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Influence of Nanofertilizers on Growth and Yield of Mulberry and its Impact on Performance of the Silkworm, *Bombyx mori* L. : An Overview

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Abstract

Sericulture significantly contributes to the Indian economy. Mulberry leaf production is the vital phenomena in sericulture. Quantitative and qualitative mulberry leaf production is necessary for attaining maximum good quality cocoons. However, farmers are utilizing conventional fertilizers without judicious manner, due to which leaching losses makes soil hazardous and the nutrients might not be sufficiently reach the plant parts. Proper nutrient management in a judicial manner is done through foliar application of nutrients, hence nanotechnology a boon to agriculture in producing nano-fertilizers to make environmental friendly and effective utilization of available nutrients. This technology reduces the cost of cultivation, increases growth and yields of mulberry and improves the quality of mulberry leaves, thereby enhances the larval, cocoon and reeling parameters of silkworm, *Bombyx mori* L. This review summarizes the current research and the future possibilities of nanotechnology in the biofortification of plant nutrition, with a focus on nanofertilizers. It also discusses the difficulties, detrimental effects on the environment and harmful repercussions of nanotechnology research done to increase crop yields. Additionally, the possible applications and advantages of fertilizers based on nanoparticles in sustainable and precision farming are covered.

Keywords : Mulberry, Non-conventional fertilizer Nanofertilizer, Silkworm (*B. mori*)

Sericulture is a science and an art of rearing silkworms for silk production. India has an opulent and complex history in silk production. Silk trade times back to 15th century. Sericulture trade offers employment to approximately 9.00 million persons in rural and semi-urban areas in our country and it is commercially attractive with its economic activity. At present, it exists as cottage and small-scale industry.

The successful harvest of quality cocoons depends exclusively on the nutrition of the silkworm (Nagesh and Devaiah, 1996). Mulberry leaves serve as ideal food and provide various nutrients to carry out the physiological activities in *Bombyx mori* L. Nutrition of silkworm is the sole factor which almost individually augments the quality and quantity of cocoon production (Laskar and Datta, 2000). Nutrition should include the essential trace elements for insect growth *viz.,* iron, nickel, copper, manganese, potassium, zinc and iodine.

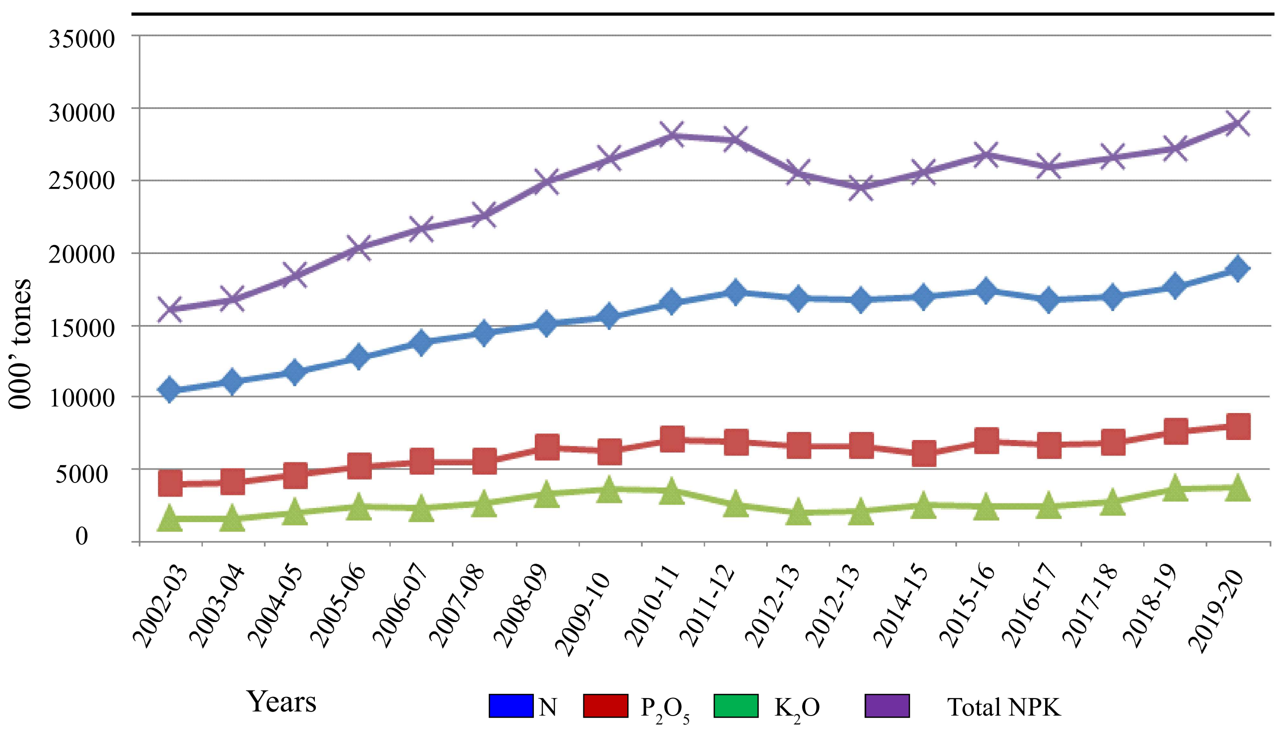
Generally, vitamins present in the mulberry leaves fulfill the minimum needs of silkworms. But the amount of vitamins present in mulberry leaves diverges on the basis of environmental conditions, usage of fertilizers in field and mulberry varieties and other field practices. The mulberry leaf quality is influenced by varied spacing's, irrigation levels, nitrogen levels, seasons and the extra foliates that are supplied exogenously through mulberry leaves (Triped *et al.,* 2009).

Fertilizers are indispensable in agricultural production system. Application of fertilizers started in the country in 1960's which closely coincided with the introduction of green revolution when fertilizer responsive varieties have been inducted in Indian agriculture. Despite fertilizer application remarkably increased the grain growth; the yields of several crops got plateaued due the low fertilizer response ratio, imbalanced fertilization, low organic matter and increased intensities of multi-micro nutrient deficiencies across the country.

In the world about 199 million tons of fertilizer consumption was recorded in 2019-2020. Among the countries, top five which are high in fertilizer consumption per capita rate are Singapore, Qatar, Hong Kong, New Zealand and Malaysia. India ranks 53rd in total fertilizer consumption with annual consumption of 28.96 million tones.

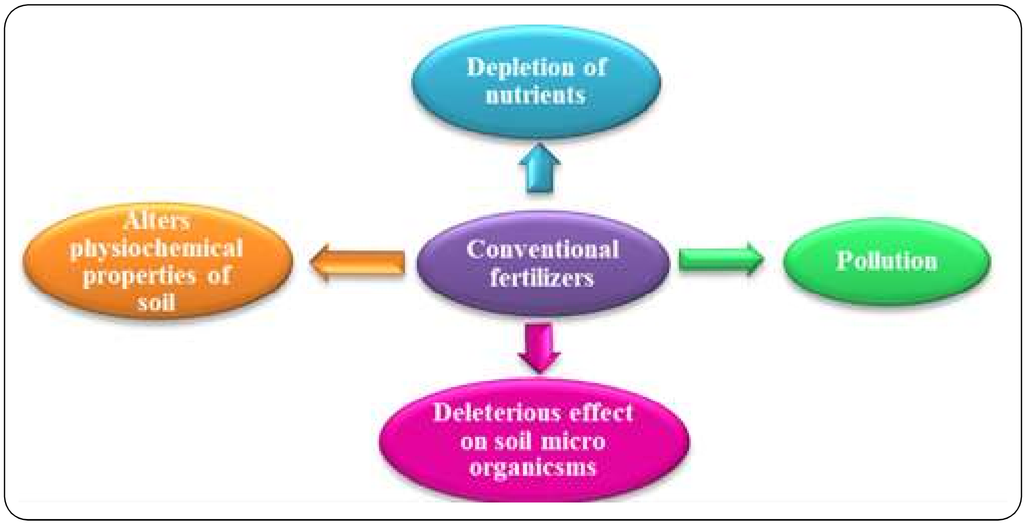
Fertilizer association of India recorded fertilizer consumption pattern in India and increasing trends were observed in the use of Conventional fertilizers, are those which are practiced by the farmers from so many years and mainly synthetic or synthesized chemical fertilizers still widely used *viz.,* di-ammonium phosphate, single super phosphate, muriate of potash, etc. for quick uptake of nutrient and to get instant results in plant growth and yield, and moving towards the conventional fertilizers where they do have their down sides.

Approximately 40-50 MT of foliage is removed from one hectare of mulberry garden at every year. Plants are capable of rejuvenate the foliage shortly by devouring soil nutrients and it causes depletion. Therefore, farmers need to replenish the soil nutrients with Recommended Dose of Fertilizer (RDF) for sustainable production of quality mulberry leaves. Continuous use of chemical fertilizer pollutes the soil and ground water by leaching of nutrients to the ground water, which alters physical and chemical properties of soil *viz.,* pH, EC, OC and less availability



Source : Fertilizer Association of India

Fertilizer consumption rate in India



Impact of conventional fertilizers

of nutrients to the plants. Excess release of fertilizers may produce toxicity and destroy beneficial micro-organisms which play vital role on soil health and plant growth.

Non-Conventional Fertilizers

Non-conventional fertilizers are those fertilizers manufactured using organic substances and nutrient enhancers of the soil, which are not traditionally practiced by the farmers (Bokhtiar and Sakurai, 2005). Any attempt to define and classify non-conventional inputs must be some what arbitrary and unsatisfactory because of certain conceptual problems as well as the difficulty of obtaining the desired statistical data. The results accruing from agricultural research by government and private industry are of course influenced significantly by the general level of knowledge on basic science and research techniques.

Adoption of new or improved techniques by farmers is not limited to agricultural extension activities and incentive payments by government. The efforts by individual farmers or farm organizations to obtain new technologies are also important. The rate and extent of diffusion will also be influenced by the communication facilities and other factors influencing the movement of people and information. The effect of improved technologies on total farm output obviously depends on the widespread diffusion of those technologies among individual farmers. Thus the non conventional inputs that promote the diffusion of new knowledge and technologies are important complements to agricultural research.

Enrichment of the mulberry leaves is one of the strategies by which cocoon and silk productivity can be increased and the quality can be enhanced and maintained by fortifying nutrient supplement

Bio-fertilizers

Foliar sprays

Coated urea

Biogas slurry

Nano fertilizer

(Krishnaswami *et al.,* 1971). Feeding of nutritionally enriched leaves with ascorbic acid, folic acids and elements like Selenium and metal, nano-particles have showed better growth and development of silkworms which also improved the economic characters of cocoons.

Nano-fertilizers are the synthesized or modified form of traditional fertilizers with the help of nanotechnology by fortifying nutrients either in single or in combination on to the adsorbents with nano-dimension. Nano-fertilizer refers to a product that delivers nutrients to crops in one of three ways the nutrient can be encapsulated inside nano-materials such as nanotubes or nanoporous materials, coated with a thin protective polymerfilm and delivered as particles or emulsions of nanoscale dimensions to improve the quality of agricultural produce (Nagula and Usha, 2016).

Nanotechnology is rapidly evolving field affords novel ways to enhance the growth and production in the field of nutrition and is expected to bring revolutionary changes in the field of agriculture. Nanoparticle, ultrafine unit with dimensions measured in nanometres (nm; 1 nm = 10^{-9}m). Nanoparticles exist in the natural world and are also created as a result of human activities. Because of their submicroscopic size, they have unique material characteristics, optical, electronic and photo catalytic properties and manufactured nanoparticles may find practical applications in a variety of areas, including medicine, engineering, catalysis and environmental remediation.

Nanoparticles are generally defined as particulate matter with at least one dimension that is less than 100 nm. This definition puts them in a similar size domain as that of ultrafine particles (air borne particulates) and places them as a sub-set of colloidal particles. A considerable fraction of the solid matter on earth can be found in the size range of colloids and nanoparticles. In the last two decades, scientists have shown that colloids and nanoparticles are present everywhere in the environment (McCarthy & Zachara, 1989 and Wiggington *et al.,* 2007). In 2008 the International Organization for Standardization (ISO) defined a nanoparticle as a discrete nano-object where all three Cartesian dimensions are less than 100 nm.

During 2011, the Commission of the European Union defined that the nanoparticles are natural, incidental or manufactured material containing particles, in an unbound state, as an aggregate or as an agglomerate and where, 50 per cent or more of the particles in the size distribution, one or more external dimensions is in the size range 1 nm-100 nm (Qureshi *et al.,* 2018). These nano-particles can be used as fertilizer for efficient nutrient management which are more eco-friendly and reduce environmental pollution (Meena *et al.,* 2017). These nano-particles developed with the help of nanotechnology can be exploited in agriculture production system. Nano-fertilizer providing greater role in crop production and several research revealed that nano-fertilizers increases the plant growth, yield and quality parameters in potato (Al-juthery *et al.,* 2019), in fig (Mustafa *et al.,* 2018), in cucumber (Merghany *et al.,* 2019) and in rice (Hiyasmin *et al.,* 2015). Plant growth and yield parameters and also benefit cost ratio was significantly highest by the foliar application of nano zinc oxide at 500 ppm compared to other treatments (Uma *et al.,* 2019).

Properties of Nanoparticles

There are various physico-chemical properties such as large surface area, mechanically strong, optically active and chemically reactive make nanoparticles unique and suitable applicants for various applications some of the important properties are listed below (Nadeem & Dirk, 2022; Juh 2007; Christian *et al.,* 2008 and Ibrahim *et al.,* 2019).

Physical Properties : They are highly mobile in the free state (*e.g.,* in the absence of some other additional influence, a 10-nm-diameter nanosphere of silica has a sedimentation rate under gravity of 0.01mm/day in water).

- Larger fractions of surface atoms
- Larger surface energy

Chemical properties : They have enormous specific surface areas (*e.g.,* a standard teaspoon or about 6 ml, of 10-nm-diameter silica nano spheres has more surface area than a dozen doubles-sized tennis courts; 20 per cent of all the atoms in each nano sphere will be located at the surface).

- Larger surface to volume ratio
- Catalytic property

Optical Properties : They may exhibit what are known as quantum effects. Thus, nanoparticles have a vast range of compositions, depending on the use or the product ;

- Surface plasmon resonance for metals
- Increased energy level spacing

Electrical Properties : They exhibit this property due to increased surface scattering ;

- Higher ionization potential
- Decreased electrical conductivity

Magnetic Properties

- The uneven distribution of electros in nanoparticles leads to magnetic property
- Transfer of ferro-magnetic to super-paramagnetic behavior

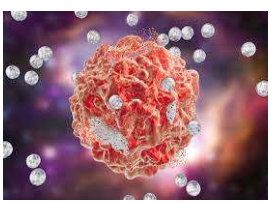
Mechanical Properties

- High elastic modulus, stress, strain, adhesion, friction and toughness
- Increased hardness and yield strength of material

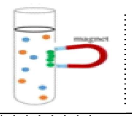
Thermal Properties : The nanoparticles have thermal conductivity higher than that of fluids in solid form, nanofluids are expected to exhibit superior properties relative to those of conventional heat transfer fluids and fluids containing microscopic sized particles.

Applications of Nanoparticles

Nanotechnology advancements have led to development of its allied fields, such as nanoparticles synthesis and suitable for various commercial and domestic applications which includes catalysis, imaging, medical applications, agriculture, biotechnology and environmental applications (Ibrahim *et al.,* 2019; Nadeem & Dirk, 2022; Ahmed *et al.,* 2021; Sonia & Deobra, 2016; Nivesh *et al.,* 2017; Heera & Shanmugam, 2015 and Rudramurthy & Kumaraswamy, 2018).

	Cancer therapy	Nanoparticles target the cancerous cells and then endocytosis or phagocytosis the cancerous cells.
	Drug delivery	Nanoparticles encasing drug increase the stability and water solubility of drug and deliver to specific tissue or cells.
	Biomolecule detection	Nanoparticles attach its surface to biomolecule and then this biomolecule can be detected by bio-tagging or labelling

	Plant Growth	The nanoparticles can be used as nano-fertilizers by encapsulating the fertilizer, positive morphological and biological responses enhance the growth, yield and quality of crop.
	Cosmetics	Nanoparticles alter properties of cosmetics including colour, transparency, solubility and chemical reactivity. Penetrate deeper into skin and deliver to more layers of skin cells.
	Diagnosis	Integrating diagnostics and therapeutic agents into single nanoparticles formulation
	Against antimicrobial resistance	Nanoparticles overcome antimicrobial resistance due to their physio-chemical properties, enabling nanoparticles to execute multiple novel bactericidal pathways to achieve antimicrobial activity
	Gene delivery	Nanoparticles provide an alternative to vectors for transferring gene in genetic engineering field.
	Enhanced immune function	Nanoparticles can stimulate immune response by acting as adjuvant.
	Antioxidant	Some of oxide nanoparticles mimic like antioxidant molecule due to their intrinsic physiochemical properties.

	Magnetic bioseparation	Magnetic nanoparticles absorb desired product on surface selectively separates from solution
• Also used as biochips in removing genetic disorders		
• Acts as catalyst to remove contaminants in environment		

Currently, many plants are being cultivated using hydroponics because of a lack of space and it has become common for crops cultivated hydroponically to be fertilized with nanofertilizers. Plants grown hydroponically display magnetic nanoparticle traces in their roots, stems and leaves; in contrast, plants grown in soil or sand do not display any such signals, indicating no uptake of the particles, which helps in growth and development of the plants.

The most frequent concern with these fertilizers is overfertilization, which can lead to environmental problems since the compounds contaminate water sources, accumulate in crop plants resulting in slow plant growth, burn foliage and increase sensitivity of plants to pests and diseases. Furthermore, by reducing soil enrichment, drawing moisture out of the soil, raising field salinity, also removing vital living organisms and organic materials that contribute to better soil quality, they fail to give plants the nutrients they need.

Nanofertilizers in Precision Farming and Sustainable Agriculture

Agrochemical use in sustainable agriculture must be kept to a minimum and creating an effective plant nutrient system with little environmental impact is crucial to this goal. Tropical and subtropical soils have a high nutrient absorption capacity, are typically highly acidic and are frequently severely lacking in nutrients. Therefore, novel and cutting-edge solutions that aim to increase crop productivity and the effectiveness of pesticide treatments, facilitate the development of effective water management systems (Ram *et al.*, 2014) and encourage the use of NFs to ensure agricultural sustainability are being provided by nanotechnological and nanoengineering techniques in order to overcome a global agricultural crisis (Kim *et al.*, 2018). By enabling a sustainable agricultural supplement, the appropriate use of nutrient sources can support global development.

A variety of application techniques are used in precision agriculture, also known as precision farming, to maximize crop protection and production in order to get the best results from targeted fertilizers. By tracking environmental variables and taking targeted action, precision agriculture has long been a desired strategy to maximize crop yields while minimizing chemical inputs, such as those of chemical fertilizers, pesticides and herbicides.

Due to the recent century's rapid population growth, there has been a constant increase in the need for food supplies. Adopting sustainable agricultural techniques that can increase crop productivity with out using excessive amounts of fertilizer is therefore vitally important. As an alternative to conventional chemical or mineral fertilizers, the use of nanomaterials to improve plant nutrition is gaining popularity in this respect. The effectiveness of macro and micronutrients in plants can be improved with the use of this technique. Comparing various nanomaterials to traditional fertilizers, agricultural production has successfully used them. Therefore, in order to preserve the basis of resources and sustain agricultural productivity, nutrient usage efficiency should be maximized.

Effect of Non-Conventional Fertilizers on Soil Properties of Mulberry Garden

The effect of nitrification inhibitors on physio-chemical properties of soil by coating the prilled urea with different nitrification inhibitors like neem oil, karanj oil and DCD (Dicyanamide) was studied by Yadav *et al.* (2016). The soil pH (7.47), bulk density (1.14 g/cm^3) and EC (0.25 µmhos/cm) were significantly low with neem oil coating, similarly available phosphorous (64.88 kg/ha) and potassium (299.88 kg/ha), porosity (45.69%) as well as water holding capacity (36.23%) were significantly highest in the neem oil coated urea.

Rohela *et al.* (2017) presented recent developments in bio-nanotechnology related to different forms of nanomaterials developed along with their application. They opined that nanofertilizers might maximize the nutrient use efficiency while minimizing the adverse effects to the environment due to use of large quantities of fertilizers in agriculture. Also they revealed that nanofertilizers released nitrogen slowly and subsequently even on the 60^{th} day where commercial fertilizer release heavily early followed by low non uniform quantities around 30^{th} day.

The effect of different sources of organic nutrients on nutrient status of S36 mulberry garden soil revealed that availability of major nutrients (208.0, 59.30 and 214.7 kg N, P and K per ha, respectively) and secondary nutrients (4.47, 3.41 and 25.40 C.mol Ca, Mg and S per kg, respectively) in the soil were significantly highest in the treatment 100 per cent recommended N through 20 per cent each of compost, *Gaena maculata,* Castor cake, Vermicompost and Urea with 10 kg each of *Azospirilllum brasilense* and *Aspergillus awamori* Bio-fertilizer along with recommended P and K through chemical fertilizer and FYM (Shashidhar *et al.,* 2017).

The effect of application of seri-waste biodigester effluent to V1 mulberry variety on soil properties growth and yield of mulberry and its impact on cocoon production (Harshitha and Chandrashekhar, 2020a). Electrical Conductivity (0.30 dS/m), organic carbon content (0.71%) and availability of macro nutrients *viz.,* available nitrogen, phosphorous and potassium (285.38, 49.82 & 186.05 kg/ha, respectively) were significantly higher in the 50 per cent Seri Bio-digester effluent with 25 per cent Bio-digester effluent and 25 per cent RDF and were lowest when they have use nothing.

These studies exhibited the positive effects of the non-conventional fertilizers. There is gap that due to the toxicity of non-conventional fertilizers over the conventional fertilizers on the soil properties and other factors which need to be addressed.

Effect of Non-Conventional Fertilizers on Growth, Yield and Quality of Mulberry

The influence of soil and foliar application of nitrogen on the yield and quality of mulberry with foliar application of 0.5 per cent nitrogen were significantly superior over the other treatments in terms of growth, yield and quality (Mancha Shetty, 1979). Foliar application of nitrogen 50 per cent through leaves and 50 per cent through soil was more effective than supply of 100 per cent of nitrogen through soil and exhibited an increased leaf yield by 42 per cent over control in Goshoerami mulberry garden. While, 100 per cent foliar application of nitrogen was highly significant over 100 per cent soil application and increased leaf yield by 52 per cent over control (Subbarao *et al.,* 1983). Further, the foliar application of 100 per cent nitrogen showed an increase in total leaf yield of Goshoerami mulberry to an extent of 46.66 per cent over soil application.

Supply of 50 per cent nitrogen through leaves and 50 per cent through soil has increased the leaf yield by 38.70 per cent over control (Fotedar and Chakrabarty, 1986). Even foliar application of urea at 0.5, 1.0 and 2.0 per cent concentrations resulted in 27.63 per cent increase (@ 0.5 per cent) in mulberry leaf yield over control (Qaiyyum *et al.,* 1991). Also found that significant improvement in quality parameters of mulberry *viz.,* moisture content, protein, carbohydrate, total sugar and starch contents over control.

The effect of urea (1, 2 & 3%) and 'Tricon' (0.05, 0.1 & 0.2%) as foliar spray on Jatinuni and M_5 mulberry resulted that nitrogen and crude protein contents were higher in Jatinuni with foliar application of 2 per cent Urea and 'Tricon' 0.2 per cent (Ali *et al.,* 1994). Further, the chlorophyll content was higher in M_5 and quantity was increased in 2 per cent Urea spray followed by 0.2 per cent 'Tricon' sprayed treatment. Further, foliar application of 0.5 per cent, 2 per cent $FeSO_4$, 2 per cent $ZnSO_4$, 2 per cent H_3BO_3 and 2 per cent $MnSO_4$ solutions significantly increased nitrogen, iron, zinc, boron and manganese contents of mulberry leaves, respectively and the balanced amount of all these nutrients in mulberry leaves were found in treatment combination of 0.5 per cent urea, 2 per cent $FeSO_4$, $ZnSO_4$, H_3BO_3 and $MnSO_4$ (Sarker and Absar, 1995).

Foliar spray of micronutrients (Iron 2%; Zinc 2% & Molybednum 2%) and urea (0.5%) given maximum leaf yield (745 kg per 1000 sq.m) when foliar spray of urea (0.5 %) with iron (2 %) was done on mulberry varieties *viz.,* S-1, S-799, C-776 and Dudhia (Yeasmin *et al.,* 1995). In another foliar application of methanol @ 5 per cent recorded higher values for the plant height (99.66 cm & 91.66 cm), leaf number per plant (183.6 & 315.0), branch no. per plant (10.66 & 10.70), leaf yield per pant (350.0 & 383.0 g/ plant), leaf area (134.5 & 58.3 cm^2), photosynthesis (12.88 & 11.41 m $mol/m^2/s$), fresh weight of 100 leaves (104.6 & 73.0 g), dry weight of 100 leaves (23.3 & 17.6 g) and leaf moisture content (84.4 & 76.5%) in two mulberry varieties S-1635 and S-1, respectively (Setua *et al*., 2009).

The effect of integrated use of bio-inoculants, FYM and inorganic fertilizers on fresh leaf yield and quality variables of M_5 mulberry had its impact on cocoon production revealed that among all the treatments maximum leaf yield (867.57g), moisture percentage (68.90%), chlorophyll 'a' (1.45 mg/g), chlorophyll 'b' (0.69 mg/g), total chlorophyll (2.14 mg/g), crude protein (18.21 mg/g), total soluble sugar (7.95 mg/g) and NPK (2.98, 1.72 and 1.33%, respectively) contents were higher when compared to standard check (Waktole Sori and Bhaskar, 2013).

Nitrification inhibitors on growth and yield attributes of mulberry (*Morus* spp.), was studied by Yadav *et al.* (2016) it was observed that plant growth, yield and quality parameters like number of shoots (10), plant height (177 cm), length of longest shoot (180 cm), number of leaves per shoot (32.18), total leaf yield (11353 kg/ha/crop) significantly highest in neem oil coated urea compared to non-coated urea.

The mulberry variety BM-9 when sprayed with different concentrations of urea solutions at 30, 45 and 60 DAP resulted that moisture (76.17%), moisture retention capacity (55.04%), crude protein (19.35%), total sugar (6.28%), reducing sugar (3.34 %) and soluble carbohydrate (10.68%) was significantly highest in the foliar application of urea @ 2.5 per cent compared to other treatments (Ahmed *et al*., 2018). Application of nano zinc oxide of 50 ppm as foliar spray recorded longer shoot height (96.63cm), more number of branches per plant (8.47), more number of leaves per shoot (18.60), more number of leaves per plant (157.15), higher leaf area (96.90 cm^2) and more leaf yield (0.46 kg/plant) (Nithya *et al*., 2018).

Foliar application of 3.5 per cent amino acid to V-1 mulberry variety recorded maximum biochemical parameters on 25th and 35th DAP on leaf moisture, carbohydrates and protein contents (74.7, 25.76 and 23.48%, respectively), chlorophyll (33.23 SPAD meter value), NPK (3.76, 0.46 & 1.53%, respectively), Calcium (1.69%), Magnesium (0.57%), Sulphur (0.49 %) and Zinc (22.75 ppm) (Deepa *et al*., 2020).

The foliar application of 0.4 per cent nano nitrogen fertilizer on 25th day after pruning + 50 per cent N, 100 per cent P and K through soil application with FYM had shown significant increase in the growth attributes *viz.,* maximum shoot height (54.42 cm), number of branches per plant (21.53 No.), number of leaves per plant (274.70 No.), total leaf area (25733.54 cm^2 /plant), leaf yield (704.64 and 928.53g /plant on 45 and 60 DAP, respectively) and quality variables *viz.,* total carbohydrates (22.79% mg), crude protein (28.44%), crude fiber (11.80%), chlorophyll 'a' (1.58), chlorophyll 'b' (0.98) and total chlorophyll contents (2.56 mg /g), leaf elemental compositions (4.55% N, 0.32% P and 1.72% K) in the V-1 mulberry variety (Pooja *et al.,* 2022a).

Foliar feeding of nanofertilizers has been widely used and accepted as an essential part of crop production, especially on horticultural crops (Pratima *et al.,* 2021).

These studies have shown a significant increase in growth parameters, nutrient composition and yield. Further Research is required to determine whether applying this in conjunction with other nanofertilizers might lower the incidence of mulberry plant diseases. Additionally, research must be carried out to determine the standard dosage of nanofertilizer. Moreover, careful consideration must be given to the adverse consequences of nanofertilizers.

Effect of Non-Conventional Fertilizers on Performance of Silkworm, *B. mori*

The cocoon weight, pupal weight and cocoon shell weight and filament length and filament denier were significantly maximum in silkworms fed on mulberry leaves sprayed with 0.5 per cent urea compared to other treatments (Mancha Shetty, 1979). The foliar spray of Urea @ 2 per cent on mulberry in relation to fertilizer dose (100, 200 and 300 kg /ha /yr) had its impact on silkworm rearing. Urea @ 2 per cent sprayed on S-41 mulberry and silkworms (NB_4D_2) were fed after two and four days after spray resulted in higher cocoon yield (370 g /dfl) with application of 200 kg per hectare per year and feeding the silkworms four days after spraying urea (Patil *et al.,* 2001).

A better larval weight and length after feeding with nitrogen supplemented mulberry leaves (Mahmood, 1989). Feeding mulberry leaves treated with nitrogen increased the growth and development of silkworm (Maqbool, 1991). The larval weight of silkworm *B. mori* was significantly highest when fed on mulberry leaves treated with 0.3 per cent nitrogen (Nabila *et al.,* 1993). Silkworms fed with mulberry leaves treated with nitrogen @ 0.2 per cent along with magnesium @ 0.15 per cent, consumed more food and were having significantly maximum larval weight compared to other treatments and untreated leaves (Zaman, 1995). Silkworms fed on mulberry leaves treated with 0.2 per cent nitrogen and 0.5 per cent manganese increased food consumption and significant increase in the larval weight (Rasool, 1995; Ashfaq *et al.,* 1998 & Etebari *et al.*, 2007). Foliar urea fertilization resulted in significantly increased weight of matured larvae (33.27g), ERR (88.92%), single cocoon weight (1.58g), single shell weight (0.25g), cocoon shell ratio (17.33%), longest filament length (972.34m), renditta(10.93) and cocoon production / 100 DFL's (69.08kg) in foliar application of 2.5 per cent urea compared to other treatments (Ahmed *et al.*, 2018). The silkworm cocoon weight was significantly and positively correlated with total soluble sugars (0.84) and total soluble protein (0.96) contents of mulberry leaf (Shilpashree and Subbarayappa, 2015). These nano-particles can be used as fertilizer for efficient nutrient management which are more eco-friendly and reduce environmental pollution (Meena *et al.,* 2017).

The nano-ZnO with nano-Cu each @ 500 ppm sprayed on V-1 mulberry exhibited the significantly superiority for full grown larval weight (40.68 g/10 larva), total larval duration (574.56 h), 5th instar larval duration (180.29 h), ERR (93.98%), silk productivity (0.62 cg/ day), cocoon weight (19.60 g/10 larva), cocoon shell weight (4.65 g/10 larva) and cocoon shell ratio (23.67%). The nano size of micronutrients and its unique property of more surface area might penetrate more efficiently and effectively when applied through foliar spray compared to foliar spray of chemical micronutrients (Pramila *et al.*, 2019).

Mulberry grown with the application of 50 per cent seri-bio-digester effluent with 25 per cent bio-digester effluent and 25 per cent recommended dose of fertilizer significantly enhanced the ERR (98.28%), maximum larval weight (38.63g/10 larvae) and cocoon parameters *viz.,* single cocoon weight (1.86g), pupal weight (1.53g), cocoon shell weight (0.30g), cocoon shell ratio (16.60%) and single cocoon filament length (915.10g) (Harshitha and Chandrashekhar, 2020b). The larval duration (4.73, 8.69 and 24.42 days for fourth instar, fifth instar and total larval duration, respectively), highest fifth instar larval weight (36.66g /10 larvae), better effective rearing rate (97.78%), cocoon yield by weight (26.36kg / 10000 worms), cocoon yield by number (9777 / 10000 worms), single cocoon weight (2.69g), single cocoon shell weight (0.63g), cocoon shell ratio (23.28%) also the reeling parameters like average filament length (1510.19 m) and filament weight (0.46 g) when *B, mori* silkworms (FC1 x FC2) fed on leaves of mulberry raised by foliar application of nano nitrogen fertilizer @ 0.4 per cent on 25th day after pruning (Pooja *et al.,* 2022b).

Non-conventional fertilizers are the best substitutes for conventional fertilizers, which cause more hazardous to the environment. Hence, in depth studies on the usage of non-conventional fertilizers also need to be studied.

Influence of Nanoparticles on Performance of Silkworm, *B. mori*

a) Influence of Nanoparticles on Feed Efficacy of Silkworm, *B. mori*

The MR_2 mulberry leaves were dipped in silver nanoparticles solution (AgNPs) at different dilutions (25, 50, 75 & 100%), then dried and fed to fifth instar larvae of CSR_2 x CSR_4 breed. The food consumption (52.76g), food utilization (49.48g), approximate digestibility (90.41%), consumption index (43.58%) and co-efficient of food utilization (91.29%) of fifth instar larvae were found significantly superior in the silkworms fed with silver nanoparticles treated mulberry leaves at 25 per cent concentration (Prabhu *et al.,* 2012).Mulberry leaved were soaked in TiO_2 nanoparticles solution at 5 mg/L for 1min and dried in air and fed to fifth instar silkworms (Suju x Minghu), then the amount of ingested food (5.31 g/ larvae), the amount of digestion (2.03 g/larvae) and percentage of ingested food (38.26%) were found significantly superior in silkworms fed with TiO_2 nanoparticles treated mulberry leaves (Zhang *et al.,* 2014).

Mulberry leaves were soaked in TiO_2 nanoparticles solution at 5, 10, 20, 40, 80 and 160 mg/l for 1min and dried in air and fed to fifth instar silkworms to study the feed efficiency of silkworm (Suju X Minghu). The silkworms group fed with TiO_2 nanoparticles at 10 mg/l treated mulberry leaves have showed significantly highest feed efficacy among other treatments and also found that TiO_2 NPs treatments improves feed efficacy of *B. mori* at lower concentrations (Li *et al.,* 2015). The mulberry leaves were treated with nanoparticles of riboflavin at 20, 40 and 60 µg/ml and fed to newly emerged larvae to know the effect of nutritional fortification of nanoparticles of riboflavin on the growth and development of mulberry silkworm, *B. mori* [(CSR6 × CSR26) × (CSR2 × CSR27)]. The consumption rate (190.923 mg/day), assimilation rate (157.201 mg/day), production rate (70.107 mg/day), metabolism rate (87.093 mg/day) and approximate digestibility (82.337%) were significantly higher in the silkworms fed with mulberry leaves treated with nanoparticles of riboflavin at 60 µg/ml. Supplementation of nanoparticles of riboflavin which enhances the proportion of food nutrients that move across the gut tissue rather than passing the food directly through the digestive system (Kamala & Karthikeyan, 2019).

The available literature has portrayed effective research work on the influence of nanofertilizers on silkworm. Exclusive studies on the feeding efficiencies with nanofertilizer raised mulberry have to be studied both in septic and aseptic conditions.

b) Influence of Nanoparticles on Growth and Development of Silkworm Larvae, *B. mori*

Silver nanoparticles (AgNPs)@ 25 per cent treated mulberry leaves (MR2) when fed to silkworms (CSR_2×CSR_4) increased the 3rd instar larval length (1.93 cm), 3rd instar larval width (0.38 cm), 3rd instar larval weight (0.12 g), 4th instar larval length (6.01 cm), 4th instar larval width (0.61 cm), 4th instar larval weight (0.54 g), 5th instar larval length (7.25), 5th instar larval width (1.33 cm) and 5th instar larval weight (3.55 g). AgNps act as vitamins to stimulate the feeding activity in the silkworms and improve the food digestibility which resulted in increased larval length, width and weight (Prabhu *et al.,* 2012).

Mulberry leaves were soaked in 5 mg/L TiO_2 nanoparticles solution and dried and fed to fifth instar silkworms. Larvae were dissected and mature silkworm's testes and ovaries were collected and stored in -80 p C. Silkworms after TiO_2 nanoparticles treatments, exhibited denser with oocytes differentiation and formation compared with those of control, indicating TiO_2 nanoparticles had strong impact on ovaries. Subsequently, the highest egg number (620 grain), egg weight ($6.49×10^{-4}$) and lowest unfertilized egg (5 grain) were also observed. It was evident that, feeding the silkworms with TiO_2 NPs is found to increase the metabolism of proteins and carbohydrates to meet the energy demand for growth and development of gonads and also formation of eggs in ovary (Ni *et al.,* 2015). Mulberry leaves were soaked in TiO_2 nanoparticles @ 100, 200, 400, 800, 1600 and 3200 mg/L and dried, later fed to silk worms (Jingsong × Haoyue) from 4th instar. The silkworms fed with mulberry leaves treated @ 400 mg/L were resulted in significantly maximum larval body weights both in 4th and 5th instar. The body weights of silkworm was increased slowly at AgNPs concentrations ≤ 200 mg/L, but the growth-promoting effect was diminished at higher (≥ 800 mg/L) AgNPs concentrations (Meng *et al.,* 2017).

The 5th instar larvae (PM x CSR_2) were fed with mulberry leaves treated with gold nanoparticles (GNPs) at different concentrations (50, 100, 200 & 300 ppm) and resulted insignificantly superior 5th instar larval duration (173.5 h), total larval duration (695 h), 3rd instar larval weight (10.81 g/30 larvae), fifth instar larval weight (47.99 g/10 larvae), silk gland weight of matured silkworm (3.31 g) and effective rate of rearing (86.25%) when silkworm fed with GNPs treated leaves @ 300 ppm concentration. It might be due to specific receptor on the region of posterior part of the silk gland. Thus 300 ppm is the specific dose and small size of gold nano probably directly stimulates the posterior receptor part of the silk gland, resulting in more content in silk glands of the silkworms (Patil *et al.,* 2016).

Mulberry leaves (V-1 variety) with nano zinc oxide @ 5, 10, 20 and 50 ppm on 25th and 35th days after pruning exhibited significantly highest larval weight (25.78 g/10 larvae), ERR (94.00%) and lowest moulting duration (91.25h) and fifth instar larval duration (7.22 days) when silkworms were fed with mulberry leaves treated with nano zinc oxide @ 20 ppm. This could be mainly due to adequate supply of zinc nanoparticles which accelerates the activity of enzymes and auxin metabolism in the plants, in turn increased the larval parameters (Nithya *et al.,* 2018). The nanoparticles of riboflavin at 60 gg/ml significantly increased larval weight (1.63g) and silk gland weight (1.67g) (Kamala and Karthikeyan, 2019).

The effect of nano zinc and nano copper nutrition on larval traits of silkworm when they fed on V-1 mulberry sprayed with nano zinc and nano copper at 25th and 35th days after pruning and then harvested mulberry leaves were fed to silkworms from 45th day after pruning. The nano-ZnOand nano-Cu each @ 500 ppm sprayed on V-1 mulberry proved as significant for full grown larval weight (40.68 g/10 larvae) and ERR (93.98%), significantly lowest total larval duration (581.61 h) and 5th instar larval duration (180.29 h). This is because that the nanoparticles have stimulated the metabolic activities in silkworm resulting in better growth and development Pramila *et al.* (2019).

MR_2 mulberry leaves were soaked in silver nanoparticles solution @ 100, 300 and 500 ppm and with spirulina @ 300 ppm for 15 min and dried in air for 10 min then fed to bivoltine double hybrid [(CSR6 × CSR26) × (CSR2 × CSR27)] silkworms from 3rd instar. The fifth instar larval length (6.2 cm), larval weight (2.4 g), silk gland weight (0.87 g) were significantly highest in the silkworms which were fed with mulberry leaves treated with silver nanoparticles and spirulina @ 300 ppm. This may be due to the nutritional supplementation of silver nanoparticles, has a direct relevance on length of larvae, weight of larvae and silk gland weight (Thangapandiyan and Dharanipriya, 2019).

Though, the studies expressed the usage of nanofertilzers have huge impact on the rearing of silkworms. However, the impact of nanofertlizer at different concentrations and nanofertilizers of different elements in varied concentrations are to be standardized in the field of sericulture.

c) Influence of Nanoparticles on Cocoon Traits of Silkworm, *B. mori*

The silkworms fed with nanoparticles of riboflavin @ 60 µg/ml treated mulberry leaves showed significantly maximumcocoon weight (1.19g), cocoon shell weight (0.27g), pupal weight (0.92g), cocoon shell ratio (22.689%), silk filament weight (0.53 mg), silk filament length (807m) and denier (3.903). This might be due to influence of nanoparticles of vitamins on growth and metabolism in silkworm larvae which inturn increased the cocoon and reeling parameters (Kamala and Karthikeyan, 2019). The cocoon weight (23.75g/10 cocoons), cocoon shell weight (4.12 g/10 shells), cocoon shell ratio (16.54%), cocoon yield (819.3g/dfl), cocoon length (3.93cm), cocoon breadth (2.36cm), cocoon filament length (867.46m), denier (4.05) and fibroin (78.07%) contents were significantly superior in silkworm fed with gold particles (GNPs) treated leaves at 300 ppm concentration. It might be due to gold nanoparticles at 300 ppm is the specific dose and small size of gold nanoparticles probably directly stimulates the silk gland, resulting in superior quality cocoons (Patil *et al.,* 2016). The effect of silver nanoparticles and spirulina on economical traits of silkworms revealed that silkworms fed with mulberry leaves treated with Ag NPs and spirulina @ 300 ppm were having significantly maximum cocoon weight (1.84g), cocoon shell ratio (41.3%), silk filament weight (0.32g) and fibroin (79.20%). This may be due to the nutritional supplementation of silver nanoparticles and food consumption, which has direct relevance on larval traits which resulted in increase in cocoon quality (Thangapandiyan and Dharanipriya, 2019).

Significantly highest cocoon length (3.66cm), cocoon width (2.40cm) and cocoon weight (2.21g) and quantity of silk production were observed in the silkworms (LNB_4 x D_2)fed with Ag NPs @ 25 per cent treated MR_2 mulberry leaves. AgNPs act as vitamins to stimulate the feeding activity in the silkworms. Therefore, AgNps can improve the food digestibility which in turn increases the cocoon length, cocoon width and cocoon weight (Prabhu *et al.,* 2011).

Silkworm fed with TiO_2 NPs treated mulberry leaves @ 5 mg/l yielded significantly superior cocoon weight (2.12g), cocoon shell weight (0.56g), cocoon shell ratio (26.32%), average cocoon filament length (1207.06m), reelability (67.57%) and neatness (98.33 points). This might be due feeding TiO_2 NP treated mulberry leaves, increased the feed efficiency of *B. mori* which in turn increased the cocoon quality and reeling parameters (Zhang *et al.,* 2014). The effects of TiO_2 NPs treated mulberry leaves on the cocoon parameters (Suju x Minghu) revealed that significantly superior cocoon and reeling parameters were found in the silkworm groups fed with TiO_2 NPs treated mulberry leaves @ 10 mg/L. This might be due to the exposure to TiO_2 NPs which promoted the growth and development of *B. mori*, probably because the TiO_2 NPs increased the digestion of food and absorption of nutrients, improved feeding efficiency thus promoting the synthesis of silk proteins (Li *et al.,* 2015)

The effect of nano zinc and nano copper nutrition on cocoon parameters of mulberry silkworms revealed that the cocoons from the silkworms fed with Nano Zn with Nano Cu @ 500 ppm sprayed mulberry leaves were found significantly superior for cocoon weight (19.64g/10 cocoons), cocoon shell weight (4.65g/10 shells), cocoon shell ratio (23.67%) and silk productivity (0.62cg/day). Nano Zn with Nano Cu @ 500 ppm might have stimulated the metabolic activities in silkworm resulting in better growth and development subsequently silk production (Pramila *et al.,* 2019). The cocoons from the silkworms fed with Nano zinc oxide @ 20 ppm sprayed mulberry leaves were given significantly superior cocoon weight (14.63g/10 cocoons), shell weight (2.49g/10 shells), cocoon shell ratio (17.98%), filament length (823.83m) and filament denier (2.45). The reason attributed was due to adequate supply of nutrients by feeding nano zinc oxide treated mulberry leaves which improved the larval traits, thereby increase in cocoon and reeling parameters (Nithya *et al.,* 2018).

The ultimate product of commercial silkworm rearing is the cocoon yield, which can be enhanced by the usage of different nanofertilizers. Further, these need to be standardized according to the type of nanoferilizers and their combination.

d) Influence of Nanoparticles on Resistance to Diseases of Silkworm, *B. mori*

The effect of silica nanoparticles on resistance against *Bm*NPV in silkworm (Nistari) was studied, by injecting the nanoparticles and *Bm*NPV complex

(@ 5 µL/ larva) to silkworm haemocoel. It was observed for dead and live insects in each group at 24, 48, 72 and 96 h and found that silica nanoparticles treated silkworms were not only healthy and controlled from infection by exhibiting prolonged the survival rate of larvae (61.03%) beyond 150 h. Due to Si NP the surface of polyhedra becomes roughened. With increasing NP concentration, the surface become more tattered and the edges roughened, leading to a general loss of the regular symmetry (Das *et al.,* 2013).

Three bacterial species (gram negative *Bacilli-I*, gram-negative *Bacilli-II* and gram-positive *Cocci*) isolated from the gut of diseased fifth instar silk worm were subjected for *in vivo* and *in vitro* anti bacterial activity of AgNPs. The sterile filter paper of 6 mm charged with AgNPs (100gL) solution and placed on agar medium for disk method and well diffusion method, then incubated at 37^0C for 24 hours. In *in vivo* study, 30 diseased larvae of fifth instar were collected of which 15 were fed with mulberry leaves as control and 15 were fed with mulberry leaves treated with AgNPs for 5 days. Later on sixth day all the larvae were dissected and their gut was removed and macerated for antibacterial assay. The results revealed that silver nanoparticle showed maximum zone of inhibition over negative control and lowest gut bacterial growth of larvae which were fed with Ag NPs treated MR_2 mulberry leaves (Prabhu *et al.,* 2013).

Mulberry leaves were soaked in TiO_2 nanoparticles @ 5 mg/L, dried and then fed to fourth instar silkworms (Jingsong × Haoyue), later the fifth instar worms were fed with mulberry leaves dipped in *Bm*NPV solution (5.6 x 10^6 polyhedra/ml). After feeding with TiO_2 nanoparticles treated mulberry leaves and *Bm*NPV for 120 hours, the silkworms found in control group were diseased and treated groups were healthy and grew well without any symptoms and also showed significant increase in survival rate of larvae (99.44%), cocooning rate (49.13%), cocoon weight (1.73 g), cocoon shell weight (0.41g) and cocoon shell ratio (23.82%). This indicated that TiO_2 NPs significantly inhibited the proliferation of *Bm*NPV in silkworm larvae (Xu *et al.,* 2015).

The fungi *viz., Beauveria bassiana* and *Metarhizium anisopliae* isolated from gut of diseased *B. mori* of fifth instar were grown in petriplates to assess the antifungal activity of silver nano particles against silkworm white and green muscardine pathogens. Later, the wells were made by puncturing them and fresh agar was poured and the nanoparticles solution is added to it (50, 75 and 100 µL). A paper disc was placed and incubated at 37°C for 24 hours to assess the growth. After incubation, zone of inhibition was recorded to check the antibiotic activity (Well diffusion method) (Ramamoorthy *et al.,* 2019). The maximum zone of inhibition was observed in silver nanoparticles @ 100 µl concentration and lowest zone of inhibition in control. The antifungal activity of silver nanoparticles is attributed to its effects on the mycelia, interact with cellular membranes and disrupting membrane potential.

The mulberry leaves were soaked in TiO_2 nanoparticles and fed to fourth instar silkworms to assess the effect of TiO_2 nanoparticles on resistance of silkworm against *Bm*CPV. Hundred grams of leaves were dipped in *Bm*CPV solution (1.1 x 10^7 polyhedra/mL) for 1 min dried and fed to fifth instar silkworms. The survival rate (98.67%), cocooning percentage (61.33%) and survival rate of cocoons (35.49%) were significantly highest in the silkworms fed with TiO_2 NPs treated mulberry leaves and less mortality compared to control. TiO_2 NPs treatment was equally effective in improving the resistance of silkworm to cytoplasmic polyhedrosis virus (CPV), inhibiting the proliferation of *Bm*CPV in silkworm (Zhao *et al.,* 2020).

Beneficial results came out by these studies with well advanced techniques; however need to study more on managing the fungal, bacterial and viral diseases of silkworms in relation to the each of nanoparticles and also with their combinations by correlating their application in the silkworm disease resistance.

Nutrition is known to play an important role on growth and overall performance of cocoon components. Nanotechnology is emerging has greatest imperative tool and possesspositive impact in advancement of functional feed which deliver nutrients effectively. Though chemical fertilizer initially yields good results, the sericulture farmers certainly experience its deterioration effect on leaf yield, as well as the quality of cocoon productivity. Therefore, promotion of non-conventional fertilizer is in need to avoid the indiscriminate usage of chemical fertilizers in mulberry garden. The non-conventional fertilizer is an alternative and is characterized by reducing the input of chemical fertilizer and combined use of non-conventional fertilizers with organic materials, for sustainable crop production, integrated use of non-conventional and organic fertilizer has proved to be highly beneficial. The non-conventional production system aims at utilization of variable resources to enrich soil to supply balanced nutrients to crop plants. Need to conduct study on nanoparticles of affordable nutrient elements for farmer's point of view.

A holistic approach should be made for creating awareness among the sericulture farmers and popularizing non-conventional fertilizer strategies to maintain soil health, improve production of quality mulberry leaf and sustainable production of silk. Chemical based inputs are much preferred by the farmers because of short term results and income. In spite of the good research efforts, the improvement in mulberry leaf yield and quality and constraints are yet to be overcome. Implementations of projects on non-conventional farming are to be intensified for sustained quality mulberry leaf production under different agro-climatic conditions of the country. The inclusion of nanomaterials in sericulture is a very new concept. Therefore, it is imperative to exploit their effects on silkworms and on silk regarding the improvement of fecundity, survival rates, management of pests and disease prevention. Also need to exploit as a novel approach towards development of new antiviral therapies.

Therefore, it is imperative that research institutes take up the task of creating an exhaustive database on the effects of non-conventional mulberry production in order to build an integrated package of practices that will guarantee success for sericulturists.

Future Perspectives of Nanofertilizers

Public access to information regarding the products and their applicability should be provided by the nanotechnology industry. New technologies should go from lab testing to controlled field trials to address the problems and challenges associated with natural antivirus programs. Such approaches could have a major positive impact on nutrition, environmental quality, food security, sanitation and sustainability. More research is required to evaluate the toxicity of recently discovered nanofertilizers and their effects on yield, crop quality, the environment and human health.

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