Pro-forma for Submission of Concluded Research Project
(To be submitted separately for each project)

1. Project code and title:

PIN-3587: Improvement of leaf quality and productivity through external application of seaweed extracts in mulberry

2. Names of the Project Investigators (including coordinator in case of collaborative projects)

Investigator	Name	Designation	Time Spent (%)
Executive Authority	Dr. Kanika Trivedy	Director	5%
Principal Investigator	Mr. Anil Pappachan	Scientist-B	50%
Co-Investigator	Dr. R. Mahesh	Scientist-B	25%
Co-Investigator	Dr. N. Chandrakanth	Scientist-B	20%

3. Duration (Date of Start) - (Scheduled Date of Completion):

1 year (October, 2016 – September, 2017)

4. Name(s) of the Institute(s) and Address:

Central Sericultural Research and Training Institute, Central Silk Board, Ministry of Textiles, Govt. of India, Berhampore – 742101, Murshidabad, West Bengal

5. A list of Objectives / Goals (clearly indicating how far they have been achieved; indicating the difficulties / reasons in case of achievement gap):

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.

6. Introduction:

Mulberry (*Morus* spp.) is one of the most important commercial crops grown extensively as food plant for silkworm. The silkworm, *Bombyx mori* L. being monophagous insect, derives 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. 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.

Unlike, synthetic chemicals, extracts derived from seaweeds are biodegradable, non-toxic, non-polluting and non-hazardous. 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. The present study was undertaken to assess the effect of seaweed extracts on leaf yield and quality of mulberry.

7. Methodology Adopted:

Field experiments were conducted at Central Sericultural Research & Training Institute, Berhampore, West Bengal with foliar application of different concentrations of seaweed extracts on mulberry. Experiments were conducted during October, 2016 - September, 2017 at the experimental plot of the institute. The existing plantation of S1635

with the spacing of 90 cm × 90 cm was used for the study which was converted in to completely randomized block design. Recommended package of practice was followed and the plantation was maintained through timely intercultural operations.

Commercially available *Ascophyllum nodosum* extract and *Kappaphycus alvarezii* extract were used in the present investigation. Initially an experiment was conducted with 12 treatments along with control to standardize the dosage and time interval of the seaweed extract for optimum performance. Required quantity of the extracts was mixed with water and was sprayed uniformly on the plants. Control plots were sprayed with water. In December 2016, Sixty days after pruning leaf yield data was recorded. Data was collected from three plants excluding border rows. 60 days after pruning data on different growth attributes *viz.*, leaf yield per plant, Shoot yield per plant, Plant height, internodal length and total shoot length per plant were recorded.

Leaf quality parameters *viz.*, Moisture content (%), Chlorophyll content and protein content were estimated. Moisture Content (%) was recorded by oven dry process. Chlorophyll content was estimated using SPAD meter, measured on the fifth fully expanded leaf from the top of the each plant. Readings were taken around the midpoint near the midrib of each leaf sample (Peng *et al.*, 1992) and averaged. Care was taken to ensure that the SPAD meter sensor fully covered the leaf lamina and the interference from the veins and midribs was avoided. Incidence of pest and diseases was recorded one week before harvest. Number of whiteflies present in the two leaves each from top, middle and bottom were counted and averaged. Diseases were scored using 0-5 scale and Percent disease index (PDI) was calculated according to FAO formula (1967);

$$\text{Percent disease index (PDI)} = \frac{\text{Sum of all disease ratings} \times 100}{\text{Total number of leaves observed} \times \text{maximum grade (5)}}$$

Six treatments were identified and later field experiments were conducted during July-September, 2017. All the experiments were conducted in completely randomized block design and all the treatments were replicated three times. Data was collected from 30 plants excluding border rows.

Bioassay on silkworm was conducted during September, 2017 with BCon 1× BCon 4 using completely randomized design using three replications. Leaves were fed to the worms from the 5th instar. Two hundred silkworm larvae were reared in each tray and each treatment was replicated thrice. Observations were recorded on larval weight, number of cocoons

harvested, weight of cocoon harvested, Single cocoon weight and single shell weight. Data was analyzed statistically to determine significant differences among different treatments. To economic impact of the treatment was calculated by partial budgeting method.

8: Observations / Results duly indicating the output in terms of adding to knowledge; know-how / new packages/ practices / processes /products / innovations developed and their utility and advantages; etc.,

E 01: *Determination of the effect of application of seaweed extracts on growth and biomass in mulberry; and standardization of the dosage and time interval for the application of seaweed extract.*

Commercially available extracts from two seaweeds *A. nodosum* and *K. alvarezii* were evaluated in the present study for assessing quantitative and qualitative effect on mulberry variety S-1635. Initially effect of single (28 Days After Pruning) and two time (21 and 28 DAP) application of three different concentrations *viz.*, 0.5 ml/L, 1.0 ml/L and 2.0 ml/L were evaluated (Table 1). Initial study revealed that application of seaweed extracts significantly increased the leaf yield (g) , stem yield (g) and total shoot length (cm) over control (Table 1) while moisture content (%) Protein content (mg/g of FW) and Chlorophyll content ($\mu g/cm^2$) were at par with control.

Table 1. Effect of seaweed extracts on the yield and quality of mulberry

Treatment		Leaf yield per plant (g)	Stem yield per plant (g)	Total shoot length per plant (cm)	Moisture Content (%)	Protein content (mg/g of FW)	Chlorophyll content ($\mu g/cm^2$)
A. nodosum extract at 21 and 28 DAP *	0.5 ml /L	152.22*	67.78*	425.60*	76.48	35.23	11.29
	1.0 ml /L	135.00*	52.22*	407.16*	68.33	41.16	10.45
	2.0 ml /L	177.78*	65.00*	460.41*	74.63	37.96	10.17
A. nodosum extract at 28 DAP	0.5 ml /L	159.50*	52.72*	471.77*	75.80	43.65	12.42
	1.0 ml /L	135.72*	58.17*	432.84*	76.26	41.16	11.30
	2.0 ml /L	120.00*	48.33	346.77	76.89	32.62	14.84
K. alvarezii extract at 21 and 28 DAP	0.5 ml /L	146.67*	62.22*	486.56*	71.00	38.55	13.87
	1.0 ml /L	141.66*	58.00*	426.02*	73.50	37.88	10.67
	2.0 ml /L	119.44*	39.33	360.94	71.68	31.73	11.81
K. alvarezii extract at 28 DAP	0.5 ml /L	118.89*	39.50	400.91	72.92	30.13	12.84
	1.0 ml /L	119.17*	43.61	445.38*	70.86	40.57	13.06
	2.0 ml /L	98.89	36.11	338.93	70.93	28.22	11.81
Water Spray (Control)		76.11	32.78	300.01	69.52	35.74	12.66
SEm±		14.53	6.65	36.14	2.6	9.13	1.23
CD at 5%		42.43	19.42	105.50	NS	NS	NS

Three treatments were selected from each seaweed extracts *viz.*, application of *A. nodosum* extract @ 0.5 ml /L and 2.0 ml /L (21 and 28 DAP), *A. nodosum* extract @ 0.5 ml

/L (28 DAP), *K. alvarezii* extract @ 0.5 ml /L and 1.0 ml /L (21 and 28 DAP) and *K. alvarezii* extract @ 1.0 ml /L (28 DAP) which were further evaluated. Results presented in the table 2 depict that application of *A. nodosum* extract @ 0.5 ml /L and 2.0 ml /L (21 and 28 DAP); and *K. alvarezii* extract @ 0.5 ml /L and 1.0 ml /L (21 and 28 DAP) significantly increased the leaf yield by 11.16 %, 16.90 %, 10.30 % and 10.86 % respectively over control (328.33 g/plant), however all the four treatments were statistically on par with each other. Leaf quality parameters *viz.*, Moisture content (%), Chlorophyll content (µg/cm^2), Protein content (mg/g of FW) were at par with control.

Table 2. Effect of seaweed extract on leaf yield and quality of mulberry

Treatment		Leaf yield per plant	Moisture Content (%)	Protein content (mg/g of FW)	Chlorophyll content (µg/cm^2)
A. nodosum extract	0.5ml on 21 & 28 DAP	365.00*	75.07	37.11	12.75
	2 ml on 21 & 28 DAP	383.83*	74.50	35.01	12.76
	0.5 ml on 28 DAP	345.00	74.40	34.38	12.63
K. alvarezii extract	0.5ml on 21 & 28 DAP	362.17*	75.27	38.63	12.03
	1.0 ml on 21 & 28 DAP	364.00*	74.23	38.86	13.00
	1.0 ml on 28 DAP	342.50	74.47	38.01	13.57
Control		328.33	73.60	35.18	12.43
SEm±		9.76	0.54	1.28	0.40
CD@ 5%		30.08	NS	NS	NS

Effect of application of seaweed extracts on the incidence of pests and foliar diseases of mulberry

Tukra incidence, whitefly incidence and Myrothecium leaf spot incidence were observed (table 3) which were either at par with control or below ETL level.

Table 3. Incidence of pests and foliar diseases

Treatment		Tukra (%)	Whitefly per leaf (No)	MLS (PDI)
A. nodosum extract	0.5ml on 21 & 28 DAP	18.80	7.13	1.47
	2 ml on 21 & 28 DAP	6.80	6.67	1.43
	0.5 ml on 28 DAP	10.23	5.73	1.43
K. alvarezii extract	0.5ml on 21 & 28 DAP	13.21	5.87	1.80
	1.0 ml on 21 & 28 DAP	16.40	5.57	2.00
	1.0 ml on 28 DAP	9.30	6.40	1.85
Control		12.62	5.83	1.03
SEm±		3.5	0.51	0.25
CD@ 5%		NS	NS	NS

Economics of the treatments (Estimated value per acre)

Treatment	Leaf yield per (Kg)	Additional Cocoon Yield (Kg)	Additional income	Quantity of Seaweed required ml/ ac	Additional Expenditure (including rearing)	Return per rupee invested
T1	1767	7.46	2089	180	1252	1.67*
T2	1859	3.43	3162	720	2012	1.57
T3	1670	11.29	960	90	597	1.61
T4	1752	6.86	1920	180	1172	1.64
T5	1762	7.26	2033	360	1319	1.54
T6	1660	3.03	847	180	601	1.41
Control	1588	-	-	-	-	-

1) Cost of *A. nodosum* extract Rs. 720/L and cost of *K. alvarezii* extract Rs. 600/L
2) Labour cost Rs. 200/day
3) Cost of cocoon production 4840/100 DFLs (excluding leaf production) and Average price of cocoon Rs. 280/kg

Economic analysis revealed that application of *A. nodosum* extract @ 0.5 ml /L at 21 and 28 DAP resulted in cost benefit ratio of 1:1.67 with 11% increase in leaf yield.

E02: *Bioassay on silkworm larvae fed with seaweed extract treated mulberry leaves*

Bioassay on silkworm was conducted during September, 2017. Larval weight (g), ERR (No.) ERR (Wt.), Single Cocoon weight (g), Single Shell Wt. (g) and Shell % were recorded (Table 4).

Table 4. Effect of seaweed extracts on silkworm rearing

Treatment		Larval weight (g)	ERR NO.	ERR Wt. (Kg)	Single Cocoon Wt. (g)	Single Shell Wt. (g)	Shell %
A. nodosum extract	0.5ml on 21 & 28 DAP	3.57	9123	13.29	1.42	0.236	16.56
	2 ml on 21 & 28 DAP	3.83	8649	12.22	1.37	0.226	16.14
	0.5 ml on 28 DAP	3.80	9404	13.17	1.35	0.223	16.44
K. alvarezii extract	0.5ml on 21 & 28 DAP	3.83	9105	12.77	1.38	0.226	16.09
	1.0 ml on 21 & 28 DAP	3.73	9193	12.60	1.43	0.236	16.80
	1.0 ml on 28 DAP	3.90	9035	13.90	1.39	0.233	16.87
Control		3.63	9228	13.95	1.49	0.246	16.32
SEm±		0.69	216	0.83	0.02	0.011	0.42
CD@ 5%		NS	NS	NS	NS	NS	NS

Larval weight (g), ERR (No.), ERR (Wt.), Single Cocoon weight (g), Single Shell Wt. (g) and Shell % were found to be at par with the control and no significant differences were observed.

9. Discussion:

Organic inputs would help to alleviate the harmful effects of agrochemicals on the environment. Seaweed extracts seem to be an excellent option for improving the productivity of mulberry using organic inputs. Application of seaweed extracts from *A. nodosum* extract @ 0.5 ml /L and 2.0 ml /L (21 and 28 DAP); and *K. alvarezii* extract @ 0.5 ml /L and 1.0 ml /L (21 and 28 DAP) significantly increased leaf yield by 11.16 %, 16.90 %, 10.30 % and 10.86 % over control (328.33 g/plant). Leaf quality parameters *viz.*, moisture content (%), Chlorophyll content ($\mu g/cm^2$) and Protein content (mg/g of FW) were at par with control. Economic analysis revealed that application of *A. nodosum* extract @ 0.5 ml /L at 21 and 28 DAP increased leaf yield by 11% with a cost benefit ratio of 1:1.67. To take up the spray in one acre field 180 ml of seaweed extract is required which would cost Rs. 130 (two applications at 0.5 ml/L at the rate of 180 L of spray solution for one time). Considering the economics and availability of the seaweed extract *A. nodosum* extract @ 0.5 ml /L at 21 and 28 DAP could be recommended further for large scale evaluation.

Present findings agrees with the observations of Tiwary *et al.* (2014) who reported that lower concentration of seaweed extract (0.5ml/L) from *Dictyota dichotoma* and *K. alvaerezii* as foliar spray was most suitable for increasing leaf yield. Maria Victorial Rani and Evanjaline (2015) observed that application of seaweed extract from *K. alvaerezii* enhanced the shoot length, number of leaves, leaf area, and 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. Similar reports were also made by Anil *et al.* (2017) where in foliar application of seaweed extract from *A. nodosum* 0.5 ml/L at 21, 28 and 35 days after pruning enhanced leaf yield per plant and average plant height in S1635 variety.

10. Inférence / Recommandations:

Application of *A. nodosum* extract @ 0.5 ml /L 21 and 28 DAP was found to be most suitable for increasing the leaf yield of mulberry. Large scale trials need to be conducted to validate the performance which was recommended by 47th RAC.

11. Applications made for patenting / commercialization if any:

Nil

12. References

Anil, P., Lungjo, B. and Trivedy, K., 2017, Effect of application of *Ascophyllum nodosum* extract on the yield and quality of Mulberry leaves, *Bioscience Discovery*, 8(2): 235-240.

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

Lowry, O.H., Rosebrogh, N.J., Farr, A.L and Randall, R.J., 1951, Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.*, 193-265.

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.

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

Peng, S., Garcia, F.V., Laza, R.C and Cassman, K.G., 1992, Leaf thickness affects the estimation of leaf N using a chlorophyll meter. IRRI Newsletter, 17(6): 19-20.

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.

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.

13. Summary:

Commercial extracts from two seaweeds *A. nodosum* and *K. alvarezii* were evaluated in the present study for assessing quantitative and qualitative effect on mulberry variety S-1635. Initially effect of single and two time application of three different concentrations *viz.*, 0.5 ml/L, 1.0 ml/L and 2.0 ml/L were evaluated. Out of 12 treatments, 6 were selected for further evaluation. Application of seaweed extracts from *A. nodosum* extract @ 0.5 ml /L and 2.0 ml /L (21 and 28 DAP); and *K. alvarezii* extract @ 0.5 ml /L and 1.0 ml /L (21 and 28 DAP) significantly increased the leaf yield ranging from 10 to 17 %, over control (328.33 g), however all the treatments were statistically on par with each other. Quality parameters were at par with the control. Bioassay revealed that rearing parameters of treatments were on par with control. Economic analysis revealed that application of *A. nodosum* extract @ 0.5 ml /L at 21 and 28 DAP gave a return of 1.67 per rupee invested on the treatment that will be further evaluated.

15. Budget utilized (In lakh rupees): 0.279

A. Non-Recurring (e.g. equipments, accessories, etc.): NA

B. B1. Recurring contingencies

Sl.	Item	Amount (Lakhs)
a.	Cultivation expenses	0.100
b.	Procurement of seaweed extract	0.050
c.	Travelling Allowance	0.100
d.	Silkworm rearing	0.100
e.	Miscellaneous	0.050
f.	Report preparation and submission of final report	0.025
	Total	**0.425**

EXPENDITURE:

S.N.	Item	Total (Lakhs)
a.	Expenditure incurred	**0.279**

Certificate

Certified that the Project work has been carried out and financial expenditure incurred for executing the Project are in accordance with the declaration / certification submitted at the time of submission of the Project Proposal and sanction obtained from time to time thereafter as per the revision made.

12. Signature of the

Principal Investigator — **Mr. Anil Pappachan**
Scientist-B

Co-Investigator (s) — **Dr. R. Mahesh**
Scientist-B

Dr. N. Chandrakanth
Scientist-B

13. Signature (with comments, if any) of Head of Division

Sri. Debasish Chakravorty
Scientist-D
Moriculture Division

14. Signature (with comments, if any) of Director / Executive authority

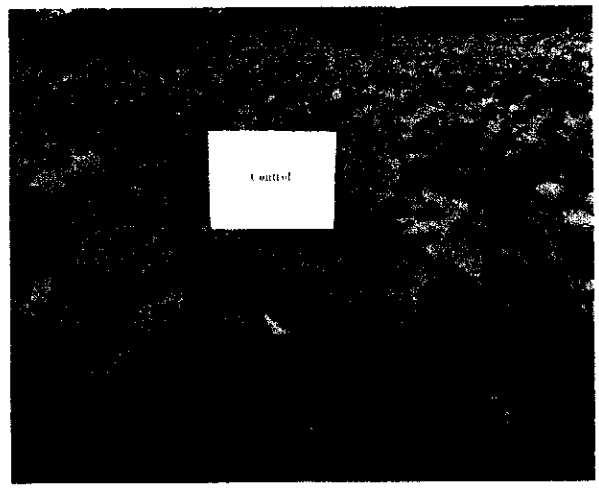
Regarding this project, it was observed that the project was concluded as per milestone successfully from October, 2016 – September, 2017. In this project application of seaweed extract from Ascophyllum nodosum @ 0.5 ml/L of water at 21 and 28 days after pruning was found most suitable for increasing leaf yield which will be further evaluated.

4/4/18

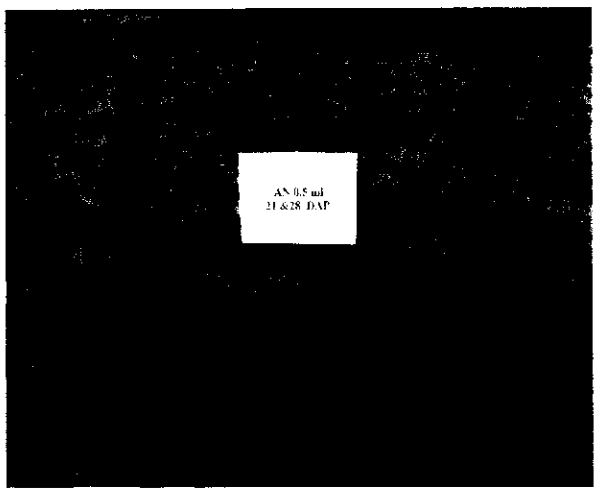
Dr. Kanika Trivedy
Director

(डॉ. कणिका त्रिवेदी)
(Dr. Kanika Trivedy)
निदेशक/Director
केन्द्रीय रेशम उत्पादन अनुसंधान एवं प्रशिक्षण संस्थान, बहरमपुर
Central Sericultural Research & Training Institute, Berhampore 742101

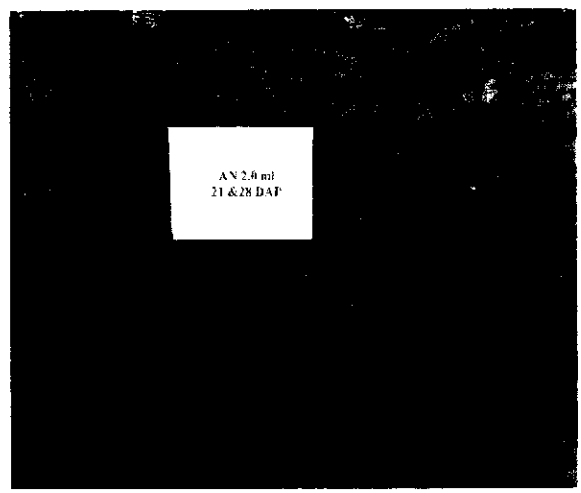
Spraying of seaweed extract



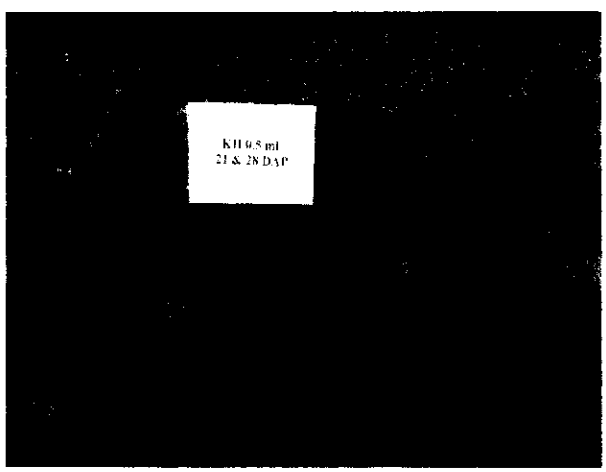
Control



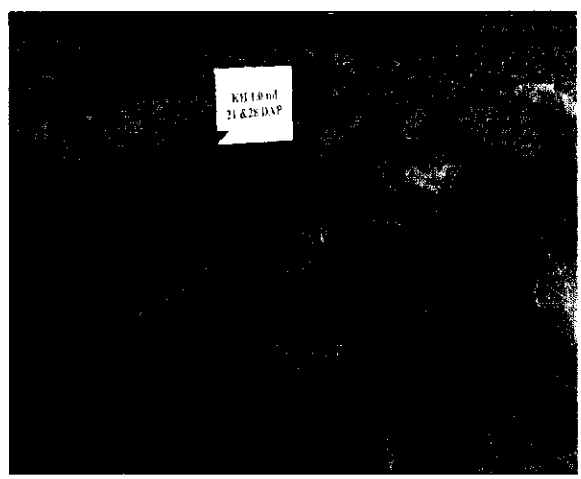
***A. nodosum* extract @ 0.5 ml /L at 21 and 28 DAP (Days after pruning)**



***A. nodosum* extract @ 2.0 ml /L 21 and 28 DAP**



***K. alvarezii* extract @ 0.5 ml /L 21 and 28 DAP**



***K. alvarezii* extract @ 1.0 ml /L 21 and 28 DAP**

#	**Observations of RAC***	**Action taken**
	• The pest incidence in treatment and control as reported by the PI is due to the natural incidence.	• Complied
	• The PI may take a study to elucidate study the biochemical principles responsible for the enhancement of growth.	• As the project is already concluded active principles will be identified in the second phase of the project
Action: Shri Anil Pappachan	• The PI was advised to specify the cost of commercial seaweed extracts used in the present project required for unit area of mulberry plantation in the concluded report.	• Quantity of seaweed required per acre and its cost is incorporated in the concluded report
	• The PI should consider taking up the same project on a larger scale considering the feasibility in relation to economics	• Will be taken up as second phase for large scale evaluation

*Minutes of the 47th RAC of CSR&TI, Berhampore: No.CSB/CSR&TI/PMCE/R-2/2017-18/2520 dated 22.30.2018