PROFORMA FOR COLLECTION OF DATA OF RESEARCH PROJECTS IN SERICULTURE

PART- I: GENERAL INFORMATION

1	Name of the Institute / University / Organization submitting the Project proposal	:	Central Sericultural Research & Training Institute, Berhampore, West Bengal
2	Status of the Institute (s)	:	N.A.
3	Name (s) and designation (s) of the Executive Authority of the institute / University forwarding the application	:	Dr. Kanika Trivedy, Director
4	Project Title	:	Assessment of designed antimicrobial peptides for mulberry protection against brown leaf spot and root rot: a biotechnological approach
5	Category of the Project	:	Applied
6	Specific Area	:	P – Plant; I – Improvement; T – Biotechnology
7	Duration	:	3 years
8	Total Cost	:	16.41Lakh
9	Is the Project single or multi-Institutional	:	Single Institutional
10	If the Project is multi-institutional, please furnish the Name, Designation and Address of the Project Coordinator.	:	N.A.

11. (a) Project summary:

Mulberry (*Morus* spp.) is a perennial tree and economically important for its foliage as nutrients to the domesticated silkworm (*Bombyx mori* L.). In India, mulberry intensively monthscultured as monocrop and garden is routinely maintained more than 20 years with repeated basal pruning for easy harvest of the leaves to feed the silkworm. Being a nutritious plant, multiplication and inoculum built-up of certain foliar /soil borne pathogens in mulberry fields are common. All commercially exploited cultivars of mulberry are susceptible to a wide range of fungal pathogens at varying degrees. The cumulative foliage loss due to the fungal disease in mulberry may reach 20%. Among these, the *Myrothecium* leaf spot (MLS) and *Fusarium* root rot (FRR =dry root rot), two important diseases caused by necrotrops, *Myrothecium roridum* and *Fusarium oxysporum* and/or *F. solani*), respectively, are most severe in the mulberry fields of eastern and north-eastern (NE) India. The former disease is predominant during summer months and affects three (out of total five) commercial crops in West Bengal and responsible for the foliage loss of ~ 12%. While, the incidence of dry root rot is being increased recently under changing global scenario and emerge out as a threat to mulberry growers in eastern and NE India. Currently, these two disease controls mainly rely

on the application of carbamate fungicides, which raises many concerns for silkworm health and the environment. Though, development of resistant cutivar(s) is considered as most sustainable and economic approach, but this time consuming (usually takes ≥ 14 years) avenue is hitherto unexplored for MLS and FRR. *In this context, the search for an alternative biotechnological approach may yield relatively rapid result*.

Plants produce specific antimicrobial peptides (AMPs) of 3- to 10-kDa mass (< 50 amino acids), cationic in nature and rich in cysteine or glycine residues. These peptides are natural antibiotics that act as an innate and/or primary defense barrier to prevent the invasion of pathogenic microorganisms by multiple interactions. Due to this multiple site of actions and broad spectrum of antimicrobial activity against pathogenic fungi, AMPs are considered as highly promising candidates for drug development and applications to control plant pathogens.

But major concerns about the use of most of the natural AMP(s) as pesticides in plant protection are: a) high production cost, b) low stability toward protease degradation, c) high haemolytic activity at their respective dose(s) of applications. To overcome these problems several design strategies have been devised in order to find shorter and more stable peptides with increasing the activity at low cytotoxicity. Indeed, >1000 such designed AMPs are available in public domains. *Several groups have proposed the use of designed AMPs in plant protection against fungal pathogens. After identification of suitable AMPs, plants have also been engineered to express such genes. Though AMPs based control of some important silkworm diseases are available, but information is scant on the application of AMPs to mulberry disease control.*

(b) Aims and Objectives

Therefore, in this study our main goals are to assess the antimicrobial potential of designed/synthetic peptides against two major fungal pathogens causing MLS and FRR in mulberry. Selected AMPs will be synthesized and tested through *in vitro* inhibition assay as plant protecting agents. Subsequently, spraying the designed peptides on the surface of infected plant organs antimicrobial activity will be assessed pathometrically in *ex vivo* to test the potential of practical utilization. For the conformation of pathogen growth suppression abilities of selected/ promising AMPs, quantification of fungal DNA will be done by qRT-PCR amplifying pathogen specific genes of *Myrothecium* and *Fusarium* spp using non-specific lebel method. In this process, internal transcribed spacer (ITS), intergenic spacer (IGS) of ribosomal DNA genes, elongation factor-1α gene (EF-1α) and trichothecenes biosynthesis genes (*MRTR-14 & 15*) will be utilized following already established methodologies.

PART-II: PARTICULARS OF INVESTIGATORS

12	a) Name		**DR SOUMEN CHATTOPADHYAY**
	Date of Birth		15-07-1961
	Sex		M
	Indicate whether Principal Investigator/ Co-investigator		**PI**
	Designation		Scientist-D
	Department		Biotechnology
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
	b) Name		**DR RITA BANERJEE**
	Date of Birth		05-04-1961
	Sex		F
	Indicate whether Principal Investigator/ Co-investigator		**CI-1**
	Designation		Scientist - D
	Department		Biotechnology
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
	c) Name		**MS PUJA MAKWANA**
	Date of Birth		
	Sex		F
	Indicate whether Principal Investigator/ Co-investigator		**CI-2**
	Designation		Scientist-B
	Department		Biotechnology
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
	d) Name		**DR KANIKA TRIVEDY**
	Date of Birth		27-08-1957
	Sex		F
	Indicate whether Principal Investigator/ Co-investigator		**Executive Authority**
	Designation		Director
	Institute/University: Address		CSR&TI, Berhampore, West Bengal
13	No. of Projects being handled by each investigator at present		
a	Dr R Banerjee		PROJECTS: 2 (AS PI-1,CO-I:1)
b	Dr. S Chattopadhyay		PROJECTS: 3 (AS PI:2, CO-I:1)
c	Ms P Makwana		PROJECT: NIL

14. Proposed Research Fellows [justification with work sharing is a must]:

Requested manpower (1 JRF) in absolutely essential to carry out the extensive studies planned for ex vivo experiment to collect disease reaction and phenotyping based selection of AMPs over multiple seasons/years and primer selection / standardization and RT-PCR works.

PART-III: TECHNICAL DETAILS OF THE PROJECT

15. **Introduction**

15.1 **Definition of the Problem**:

(a) Origin of the project:

Mulberry (*Morus* spp), the solitary host plant of domesticated silkworm (*Bombyx mori*) is cultivated in ~219819ha area across the country. Though India occupies 2nd position in global silk scenario by producing ~ 28mt of raw silk, however quality silk (bivoltine; 3870ton) production is far reaching the grade of international demand. The paucity of quality mulberry foliage is considered as one of the major impediments in silk quality improvement (Datta and Nanavathi 2005). The pathogen induced qualitative and quantitative deterioration of foliage is one of the major bottlenecks of mulberry improvement. The foliage loss due to major fungal pathogens in mulberry often goes upto 20%; productivity and quality loss of silkworm cocoon is even more (Sengupta 1990).

Brown leaf spot (MLS) caused by *Myrothecium roridum* is one of the major diseases of mulberry. The MLS pathogen is a necrotroph and produces a group of non-host selective macro-cyclic toxin, trichothecenes, and a potent inhibitor of protein synthesis (Jarvis et al 1985). The disease appears during April to October covering three major cocoon crops in West Bengal. It is responsible for foliage loss of 10-12% during peak season of incidence (Qadri et al 1999). Presently growers are relying upon application of carbamate fungicide to control the disease; however, it needs a safe period before use, as silkworm feed and hazardous to soil flora/fauna. On the other hand, use of fungi-toxic botanicals against MLS is still at infancy (Chattopadhyay et al 2002).

Fusarium root rot (FRR = dry root rot) caused by *F. solani* and *F. Oxysporum* is a major soil borne disease in the southern states of India and affecting plant growth, survivability and leaf yield in mulberry (Gupta 2001). FRR also affects the sprouting of stem cuttings and growth of saplings. High soil moisture and poor soil health increases FRR severity. The disease is responsible for ~14%reduction in leaf yield (Sharma and Gupta 2005). Currently, it is emerging as a major disease in eastern/north-eastern India under

changing global scenario. During initial level of infection, Carbendazim 50% WP and Mancozeb 75% has been suggested as chemical control.

AMPs are small molecular weight protein with broad spectrum antimicrobial activity against various microorganisms, including bacteria, viruses and fungi. In this study, we examine possible utilization of synthetic AMPs to enhance resistance to MLS and FRR in mulberry by:

- ✓ *In vitro testing of synthetic AMPs against MLS and FRR by inhibition zone assay*
- ✓ *Spraying of AMPs to infected plants and assessment of disease protection potential of AMPs ex vivo.*
- ✓ *Assessment of pathway specific cyclic trichothecens biosynthesis genes and pathogen specific nuclear encoded ribosomal ITS or IGS regions for MLS and FRR pathogens detection and quantification using by qRT-PCR.*
- ✓ *Assessment of toxicity (hemolytic activity) of the selected AMPs against silkworm.*

b) Expected outcome:

a. Identification of suitable synthetic AMPs against MLS and FRR infection/progression in mulberry for commercial utilization

b. Development of transgenic plant for enhancement of resistance to MLS and FRR by overexpression of genes coding for desirable AMPs into commercial cultivar(s) without loss of existing desirable traits.

15.2 **Rationale of the Study:**

Among the major foliar diseases of mulberry, brown leaf spot (MLS; c.o.*Myroyhecium roridum*) is predominant during summer months in Easter and NE India. The disease and affects 3 silkworm rearing seasons (out of total 5) in West Bengal. Similarly, *Fusarium* root rot (FRR;c.o. *Fusarium solani* and *F oxysporum)* is another threat to mulberry growers which emerges as a dreaded disease under the present changing global scenario. Presently, carbamate fungicides (methyl-benzimidazole-carbamate and/or ethylene-bis-benzimidazole carbamate) are applied to the foliage or soil to control these two diseases. However, use of carbamate fungicide(s) has several limitations as it is costly, needs a specific waiting period before silkworm consumption, prone to develop resistant pathogen strain and environmentally undesirable. Though biological control of MLS and FRR pathogens was also reported on the laboratory condition, but commercial application of such agents is still at

infancy. Moreover, there is no resistant mulberry cultivar available for commercial utilization against MLS and FRR.

In this back drop intervention of biotechnological approaches / newer avenues of disease control may rapidly enhance the quality of plant products and/or prevent, reduce or eliminate constraints to plant productivity caused by biotic stresses.

Plant antimicrobial peptides (AMPs) have been subject of interest as strong candidates for plant protection application due to minimal possibilities to emergence of resistance against these (Montenisons 2007). AMPs are short-sequenced peptides with generally fewer than 50 amino acid residues. Ideally, useful AMPs should have specific properties to supress pathogenic micro-organisms growth with lack of toxicity against host cells, less prone to low proteolytic degradation and low cost and for this reason, the search for novel peptide sequences with improved properties is an area of great interest. Indeed, many of such designed, short-sequence peptides (at present >840 nos) are reported against many plant pathogenic organisms. Therefore, detection of potential synthetic AMPs against two major diseases of mulberry *viz*. MLS and FRR has been considered as an important step for direct commercial use.

15.3 **Relevance to the current issues and expected outcome:**

The present project would plan to search of designed / synthetic AMPs as a good alternative to current fungicides against MLS and FRR in mulberry. The goals of the project are:

- design of a set of well-established antimicrobial peptides,
- assessment of their potential use as plant protecting agents of MLS and FRR *in vitro* inhibition assay,
- Assessment of selected AMPs based disease suppression by spraying on the surface of infected plants through *ex vivo* experiments,
- Quantification of fungal DNA from the AMP treated systems by the amplification of suitable unique pathogen specific candidate gene(s), and
- Assessment of the utilization potential of identified designed AMPs through the hemolytic activity on silkworm.

The expected deliverables from this study are:

- ✓ Identification of synthetic AMPs to control MLS and FRR as a sustainable strategy in mulberry crop protection and which may expectedly represent an alternative or complement to the existing fungicides.

Create a suitable platform to develop mulberry cultivar resistance against MLS and FRR by expression of antimicrobial peptides.

15.4 Objectives:

- *Assessment of disease protection potential of synthetic AMPs against Myrothecium leaf spot and Fusarium root rot of mulberry in vitro*
- *Assessment of disease protection potential of selected AMPs against Myrothecium leaf spot and Fusarium root rot of mulberry ex vivo*
- *Determination of disease suppression ability of selected AMPs using quantitative PCR.*

16. **Review of status of Research and Development on the subject**

16.1 **National Status**:

Important mulberry diseases

Mulberry is a perennial tree and propagated mainly by stem cutting clones. Its foliage is the unique food of domesticated silkworm *Bombyx mori* L. Fungal diseases of mulberry create challenging problems in commercial sericulture because they are difficult to control, and often result in sudden, devastating economic losses. Apart from quantitative loss, quality deterioration in foliage production due to infestation of various diseases is also a major concern amongst mulberry-biologists (Govindiah et al 2005). Uptill, advocated disease control is mainly based on carbamate fungicides, which are costly and under restrictions in many countries due to their adverse effects on non-target organisms. Therefore use of carbamate fungicides is impractical from a biosafety view and is not economical for marginal sericulture farmers. In this context, there is a need to search alternative avenue(s) to control major mulberry diseases. Synthetic / designed antimicrobial peptides (AMPs) may be a cost-effective alternative to complement the existing practices.

In eastern and NE India MLS (c.o. *Myrothecium roridum*) and dry root rot (c.o. *Fusarium solani/ F.oxysporum*) are two major diseases of mulberry. The former pathogen is responsible for the necrotrophic spot development, yellowing and premature defoliation of leaves; while root rot affecting cutting-survivability, plant growth and foliage yield (Gupta 2001).Presently available mulberry cultivars are prone to these two diseases in varying degree. Though host resistance is considered as the most sustainable and economical control strategy, but development of disease resistant mulberry through classical breeding has several limitations, like lack of sex-compatibility between desired genotypes, highly heterozygous plant behavior and long-time requirement (~14 years).

Myrothecium leaf spot:

The fungus *Myrothecium roridum* is wide spread in temperate and tropical regions of the world and has been reported to pathogenic to many important plants including mulberry (Kuti et al 1987; Murakami et al 2000). The MLS appears in late summer under suitable weather conditions (30-32ºC temperature, 80-90% humidity and >10 rainy days /month) and spreads until late autumn. The disease appears in the form of large, irregular/circular, linear tan-colored spots with dark margins on the leaf surface. The spots later become necrotic and greyish brown. This ultimately results in yellowing of leaf and premature leaf fall (Chattopadhyay et al 2006). It is reported that Myrotoxin-B, primary mycotoxin metabolites of *M. roridum* belongs to trichothecenes which are chemically group of sesquiterpenoid-epoxides and play roles as infection factor or virulence factor of *M.roridum* (Murakami and Shirata 2005). The disease is soil borne and the foliage loss due to MLS goes up to 10-12% with substantial reduction in nutritive value (Qadri et al 1999) due to rapid loss of protein, total and reducing sugar and chlorophyll contents. MLS can be controlled by application of carbendazim (0.1% active ingredient) and mancozeb (0.2% active ingredient), but they are under restrictions due to adverse effects on soil flora and fauna. Moreover, resistance to carbendazim and mancozeb has been reported in pathogens of different field crops in India (Gangawane 1990). Reports are also available on the use of botanicals to control MLS, but direct field applications of such molecules are still in infancy.

Fusarium or dry root rot:

Fungi of the genus *Fusarium* are worldwide, occurring plant pathogens which cause severe damages to numerous cultivable plants (Li et al 2008). *Fusarium* caused diseases can destroy crops within several weeks and the infection leads to quality losses in two different aspects: besides the reduced yield the fungus produces various toxic metabolites while colonizing the plant. These mycotoxins heavily impair the quality of the harvest (McMullen et al., 1997). *Fusarium oxysporum* is a soil-borne facultative parasite present worldwide. The species includes nonpathogenic and pathogenic strains, the latter causing vascular wilt and root rot on many economically important crops including mulberry.

Root rot is a serious problem during mulberry cultivation in almost all the sericulture countries. The attack of root rot is always patchy in the fields and mulberry plants are quite resilient due to extensive root system that enables the plant to remain standing and asymptomatic even when heavily infected. Therefore, adoption of remedial measures for root rot control is not always in time. Among the root rots, dry root rot (c.o *F. solani* and *F oxysporum*), black root rot (*Lasiodiplodia theobromae*) and charcoal root rot (*Macrophomina*

phaseolina) are reported from various mulberry gardens of India (Sharma et al 2003). *But in the recent years, dry root rot emerges as a dreaded disease, especially to the young (5-10 years old) plants in the eastern and NE states.* The FRR is responsible for ~14 % loss in leaf production (Philip et al 1995). Most *Fusarium* species are widely distributed in substrates such as soil, on subterranean and aerial plant parts, plant debris, and on dead organic matter and usually infected broad host ranges. Diseased saplings cause secondary infection and spreads rapidly through contaminated soil, irrigation and farm implements (Philip et al 1996). The symptoms of FRR are associated with white mycelial stands on the root surface leading to the rotting of roots, and results in sudden withering of leaves, followed by defoliation and death of plants. Besides, it affects mulberry plantation mainly at nursery at the initial stage of development. The stem-cuttings decay and rot resulting in the failure of cuttings to sprout, or wilting or death of sprouted cuttings (Sharma et al. 2003).

Chemical control by root –dipping of saplings in carbendazim / mancozeb has been the suggested prior to transplantation. In spite of their effectiveness, chemicals are not advocated for controlling the soil borne plant pathogens in view of their prohibitive cost and threat to beneficial soil microflora, hence the interest is shifting towards biological control in other plants. In this line, bio-fungicide 'Raksha' in integration with carbendazim are also recommended to control the disease (Philip et al 1996). But the approach h yielded very little impact on the commercial level.

Necessity to confirm MLS and FRR pathogen biomass using quantitative PCR:

Fusarium oxysporum is a complex of species that comprises morphologically indistinguishable formae speciales, which may be pathogenic or non-pathogenic strains (Farnandez et al 2005).Most of them are morphologically similar anamorphic fungi with multiple phylogenetic origins (Bogale et al 2006). This species complex is well represented among fungal communities in different soil types worldwide and considered as a common member of the fungal communities in the plant rhizosphere (Gordon and Martyn 1997).However, within this species complex some strains can cause devastating wilts and root rots diseases on a large number of crop plants of high economical importance. These phytopathogenic fungal strains show a high level of pathogenic specificity to host species and cultivars, on which basis they are classified into more than 100 formae specialis (Edel et al1995). On the other hand, the fungus *Myrothecium roridum* is wide spread in temperate and tropical world and has been reported pathogenic to many important crop species, including mulberry (Tulloch 1972; Murakami and Shirata 2005). The species is responsible for leaf spot and fruit decay in many plants. In addition to its broad host range of pathogenicity, the fungus produces secondary

metabolites (tricotheciecenes), which have pathotoxic, cytotoxic and cytostatic effects (Bean et al 1984). But these trichothecene mycotoxins are produced by several filamentous fungi including *Myrothecium* and *Fusarium* spp (Jarvis 1991).

Uptill, detection of FRR and MLS pathogens in mulberry is largely depend upon morphological basis using symptomatology and analysis of fungal growth in specific culture media (Govindaiah et al 2005). It requires extensive knowledge of classical taxonomy and are frequently laborious, time-consuming and difficult for a species like *Myrothecium* and *Fusarium* , where either different formae specialis or broad host specificity involving common toxin(s) generation possibilities are involved. Moreover, race specificity of Fusarium species during mulberry FRR is also not ascertained.

Quantitative real-time PCR (RT-PCR) technology provides conclusive results as it can discriminate between closely related organisms and is therefore appears as a versatile method for the accurate, reliable, and high throughput quantification of target DNA. (Garrido et al. 2009).Nowadays, a wide range of plant pathogens can be detected and quantified by real-time PCR methods in numerous hosts or environmental samples (Schena et al. 2013). Basically, there are two common methods for the detection of products in RT-PCR: first, non-specific fluorescent dyes that intercalate with any double-stranded DNA; and second, sequence-specific DNA probes consisting of oligonucleotides that are labelled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence (Mirmajlessi et al 2015).Indeed, RT-PCR technique based on the former approach is successfully utilized to detect and fungal biomass estimation of *Fusarium oxysporum* (Rispai and Rubiales 2014), *F solani* (Huang et al 2012) and *M roridum* (Han et al 2014) utilizing specific primers. *But detection and fungal growth estimation of MLS and FRR pathogens of mulberry is not reported.*

Alternative approaches for the control of plant diseases using antimicrobial peptides (AMPs):

Plant diseases caused by viruses, bacteria and fungi affect crops, and are responsible for significant losses or decrease the quality and safety of agricultural products. Their control relies mainly on chemical pesticides and all pathogenic microorganisms require the continued use of such chemicals for control (Agrios 2005). However, stringency in the pesticide registration has been increasing pervasively in all countries. As a result, several pesticides have been banned and some plant diseases of economic importance are managed with difficulty due to the lack of effective compounds. Besides, crop breeding programs struggle to release new cultivars in which complete disease resistance is achieved, and usually such resistance becomes quickly overcome by the targeted pathogens.

Plant antimicrobial peptides are being considered as a good alternative to current fungicides and different efforts have been initiated for their application in plant disease control (Macros et al 2008). However, application of designed peptides in mulberry disease control is yet to be explored.

16.2 **International Status**

Antimicrobial peptides (AMPs)

Protection of plants against infectious microorganisms depends on both constitutive and induced defense mechanisms. All plant organs express AMPs constitutively or in response to microbial challenges. They exhibit a wide range of functions ranging from direct antimicrobial properties to immunomodulatory effects (Nawrot et al 2014). AMPs are a part of the nonspecific host defense system of plants and active against different types of microorganisms. AMPs are produced by all species of life and represent key components of the innate immune system, providing a fast acting weapon against invading pathogens (Hegedus and Marx 2013).Structurally, AMPs are ~12–50 amino-acid long, carry a net positive charge of >2, and are composed of ~50% hydrophobic amino acids. Many of them adopt an amphipathic structure only in membrane environments, which is considered to be a prerequisite for their lytic activity (Shai and Oren 2001). It was suggested that AMPs might account for upto 2%–3% of the genes predicted in plant genomes (Marcos et al 2008).

Diversity of AMPs:

Plant antimicrobial peptide have been isolated from roots, seeds, flowers, stems, and leaves from a wide variety of species and have demonstrated activities towards phytopathogens, as well as against organisms pathogenic to human, viruses, bacteria, fungi, protozoa, parasites, and neoplastic cells (Montesinos 2007).

AMPs can be classified as either ribosomal or non-ribosomal according to their mode of synthesis by the cells. Ribosomal peptides are gene-encoded peptides usually resulting from the cleavage of a pro-protein. While, non-ribosomal peptides are assembled by multi-modular enzymes called non-ribosomal peptide synthetases (NRPS).

AMPs are also classified according to their origin, composition, secondary structure and predominant amino acids. Structural diversity wise AMPs are divided into five structure classes: α-helical structure, β-sheet structure, mixed helical/β-sheet structure, irregular structure, and those incorporating a macro cyclic structure (Nakatsuji and Gallo 2011).

According to the amino sequences and secondary-dimensional structure, there are nine classes of AMPs (table -1 for detail). Among them eight classes belonging to cysteine-

rich peptides with 4 to 12 cysteine residues involved in disulfide bridges and one class of cysteine free peptides that is rich of Histidine /Glycine or Glycine. There are a significant diversity in both the size and charge of molecular within each of the classes and between the classes.

Despite their high sequence diversity, AMPs also share certain common structural characteristics such as:

(i) *amino acid composition:* the residues most abundant in AMP are cationic (arginine and lysine) and hydrophobic (tryptophan, phenylalanine, leucine, and isoleucine);

(ii) *net charge:* most AMP are positively charged at physiological pH, although a minor subgroup includes anionic peptides;

(iii) *amphipathicity:* conferred by their amino acid composition and arrangement;

(iv) *structures and conformations*: including α-helices, β-sheets and nonconventional structures (latter one is specially abundant in short AMP).

Table 1: *Origin and structure properties of major classes of plant AMPs (after Yili et al 2014)*

Type	Number	Origin	Number of amino acids	Structural property
Shepherins	2	*Capsella bursa-pastoris*	28~38	Rich of His, Gly (cysteine free) His, Gly, linear or rule less loop
Cyclotides	45	*Caryophyllaceae* and *Annonaceae*	28~37	Three disulfide bridges, CCK (cyclic cystine knot) bonded with the β-sheet
Snakins	2	*Solanum tuberosum*	63~66	Six disulfide bounds
Thionins	6	*Brusels sprouts* (cabbage) and oats, seeds of the Santalaceaes plants, leaf of barley, *Phoradendron tomentosun*, etc.	45~47	Three or four disulfide bridges, r-shaped molecular, the vertical, stem consists of a pair of anti-parallel α-helicas, and the horizontal arm consist of a pair anti-parallel β-sheet
Defensins	80	Barley, wheat and maize, sorghum, oil spinach, beet, mustard, chile, turnip greens, radish, silkworm, *Clitoria ternatea* L., potato, Yunnan Bean, sunflower, *Aesculus hippocastanum*, etc.	45~54	Four disulfide bridges, three-dimensional structurewith triple-stranded, anti parallel β-sheet and a single α-helix lying in parallel with the β-sheet
Lipid transfer proteins	10	Barley, wheat and maize, radish, onion, *Arabidopsis thaliana*, rubber, rapeseeds, radish seed, embryos of grape vine, etc.	90~93	Three or four disulfide bridges, four helices linked by three loops, a C-terminal tail without regular structure, the fold is stabilized by four disulfide bonds and helices enclosed. Internal hydrophobic cavity in which can be inserted by the aliphatic chain
Heveins	9	Amaranth, *Pharbitis nil* (Linn.) *Choisy*, *Saintpaulia ionatha*, *Euonymus* L., etc.	29~44	Three, four or five disulfide bridges Three disulfide bridges, short triple-stranded

Mode of action of AMPs:

AMPs have been demonstrated to inactivate prokaryotic cells by targeting a number of essential primary or secondary metabolic processes at extracellular, plasmamembrane,

and/or intracellular sites (Yount and Yeaman2013). Most of the AMPs rapidly permeate and destroy the cell membrane integrity in contrast to conventional pesticides, which act on specific targets such as enzymes or DNA (Makoviitzki et al 2006). AMPs act by formation of membrane pores, resulting in ion and metabolite leakage, depolarization, interruption of the respiratory processes, and cell death. Additionally many AMPs suppress the nucleic acid and protein syntheses and induced programmed cell death (Brogden 2005). Majority of AMPs are cationic and have the ability to adopt an amphipathic conformation in which positively charged and hydrophobic groups segregate onto opposite faces of an α-helix, β-sheet or other tertiary structures (Bechinger 2004). Amphiphilicity is thought to be the key requisite for AMPs to interact with their primary target, the microbial cell membrane (Ferre et al 2006). AMPs do not interact with a specific receptor, rather disturb membrane bilayer integrity, either by disruption or pore formation, probably through peptide-phospholipid interactions. Therefore, *possibility of microbial resistance is very less than that observed with available pesticides* (Zasloff 2002).

Exploiting AMPs in plant disease control:

As demands for a better control of various diseases increase, AMPs come into focus. Several hundreds of natural AMPs have been reported form microorganism to animals including plants and several thousands have also been synthesized / designed *de novo* from different systems. To date, a multitude of gene constructions with coding sequences of AMPs have been expressed in different animal systems leading to various extents of protection against fungal and bacterial pathogens (Munoz and Macos 2006). The most comprehensive AMPs database to date, named ADAM, is publically available and currently contains 7007 unique peptide sequences and 759 structures (http:// bioinformatics. cs. ntou. edu. tw/ ADAM/ links. Html). Other AMP databases are exists including phytAMP data base (Hammami et al 2008).

Among plant antimicrobial peptides, thionins were the first whose activity against plant pathogens was demonstrated *in vitro* (Stec 2006). Subsequently, several families of cysteine-rich peptides have been characterized, including defensins, lipid transfer proteins, hevein-type peptides, knottin-type peptides , and others (Bechinger 2004). Plant AMPs are small (6-70 residues), positively charged with a high portion of hydrophobic residues (about 30%) that allow them to fold into an amphiphilic structure with distinct patches of hydrophobic and positive charged amino acids (Hancock and Sahl 2006). These structural features ensure effective interaction with plasma membranes of pathogenic microorganisms assumed to be their primary target.

Synthetic or designed AMPs:

Exploitation of AMPs obtained from living organisms is difficult in many cases because of the low amounts of natural presence. In fact major concerns about the use of natural AMPs as pesticides in plant protection are: a) high production cost (Motesinos and Bardaji 2008), b) low stability toward protease degradation (Badosa et al 2009), c) high haemolytic activity (Broekaert et al 1997) and d) other cytotoxicity at relatively higher dose of applications. To overcome these problems several design strategies have been devised in order to find shorter and more stable peptides with increasing the activity at low cytotoxicity. These strategies include the juxtaposition of fragments of natural antimicrobial peptides, the modification of natural peptides, and the *de novo* design of sequences maintaining the crucial features of native AMPs. Indeed, >1000 such designed AMPs are available in public domains; many of which have antimicrobial activity against a broad spectrum of pathogens under *in vitro* and *in planta* conditions. Most of the AMP assayed *in vitro* has 50% inhibitory and minimum completely inhibitory concentrations (IC_{50} and MIC, respectively) in the range of 1 to 20μM (Marcos et al 2008). Some important reports of synthetic / designed animal or plant originated peptides and their potential uses in phytopathogens control are given below.

Application potential of synthetic or designed AMPs in plant disease control*:*

Several synthetic AMPs based on compounds of animal or plant origins have been reported with broad spectrum of phytopathogens control potentials. Cecropins, magainins, and melittin are well-characterized membrane active AMP whose toxic properties have made their practical use difficult (Bechinger 2004). Cecropins, among the first, where the ratio of activity against microbes vs toxicity to plant cells were modulated. Two sequence analogs of cecropin-B with reduced toxicity to plant protoplasts, SB-37 and Shiva-1, were engineered and exhibited several phyto-pathogenic microorganisms (*Xanthomonas campestires*, *Pseudomonas syringe* and *Erwinia caratovora*) growth in tomato with a lethal concentration range of 0.1 – 4.5μM (Jayens et al 1993). A synthetic substitution analogue of magainin, MSI-99 inhibited the growth and spore germination of *Fusarium oxysporum* f.sp. *cubense*, *Sclerotinia sclerotiorum*, *Alternaria alternata* and *Botrytis cinerea* (Alan and Earle 2002). Two analogues of cecropin-B, D4E1 and MB39 inhibits *Verticillium dahliae*, *Fusarium moniliforme*, and the bacterial pathogens *Pseudomonas syringae* pv. *tabaci* and *Xanthomonas campestris* pv. *malvacearum* (DeLucca and Walsh 1999).

Fusions of fragments of natural AMP have been designed in an attempt to increase the efficacy than the parental AMPs. A well-documented instance is that of cecropin: melittin hybrids. Pep3, a cecropin-melittin hybrid, is active against two *Fusarium* species, (Cavallarin

et al 1998). Other examples are peptides CEMA and its derivative MsrA1(Osusky et al 2000) or Pep3 which are active against different phyto-pathogenic fungi. Likewise, cecropin A:magainin hybrids have been designed that show antibacterial activity, but do not produce hemolysis (Lee et al 2004). Synthetic analogs of natural AMP with greater resistance to *in vitro* degradation with same pathogen control abilities have also been described. Examples include MB39 from cecropin-B, Pep3 from cecropin-A, and Myp30 from magainin-2. These synthetic AMPs are active against wide group of microbial and fungal plant pathogens (Marcos et al 2008). Derivatives of tachyplesin are active against two *Fusarium* species (Rao 1999). It has been demonstrated that two synthetic peptides derived from the bovine protein lactoferricin (LfcinB20-25 and LfcinB17-31) have broad antimicrobial properties against *Alternaria alternata, Fusarium oxosporum* and *Magnaporthe grisea.* Pe4-1, an isomorph of penaedin from white shrimp inhibits several plant pathogenic bacteria and *Fusarium oxysporum* (Cuthbertson et al 2004). Peptides GR7 and SA3 were further derived from synthetic AMP ESF1, and showed higher amphipathicity and net charge with reduced inhibitory concentration to dry rot pathogen *F. oxysporum* growth down to 0.1μM (Dykes et al 1998). A similar rational design approach was taken to develop D4E1 and D2A21. The activities of these peptides were compared with those of cecropin B and magainin II, showing that they are more active and have no adverse effect on pollen and seed germination (Jacobi et al 2000).

Though the reports are relatively less still derivatives of plant originated AMPs have also been prepared and tested for their phyto-pathogen control abilities. Rs-AFP2, a peptide derived from radish defensin, inhibits growth of several fungi (De Samblanx et al 1996). Two hepta-peptide derivatives 77-3 and 77-12 (PEP6), showed broad spectrum anti-fungal activity against *F. sambucinum* (dry rot pathogen of potato), *Fusarium oxysporum* f. sp. *Lycopersici* (root rot of tomato), *Rhizoctonia solani* (root rot of many species), *Ceratocystis fagacearum*, and *Pythium ultimum* (Reed et al 1997; Gonzalez et al 2002). Three synthetic AMPs of CECMEL11 library, BP15, BP22 and BP25, reduced the growth and sporulation of *Stemphylium vesicarium* (brown spot of pear) significantly at <50μM concentration (Puig et al 2014). Antimicrobial synthetic peptide D2A21, altered the conidial plasma membrane of three ascomycetes *Gremmeniella abietina*, *Ophiostoma ulmi* and *Nectria galligena* and basidiospores of *Cronartium ribicola* (Rioux et al 2000).D32R, an analogue of the thionin, is active against the plant pathogenic fungai *F. oxysporum, Botrytis cinerea*, and plant pathogenic bacteria *X. campestris* pv. *translucens* and *C. michiganensis* (Vila-Perell et al 2003). ESF1 is active against several fungi including *Cryphonectria parasitica*, *F. oxysporum*, *Phytophthora infestans* and *Alternaria solani*, and rot-causing bacteria *E. carotovora* (Ali and

Reddy 2000). PAF26, a synthetic antifungal hexapeptide, is active against *Penicillium italicum*, *P. digitatum* and *Botrytis cinerea*. Synthetic cyclic peptides active against plant pathogens are less abundant. The cyclodecapeptide BPC194 is active against the plant pathogenic bacteria *E. amylovora*, *Pseudomonas syringae* and *X. vesicatoria* and was obtained in an extended survey from tetra- to decapeptides using combinatorial chemistry (Monroc et al. 2006).

Only a few synthetic AMPs (ribosomal) underwent functional validation *in planta* for resistance to plant pathogens and they all originate from animal species. A synthetic substitution analogue of magainin, MSI-99, exhibited disease resistance in transgenic tobacco and banana. This peptide inhibited the growth and spore germination of *Fusarium oxysporum, Sclerotinia sclerotiorum*, *Alternaria alternate, Mycosphaerella musicola* and *Botrytis cinerea* (Chakraborti et al 2003). Pathogen-induced expression of a cecropin A-melittin hybrid peptide, CEMA in tobacco enhanced the resistance to *Fusarium solani* (Yevtushenko et al 2005). Transgenic cotton and poplar plants expressed with the synthetic antimicrobial peptide, D4E1, exhibited the growth and /or sporulation suppression of *Fusarium verticillioides* and *Verticillium dahlia* (Rajasekaran et al 2005) and, *Agrobacterium tumefaciens*, *Xanthomonas populi* and *Hypoxylon mammatum (Mentag et al 2003)* respectively. MsrA2, a synthetic derivative of cationic AMP dermaseptin B1, elicited strong antimicrobial activities against various phyto-pathogenic fungi and bacteria *in vitro*. To assess its potential for plant protection, MsrA2 was expressed at low levels (1–5 µg/g of fresh tissue) in the potato plant and tuber tissues. Subsequent challenges to transgenic potato with a variety of highly virulent fungal phytopathogens—*Alternaria*, *Cercospora*, *Fusarium*, *Phytophthora*, *Pythium*, *Rhizoctonia* and *Verticillium* species—and with the bacterial pathogen *Erwinia carotovora* showed an unusually broad-spectrum resistance to infection. MsrA2 profoundly protected both plants and tubers from diseases such as late blight, dry rot and pink rot and markedly extended the storage life of tubers (Osusky et al 2004). Overall information shows the emerging potential of the synthetic AMPs based control of plant diseases through transgenic approach.

Several groups have proposed the use of designed AMPs in plant protection, mainly against bacterial and fungal pathogens. After identification of suitable AMPs, plants have also been engineered to express such genes. Though AMPs based control of some important silkworm diseases are available, but information is scant on the application of AMPs to mulberry disease control excepting the report of cyclic peptide iturin-A2 based control of mulberry anthracnose (Hiradate et al 2002).

16.3 **Importance of the proposed project in the context of current status**:

Fungal diseases are mostly the root causes for quantitative/qualitative impairment of foliage production in mulberry. MLS is a dreaded foliar disease and affects important silkworm rearing seasons. Similarly, FRR infestation is responsible for poor leaf yield and even death of plants. AMPs have successfully been implicated in plant disease control for replacing conventional treatment methods that are polluting and hazardous to the environment and to human health. Nevertheless, AMPs based controls of some important silkworm diseases are available, but information is scant on the application of AMPs to mulberry disease control. Therefore, in the present work we proposed followings:

- ✓ Selection of designed/ synthetic AMPs for their potential use as plant protecting agents against MLS and FRR *in-vitro* inhibition assays.
- ✓ Spraying the designed peptides on the surface of infected leaves / roots to demonstrate their antimicrobial activity directly on mulberry to displays a way of practical application.
- ✓ Confirmation of promising AMPs mediated suppression of fungal growth by RT-PCR using specific primers for each fungus.
- ✓ Testing of hemolytic activity or toxicity of short-listed AMPs to silkworm.

16.4 **Anticipated Products, processes/Technology, Packages/ Information or other outcome from the project and their expected utility**:

Anticipated product: In-vitro and *ex-vivo* assessment of selected AMPs may provide a complementary and sustainable control strategy against MLS/FRR, two major fungal diseases of mulberry .

Generation of useful information: Assessment of selected AMPs ability to suppress the MLS and FRR pathogen growth by qRT-PCR using tricothecens biosynthetic gene(s) and/or specific internal transcribed spacer (ITS) regions expectedly confirm the results and provide suitable platform for transgenic overexpression of identified AMP to develop MLS and FRR resistant lines.

16.5 **Expertise available with proposed investigation group/ institution on the subject of the project*:**

Name of the Scientists	Designation	Experience
Dr S Chattopadhyay	Scientist-D	As a PI of the proposal, he has 24 years of research experience in various field of mulberry crop improvement. He has expertise in the areas of- a) screening of mulberry genetic resources using random molecular markers, b) analysis of various enzymes and cellular constituents of mulberry, and c) pathometrical analysis of various mulberry diseases including MLS. He has >49 publications in different International and National level peer review journals. He had concluded a DBT funded network project on-Identification of DNA markers associated with powdery mildew disease in mulberry as PI and instrumentally associated with two DBT supported projects (collaboratively with CCMB) to identify SCAR-markers associated with powdery mildew resistance and development of linkage map of mulberry.
Dr R Banerjee	Scientist-D	As a Co-I-1 of the proposal, she has about 22 years research experience in the field of plant breeding and genetics. She has expertise in the areas of a) characterization of mulberry germplasm materials using anatomical, morphological and molecular tools as well as b) various aspects of conventional/targeted breeding in crop plants and C) disease resistance breeding of mulberry for BLS and other diseases. She has more than 40 publications in International and National peer review journals. She is instrumentally associated as PIs with two DBT supported project (collaborative with CCMB) on development of mulberry linkage map and powdery mildew SCAR marker development and one in-house project on development of bacterial leaf spot resistant mulberry.
Ms P Makwana	Scientist-B	As a CO-I-2 of the proposal, she has 3 years of research experience in various fields of silkworm molecular genetics, physiology and rearing technology. She has expertise (relevant to the proposal) in the areas of- bioassay of various silkworm breeds / hybrids and determination of genotoxicity of different nano-emmusions. She has already published couple of research papers in different International and National level peer review journals.

** Dr Somnath Bhattacharya, Associate Professor, Dept of Genetics & Plant Breeding, BCKV, Nadia agreed to provide all kind of technical supports / laboratory assistance for RT-PCR based assessment.*

17. **Work Plan**:

17.1 **Methodology**:

I) Activity-1: ***Assessment of disease protection potential of synthetic AMPs against MLS and FRR in vitro***

a) *Designing of peptides*

Synthetic (~20nos) short peptides originated from ccecropin, alpha-theonin, magainin and melittin groups (as they are mostly toxic to wide group of plant fungal pathogens at low concentrations) will be synthesized from the available sequences in public domains. Until and unless specifically required, all lyophilized peptides will be dissolved in sterile double-distilled water at a concentration of 1 mg/ml and kept at –25°C until use (Alan and Earle 2002).

Table 2: *Synthetic short sequence (< 50AA)AMPs reported working well against wilt, rot and leaf spot fungal phytopathogens and majority of them used in expression studies in plants*

Compound	Size	Source	Active against	Hemolytic activity (µg /ml)
PEP6	6	Synthetic	*F oxysporum; R solani*	>200
ESF1	20	Magainin analog	*F oxysporum; A solani*	>200
PAF26	6	Synthetic	*Botrytis cinerea; Penicillium spp*	>175
D4E1	17	Cecropin analog	*Verticilliumspp; Fusarium spp.*	>200
MSI-99	23	Magainin analog	*A solani; Phytopthora spp*	>224
Rs-AFP2		Plant defensin derivative	Many fungi	>200
D32R	47	Plant theonin derivative	*F oxysporum; R solani; Microspherella spp.; X. campestris*	>175
PPD1	5	Synthetic	*F. oxysporum; R solani; Pythium ultimum*	>200
77-3	7	Synthetic	*F oxysporum; R solani ; F. sambucinum*	>224
77-12	7	Synthetic	*F oxysporum; R solani ; F. sambucinum*	>200
P-18	18	cecropin -magaininhybrid	*Trichosporon beigelii, Aspergillus flavus and Fusarium oxyspovrum*	>200
Pep1	15	cecropin - melittin hybrid	*F oxysporum; Microspherella spp*	>200
10R	13	Indolicidin analog	group of wilt, rot and leaf spot fungi	
11R	13	Indolicidin analog	do	>200
MsrA2	32	derivative of dermaseptin B1	*Alternaria, Cercospora, Fusarium, Pythium, Rhizoctonia and Verticillium spp.*	>200
CEMA	29	cecropinA-melittinhybrid	Group of soft rot and dry rot fungi	>200
LfcinB17-31	15	lactoferrin derivative	*F oxysporum; R solani; Microspherella spp.; X. campestris*	>200

b) Fungal pathogens and growth media

i. Viable propagules of MLS will be isolated and cultured on potato dextrose agar (PDA) plates using aseptic procedures to avoid contamination according to Chattopadhyay et al (2002).

ii. Pathogenic wild-type isolets of *Fusarium* spp will be isolated from the infected mulberry plants during their active stage of growth. Conidial suspension will be cultured according to the method of Freeman et al (2001).

c) Detection of antifungal activity

In vitro inhibition assays will be performed in sterile flat-bottom plates. About 20µL of each concentration will be loaded per well. Spores of MLS and FRR pathogens will be collected from sporulating cultures in water and spore concertation will be determined by haemocytometer adjusted to suitable concentration in 2% PDA and/or malt extraction media. Fungal spore will be added per well by adjusting final AMP concentrations from 0 to 100µg/ml. After incubation at room temperature for 3d on a rotary shaker fungal growth will be determined by measuring OD and

visual screening of the plates. The Minimum Inhibitory Concentration (MIC) will be taken as the lowest peptide concentration without growth at the end of the experiment- after 48 hours. MIC will be calculated with the average of three replications. Three replicates of each strain and peptides will be used. Water will be used as positive control instead of peptide and negative controls will contain peptides without the fungal pathogen. Potential of pathogen control abilities of AMPs will be confirmed using thin agarose radial diffusion assay of Hultmark et al slightly modified by Nordeen et al (1992).

II) Activity-2: ***Assessment of disease protection potential of selected AMPs against MLS and FRR ex vivo***

a) To determine the anti-fungal activity of AMPs against MLS pathogen, 60 days old potted plants will be inoculated by measured spore suspensions according to the method of Chattopadhyay et al (2006). For FRR, mulberry saplings will be inoculated with the pathogen propagules suspension using root-dip method of Freeman and Rpdriguez (1993). Appropriate plant tissues will be harvested at periodic interval of 24h of peptide treatment. Disease symptoms will be evaluated using standardized methods, severity index will be calculated and compared with the respective controls.

b) *Disease assessment*

MLS disease reaction of sprayed leaves and controls will be scored using Horsefall-Cowling (1978; 0 to 10 points) scale giving equal importance on Disease severity index (DSI), Disease incidence (DI) and area under disease progression curve (AUDPC) according to Chattopadhyay et al. (2011). FRR will be rated according to the method of Sharma and Gupta (2005). Three replicates of each treatment along with control will be maintained in both the diseases.

III) Activity-3: ***Determination of disease suppression ability of selected AMPs using quantitative PCR***

a) DNA extraction from fungi and plants

DNA from 50 to 100 mg of respective fungal cultures, as well as from 50 to 100 mg of control or AMP treated diseased mulberry tissues will be isolated according to already established methods in the laboratory. Integrity of the DNA was checked on a 0.8% agarose gel and concentration was determined with a NanoDropTM 1000 spectrophotometer (Thermo Scientific) at 260 nm.

b) Determination of fungal biomass

The extents of respective plant part(s) colonization by *M. roridum* and *F. oxysporum* (and *F solani*) will be determined using quantitative PCR. In this process, 5 to 10ng of

total DNA collected from AMP treated and untreated samples will be used. Amplifications will be performed in 5µL of 2X SYBR green JumpStart mix (Sigma–Aldrich, India) with 10pmol of the respective oligonucleotides using an Mx3000P thermal cycler (Stratagene, La Jolla, CA, USA). Detectable fungal DNA will be therefore considered as an indication of bacterial growth. As race / strain specificity of *Fusarium oxysporum* and *Myrothecium roridum* is not yet ascertain in mulberry, aseptically grown mulberry saplings in glasshouse will be challenged by local isolates of these pathogens and mentioned primers sequences (table-3) will be used. The RT-PCR protocols of Fernandez et al (2010) and Maghighi and Shahdoust (2015) will be followed for FRR and MLS pathogens, respectively.

Table 3: Specific primer sequences to be used to detect MLS and FRR phytopathogens in mulberry

Pathogen	Primer	Sequence	Remarks
Myrothecium roridum	ITS1	5'-TCC GTA GGT GAA CCT GCG G-3'	Specific Internal Transcribed Spacer (ITS)
	ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	do
	ITS5	5′-GGAAGTAAAAGTCGTAACAAGG-3'	do
	ITS4	5'-TCC TCC GCT TAT TGA TAT GC-3'	do
	MRTR15 #446	5'-TCCTTTCAACATCCAGAG-3'	trichodiene synthase gene
	MRTR15 #508	5'-TCGACATGTGAAGCCTGG-3'	do
Fusarium oxysporum	FocTR4-F	5´-CACGTTTAAGGTGCCATGAGAG-3´	Specific Inter-Genic Spacer (IGS)
	FocTR4-R	(5′-GCCAGGACTGCCTCGTGA-3′	do
	Foc-1	5′-CAGGGGATGTATGAGGAGGCT-3′	do
	Foc-2	5′GTGACAGCGTCGTCTAGTTCC-3′	do
	EF1-F	3′-ATGGGTAAG GARGACAAGAC-5′	specific translation elongation factor 1-α (TEF)
	TEF-WI-R	3′- GCTCACGRGTCTGGCCATCCTTG′	do
	GYCF1	5′CTCCGGATTTCTGGAGACTTG-3′	dp
	GYCR4C	5′-ACTATCGTGTGCCGGGGTTGGC-3′	do
	Fc-1	5′CATACCACTTGTTGCCTC 3′	Specifically designed primers based on ITS1
	Fc-2	5′ATTAACGCGAGTCCCACC3′	do

IV) Activity-4: *Determination of the hemolytic activity of the selected AMPs against silkworm:*

Hemolytic activity / toxicity to silkworm by the identified promising AMPs will be tested according to the standardized protocols after isolation of hemolymph according to the method of Asada et al (1981). The hemolytic activity of the AMPs will be evaluated by determining the hemocyte integrity of fresh hemolymph using trypan blue dye (Philip 1973).

V) *Statistical analysis:*

All spraying experiments will be conducted in at least three biological replicates.

Quantitative estimation of all parameters will be determined using genetic model for 'Statistica' version 8.0 software (Statsoft Inc., Tulsa, OK, USA).

Establishment of correlation between AMP *in planta* traits and hemolytic activity of silkworm by Pearson's correlation coefficients using genetic model for 'Statistica' version 8.0 software (Statsoft Inc., Tulsa, OK, USA)

17.2 **Organization of Work Elements**

Name of Scientists	Designation	Time	Organization of work elements
1. Dr S Chattopadhyay	PI	45%	• Fungal culture and test of antifungal potential of designed AMPs through *in-vitro* assay • Determination of antimicrobial peptide activity on plant surface (30%) • Estimation of fungal biomass using qPCR • Compilation of analyzed data and all sorts of report writing
2. Dr R Banerjee	CI	35%	• Determination of antimicrobial peptide activity on plant surface (70%) • Isolation of fungal DNA • Analysis of data using 'Statistica'
3. Ms Pooja Makwana	CI	20%	• Assessment of hemolytic activity on silkworm

17.3 **Proprietary / Patented items, if any, expected to be used for this Project**:

Nil

17.4 **Suggested plan of action for utilization of the expected outcome from the project:**

- Identified promising AMPs will be recommended as a complementary process to currently used fungicides based control of MLS and FRR.
- Quantification of fungal DNA and sequencing of trochothecenes gene(s) and ITS region for MLS and FRR and deposit the information for web enabled database for future use.

17.5 **Time Schedule of activities giving milestones**:

#	Activity	Milestones	Expected date of	
			Starting	**Completion**
1.	Assessment of disease protection potential of synthetic AMPs against MLS and FRR *in vitro*	Procurement of equipment /chemicals/ synthesis of peptides/engagement of JRF	Beginning of 1st quarter	End of 2nd quarter
		Isolation and single spore culture of MLS and FRR pathogen propagules	Beginning of 3rd quarter	End of 4th quarter
		Testing of *in vitro* antifungal potential of AMPs	Beginning of 4th quarter	End of 7th quarter
		Assessment of effective doses of AMPs on growth and sporulation of fungal pathogens *in vitro*	Beginning of 5th quarter	End of 8th quarter
2	Assessment of disease protection potential of selected AMPs against MLS and FRR ex	Establishment of potted plants for inoculum based assay.	Beginning of 6d quarter	End of 7th quarter
		Ex vivo assessment of MLS and FRR	Beginning at 7th	End at 10th

	vivo	disease control abilities of AMPs by phenotyping	quarter	quarter
3	Determination of disease suppression ability of selected AMPs using quantitative PCR	Isolation of fungal and plant DNA from the infected plants treated with selected AMPs	Beginning of 7th quarter	End of 7th quarter
		Standardization of RT-PCR procedure and primer selection	Beginning of 7th quarter	End of 9th quarter
		Detection of pathogen DNA and quantification of fungal biomass using RT-PCR [*work will be done in the Molecular genetics laboratory of VCKV, Kalyani, Nadia under the supervision of Prof. Somnath Bhattacharya*]	Beginning of 9th quarter	End of 11th quarter
4.	Determination of the hemolytic activity of the selected AMPs against silkworm	Collection of silkworm hemolymph from 5th instar larvae of *Bombyx mori* after treatment of selected AMPs	Beginning of 10th quarter	End of 10th quarter
		Measurement of hemolytic activity using selected AMPs	Beginning of 10th quarter	End of 11th quarter
5	*Data analysis, compilation of results, report preparation and submission of report*		Beginning of 12th quarter	End of 12th quarter

17.6 **Project Implementing Agency /Agencies**:

Name of the agency	Address of the agency	Proposed Research Aspects	Proposed Amount	Cost Sharing %
Central Silk Board	Central Silk Board, Ministry of Textiles, Govt. of India, Bangalore		16.41	100%

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PART-IV: BUDGET PARTICULARS

18. **BUDGET** (in Lakh): [In case of multi-institutional projects, the budget details should be provided separately for each of the Institute]

A. Non-Recurring	**Year 1**	**Year 2**	**Year 3**	**Total**
Microplate reader	5.0	-	-	5.0
B. Recurring	**Year 1**	**Year 2**	**Year3**	**Total**
B.1 Manpower (1JRF)	1.58	1.58	1.85	5.01
B.2 Consumables	1.5	1.6	1.0	4.1
B.3 Travel	0.3	0.3	0.2	0.8
B.4 Contingency	0.5	0.6	0.4	1.5
Sub Total (B.1+B.2+B.3+B.4)	3.88	4.08	3.45	11.41
Total	8.88	4.08	3.45	16.41

Manpower:

Requested manpower (1 JRF) in absolutely essential to carry out the extensive studies planned to be carried out fungal culture, ex vivo experiment to collect disease reaction and phenotyping based selection of AMPs over multiple seasons/years, primer selection / standardization and RT-PCR works.; establishing/standardizing DNA analysis capability at CSRTI.

Consumables:

The proposed work is experiment-intensive involving huge numbers inoculums preparation, designing of AMPs, PCR reactions, DNA-amplicon separations, therefore needs relatively high consumable support.

Contingency

The proposed study requires intensive equipment support. The contingency amount may be utilized for repairing of equipment and other miscellaneous expenditure.

PART-V: EXISTING FACILITIES

19. Available equipment and accessories to be utilized for the project:

SI. No.	**Name of the Equipment/ Accessory**	**Make**	**Funding Agency**	**Year of Procurement**
1	Palm Cycler	Genetix	CSB	2013
2	Nanodrop spectrophotometer	Thermafisher	CSB	2016
3	Ultra-freezer (-80°C)	Thermo	CSIR	2006
4	Medium speed cold centrifuge	Remi	CSB	2002
5.	Spinwin	Tarson	CSB	2015
5	Quick freezer	Remi	DBT	2011
6	Horizontal gel electrophoresis set with power pack	Atto Corporation	DBT	2007
7	Gel documentation system	Vilber Lourmat(France)	CSB	2012
8	Gel electrophoresis unit	Tarson	DBT	2011

PART-VI: DECLARATION / CERTIFICATION

It is certified that

a. The research work proposed in the project does not in any way duplicate the work already done or being carried out elsewhere on the subject.

b. The same project has not been submitted to any other agencies for financial support.

c. The emoluments for the manpower proposed are those admissible to persons of corresponding status employed in the institute/ university or as per the Ministry of Science & technology guidelines (Annexure-III).

d. Necessary provision for the project will be made in the Institute in anticipation of the sanction of the scheme.

e. If the project involves the utilization of genetically engineered organism , it is agreed that we will ensure that an application will be submitted through our institutional bio-safety committee and we will declare that while conducting experiments, the bio-safety guidelines of the Department of Biotechnology would be followed in toto.

f. If the project involves field trials / experiments / exchange of specimens etc we will ensure that ethical clearances would be taken from the concerned ethical committees of Biotechnology before implementing the project.

g. It is agreed by us that any research outcome or intellectual property right(s)on the interven (s) arising out of the project shall be taken in accordance with the instructions issued with the approval of the Ministry of Finance . Department of Expenditure as contained in annexure-V

h.. We agree to accept the terms and conditions as enclosed in Annexure-IV. The same is signed and enclosed.

i. The institute agrees that the equipment, the basic facilities and such other administrative facilities as per terms and conditions of the grant will be extended investigators through out the duration of the project .

j. The institute assumes to undertake the financial and other management responsibilities of the project.

Signature of Executive Authority of
Institute with Seal & date

Signature of Project Co-ordinator with Seal & date
[Applicable for inter-institutional Projects only]

Signature of Principal Investigator & date	**Signature of Co-Investigator-I & date**	**Signature of Co-Investigator-II & date**

CURRICULUM VITAE of Principal investigator

Name	**Soumen Chattopadhyay** Scientist -D Biotechnology Laboratory Central Sericultural Research & Training Institute Berhampore 742 101 E-mail: soumenchatto@rediffmail.com
Date of Birth	15th July, 1961
Nationality	Indian
Sex	Male
SC / ST	No

Educational (Post graduation onwards and professional career):

Sl.No	Institution Place	Degree Awarded	Year	Award / prize / Certificate
1	University of Kalyani West Bengal, India	M. Sc. (Botany)	1985	**First Class** **Specialization**: Plant Physiology & Biochemistry. **Dissertation topic:** Partial purification and characterization of α-amylase from different rice genotypes
2.	University of Calcutta, West Bengal, India	Ph. D (Botany)	1991	**Thesis topic:** Studies on polyamines in relation to source and sink organ in plants **Supervisor:** Prof. Bharati Ghosh, Dept. of Botany, Bose Institute, Kolkata

Position and Honors

Post Doctoral Fellow / SRF / JRF Dept. of Botany, Bose Institute 93/1 A. P. C Road , Kolkata 700 009 1985 – 1988 JRF 1988 - 1990 SRF 1991 - 1992 RA ***Enjoyed following research fellowships:*** • RA-ship in DBT funded "Centre for Plant Molecular Biology", Bose Institute, Kolkata • CSIR Ad hoc SRF, New Delhi • ICAR , New Delhi • Bose Institute , Kolkata	**Major exposure during research fellowships:** **a)** Elucidation of polyamine metabolic pathways in rice, pea and black gram. **b)** Evaluation of polyamine induced changes in source and sink organ photosynthesis. **c)** Evaluation and characterization of argininine decarboxylase activity during water deficit stress in rice.

Professional Training undergone:

Topic of professional training	Organised by and venue	Period
Post M Sc course work	Bose Institute, Kolkata	06 months
SRA Foundation course	CSR&TI, Central Silk Board Berhampore,	06 months
Computer application	Murshidabad Institute of Technology, Berhamore, Murshidabad	06 days
Statistical methods for Sericulture research	Central Silk Board, Bangalore and IIM, Bangalore	04 days
Dimension of Nanotechnology: Science, Technology and Society	DST-NIAS workshop, National Institute of Advance studies, IISc-campus, Bangalore	06 days

Membership of Professional Societies:

(a) Member Plant Physiology Forum, India
(b) Life member Indian Association of Cultivation of Sciences, Kolkata
(c) Member The Indian Science Congress Association, Kolkata
(d) Member National Academy of Sericulture Sciences
(e) Member Indian Society of Genetics & Plant Breeding, New Delhi

Publications (Numbers only):

Books : 2 (chapters) Research Papers, Reports : 40 General articles : 2
Patents : 1 Others (Please specify) : Nil

Selected peer-reviewed publications:

1. **Chattopadhyay S**, Maitra N, Ghosh B, Sen SP (1988) Effect of polyamines on photosynthesis of source and sink organs in rice (*Oryza sativa* L.). *Plant Cell Physiology* (Springer) 29: 1207 – 1213.
2. **Chattopadhyay S**, Ghosh B (1990) Effect of spermine on chloroplastic metal ion efflux of source and sink organs. *Phytochemistry* (Great Britain) 29: 45 – 48.
3. **Chattopadhyay S,** Chattopadhay S, Ghosh B (1992) Retardation of source and sink organ senescence in rice by spermine. *Indian Journal of Experimental Biology* (CSIR) 30: 231 – 234.
4. Lahiri K, **Chattopadhyay S**, Chattopadhyay S, Ghosh B (1993) Biochemical changes in nodules of *Vigna mungo* during vegetative and reproductive stages of plant growth in the field. *Annals of Botany* (Oxford) 71: 485 – 488.
5. **Chattopadhyay S**, Das C, Sengupta T, Ghosh JK, Das KK, Sen SK, Pavankumar T (1996) Evaluation of leaf gas exchange parameters of five Chinese germplasms in Indian tropical conditions. *Sericologia* (France) 36: 723 – 726.
6. **Chattopadhyay S**, Maitra N, Lahiri K, Ghosh B (1996) Retardation of photosynthesis by polyamines during stipule and pod development in pea. *Photosynthetica* (Springer) 32: 629-633.
7. **Chattopadhyay S**, Maji MD, Pratheesh Kumar PM, Das KK, Saratchandra B (2002) Response of mulberry brown leaf spot fungus *Myrothecium roridum* to different plant extracts. *International Journal of Industrial Entomology* 5: 183 – 188.
8. Krishnan N, **Chattopadhyay S**, Kundu JK, Chaudhuri A (2002) Superoxide dismutase activity in haemocytes and haemolymph of *Bombyx* mori following bacterial infection. *Current Science* 83: 321 – 325.
9. Lahiri K, **Chattopadhyay S**, Ghosh B (2004) Correlation of endogenous free polyamine levels with root nodule senescence in different genotypes in *Vigna mungo L. Journal of Plant Physiology* (Elsevier) 161: 563 – 571.
10. **Chattopadhyay S**, Krishnan N, Maji MD (2006) Peroxidase activity during leaf infection of mulberry (*Morus alba* L) with brown leaf spot fungus *Myrothecium roridum*. *International Journal of Industrial Entomology* 12: 21-28.
11. **Chattopadhyay S**, Ali KA, Doss SG, Das NK, Aggarwal RK, Bandopadhyay TK, Sarkar A, Bajpai AK (2010) Evaluation of mulberry germplasm for resistance to powdery mildew in the field and greenhouse *Journal of General Plant Pathology* (Springer) 76:87–93.
12. **Chattopadhyay S**, Ali KA, Doss SG, Das NK, Aggarwal RK, Bandopadhyay TK, Sarkar A, Bajpai AK. (2011) Association of leaf micro-morphological characters with powdery mildew resistance in field-grown mulberry (Morus spp.) germplasm. *AoB PLANTS* (Oxford) plr002 doi:10.1093/aobpla/plr002.
13. **Chattopadhyay S**, Tikader A, Das NK (2011) Nondestructive, simple, and accurate model for estimation of the individual leaf area of som (*Persea bombycina*). *Photosynthetica* (Springer) 49: 627-632.
14. **Chattopadhyay S,** Doss SG, Halder S, Ali KA, Bajpai AK (2011) Comparative micro-propagation efficiency of diploid and triploid mulberry (*Morus alba* cv. S1) from axillary bud explants. *African Journal of Biotechnology* (Academic Journal) 10: 18153-18159
15. **Chattopadhyay S** , Sangma CD, Tikader A, Rajan RK, Bindroo BB (2014) Assessment of som (*Persea bombycina* Kost.) clones for resistance against leaf spot pathogen *Phyllosticta persae* under field condition. *Tropical Plant Pathology* (Brazil) 39: 259-264.
16. R Banerjee, **S Chattopadhyay,** Saha AK (2016) Genetic Diversity and Relationship of Mulberry Genotypes Revealed by RAPD and ISSR Markers. *J Crop Improvement* (in press)

Research Support

Ongoing/Completed Research Projects: ***last 10 years***

Sl No.	Title of Project	Funding Agency	Amount (Rs. in lakh)	Date of sanction and Duration
1	Assessment of promising powdery mildew resistant mulberry lines for prospective commercial use	CSB	3.32	2015-2018
2	Development, characterization and validation of expressed sequence tag derived microsatellite markers for mulberry (*Morus* spp.)	CSB	2.95	Sept 2014 3 years
2	Development of DNA markers based genetic linkage map of mulberry and QTL analysis for agronomically important *planta* traits	DBT (with CCMB)	22.07*	March 2011 3 years
4	Development, validation and utilization of SCAR marker(s) for powdery mildew (*Phyllactinia corylea*) resistance in mulberry	DBT (with CCMB)	15.54*	November 2009 (4.5years)
5	Identification of DNA markers for disease and pest resistance in mulberry (Morus spp.)	DBT [Network project with 4 other Centers]	9.03*	Dec 2005 3.5 years.
6	Development of high frequency regeneration protocol from leaf disc explants in mulberry.	CSB	12.45	Jan. 2006. 3 years
7	Field evaluation of acaciasides for the control of root knot and foliar mulberry diseases.	Ministry of textiles [Collaborative project with Dept. of Zoology, Visva Bharati]	6.8	July 2004 3 years
8	Induction and preliminary characterization of systemic resistance in mulberry against *Myrothecium roridum* infection	CSB	9.45	Apr., 2005 3 years

CSR&TI, Berhampore component.

CURRICULUM VITAE of Co- investigator-I

Name	**Rita Banerjee** Scientist -D Biotechnology Laboratory Central Sericultural Research & Training Institute Berhampore 742 101 E-mail: rita_csb@rediffmail.com
Date of Birth	15th April, 1961
Nationality	Indian
Sex	Female
SC / ST	No

Educational (Post graduation onwards and professional career):

Sl.No	Institution Place	Degree Awarded	Year	Award / prize / Certificate
1	Calcutta University West Bengal, India	M. Sc. (Genetics& Plant Breeding)	1985	**First Class** **Specialization**: Genetics& Plant Breeding **Dissertation topic:** Genetic parameters and path-coefficient analysis in Rosselle (*Hibiscus sabdariffa* L.)
2.	Bidhan Chandra Krishi Viswavidyalaya, West Bengal,India	Ph. D (Genetics& Plant Breeding)	1991	**Thesis topic:** Assessment of mutagenic effects of mytomycin and streptomycin on tossa jute (*Corchorus olitorius* L) **Supervisor:** Prof. S.C.Rakshit, Dept. of Genetics & Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, West Bengal

Position and Honors

Post Doctoral Fellow / SRF / JRF Dept. of Genetics & Plant Breeding, Bidhan Chandra Krishi Viswavidyalaya, West Bengal 1985 – 1988 JRF 1988 - 1990 SRF ***Enjoyed following research fellowships:*** • Hindusthan Lever Ad-hoc SRF in the project entitled "Evaluation of male sterile lines in jute by genetic manipulations", BCKV, West, Bengal • Hindusthan Lever Ad hoc JRF	**Major exposure during research fellowships:** **d)** Induction of male sterility in jute by chemical mutagens. **e)** Detection and isolation of photoperiodic insensitive lines. **f)** Evaluation and characterization of mutagen treated population for important yield traits

Professional Training undergone:

Topic of professional training	Organized by and venue	Period
Intensive Bivoltine Training	CSR&TI, Central Silk Board Berhampore,	45days
Intensive Bivoltine Training	CSR&TI, Central Silk Board Mysore,	45 days
Summer School Training	SSTL, Central Silk Board, Kodathi, Bangalore	10 days
Computer application	Murshidabad Institute of Technology, Berhamore, Murshidabad	06 days
Basic techniques and application procedures of DNA markers	Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal	20 days
Basic training programme /exposure to molecular biology techniques	SBRL, Central Silk Board, Kodathi, Bangalore	10 days
Exposure training on molecular biology at lab	SBRL, Central Silk Board, Kodathi, Bangalore	03 days
Training workshop on "Winning Research Proposals"	NAARM, Hyderabad	23 day

Membership of Professional Societies:

a) Life Member National Academy of Sericulture Sciences

b) Member Indian Society of Genetics & Plant Breeding, New Delhi

c) Publications (Numbers only): Research Papers, Reports : 40 General articles : 06 Book chapter : 01; Brochures:01

Selected peer-reviewed publications (In chronological order) :

1. **Banerjee R**, S Chattopadhyay, A K Saha and S. Nirmal Kumar(2014) *Archives Of Phytopathology And Plant Protection*(Taylor & Francis), DOI: 10.1080/03235408.2013.868693
2. **Banerjee R**, S Chattopadhyay,N K Das, S G Doss, A K Saha and S. Nirmal Kumar(2014) *Journal of Crop Improvement*(Taylor & Francis), 28:305–323
3. **Banerjee, R.**, Chattopadhyay, S., Sarkar**,** S., L.alitha, L.,Saha A.K. and Bindroo, B.B (2013) Proceedings of International Seminar on Bioresources and Human Sustenance, pp.153-159. Cotton College, Guwahati, Assam.
4. Chattopadhyay, S., Ali, K.A. , Doss, S. G., **Banerjee, R.,** Saha, A K., Sarkar, A. And Bindroo, B. B. (2013) Proceedings of international seminar on bioresources and human sustenance PP.143-152. COTTON COLLEGE, GUWAHATI, ASSAM
5. **Banerjee, R.,** Das, N.K., Doss, S.G., Saha, A.K., Bajpai, A.K and Bindroo B.B. (2012) *Eur. J Pl. Pathol*. (Springer) doi: 10.1007/s10658-011-9894-z
6. **Banerjee, R.**, Roychowdhuri, S., Sau, H., Das, B.K., Saha, A.K., Saratchandra, B. And Bajpai, A.K. (2011) *J. Crop Improvment* (Taylor & Francis) doi: 10.1080/15427528.2011.583715
7. **Banerjee, R**., Ghosh, S., Doss, S.G., Saha, A.K., Bajpai, A.K and Khatri, R.K. (2011) *Ind.J. Genet* 71(4): 392-396.
8. **RitaBanerjee**, Manas Dev Maji,Pannalal Ghosh&Amitabh Ssarkar(2009). *Archives of Phytopathology and Plant Protection*. (Taylor & Francis) 42:291-297.
9. **Banerjee R,** Das NK, Maji MD, Mandal K, Bajpai AK (2009). *Indian. J. Genet*.69:292-296.
10. **Rita Banerjee**, Sukhen Roychowdhuri, Haradhan Sau, Bimal Kumar Das, Pannalal Ghosh & Beera Saratchandra (2007). *Journal Of Genetics And Genomics*. (ELSIVIER)34:691-697.
11. **R.Banerjee**, S.Roy Chowdhuri, H.SAU, B.K.DAS, P.L.GHOSH AND A.SARKAR (2007) *The Iindian Journal* of Agricultural *Research*78:142-145.
12. **Banerjee R,** Chakroborty SP, Das BK (2006). *INDIAN. J. GENET*.66:134-136.
13. Mondal BK, Dhara MC, Mondal BB, Das SK And **Nandy R** (1989) Effect of intercropping on the yield components of rice, mungbean soybean, peanut and blackgram *J. Agronomy*.162:34:34
14. Mondal BK., Dhara MC, Mondal BB, Das SK and **Nandy R** (1990) Rice, Mungbean Soybean, Ricebean and Blackgram when grown as sole and intercrops. *Agronomy J.* 82:1063-66.
15. **Rita Banerjee,** S Ghosh, SG Doss, AK Saha, AK. Bajpai and RK Khatri (2011) Morphological, anatomical and molecular characterization of full-sib pseudo-F_2 (F_1) progenies in mulberry with resistance to bacterial leafspot (*Xanthomonas campestris* pv. *mori*) *Indian J. Genet.*, **71**: 356-362.
16. **R Banerjee,** S Chattopadhyay, Saha AK (2016) Genetic Diversity and Relationship of Mulberry Genotypes Revealed by RAPD and ISSR Markers. *J Crop Improvement* (in press).

Research Support : Ongoing/Completed Research Projects: ***last 10 years***

#	Title of Project	Funding Agency	Amount (Rs. in lakh)	Date of sanction and Duration
1.	Development of DNA marker based genetic linkage map of mulberry and QTL analysis for agronomically important *planta* traits-PI	DBT [Collaboration with CCMB, Hyderabad	22.07*	2011-2014
2	Development, validation and utilization of SCAR markers for powdery mildew (Phyllactinia corylea) resistance in mulberry-PI	DBT [Collaboration with CCMB, Hyderabad]	15.54*	2009-2014
3	Identification of DNA markers associated with bacterial leaf spot resistance in mulberry-PI	CSB	5.55	2013-2015
4	Screening of germplasm and raising of progeny towards development of disease resistant mulberry against bacterial leaf spot-PI	CSB	10.67	2005-2010
5	Development of weather based forewarning system of mulberry diseases-CI	CSB	39.51	2005-2008
6	Development, characterization and validation of expressed sequence tag derived microsatellite markers for mulberry (*Morus* spp.)-CI	CSB	2.95	2014-2017
7	Assessment of promising powdery mildew resistant mulberry lines for prospective commercial use-CI	CSB	3.32	2015-2018

CSR&TI, Berhampore component.

CURRICULUM VITAE of Co- Investigator-II

Name	**Pooja Makwana** Scientist -B Biotechnology Laboratory Central Sericultural Research & Training Institute Berhampore 742 101 E-mail: pooja.may16@gmail.com
Date of Birth	16th May 1989
Nationality	Indian
Sex	Female
SC / ST	No

Educational (Post graduation onwards and professional career):

Sl.No	Institution Place	Degree Awarded	Year	Award / prize / Certificate
1	VIT University, Vellore (T.N.), India	M. Sc. (Biomedical Genetics)	2012	**First Class** **Specialization**: Biomedical Genetics **Dissertation topic:** Genotoxicity study of Neem oil Nanoemulsion on Peripheral Blood Mononuclear cells(PBMCs)
2.	Mysore University, Mysore (Karnataka), India	Ph. D (Biotechnology)	Pursuing	**Thesis topic:** Cytotoxicity and detoxification response in the silkworm *Bombyx mori* induced by uzifly *Exorista bombycis* **Supervisor:** Dr. A. R. Pradeep, Scientist-D, SBRL, Bangalore

Position and Honors

2013 – 2015 JRF/SRF- in DBT funded project- "Host-parasite interaction: Transcriptome responses of the silkworm Bombyx mori against parasitism"	**Major exposure during research fellowships:** **g)** Microarray analysis and gene expression studies by quantitiative PCR. **h)** Handling of SDS-PAGE, ELISA, HPLC and Real Time PCR. **i)** Cytotoxicity assays on hemocytes of silkworm in *in vitro* system.

Professional Training undergone:

Topic of professional training	Organized by and venue	Period
Foundation Training for newly inducted Scientist-B	CSB, Central office. Bangalore	15days

Other:
Worked on a mini project- "Environmental and Genetic factors in determining the severity and progression of Spinocerebellar Ataxia in Vellore/India" under the Dr. Sonali Sengupta in 3rd International Science, Engineering and Technology Conference (2011).

Selected peer-reviewed publications (In chronological order) :

1. Pradeep AR, Anitha J, Panada A, **Pooja M**, Awasthi AK, Geetha NM, Ponnuvel KM, Trivedy K (2015) Phylogeny of host response proteins activated in silkworm *Bombyx mori* in response to infestation by dipteran endoparasitoid revealed functional divergence and temporal molecular adaptive evolution. J Clin Cell Immunol. 6:370. Doi:10.4172/2155-9899.1000370.
2. Jerobin J, **Makwana P**, Suresh KRS, Rajiv S, Mukherjee A, Chandrasekaran N (2015) Antibacterial activity of neem nanoemulsion and its toxicity assessment on human lymphocytes in vitro. Internat J Nanomedicine, http://dx.doi.org/10.2147/IJN.S79983.
3. Rajiv S, Jerobin J, Saranya V, Nainawat M, Sharma A, **Makwana P**, Gayathri C, Bharath L, Singh M, Kumar M, Mukherjee A and Chandrasekaran N (2015) Comparative cytotoxicity and genotoxicity of cobalt (II, III) oxide, iron (III) oxide, silicon dioxide, and aluminum oxide nanoparticles on human lymphocytes in vitro. Human and Experimental Toxicology, DOI: 10.1177/0960327115579208.