IMPROVEMENT OF LEAF QUALITY AND PRODUCTIVITY THROUGH EXTERNAL APPLICATION OF SEAWEED EXTRACTS IN MULBERRY

PROJECT: PIN-3587
SUBMITTED TO

CENTRAL SILK BOARD
MINISTRY OF TEXTILES (GOVERNMENT OF INDIA)
BTM LAYOUT, MADIVALA
BANGALORE – 560 068

October, 2016- September, 2017

SUBMITTED BY

Mr. ANIL PAPPACHAN
SCIENTIST-B

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CENTRAL SERICULTURAL RESEARCH & TRAINING INSTITUTE,
CENTRAL SILK BOARD, MINISTRY OF TEXTILES: GOVT. OF INDIA,
BERHAMPORE - 742 101, WEST BENGAL, INDIA

PART I: GENERAL INFORMATION

1. **Name of the Institute / University /Organization submitting the Project Proposal:**

Central Sericultural Research &Training Institute, Berhampore -742101, Murshidabad, West Bengal.

2. **Status of the Institute (s):**

Research & Development of Mulberry sericulture

3. **Name (s) and designation(s) of the Executive Authority of the Institute/ University forwarding the application:**

Dr. Kanika Trivedy, Director, Central Sericultural Research & Training Institute, Berhampore - 742101, Murshidabad, West Bengal.

4. **Project Title:**

Improvement of leaf quality and productivity through external application of seaweed extracts in mulberry

5. **Category of the Project:** Applied

6. Specific Area: Enhancing mulberry leaf quality and productivity with organic inputs

7. Duration: 1 year (October, 2016 – September, 2017)

8. Total Cost: Rs. 42,500.00

9. **Is the Project single Institutional 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.

11a). Summary of the Project Proposal:

Disadvantages of chemical inputs have led farmers turning towards organic sources. To meet increasing demand of organic inputs, among many viable options, one option is the use of seaweed extracts as a source of nutrients and hormones. Use of seaweed formulations as biostimulants in crop plants is well established. In agriculture and horticulture, application of seaweed has proved beneficial for the increased growth and yield, delayed senescence, improved plant vigour, quality and quantity of produce. Seaweed extracts are applied to crops as root dips, soil drenches or foliar sprays. Seaweed extracts contain major and minor nutrients, amino acids, vitamins, and also cytokinins, auxins, and ABA like growth substances. Most of

these compounds have synergistic effect and their individual application may be less effective. However, the biostimulatory potential of many of these products has not been exploited in sericulture due to the lack of scientific data on this aspect.

This study is formulated to evaluate effect foliar application of *Ascophyllum nodosum* and *Kappaphycus alvarezii* extracts in enhancing growth, yield, and quality of mulberry leaf in the field. Commercially available new seaweed extracts will be applied at various concentration and time interval to standardize the dosage and interval of application. Data on yield, quality parameters and disease incidence will be recorded and statistically analyzed. Treatments giving promising results in first crop will be identified to standardize the dosage and time interval and will be carried forward to confirm consistency. Bioassay on silkworm larvae fed with seaweed extract treated mulberry leaves will be conducted in the last crop.

11b). Objectives:

i. To determine the effect of application of seaweed extracts on growth and biomass in mulberry leaves.
ii. To standardize the dosage and time interval for the application of seaweed extract.
iii. To determine the effect of application of seaweed extracts on foliar diseases of mulberry.
iv. To perform bioassay on silkworm larvae fed with seaweed extract treated mulberry leaves.

PART II: PARTICULARS OF INVESTIGATORS

12.

i)	Name	**ANIL PAPPACAHN**
	Year of birth	1989
	Sex	Male
	Principal Investigator or CI	**Principal Investigator,**
	Designation & Department	Scientist-'B', Moriculture-II
	Institute / University : Address	CSR&TI., CSB., Berhampore - 742 101, (WB).
ii)	Name	**Dr. R. Mahesh**
	Year of birth	1985
	Sex	Male
	Principal Investigator or CI	**Co-Investigator (1),**
	Designation & Department	Scientist-'B', Moriculture-I
	Institute / University : Address	CSR&TI., CSB., Berhampore - 742 101, (WB).

iii)	Name	**N. Chandrakanth**
	Year of birth	1986
	Sex	Male
	Principal Investigator or CI	**Co-Investigator (2),**
	Designation & Department	Scientist-'B', Silkworm Breeding and Genetics,
	Institute / University : Address	CSR&TI., CSB., Berhampore - 742 101, (WB).

13. No. of Projects being handled by each investigator at present:

Principal Investigator: Nil
Co- Investigator 1: Nil
Co- Investigator 2: Nil

14. Proposed Research Fellows: Nil

PART III: TECHNICAL DETAILS OF PROJECT

15. Introduction: Project Background

Mulberry is one of the most important commercial crops grown extensively as food plant for silkworm. Mulberry (*Morus* spp.) is a perennial and high biomass producing plant, continues to grow throughout the year in tropics. The continuous production of mulberry for a long time results in gradual reduction in leaf yield and quality (Rashmi *et al*., 2009). In India, mulberry is cultivated in about three lakh hectares in different agro climatic conditions varying from temperate to tropical. The silkworm, *Bombyx mori* L. being monophagous insect, derives almost all the nutrients for growth and development from the mulberry leaf. It has been estimated that, nearly 70% of the silk proteins are derived from mulberry leaves. Hence, silkworms should be fed with good quality mulberry leaves in abundant quantity for the successful cocoon production (Vijaya *et al*., 2009).

The leaf yield and quality of mulberry depends on the soil type, plant variety, and availability of plant nutrients and agro-ecological conditions, which reflects on the quality of silk production. In India, mulberry contributes to an extent of 38.20 per cent for successful cocoon crop production (Miyashita, 1986). Though synthetic chemicals, fertilizers, pesticides, herbicides, growth promoters and other inputs enhance productivity of mulberry but they adversely affect the ecosystem. So, it is essential to standardize the techniques for mulberry cultivation and also technological innovations are to be exploited to achieve the objective of higher mulberry productivity with organic inputs like seaweed.

Seaweed is a macroscopic, multicellular, marine alga that lives near the seabed. Extracts from marine seaweeds could replace synthetic chemicals because they contain high levels of organic matter, micro nutrients, vitamins and fatty acids and also growth regulators such as auxins, cytokinin and gibberellins. Unlike, synthetic chemicals, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous. Their beneficial effects include enhanced root growth, leaf growth, yield, tolerance to different plant stresses

and increase in plant resistant to infections or insect attack.

There is a long history of coastal people using seaweeds to fertilize soil for crop production but due to bulky nature; these are not usually carried very far. Algal biomass has also been composted and then used for growing crops on various types of soil (Mc Hugh., 2003). Seaweed extracts are considered as an organic agriculture input as they are environmentally benign and safe for the health of animals and humans (Khan *et al*., 2009). Application of seaweed extract as organic biostimulant is fast becoming accepted practice in agriculture due to its beneficial effect (Russo and Beryln., 1990 and Verkleij., 1992).

Seaweed polysaccharides and oligosacchairdes derived from green, brown and red seaweeds (marine macroalgae) corresponding to ulvans, alginates, fucans, laminarin and carrageenans act as elicitors that bind to receptors on plasma membrane and probably trigger an initial oxidative burst at local level and/or help in the activation of salicylic (SA) and/or jasmonic acid (JA) and/or ethylene signaling pathways at systemic level.

Seaweed extracts contain plant growth regulators (PGR) like auxins, cytokinins, and gibberlins which control the growth and structural development of mulberry. Auxins regulate development of plant roots and buds. Cytokinins promote growth *via* rapidly speeding up the process of cell division. When they are applied to foliage the leaves rejuvenate stimulating photosynthesis. Thus they stay green longer, delaying senescense. Seaweeds enhance photosynthesis *via* increasing a plants chlorophyll levels. By increasing the level of chlorophyll, plant would be able to efficiently harness the sunlight increasing yield. Seaweed extracts also stimulate beneficial soil microbial activity, particularly in the pockets of soil around the feeder roots resulting in a substantially larger root mass, where the beneficial fungi known as "mycorrhizae" make their home.

However studies on utilization of various seaweed extracts in sericulture is scarce and it is necessary to study the effect of seaweed extracts in enhancing the quality and productivity of mulberry leaves for the optimal utilization of seaweeds in sericulture.

15.1 DEFINITION OF THE PROBLEM:

The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable agriculture. Chemical agriculture has made an adverse impact on not only soil but also on the beneficial soil microbial communities and the plants cultivated in these soils. Organic farming is proving as a remedy to the ills of modern chemical agriculture. To meet the increasing demand of organic fertilizer many viable options have to be explored (Chhaya, 1997) and one such option is the use of seaweed extracts as source of nutrient (Zodape, 2001).

Brown seaweeds are the second most abundant group comprising about 2,000 species which reach their maximum biomass levels on the rocky shores of the temperate zones. They are the type most commonly used in agriculture (Blunden and Gordon 1986) and, among them, *Ascophyllum nodosum* (L.) is the most researched (Ugarte *et al.,* 2006). Besides *A. nodosum,*

other brown algae such as *Fucus* spp., *Laminaria* spp., *Sargassum* spp., and *Turbinaria* spp. are used as biofertilizers in agriculture (Hong *et al.,* 2007). *Kappaphycus alvarezii*, which is widely cultivated for its carageenan content, has not been much tried as a source of organic matter and nutrients. Therefore present study attempts to evaluate the potential of *Ascophyllum nodosum* and *Kappaphycus alvarezii*, in enhancing the growth, yield and quality of mulberry under field condition.

15.2 ORIGIN OF THE PROPOSAL / RATIONALE OF THE STUDY:

Application of manures, fertilizers and plant growth stimulants is one of the important ways for increasing mulberry leaf yield. Synthetic chemicals, fertilizers, growth promoters and other outputs though enhance productivity of mulberry they adversely affect the ecosystem (Patil *et al.,* 2006). The use of chemicals in plant growth promotion and disease management leads to environmental hazards and development of new races of pathogen. Future strategy for sericulture would be use of natural compounds like that is present in seaweeds. Since these seaweed are abundantly available, there is further scope to utilize the available seaweed extracts and further identify new seaweed molecules that stimulate mulberry growth.

Liquid extracts obtained from seaweeds has recently gained much interest as soil and foliar spray for inducing shoot growth and yield in orchards and horticultural plants. Seaweed extracts were found superior than chemicals because of the presence of high level of organic matter, micro nutrients, vitamins and fatty acids and also growth regulators such as auxins, cytokinin and gibberellins (Crouch and Van Staden., 1994).

Some 15 million metric tonnes of seaweed products are produced annually (FAO., 2006), a considerable portion of which is used for nutrient supplements and as biostimulants or biofertilizers to increase plant growth and yield. Seaweed extract (SWE) is a new generation of natural organic fertilizers containing highly effective nutrients and promotes faster growth and increase the yield of many crops. Seaweeds also provide protection against broad range of pathogens attacking plants. Although many of the various chemical components of seaweed extracts and their modes of action remain unknown, it is possible that these components exhibit synergistic activity (Fornes *et al.,* 2002 and Vernieri *et al.,* 2005).

Beneficial effects of seaweed extracts include enhanced leaf growth, plant yield, root growth, tolerance to different plant stresses and increase in plant resistant to infections or insect attack (Maria Victorial Rani and Revathy., 2009; Maria Victorial Rani *et al.,* 2011 and Usha *et al.,*2013). Moreover application of seaweed extract increased chlorophyll content of the leaves (Thirumaran *et al.,* 2009). Seaweed components such as macro and microelement nutrients, amino acids, vitamins, cytokinins and auxins affect cellular metabolism in treated

plants leading to enhanced growth and crop yield (Stirk *et al.,* 2003; Crouch and Van Staden, 1992). According to Crouch and van Staden 1993, Seaweed extracts are bioactive at low concentrations (diluted as 1:1000 or more).

Marine algal seaweed species are often regarded as an underutilized bioresource in sericultre, though many have been used as a source of food, industrial raw materials, and in therapeutic and botanical applications for centuries. Moreover, seaweed and seaweed-derived products have been widely used as amendments in crop production systems due to the presence of a number of plant growth-stimulating compounds. However, the biostimulatory potential of many of these products has not been fully exploited due to the lack of scientific data in this area in mulberry cultivation. Seaweeds are considered to be renewable bioresource in sustainable agricultural systems (Khan *et al.,* 2009).

Nutrients present in the soil are not well absorbed in deep rooted plants and translocation of nutrients to shoot is sluggish under adverse soil condition which favour soil fixation of nutrients. Mulberry (*Morus* spp.) as a deep rooted high biomass producing foliage crop, responds well to foliar nutrition. Foliar application in right time increases level of absorption in specific nutrients to the leaf during growth and development (Narahari *et al*., 2001). It is likely that mulberry would benefit from foliar application of seaweed extracts.

"An 'appropriate technology' is usually characterized as small scale, energy efficient, environmentally sound, labor-intensive, and controlled by the local community. It must be simple enough to be maintained by the people who use it. In short, it must match the user and the need in complexity and scale and must be designed to foster self-reliance, cooperation, and responsibility." (Amadei, 2004)

Present study is planned to evaluate the efficacy of different concentrations of newly available seaweed extracts (*Ascophyllum nodosum* and *Kappaphycus alvarezii*) in enhancing the growth, yield and quality of mulberry under field condition.

15.3. RELEVANCE TO THE CURRENT ISSUES AND EXPECTED OUTCOME:

Disadvantages of chemical inputs are turning farmers towards organic sources. To meet increasing demand of organic inputs, among many viable options, one option is the use of seaweed extracts as a source of nutrients and hormones. Seaweed extracts application is expected to minimize the hazardous effects of synthetic chemicals and nutrients in the ecosystem.

External application of seaweed extract is expected to improve the quality and quantity of mulberry leaves organically which in turn would have a direct bearing on the yield and the quality of cocoon.

15.4 OBJECTIVE:

The present proposal aims to study the effect of application of new seaweed extracts (*Ascophyllum nodosum* and *Kappaphycus alvarezii*) on growth and biomass in mulberry leaves. This would also help to standardize the dosage and time interval for the application of seaweed extracts by identifying best performing treatments. It is also essential to determine the effect of application of seaweed extracts on foliar diseases of mulberry since there are many reports of seaweeds significantly minimizing the disease incidence in various crops. A bioassay would be performed to determine the effect of seaweeds on silkworm larvae.

16. REVIEW OF STATUS OF RESEARCH AND DEVELOPMENT ON THE SUBJECT

16.1 International status:

Foliar application of commercially available seaweed liquid extract (Kelpak 66) enhanced bean yield by 24% (Nelson and Van Staden, 1984).

Application of a low concentration of *Ascophyllum nodosum* extract to soil or on foliage of tomatoes produced leaves with higher chlorophyll content than those of untreated controls. This increase in chlorophyll content was a result of reduction in chlorophyll degradation, which might be caused in part by betaines in the seaweed extract (Whapham *et al*., 1993).

Field studies on winter barley (*Hordeum vulgare* cv Igri) had shown that application of seaweed extract (Maxicrop) improved winter hardiness and increased frost resistance (Burchett *et al.,* 1998).

Foliar sprays of *Ascophyllum nodosum* extract reduced *Phytophthora capsici* infection in Capsicum and *Plasmopara viticola* in grape (Lizzy *et al.,* 1998).

Commercial formulations of *Ascophyllum* extracts (Seasol) improved freezing tolerance in grapes. Grapevines sprayed with Seasol (0.8%) showed a reduction in leaf osmotic potential, a key indicator of osmotic tolerance. The treated plants showed an average osmotic potential of -1.57 MPa after 9 days of seaweed extract treatment, whereas it was -1.51 MPa in untreated controls (Wilson, 2001).

Soil application of liquid seaweed extracts to cabbage stimulated the growth and activity of microbes that were antagonistic to *Pythium ultimum*, a serious fungal pathogen that caused damping-off disease of seedlings (Dixon and Walsh 2002).

Treatment of alfalfa with the algal extracts prior to pathogen challenge resulted in an increased resistance to *Colletotrichum*. cDNA array revealed that the algal extract caused upregulation of 152 genes, mostly plant defense genes such as those involved in phytoalexin, PR proteins, cell wall proteins, and oxylipin pathways (Cluzet *et al.,* 2004).

Foliar application of commercial liquid seaweed extract from *Ascophyllum nodosum*

(Acadian Seaplants Limited), supplemented with BA and IBA, enhanced the number of propagules (crown divisions) per plant in the ornamental herbaceous perennial *Hemerocallis* sp. (Leclerc *et al.,*2006).

Norrie and Keathley (2006) have reported that *Ascophyllum nodosum* extracts showed positive effects on the yield of 'Thompson seedless' grape (*Vitis vinifera* L.) consistently over a 3-year period. They observed that *A. nodosum* extract treated plants always outperformed (in terms of berries per bunch, berry size, berry weight, rachis length, and the number of primary bunches per plant) the control maintained under the regular crop management program, and resulted in improved fruit size (13% increase), weight (39% increase), and yields (60.4% increase over the control).

Extracts of seaweed *Ascophyllum nodosum* have been shown to affect the root growth of *Arabidopsis* at very low concentrations (0.1 g / L), whereas plant height and number of leaves were affected at concentrations of 1 g / L (Rayorath *et al.,* 2008).

Foliar application of commercially available seaweed and mixture of free amino acids increased growth characteristics *viz.,* plant height, stem diameter, number of branches and leaves/ plant, total leaf area /plant, dry weight of shoots and specific growth rate in common bean plant (*Phaseolus vulgaris* L.). Treatments also significantly increased Photosynthetic pigments, total chlorophyll SPDS, total carbohydrates and crude protein in leaves of common bean (Zewail, 2014).

16.2.1. National status (in Mulberry):

Significant increase in mulberry leaf yield was recorded when 0.5 ml / L of seaweed extract from *Dictyota dichotoma* and *Kappaphycus alvaerezii* was applied twice as foliar spray at Central Sericulture Research and Training Institute , Berhampore, West Bengal, India (Tiwary *et al.,* 2014).

Application of seaweed *Kappaphycus alvaerezii* enhanced the shoot length, number of leaves, leaf area, circumference of stem, root growth and total biomass by 107%, 100%, 135%, 91%, 140% and 140% respectively at third month after the application in comparison with control. Treatment also increased total soluble protein, carbohydrate and total free amino acid contents. Length and weight of *Bombyx mori* larvae reared on seaweed extract treated leaves were significantly higher over the control. Increase of leaf biomass rich in total soluble protein, carbohydrate and total free amino acid by seaweed extract application is a desirable feature in Sericulture industry which was obtained by *Kappaphycus alvaerezii* application (Maria Victorial Rani and Evanjaline, 2015).

16.2.2. National status (in other crops): Foliar spray of *Kappaphycus alvarezii* sap (5.0%), increased yield of tomato fruit (60.89%) as compared to control, by increasing number of fruits per plant and size of fruit. With application of spray, fruit quality, macro and micro elements content increased over control. Nutrient uptake by fruit and shoot was improved with foliar application and treated plants showed resistance to leaf curl, bacterial wilt and fruit borer (Zodape *et al.,* 2011).

Application of seaweed extracts prepared from *Gracilaria corticata* var.*corticata* and *Sargassum wightii* significantly promoted the growth of chilly plants. Seedlings which received full dose of both seaweed extracts and 50 % recommended dose of fertilizers + 50 % extracts showed more growth than plants applied with 50% *S. wightii* sap and 50% *G. corticata* sap compared to control. Application of seaweed extracts reduced 50 % fertilizer requirement in chilly (Arunkumar *et al.,* 2015).

Kappaphycus alvarezii and *Gracilaria edulis* extracts were very effective in enhancing the yield, growth and improved the quality of the produce in black gram. The yield of seed had increased by 47.52% and 42.52% with the application of *K. alvarezii* and *G. edulis* extracts, respectively when applied at 10% concentration. Number of pods/plant, pod weight, seed weight/plant and test weight of seed and quality of seeds were augmented due to the application of seaweed extracts (Ganesh *et al.,* 2015).

16.3 IMPORTANCE OF THE PROPOSED PROJECT IN THE CONTEXT OF CURRENT STATUS: The recent challenges to food production due to the increasing occurrence of biotic and abiotic stresses is likely due to climate change and will further reduce yields and/or will have an impact on crops in the 21st century (IPCC., 2007). Therefore, research into developing sustainable methods to alleviate these stresses should be a priority. Various studies have shown that seaweed extracts protect plants against a number of biotic and abiotic stresses and offers potential for field application. Further seaweed extracts are considered as an organic farm input as they are environmentally benign and safe for the health of animals and humans.

The proposed project is a unique need-based eco-friendly initiative among various mulberry quality improvement strategies. Currently there is a global drive towards organic agriculture including organic silk. In sericulture, systematic study on utilization of seaweed is yet to be initiated and this study is expected to throw some light on the subject.

16.4 ANTICIPATED PRODUCTS, PROCESSES/ TECHNOLOGY PACKAGE INFORMATION OR OTHER OUTCOME FROM THE PROJECT AND THEIR EXPECTED OUTCOME:

It is anticipated that foliar application of seaweed extract would improve the quality and quantity of mulberry leaves organically, at affordable cost to the small and marginal sericulture farmers.

16.5 EXPERTISE AVAILABLE WITH PROPOSED INVESTIGATION GROUP / INSTITUTION ON THE SUBJECT OF THE PROJECT:

To carryout experiments drawn under the project required expertise and infrastructure facilities are available at the Institute. Principal Investigator has been working on the subject since last two years. Besides all the investigators are adept in running research projects/ programs as principal investigators/ Co investigators.

16.6 LIST OF FIVE EXPERTS IN INDIA IN THE PROPOSED SUBJECT AREA:

Sl. No.	Name	Designation	Address
i.	Dr. Asok Kr Biswas	Professor	Department of Botany, University of Calcutta dr.biswasak@yahoo.co.in
ii.	Dr. P.K Banerjee	Professor	Department of plant physiology, BCKV West Bengal
iii.	Dr. Malay Kumar Adak	Professor	Kalyani university , West Bengal
iv.	Dr. M.S. Sheshshayee	Professor	Department of plant physiology, UAS, GKVK, Bangalore Krisna4@bgl.vsnl.net.in
v.	Prof. Govindaiah	Professor	College of sericulture , Bangalore dr.govindaiah@gmail.com

17. WORK PLAN

17.1. Methodology

Commercially available new seaweed extracts will be applied at various concentration and time interval to standardize the dosage and interval of application. Extracts from *Ascophyllum nodosum* and *Kappaphycus alvarezii* will be evaluated. Data on yield, quality parameters and disease incidence will be recorded and statistically analyzed. Treatments giving promising results in first crop will be identified to standardize the dosage and time interval and will be carried forward to confirm consistency. Once the best performing treatments are identified bioassay on silkworm larvae fed with seaweed extract treated mulberry leaves will be conducted in the last crop.

17.1.1. Experimental details

Variety	:	S-1635	Experiment design	:	RBD
Treatments	:	13	Replications	:	3

17.1.2 Treatment details

T_1	:	Foliar application of *Ascophyllum nodosum* extract at 0.5 ml /L at 21 and 28 days after pruning (DAP)
T_2	:	Foliar application of *A.nodosum* at 1.0 ml /L at 21 and 28 DAP
T_3	:	Foliar application of *A.nodosum* extract at 2.0 ml /L at 21 and 28 DAP
T_4	:	Foliar application of *A.nodosum* extract at 0.5 ml /L at 28 DAP
T_5	:	Foliar application of *A.nodosum* extract at 1.0 ml /L at 28 DAP
T_6	:	Foliar application of *A.nodosum* extract at 2.0 ml /L at 28 DAP
T_7	:	Foliar application of *K. alvarezii* extract at 0.5 ml /L at 21 and 28 DAP
T_8	:	Foliar application of *K. alvarezii* at 1.0 ml /L at 21 and 28 DAP
T_9	:	Foliar application of *K. alvarezii* extract at 2.0 ml /L at 21 and 28 DAP
T_{10}	:	Foliar application of *K. alvarezii* extract at 0.5 ml /L at 28 DAP
T_{11}	:	Foliar application of *K. alvarezii* extract at 1.0 ml /L at 28 DAP
T_{12}	:	Foliar application of *K. alvarezii* extract at 2.0 ml /L at 28 DAP
T_{13}	:	Water Spray (Control)

17.1.3. Seaweed extracts details

Commercially available new seaweed extracts from *Ascophyllum nodosum* and *Kappaphycus alvaerezii* will be purchased and applied at the specified concentration and time interval.

17.1.4. Irrigation management

In the treatment plots irrigation will be done as per standard schedule.

17.1.5. Nutrient management

FYM and NPK will be applied as per standard RDF under package of practices.

17.1.5. Biometric observation to be recorded on:

a) Growth parameters :

- Number of shoots/ plant
- Total Shoot Length (cm)
- Internodal length (cm)
- Leaf yield/ plant (g)
- Shoot yield/plant (g)

b) Leaf quality parameters :

- Soluble protein content
- Moisture content (%)
- Chlorophyll content (SPAD)

c) Recording diseases Incidence :

Incidence on major foliar diseases like *Myrothecium* leaf spot, Powdery mildew**,** Rust and Bacterial leaf spot will be recorded.

Grade	% leaf lamina covered by the symptom
0	No infection
1	0-5% leaf lamina covered by the symptom
2	6-10% leaf lamina covered by the symptom
3	11-25% leaf lamina covered by the symptom
4	26-50% leaf lamina covered by the symptom
5	50% above leaf lamina covered by the symptom

Percent disease index (PDI) will be calculated according to FAO formula (1967)

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all disease ratings X 100}}{\text{Total No. of leaves observed} \times \text{maximum grade (5)}}$$

d) Recording pest incidence

Pest	Observation to be taken from leaf	ETL
1) Thrips	4,5,6 and 7	20/leaf
2) Whitefly	Top 2, middle 2 and bottom 2	20/plant
3) Mealy bug (Tukra)	% infestation = $\frac{\text{No of Tukra infested shoots} \times 100}{\text{Total no of shoots}}$	10%

e) **Bioassay of silk worm**

- Assessment of rearing traits and cocoon parameters

Economics

- Cost of cultivation of mulberry crop per hectare per year with foliar application of seaweed extract and without foliar application of seaweed extract.
- Net return per hectare per year with foliar application of seaweed extract.
- Benefit cost ratio of the treatment.

17.2. Organization of Work Elements

Sl.No	Name of the scientist	Time allocation	Work to be done
1	Mr. Anil Pappachan, PI	50%	i. Selection and establishment mulberry plantation for the experiment. ii. Arrangements of inputs for conduct of the experiments. iii. Maintenance of experiment plots as per schedule iv. Estimation of Moisture content, soluble protein content and chlorophyll content in leaves
2	Dr. R. Mahesh, CI-1	30%	Agronomic practices and maintenance of experimental plot
3	Mr. N.Chandrakanth, CI-2	20%	Bioassay: Silk worm rearing, recording growth, development and yield (as per standard methodology of silkworm bioassay)

Established plantation of mulberry variety S-1635 belonging to Agronomy/ Farm Management Section of CSR&TI, Berhampore will be selected and utilized as per requirements of the experiment.

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

Commercially available new seaweed extract will be purchased and sprayed as per experimental requirements.

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

It is anticipated that affordable technology will be developed for boosting mulberry leaf yield of small and marginal farmers organically utilizing commercially available seaweed extract.

17.5 TIME SCHEDULE OF ACTIVITIES GIVING MILESTONES

Sl. No.	Milestone/ Activity	Expected Date of		Expected Outcome/visible/ measurable indicator
		Starting	Completion	
1.	Imposing treatments in the field and collection of data and analysis	October 2016 to August 2017		Identification of best performing treatments for standardization of dosage and time interval, confirmation of consistent performance
2.	Brushing of silkworm, observations on growth development and yield.	August 2017 To September 2017		Bioassay of silk worm fed with seaweed extract treated mulberry leaves

17.6. Program implementation Agency / Agencies:

Name of the Agency	Address of the Agency	Proposed Research Aspects	Proposed Amount (Rs. in Lakh)	Cost sharing
Central Silk Board	Central Silk Board, Bangalore - 560 068	Applied	0.425	100%

PART IV: BUDGET PARTICULARS:

(Excluding salary of Scientists, Technical Staff and wages of Field workers/ Time scale field workers).

18. Budget (in Lakh Rupees): Rs. 0.425 lakhs (Single-institutional project)

S. No.	Particulars	2016-17 Financial year (lakhs)	2017-18 Financial year (lakhs)	Sub total
A	**Non recurring contingencies** (e.g. equipments, accessories, etc. required/ available at the Institute).			
-	-	-	-	-
B1	**Recurring contingencies**			
a.	Cultivation expenses including fertilizer and pesticides etc., @ Rs. 10,000/unit/year	0.050	0.050	0.100
b.	Procurement of seaweed extract	0.050	-	0.050
c.	Travelling Allowance	-	0.100	0.100
d.	Silkworm rearing	-	0.100	0.100
e.	Miscellaneous	0.025	0.025	0.050
f.	Report preparation and submission of final report	-	0.025	0.025
	Total	**0.125**	**0.300**	**0.425**

B2. Manpower: 2 STA/TAs from CSR&TI pool

Sl. No.	Position	Nos.	Consolidated Emoluments	1st year	2nd year	Total
1	JRF/SRF/RA	-	-	-	-	-
2	HRA	-	-	-	-	-
3	CCA	-	-	-	-	-
Sub-total B1:				**-**	**-**	**-**

PART V: EXISTING FACILITIES:

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

Sl. No.	Name of the Equipment/ Accessories	Make	Model	Funding Agency
1.	UV-VIS Spectrophotometer	Sysronics	UV-VIS Spectrophotometer	CSB
2.	Hot Air Oven			CSB
3.	Electronic Balance	Anamed	M-300DR	CSB
4.	Centrifuge	Remi	R-4C	CSB
5.	Computer with printer			CSB

PART VI: REFERENCES

Amadei, B., 2004., "Engineering for the Developing World". National Academy of Engineering. *The Bridge*., 34 (3): 24-31.

Arunkumar. K., Kamalarasan, M and Archanadevi, J., 2015., Growth promoting effect of two seaweed extract on chilly, *Capsicum annuum* L. var. PMK 01. *Phykos*., **45** (2): 1-8.

Blunden, G., Jenkins, T and Liu, Y., 1997., Enhanced leaf chlorophyll levels in plants treated with seaweed extract, *J Appl Phycol*., **8**:535-543.

Burchett, S., Fuller, M.P and Jellings, A.J., 1998., Application of seaweed extract improves winter hardiness of winter barley cv Igri. The Society for Experimental Biology, Annual Meeting, The York University, March 22-27., Experimental Biology Online. Springer ISSN., 1430-34-8.

Chhaya, N.D., 1997., Minding our marine wealth, an appraisal of Gujarat coastal resources. pp.30-31.

Cluzet, S., Torregrosa, C., Jacquet, C., Lafitte, C., Fournier, J., Mercier, L., Salamagne, S., Briand, X., Esquerre´-Tugaye´, M.T. and Dumas, B., 2004., Gene expression profiling and protection of *Medicago truncatula* against a fungal infection in response to an elicitor from the green alga *Ulva* spp. *Plant Cell Environ*., **27**:917-928.

Crouch, I.J and Van Staden, J., 1992 ., Effect of seaweed concentrate on the establishment and

yield of greenhouse tomato plants. *J. Appl Phycol..,* **4**:291-296.

Crouch, I.J and Van Staden, J., 1993, Evidence for the presence of plant growth regulators in commercial seaweed products. *Plant Growth Regul.,* **13**:21-29.

Crouch I.J and Van Staden, J., 1994., Commercial seaweed products as bio stimulants in horticulture. *J. of Home and Consumer Horti*., **1**: 19-76.

Dixon, G.R and Walsh, U.F., 2002., Suppressing *Pythium ultimum* induced damping-off in cabbage seedlings by biostimulation with proprietary liquid seaweed extracts managing soil-borne pathogens: a sound rhizosphere to improve productivity in intensive horticultural systems. Proceedings of the XXVIth International Horticultural Congress, Toronto, Canada, 11-17 August., pp 11-17.

FAO., 1967., Crop losses due to diseases and pests. Food and Agriculture Organization, Rome,.

FAO., 2006., Yearbook of fishery statistics, Food and Agricultural Organisation of the United Nations, Rome., Vol 98 (1-2).

Fornes, F., Sa`nchez-Perales, M and Guadiola, J.L., 2002., Effect of a seaweed extract on the productivity of 'de Nules' clementine mandarin and navelina orange, *Bot Mar.,* **45**:486-489.

Fornes, F., Sanchez-Perales, M and Guadiola, J.L., 2002., Effect of a seaweed extract on the productivity of 'de Nules' clementine mandarin and navelina orange., *Bot Mar.*, **45**: 486-489.

Ganesh, R.J., Doongar, R. C., Khadse, V.A. and Sudhakar, T. Z., 2015., Utilization of seaweeds in enhancing productivity and quality of black gram [*Vigna mungo* (L.) Hepper] for sustainable agriculture., *Indian Journal of Natural Products and Resources*., **6**(1) : 16-22.

Hong, D.D., Hien, H.M and Son, P.N., 2007., Seaweeds from Vietnam used for functional food, medicine and biofertilizer., *J Appl Phycol*., **19**:817-826.

IPCC (Intergovernmental Panel on Climate Change)., 2007., Summary for policymakers. In: Parry ML, Canziani OF, Palutikot JP, van der Linden PJ, Hanson CE (eds) Climate change 2007: impacts, adaptation and vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK.

Khan Wajahatullah,, Usha, P. Rayirath, Sowmyalakshmi Subramanian., Mundaya, N., Jithesh, Prasanth Rayorath, D. Mark Hodges, Alan T. Critchley, James S. Craigie, Jeff Norrie and Balakrishan Prithiviraj., 2009., Seaweed Extracts as biostimulants of Plant Growth and Development. *J. Plant Growth Regul*., **28**:386-399.

Leclerc, M., Caldwell, C.D., Lada, R.R and Norrie, J., 2006., Effect of plant growth regulators on propagule formation in *Hemerocallis* spp. and *Hosta* spp. *Hortic Sci.* **41**:651-653.

Lizzi, Y., Coulomb, C., Polian, C., Coulomb, P.J and Coulomb, P.O., 1998, Seaweed and mildew: what does the future hold? Laboratory tests have produced encouraging results. *Phytoma.*, **508**:29-30.

Maria Victorial Rani, S and Revathy, D., 2009., A study on utilization of seaweeds in the remediation of heavy metal toxicity. *International journal of Basic and Applied biology,* **3**:16-21.

Maria Victorial Rani, S., Usha R and Mary Josphine, M., 2011., Seaweeds as an alternative natural resource for mitigating Cu/Pb stress in *Dolichus biflorus* Linn. *Seaweed Res.Utilin.*, **33**(1&2): 133-142.

Maria Victorial Rani, S and Michael Evanjaline, R., 2015., Effect of *Kappaphycus alvaerezii* SLF on Growth and Biochemicals in *Morus Alba* L. and *Bombyx Mori* L. *International Journal of Research Studies in Biosciences.,* **3**(12):47-52.

Mc Hugh, D.J., 2003., A Guide to the Seaweed Industry, FAO Fisheries Technical Paper No. 441, Food and Agriculture Organization, Rome., 105.

Miyashita, Y., 1986. A report on mulberry cultivation and training methods suitable to bivoltine rearing in Karnataka, pp. 1-7.

Narahari, R., Krishna, B.V., Chaluvachari, M and Bongale, U.D., 2001. Studies on the effect of commercial foliar spray formulations on growth yield and quality of mulberry. National Seminar on Mulberry Sericulture Research in India. pp: 76-77.

Nelson, W.R and Van Staden, J., 1984., The effect of seaweed concentrate on wheat culms. *J. Plant Physiol.* **115**:433-437.

Norrie, J and Keathley, J.P., 2006., Benefits of *Ascophyllum nodosum* marine-plant extract applications to 'Thompson seedless' grape production. Proceedings of the Xth International Symposium on Plant Bioregulators in Fruit Production, 2005. *Acta Hortic.,* 727:243-247.

Patil S.S., Kengar S.B. and Sathe T.V., 2006., A new vermiwash model for sustainable agriculture. Proceedings of the Asia Pacific Congress of Sericulture and Insect Biotechnology, (SIB'06), *Korea.*

Rashmi, K., Shankar, M.A., Shashidhar, K.R and Narayanaswamy, T.K., 2009., Growth and foliar constituents of mulberry (M5) cultivated under organic based nutrient management. *Int.J.Indust.Entomol.*, **19** (1):165-169.

Rayorath, P., Narayanan, J.M., Farid, A., Khan, W., Palanisamy, R., Hankins, S., Critchley, A.T and Prithiviraj, B., 2008., Rapid bioassays to evaluate the plant growth promoting activity of *Ascophyllum nodosum* (L.) Le Jol. using a model plant, *Arabidopsis thaliana* (L.) Heynh. *J Appl Phycol.,* **20**:423-429.

Russo, R.O and Beryln, G.P., 1990., The use of organic biostimulants to help low inputs, *J. Sust Agric.*,1: 19-42.

Stirk, W.A and Novak, M.S. and Van Staden, J., 2003., Cytokinins in macroalgae. *Plant Growth Regul.* **41**:13-24.

Thirumaran, G., Arumugam, M., Arumugam, R and Anantharaman., 2009., Effect of seaweed liquid fertilizer on growth and pigment concentration of *Cyamopsis tetragonolaba* L. Taub. *Am-Euras. J. Agron.,* **2** (2): 50-56.

Tiwary, P.K., Ghosh, M.K and Nirmal Kumar., S., 2014., effect of foliar application of seaweed extracts on quality and quantity improvement in mulberry. 23rd international congress on sericulture and silk industry, pp-14.

Ugarte, R.A., Sharp, G and Moore, B., 2006., Changes in the brown seaweed *Ascophyllum nodosum* (L.) Le Jol. Plant morphology and biomass produced by cutter rake harvests in southern New Brunswick, Canada., *J Appl Phycol.,* **18**:351-359.

Usha R., Maria Victorial Rani, S., Mary Josephine, M and Flora, G., 2013., Effect of *Padina pavonia* extract on soil fertility and biochemical profile of *Vigna unguiculata* under induced lead stress, *Seaweed Res. Utilin.*, **35**(1&2): 129-137.

Verkleij, F.N., 1992., Seaweed extracts in agriculture and horticulture: a review, *Biol Agric Hort*, **8**: 309-324.

Vernieri, P., Borghesi, E., Ferrante, A and Magnani, G., 2005., Application of biostimulants in floating system for improving rocket quality., *J Food Agric Environ.*, **3**: 86-88.

Vernieri, P., Borghesi, E., Ferrante, A and Magnani, G., 2005., Application of biostimulants in floating system for improving rocket quality. *J Food Agric Environ.,* **3**:86-88.

Vijaya, D., Yeledhalli, N.A., Ravi, M.V., Nagangoud, A and Nagalikar, V.P., 2009., Effect of fertilizer levels and foliar nutrients on M-5 mulberry leaf nutrient content, quality and cocoon production. *Karnataka J. Agric. Sci.,* **22**(5): 1006-1012.

Whapham, C.A., Blunden, G., Jenkins, T and Hankins, S.D., 1993., Significance of betaines in the increased chlorophyll content of plants treated with seaweed extract. *J. Appl Phycol.* **5**:231-234.

Wilson, S., 2001., Frost management in cool climate vineyards. In: University of Tasmania Research Report UT 99/1, Grape and Wine Research and Development Corporation.

Zewail, R.M.Y., 2014., Effect of seaweed extract and amino acids on Growth and productivity and some Biocostituents of common bean (*Phaseolus vulgaris* L.) plants. *J. Plant Production, Mansoura Univ.,* **5** (8): 1441 - 1453.

Zodape, S.T., 2001., Seaweeds as a biofertilizer. *J. Sci. Ind. Res*., **60**: 378-382.

Zodape, S.T., Abha Gupta., Bhandari, S.C., Rawat, U.S., Chaudhary, D.R., Eswaran, K and Chikara, J., 2011., Foliar application of seaweed sap as biostimulant for enhancement of yield and quality of tomato (*Lycopersicon esculentum* Mill.). *Journal of Scientific and Industrial research*.,**70** : 215-219.

PART VII: BIODATA OF PROJECT CO-ORDINATOR / PRINCIPAL INVESTIGATOR / CO-INVESTIGATOR (S)

Principal investigator

1. Full Name (in Block letters): Mr. ANIL PAPPACHAN
2. Designation: Scientist-B
3. Department/Institute/University Address for communication: Moriculture-II, Central Sericultural Research & Training Institute, Institute, Berhampore -742 101, Dist. Murshidabad, West Bengal
4. Date of birth: 02.01.1989
5. Sex: Male
6. Education (Post Graduation onwards & Professional Career):

Name of the University	Degree Passed	Year of Passing	Subject taken with specialization	Class/ Division
ANGRAU, Hyderabad	M.Sc (Ag)	2013	Agriculture (Plant Pathology)	1st

Memberships/fellowship: -

Patents: (Not required for in-house personnel)

Publications (Number only):

Books: -Nil

Research Papers / Reports: 6 full papers, 1 abstract

General articles: Nil

Project(s) submitted / being pursued / carried out by Investigator:

Sl.No.	Title of the Project	Funding agency	Duration From and To	No of Scientists /Associates working under the project	Total approved cost of the project (Rs.in lakh)
Nil	Nil	Nil	Nil	Nil	Nil

Highlights of outcome / progress of the project (s) handled during the past 10 years their outcome and utilization (in 200 words): NIL

List of important publications:

1) **Anil Pappachan**., R. Sarada Jayalakshmi Devi., S. Bommalinga., K.B. Palanna., 2015., Management of leaf late leaf spot (*Phaeoisariopsis personata*) disease in groundnut with fungicides., *Environment and Ecology*., **33** (3) : 1147-1150.

2) **Anil Pappachan.**, R. Sarada Jayalakshmi Devi and Shreeshail Sonyal., 2015., Effect of weather parameters on development and progress of late leaf spot (*Phaeoisariopsis personata*) disease in groundnut. *Journal of Pure and Applied Microbiology*., **9** (4) : 2987-2990.

3) Shreeshail Sonyal., **Anil Pappachan**., K. B. Palanna., Mahesha, H. S., Manjunath, S. Hurakadli and Madhu S. Giri., 2015., Effect of antagonists against *Ceratocystis fimbriata* ELL. and Halst. causing wilt in pomegranate., *International journal of pure and applied bioscience.,* **3** (4): 18-22.

4) Shreeshail Sonyal., **Anil Pappachan.**, K. B. Palanna., Mahesha, H. S., Manjunath, S. Hurakadli and Madhu S. Giri., 2015., Survivl ability of *Ceratocystis fimbriata* causing pomegranate wilt in different temperature and Hydrogen ion concentration (pH)., *International journal of pure and applied bioscience.,* **3** (4): 49-53.

5) Shreeshail Sonyal., V.B. Nargund., **Anil Pappachan.**, V.I. Benagi., K.B. Palanna., Madhu S. Giri., H. Shivalingappa., H.S. Mahesha., Devanshu Dev., M.E. Puneeth and Yallappa Jagarkal., 2016., Studies on interaction between *Ceratocystis fimbriata* and *Meloidogyne incognita* on pomegranate wilt complex., *Journal of Pure and Applied Microbiology.,***10** (1): 197-201.

6) S. Bommalinga., K. B. Palanna., **Anil Pappachan**., N. S. Somaning., N. G. Ravichandra., B. Manjunatha., 2015., Effect of bio-agents on population and reproductionof root-knot nematode *Meloidogyne incognita* infecting bell pepper., *Environment and Ecology*., **33** (1B) : 453-457.

Abstract

1) **Anil Pappachan**, R. Sarada Jayalakshmi Devi and S. Khayum Ahammed., 2014., Effect of microclimate on development and progress of late leaf spot disease in Groundnut ., National symposium on plant health for sustainability in the field and horticultural crops., 18-20 November., pp-65.

CO- INVESTIGATOR - 1

1. Full Name (In Block letters): Dr. R. MAHESH

2. 2. Designation: Scientist-B

3. Department/Institute/University Address for communication: Moriculture-I, Central Sericultural Research & Training Institute, Institute, Berhampore - 742 101, Dist. Murshidabad, West Bengal

4. Date of birth: 25.05.1985

5. Sex: Male

6. Education (Post Graduation onwards & Professional Career):

Name of the University	Degree Passed	Year of Passing	Subject taken with specialization	Class/ Division
Tamil Nadu Agricultural University	M.Sc (Ag)	2009	Agriculture (Agronomy)	1st
Tamil Nadu Agricultural University	Ph.D (Ag)	2016	Agriculture (Agronomy)	1st

Memberships/fellowship: -

Patents: (Not required for in-house personnel)

Publications (Number only):

Book	:	1
Technical bulletin	:	4
National article published	:	3
Articles in the proceeding of national conferences	:	5
Book chapters	:	5
Training attended	:	1
Leaflets	:	1
International Magazines	:	2
Conferences attended (National)	:	7
Abstract published	:	6
Newspaper cuttings published	:	3

Project(s) submitted / being pursued / carried out by Investigator:

Sl.No.	Title of the Project	Funding agency	Duration From and To	No of Scientists /Associates working under the project	Total approved cost of the project (Rs.in lakh)
Nil	Nil	Nil	Nil	Nil	Nil

Highlights of outcome / progress of the project (s) handled during the past 10 years their outcome and utilization (in 200 words) : NIL

CO- INVESTIGATOR - 2

1. Full Name (in Block letters): Shri. N. CHANDRAKANTH
2. Designation: Scientist – B
3. Department/Institute/University Address for communication: Central Sericultural Research & Training Institute, Berhampore -742 101, West Bengal.
4. Date of birth: 24.04.1986
5. Sex: Male
6. Education onwards & (Post Graduation onwards & Professional Career)

Name of the University	Degree Passed	Year of Passing	Subject taken with specialization	Class/ Division
Punjab Technical University, Jalandhar	M. Sc.	2009	Biotechnology	1st
University of Mysore, Mysore	Ph.D.	2016	Biotechnology (Submitted)	-

Publications (Number only): 12
Books: 01
Research Papers, Reports: 11
General articles: Nil

Project(s) submitted / being pursued / carried out by Investigator:

Sl.No.	Title of the Project	Funding agency	Duration From and To	No of Scientists /Associates working under the project	Total approved cost of the project (Rs.in lakh)
Nil	Nil	Nil	Nil	Nil	Nil

Highlights of outcome / progress of the project (s) handled during the past 10 years their outcome and utilization (in 200 words) : NIL

PART VIII: 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 committee we will declare that while conducting experiments, the bio-safety guidelines of the Department of Biotechnology would be followed in total.

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 / competent authorities and the same would be conveyed to the Department of Biotechnology before implementing the project.

g. It is agreed by us that any research outcome or intellectual property right(s) on the invention (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 to investigators through out the duration of the project.

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

1. Signature of Executive Authority of Institute with Seal

2. Signature of Principal Investigator

3. Signature of Co-Investigator - 1

4. Signature of Co-Investigator-2