants. Consumer awareness of the consequences of residual toxicity and increasing resistance of insects to storage insecticides has led the researchers to evaluate alternative strategies to protect stored products. One such alternative is the use of Diatomaceous Earths (DEs), composed mainly of amorphous silica and derived from fossilized phytoplankton. Earlier formulations of DE were not widely accepted because of their adverse effects on bulk density (volume : weight ratio) of grains, which is a very important physical property of grain mass. In the last decade, several improved DE formulations have been successfully evaluated against several stored-product pest species.

DE becomes more effective against insects if it possesses high amorphous silica content with uniform size distribution.[24,25,28–32] This cue led us to investigate the entomotoxicity of amorphous surface-functionalized silica nanoparticle (SFSN). We investigated the potential of SFSN in comparison with its bulk counterpart (having a wide size distribution in micrometer range) against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). *S. oryzae* is a very important insect pest damaging stored grains worldwide. It is classified as a primary pest which means that insects are capable of infesting unbroken grain kernels. Larvae of *S. oryzae* develop within the kernel and, therefore, are protected from grain protectant residues on the exterior of the kernel. *S. oryzae* is becoming resistant to phosphine and conventional insecticides such as pyrethroids.[22]

Hydrophilic spherical SFSNs of different size range were synthesized in the laboratory by sol–gel method from aqueous alcohol solution of silicon alkoxide by slightly modifying the Stober process.[39] We also used custom made (15–20 nm), surface functionalized hydrophilic, lipophilic, and hydrophobic SFSN as controls. Experimental results showed that on day 1, 0.5 g kg^{-1} dose was not at all effective on the insects. However, hydrophilic SFSNs (both custom made and modified Stober) showed considerable insecticidal property at 1 g kg^{-1} dose or above. At 0.5 g kg^{-1}, lipophilic SFSN was most effective causing mortality of almost 50% insects on day 2. Application of hydrophilic SFSN at 1 g kg^{-1} could kill more than 80% of the insects. Greater than 90% mortality was obtained with all the SFSNs when dosage rate was 2 g kg^{-1}. Bulk-sized commercially available silica caused only 23% mortality at this dose. After day 4, more than 90% *S. oryzae* died when hydrophilic SFSN (both types) was applied at the dose of 1 g kg^{-1}. 70% insect mortality was found with application of lipophilic and hydrophobic SFSN at this dose. Hydrophilic SFSNs were not very effective at the lowest dose. After 7 days of exposure, 95% and 86% mortality were obtained with hydrophilic and hydrophobic SFSNs at 1 g kg^{-1}, respectively. Nearly 70% of the insects were killed when the rice was treated with lipophilic SFSN at 1 g kg^{-1}. Almost all insects became dead when SFSNs were applied at a dose of 2 g kg^{-1}. Bulk-sized silica caused only 34% insect mortality even at the highest dose. At the end of 2 weeks, nearly 90% of the adults died when hydrophobic and lipophilic SFSN were applied at the rate of 1 g kg^{-1} (in the case of hydrophilic SFSN mortality was 96%). Hydrophobic and lipophilic SFSN could kill 70% of the insects at the lowest dose. Approximately 40% insects were killed when bulk silica was applied at a dose of 2 g kg^{-1}. According to the Tukey's test criteria, we found that custom made hydrophilic SFSN and 20–30 nm modified Stober SFSN were equally effective on *S. oryzae* at 95% level of significance. No new progeny were found in rice treated with SFSN even after 2 months of the treatment.[9,18] Earlier researchers successfully applied nano alumina against two stored grain pests *S. oryzae* and *Rhyzopertha dominica* (F.).[40] However, nano alumina in ground water inhibits the growth of carrot, cabbage, cucumber, corn, and soybean.[41] Our study presented the entomotoxic potential of SFSN which has no adverse effect on plant growth; rather silica enhances structural rigidity and strength of plant (N. Debnath and A. Goswami, unpublished data). Insect mortality due to SFSN treatment was obtained at dose rates almost comparable with those of commercially available DE formulations ranging from 500 to 5,000 mg per kg.[42] SFSN does not affect the looseness and bulk density of grain mass like DE even with the highest dose used in our bioassay. One exciting finding from these experiments is that no fresh insect infestation is found in the SFSN-treated stored rice even after 2 months of treatment. SFSN nanocides can be removed by conventional milling process unlike sprayable formulations of conventional pesticides, leaving residues on the stored grain. Therefore, SFSN has an excellent potential as stored grain as well as seed protecting agent if applied with proper safety measures. This study could lead to opening up newer pathways of using nanomaterial-based technology in Indian pesticide industry. SFSNs have been patented and the technology is now undergoing through the process of pre-commercial evaluation through the BIPP program of Department of Biotechnology, GoI. We have also generated nano-encapsulated complex of a popular but neurotoxic insecticide, Acephate.[16] The material has shown efficacy at much lower dose than the conventional formulations (S. Pradhan and A. Goswami, unpublished data). DNA microarray data on the effect of nanosilica in *Drosophila melanogaster* have been generated and currently we are confirming the results by RT-PCR (N. Debnath and A. Goswami, unpublished data).

In collaboration with NIMR (National Institute of Malaria Research, New Delhi, India), ICMR, we have shown recently that various kinds of nanosilica could effectively control different kinds of mosquito larvae (Figs. 6 and 7).

We have also developed mesoporous silica and mesoporous carbon nanoparticles where agrochemicals including insecticides and fungicides can be loaded and delivered as per the need of the

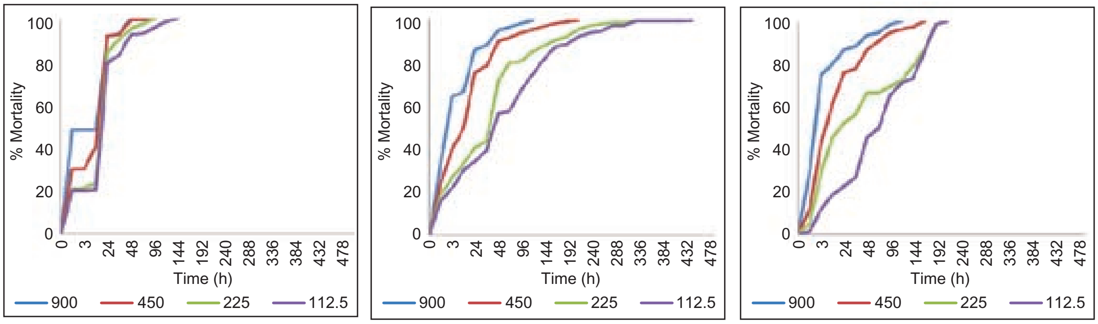

Figure 6: Cumulative mortality of mosquito larvae after exposure to hydrophobic nanosilica at different time intervals. Left, middle and right panels represent effect on *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus* respectively. Data represent means from five replicates, each containing 25 larvae. Colored lines indicate dose in ppm (Goswami, Barik and Raghavendra, unpublished data).

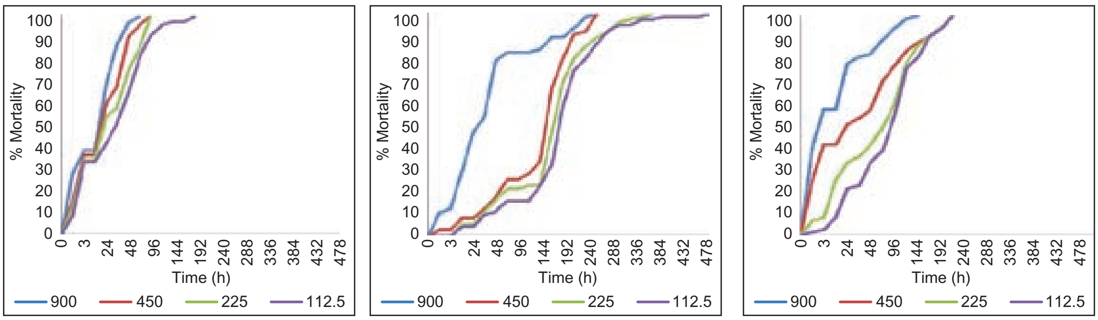

Figure 7: Cumulative mortality of mosquito larvae after exposure to hydrophilic nanosilica at different time intervals. Left, middle and right panels represent effect on *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus* respectively. Data represent means from five replicates, each containing 25 larvae. Colored lines indicate dose in ppm (Goswami, Barik and Raghavendra, unpublished data).

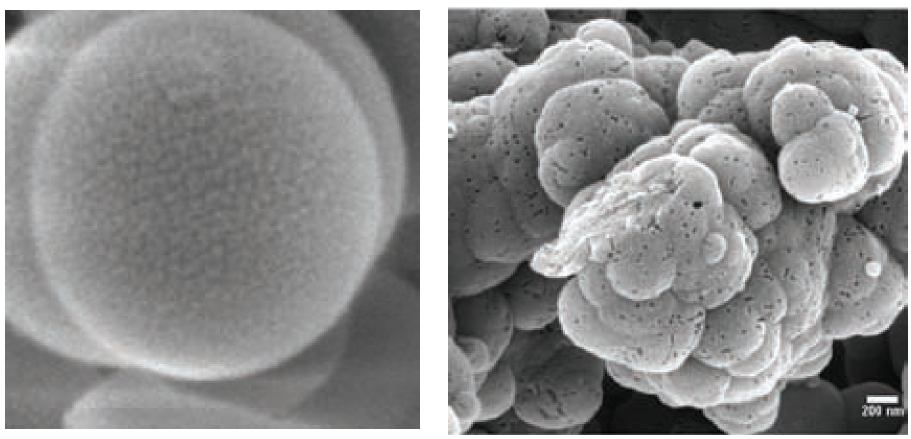

Figure 8: Mesoporous silica (left panel) and carbon (right panel) nanoparticles (S. Mitra and A. Goswami, unpublished data).

system (S. Mitra and A. Goswami, unpublished data; Fig. 8).

3 Future Prospects

In order to study mode of action of nanomaterials in various model systems, we are currently developing a number of specialized nanoparticles. For example, carbon based nanostructures especially carbon quantum dots with their emergent nanolights are attracting enormous attention to the researchers. High photoluminescence property (PL), tunable PL property over a long range of excitation wavelength, high photostability without photobleaching is the major advantages of carbon quantum dots. Contemporary to other heavy metal quantum dots, carbon quantum dots (CQDs) exhibit low toxicity and environmental friendliness; allowing the wide spread biology based applications of CQDs. Therefore developments of CQDs are producing potential future for their use in energy conversion, energy storage, bio-imaging, drug delivery, catalysis, sensors, diagnostics and composites design.

With the available various synthetic methods; microwave assisted pyrolysis, combustion method, laser ablation method, and oxidation by using strong acid are well established methods for the production of CQDs. Interestingly multi-structure nanocarbons can be synthesized by combustion of carbon xerogel with just varying the concentration of nitric acid. Multi-structured nano-carbons include CQDs, nano-cube, nano-fullerenes and carbon nano-leaf. The most fascinating aspect is to synthesize crystalline nano-fullerene starting from an amorphous carbon xerogel at a particular time period of the reaction. Prolonged reaction results in the destruction of crystallinity with aggregation to porous nano-leaf like structure which is introduced into the carbon family for the first time. All these versatile nano structures posses PL property and can be used as a bio-imaging agent with their future promising aspect in agro-chemical delivery and a tracer for intracellular trafficking of the nanomaterials (Fig. 9).[13]

Zinc oxide nano particles are widely known for its antimicrobial property, when the fabricated zinc oxide nano particles are allowed to react chemically with carbon nanoparticles, a nano composite or nanohybrid is produced. Therefore the nanohybrid is now capable of producing good PL property and can be used as a fluorescent antimicrobial agent. The advantages of this sort of chemical grafting are (a) Carbon nanoparticles or CQDs are nontoxic, (b) The whole process is cost effective, and (c) PL property is restored for long time in comparison to other organic fluorophores. However antimicrobial property is less in comparison to native zinc oxide nano particle due to the reduction in effective surface area of zinc oxide.[8]

Gold nanoparticles (GNPs) and silver nanoparticles (AgNPs) are widely known for their biological applications. It is unworthy to mention that GNPs are the safest nanoparticles which are used throughout. Retrieving its nontoxic nature GNPs found their potential application from sensors to catalyst; agrochemical delivery to gene delivery. The benign of alternative routes to synthesize GNPs and AgNPs are still considered to be the challenge in materials research. Now CQDs are known to be a good electron donor as well as good

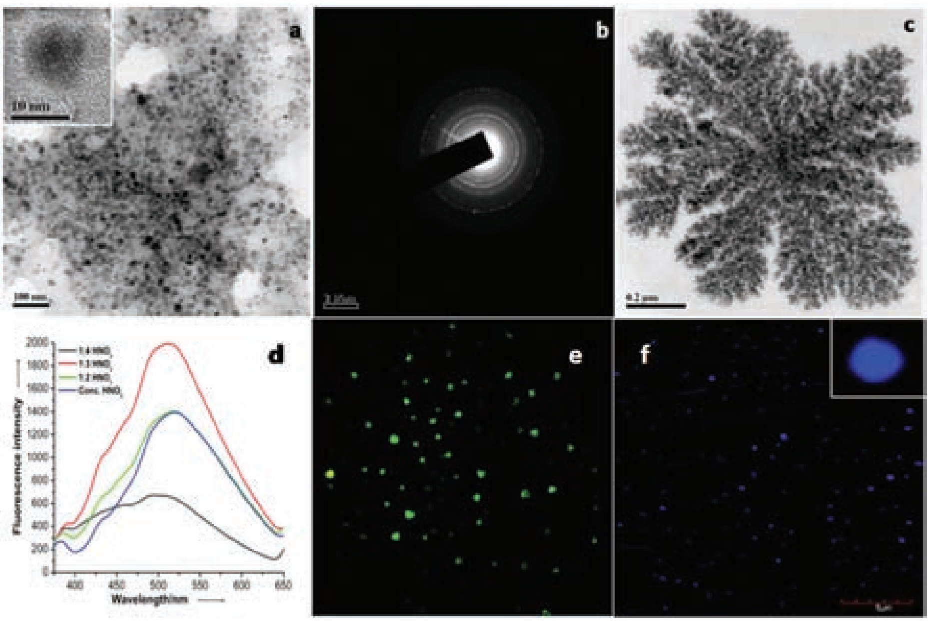

Figure 9: (a) HR-TEM image of nano-fullerene obtained during combustion reaction with 1:3 HNO_3; inset signified its high resolution image, (b) SAED pattern of nano-fullerene justified its crystalline nature, (c) HR-TEM image of carbon nano leaf after prolonged reaction, (d) PL spectra of the product at different reaction conditions, (e) Fluorescence microscopic image of nano-fullerene incubated *S. aureus* (pathogenic strain which affects veterinary animals and humans) at 350 nm excitation wavelength, (f) Confocal microscopic image of nano-fullerene incubated *S. aureus* at 400 nm excitation wavelength; inset signified single bacterial cell.

electron acceptor; hence CQDs are being used in catalyst-photocatalyst design. In search of new CQDs based catalyst design we are successful in synthesizing matrix embedded carbon quantum dots (MCQDs) by a simple microwave assisted fabrication. Dispersion of very small sized quantum dots over the matrix allows its larger surface to be used for catalysis and CQDs reward the PL property of MCQDs. MCQDs are successfully used for the production of GNPs and AgNPs (Fig. 9). In justification of a mechanism for the formation of GNPs and AgNPs we speculate that the positively charged metal ions are attracted to the surface of the matrix through the interaction with the carbonyl groups engrafted on to the surface of MCQDs and bring them to a close proximity. Then the CQDs being good electron donor and acceptor shuttles the electron from reducing agent to metal ions resulting in the formation of corresponding nanoparticles. In a nut shell, CQDs can be used as bioimaging agent, composite design and in catalysis which we have already established, however other challenges are still open to be fulfilled by CQDs or CQD based nanomaterials (Fig. 10).[12]

Throughout the world approximately four hundred companies were active in R&D in products containing nanomaterials. It is estimated that this number is going to touch the 1000 mark in 2016. USA, Japan, China and EU are the major players. Market research analysis done by Business Communications Co. showed that the total market share of nanotechnology companies was 7.6 billion US$ in 2003 and reached to one trillion US$ during 2010.[43] The report also highlighted that full potential of nanotechnology research and product development has not been realized so far. The R&D is still at the rudimentary stage and so there is large scope in this direction.

Nanofood is defined as food produced using nanotechnology tools. This definition does not encompass atomically modified food or food produced by nanomachines. Although scientists have predicted that molecular foods could be created using nanomachines, but the chances of development of such kinds of foods are remote. Nanotechnology is currently used for changing the existing food processing system, increasing the biosafety towards a healthy food culture, enhancing nutritional quality of foods, improving the digestive capability and increased food absorbance towards a healthy nutrient packed food culture.

Helmuth Kaiser Co. has prepared a recent report which estimated that size of the nanofood market has increased from 2.6 billion US$ in 2004 to 7.0 billion US$ in 2006. The figure has reached to 20.4 billion US$ in 2010. The report also points out that Asia with approximately 50% of the world population will be the largest market for nanofood industries (Fig. 11).[44]

Nanofood manufacturers as well as R&D companies were highly conservative in advertising about the details of their research programs.

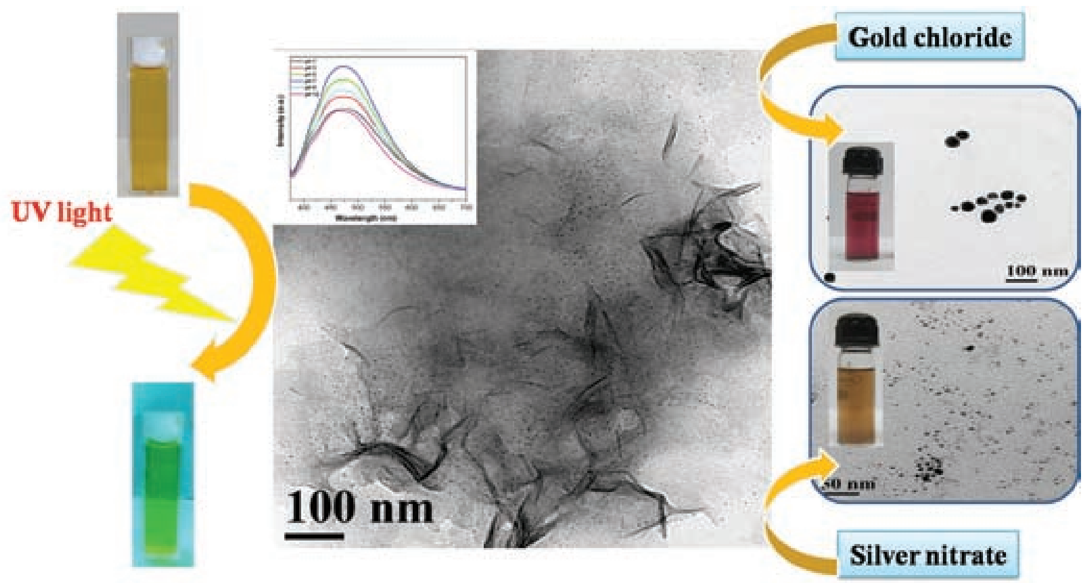

Figure 10: Schematic representation of MCQDs enrouting gold and silver nanoparticles. Originally brown colored MCQDs exhibits bright green color under the exposure of UV radiation, PL spectra is shown in the inset. HR-TEM image of MCQDs justified distribution of carbon quantum dots on to the surface of the matrix; this matrix brings the metal ion to a close proximity towards CQDs which then shuttles electron from reducing agent producing GNPs and AgNPs.

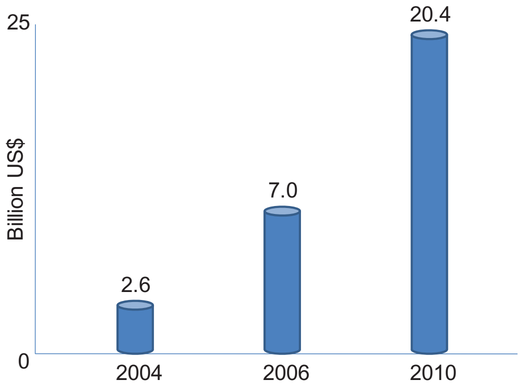

Figure 11: World nanofood market trend analysis.[44]

But now they have opened up such information in the public arena after they have completed the biosafety trials satisfactorily. Some of the companies are aggressively pursuing the research and product development to preserve their market dominance. In this review we will highlight following areas where a considerable amount of progress has been made- smart packaging materials for food, food preservatives on demand & interactive food. Interactive food principle is built on the concept of on-demand food which allows customers to modify food depending on the nutritional and other needs like taste, color enhancers and flavor etc. In these products nanocapsules containing taste, flavor or color enhancers or added nutritional elements (such as vitamins), would remain dormant in the food and only be released when triggered by the consumer.[45] International major food companies like Nestle, Kraft, Heinz and Unilever are poised to market such products within the next five years.

Smart packaging of food products for increasing the shelf life of food products is an urgent need of several food giants & growing faster than predicted and has already shown signs of maturity. Worldwide food packaging market was worth of 1.1 billion US$ in 2006 and reached 3.7 billion US$ in 2010–2011. Extensive market research work done by Frost and Sullivan Co. showed that customers are demanding smart packaging for protecting quality shelf life including freshness, safety and convenience suiting the changing lifestyle all over the world. These are the major reasons driving R&D for novel methods of food packaging.[46]

Nanotechnology has offered new nanomaterials which would be able to repair small holes or tears, respond to changing environmental conditions like that of temperature and moisture and finally alert consumers or sellers if the food is spoiled or contaminated. The future projections of nano-packaging industry look highly promising in the following areas- modification of the permeation properties of foils, increasing the mechanical, thermal, chemical and microbial barrier properties, development of active antimicrobial and antifungal surfaces and developing sensors and signal systems for changes in microbial loads and biochemistry of packaged food items.

There are several examples of recent developments in smart food packaging arena. Kraft Foods Inc. in collaboration with researchers at Rutgers University is developing an electronic tongue which consists of an array of sensors and these sensors can sense the volatile gases emanating from packaged foods. The major advantage of such sensors is that if the food spoils, the color of the sensor strip will change. Kraft Foods Inc. has also established a consortium of 15 universities in EU for producing such interactive foods.[47]

Bayer Polymers Inc. has already developed a packaging film called Durethan KU2-2601, which are lighter, stronger and more heat resistant than those currently available in the market. The added benefit of this film is that it significantly reduces entrance of oxygen and other gases. Company has termed this film as hybrid system and has a large number of impregnated silicate nanoparticles. This film apart from blocking entry of different gases also massively reduces exit of moisture thereby preventing the food from drying out quickly.[48]

Voridan Inc. in collaboration with Nanocor Inc. has developed a clay nanoparticle based nanocomposite called Imperm. Bottle made form this material is expected to revolutionize the Brewery industry. Breweries around the world uses heavy glass bottle or cans to ship beers or other alcoholic drinks. This is simply because alcohol in beer reacts with plastic. Imperm will be much lighter and cheaper than metal cans. Imperm bottles and cans also minimize the loss of CO_2 from beer and ingress of oxygen to the bottle. The company claims that Imperm containers keeps beer fresh and increases the shelf life to a maximum of six months.[49] Imperm has been used by Miller Brewing Inc. Honeywell Inc. has developed a special nanocomposite based polymer for engineering the surface of the plastic bottle which gives extended shelf life of alcohol even up to twenty six weeks. The product called Aegis nylon 6, is a nanocomposite based barrier layer put on plastics and has been used by South Korean Hite Brewery Inc. in its 1.6 liter Hite Pitcher beer bottle since 2003.[50]

Kodak Inc. is developing an antimicrobial film that has the ability to absorb O_2 from the contents of package, thereby preventing food from deterioration. Agromicron plans to market a NanoBioluminescence Detection Spray under the name

BioMark for detecting food pathogens like *Salmonella* and *E. coli*. Currently the company is designing spray formulations so that the spray can be utilized in ocean freight containers.[51]

Apart from packaging industry, nanomaterials are now heavily used by the interactive food industry. Unilever has been able to reduce size of the nano-emulsion particles that give ice cream its texture. The product is supposed to use 90% less of the emulsion and reduce fat content significantly (1% from 16%).[52]

Tip-Top Up bread from one of the top selling bread manufacturers in Western Australia has incorporated nanocapsules containing tuna fish oil in the bread. The nanocapsules are designed such a way that the capsule will break in stomach and release its content. As a result consumers can avoid the unpleasant taste of the tuna fish oil.[53] Nutralease Inc. has successfully used NSSL technology to deliver nutraceuticals in the body. The nanoparticles containing lipid shells and nutrients entrapped in the core are approximately 30 nm in size. The company has developed nanoparticles which can deliver lycopene, beta-carotene, lutein, phytosterols, CoQ10 and DHA/EPA etc. Nutralease products enter the blood stream from intestine via perisorption easily and thus increasing the bioavailability index. Using this technology Shemen Industries Co. is marketing Canola active oil which the company claims would reduce cholesterol by competing with bile solubilization intake into the body by approximately 14%.[54]

Biodelivery Sciences International Inc. has developed a 50 nm coiled nanoparticles which deliver nutrients such as vitamins, lycopene and omega fatty acids more efficiently in the body without compromising the taste, smell and color of the food items.[55] Oilfresh Corporation has launched a product which contains nanoceramic based nanoparticles. This product would reduce oil use significantly in cooking as the product heats up quickly and reduces the energy required for deep and pan frying.[56] Aquanova Inc. has developed a series of products, NovaSOL Sustain which contains 30 nm size micelles containing CoQ10 which reduces fat and α-lipoic acid for generation of satiety feeling. Similarly NovaSoluE and NovaSluC deliver Vitamin E and C respectively.[57]

Different funding agencies of GoI should therefore form study groups which will monitor the growth of the nanotech industries worldwide and prepare an inventory of the products, in the priority areas, which could be developed in India. Indian nanotech industries would need strong support from the nanoscience research on soft matter in near future. Currently, very few Indian nanoscientists work on soft materials which would be applicable to biology. Therefore, it is high time that we strengthen research in this direction to garner long term benefits from nanotechnology.

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