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Foliar application of *Caulerpa racemosa* seaweed extract as bio-stimulant for enhancement of growth and yield of blackgram (*Vigna mungo* L.)

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Abstract

Field experiments were carried out to identify the effect of foliar spraying of *Caulerpa racemosa* seaweed extract on growth and yield of blackgram. Foliar sprary @ 3 % given at vegetative (VS), flowering (FS) and combinations of vegetative and flowering stages (VS+FS) improved the growth and yield parameters The effect more evident with *Caulerpa racemosa* as evidenced through increase in dry weight, Leaf Area Index, total chlorophyll, Crop Growth Rate and Relative Growth Rate) and yield attributes (Number of pods plant $^{-1}$,1000 seed weight and seed yield plant $^{-1}$) against unsprayed and water sprayed plants. Application of 3 % *Caulerpa racemosa* extract at combinations of both stages (VS +FS) highly accelerate the growth attributes thereby increased seed yield (74%) compared to control.

Key words: *Caulerpa racemosa*, Foliar spray, growth attributes, Seed yield, Blackgram

In modern Agriculture, extensive application of chemical fertilizers caused imbalance of soil nutrients. So, search for natural organic inputs for sustainable crop productivity has been emphasized. Bio-fertilizers are a 100 % natural organic fertilizer that helps to provide all the nutrients to the soil required for the plants. Bio-fertilizers based on renewable energy sources are most effective supplement to chemical fertilizers. Eco-friendly and help to curtain high

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investment needed for chemical fertilizers. Seaweeds are rich source of growth promoting substances (Sylvia et al., 2005) such as IAA, kinetin, zeatin and gibberellins (Zodape et al., 2010) auxins and cytokinins (Zhang and Ervin, 2004); metabolic enhancers (Zhang and Schmidt, 1997) ; macro and micro elements (Strik et al., 2003), amino acids, vitamins and beneficial results from their use in crop plants like early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress and enhanced post harvest shelf life of seeds (Hankins and Hockey, 1990; Bluden, 1994; Booth, 1965). Foliar application of growth regulators constitutes one of the important techniques that caters to seed nutrition at the most vulnerable stage i.e., seed filling. Plant growth promoters are modify the crop growth and developmental patterns by exerting profound effects in physiological process like pod development and also eliminates the losses through leaching and fixation and helps in regulating the uptake of nutrients by plants and foliar application of seaweed extracts enhanced the chlorophyll level in plants (Bluden et al., 1991). With this background studies were formulated with foliar application of *Caulerpa racemosa* seaweed extract on growth and yield attributes of blackgram.

Materials and Methods

The marine green algae *Caulerpa racemosa* was collected from Mandapam coast, Tamil Nadu, India were washed with sea water initially to remove macroscopic epiphytes and sand particles finally with fresh water to remove adhering salt then shade dried for 4 - 5 days followed by oven drying at 40^{o}C for 24 h and powdered from which 100g powder was taken and 100 ml of alcohol was added then kept it for overnight with intermittent stirring and extracted through rotary evaporator with 40^{o}C and 45 rpm and liquid fertilizer collected and stored in air tight container (100 % extract – from which 3 % spray fluid was prepared by dilution with water).

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Field experiments were conducted at Agricultural College and Research Institute, Madurai, TamilNadu, India during February 2013 with all recommended packages of practices for blackgram cv.VBN 4. Foliar application was given with following treatments; Control (T_1), Water (T_2), *Caulerpa racemosa* 3 % extract was given at Vegetative stage (VS) spraying @ 30 Days After Sowing (T_3), Flowering stage (FS) (T_4) spraying @ 30 Days After Sowing and combinations of VS+ FS (T_5) and evaluated for growth attributes such as Plant height (cm), Dry matter production (g), Leaf area index (LAI), Relative Growth Rate (RGR), Crop Growth Rate (CGR) , Total chlorophyll (SPAD) and yield parameters such as number of pod plant $^{-1}$,1000 seed weight (g) and seed yield plant $^{-1}$.The experimental design was Randomized Design (FCRD) with three replicates. Significance of mean differences of the variable mean was separated using LSD at P=0.05 using ANOVA.

Gas Chromatography – Mass Spectrum Analysis (GC – MS)

Gas chromatography – Mass spectrum analysis technique was used in this study to identify the phytocomponents in the extract. GC – MS technique was carried out at Indian Institute of Crop Processing Technology (IICPT) Thanjavur, Tamil Nadu.

Identification of components

Interpretation of mass spectrum GC - MS was conducted using the database of National Institute Standard and Technique (NIST) having more than 62, 000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library (NIST Version – Year 2005). The name, Molecular Weight, Structure of the component of the test material was ascertained.

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Results

The growth parameters *viz.*, plant height, dry matter production, leaf area index, total chlorophyll, relative growth rate, crop growth and yield parameters were higher in seaweed sprayed plants than control and water. Foliar application of *Caulerpa racemosa* (3%), at vegetative and flowering stages gave increase in Leaf Area Index (2.89), Total chlorophyll (46.62), Crop Growth Rate (3.47 g $m^{-2}d^{-1}$), Relative growth rate (3.73 mg $g^{-1}d^{-1}$) number of pods plant $^{-1}$(61.66), 100 seed weight (4.19 g) and seed yield plant $^{-1}$ (16.21 g) than spraying at vegetative and spraying at flowering stage. The lowest values were observed in control (1.41, 34.90, 2.70 g $m^{-2}d^{-1}$, 3.02 mg $g^{-1}d^{-1}$, 45.67, 3.85 g and 9.31 g respectively for Leaf Area Index, Total chlorophyll, Crop Growth Rate, Relative growth rate, number of pods plant $^{-1}$, 100 seed weight and seed yield plant $^{-1}$. Significant increase in the yield has been observed in foliar application at vegetative and flowering stage in *Caulerpa racemosa.* The percentage increase over control was 74 %.

Seaweed contains many major plant nutrients, trace elements and anti oxidants as observed through GC-MS – studies. *Caulerpa racemosa* seaweed extract contains eighteen components, their active principle, Molecular Weight (MW), Concentration (%), Molecular Formula (MF), Retention Time (RT) and bioactivity were presented in Fig 1 and Table 2 &3.The Prevailing compounds were β – Sitosterol (28.57%), n-Hexadecanoic acid (15.57%), Phytol (13.65 %), Stigmastan - 3- ol, 5-chloro -acetate , (3á, 5á) (8.48 %), 3,7,11,15 – Tetramethyl – 2-hexadecane - 1- ol (7.57%) and Stigmastan – 6, 22 – dien, 3, 5 – dedihydro (6.35). These compounds may greatly accelerate growth attributes thereby yield (Munda and Gubensk, 1975; Yamamoto, 1979; Strik et al., 2003; Sylvia et al., 2005 and Zodape et al., 2009).

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Discussion

Occurrence of growth regulators and their effect alter the physiological attributes (Temple and Bomke, 1989) and presence of growth promoting substances including auxins, gibberellinfs in seaweed (Zhag et al., 1997) have been reported. The increase in chlorophyll due to application of seaweed has been reported by (Blunden,1994 ; Venketraman Kumar et al., 1993 in blackgram; Asir Selin Kumar and Saravana babu, 2004 in paddy; Pise and Sabale, 2010 in fenugreek; Veera gurunathan et al., *2011in* capsicum; Gandhiappan and Perumal, 2001 in sesame). Higher chlorophyll content accompanied by higher leaf area index observed in the present study should have delayed senescence and lead to higher nutrient uptake through roots ultimately favouring the yield attributes as reported by Crouch and Van staden (1993). To achieve the highest yield, physiological parameters like Leaf Area Index, Crop Growth Rate and Relative Growth Rate must be higher to intercept more solar energy for higher dry matter production (Asir Selin Kumar and Saravana babu, 2004).

Increase in seed yield was mainly due to increase in number of pods as well as 100 seed weight. This is in conformity with several scientist reported Hamblin,1991 in *Phaseolus aurus*, ; Kavipriya et al., 2011in Green gram; Gandhiyappan and perumal, 2001in Sesame; Temple, 1989 in beans; Crouch and Van Staden, 1992 in tomato; Pise and Sabale, 2010 in fenugreek .

Mainly *Caulerpa racemosa* contain phytol, Squalene, β stigmasterol and Stigmastan these compounds greatly accelerate growth attributes thereby increase in yield.

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Conclusion

It could be inferred that foliar application of 3 % *Caulerpa racemosa* seaweed extract at critical growth phases viz., vegetative and flowering stages enhances the physiological and yield attributes in blackgram.

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References:

R.Asir Selin Kumar & S. Saravana Babu (2004). Effect of seaweed extract on oxidizing enzymes during the senescence of *Oryza sativa* var. Ambai 16, *Seaweed Res Utiln*, 23(1&2): 177-180.

G. Blunden (1994). Agricultural uses of seaweeds and seaweed extracts, *In*: Seaweed resources in Europe: Uses and potential. M D Guiry and G Bluden (eds). Wiley, Chicester 65- 81.

G. Blunden Jenkins T & Y. W, Liu (1991).Enhanced chlorophyll levels in plants treated with seaweed extract, *J. Appl. Phycol.*, 8**:** 535-543.

G. Blunden (1994).Agricultural uses of seaweeds and seaweed extracts. In**:** Seaweed resources in Europe: Uses and potential. M.D.Guiry and G.Bluden (eds). Wiley, Chicester. pp:.65- 81.

E. Booth (1965).The manural value of seaweed, *Bot Mar,* 8 : 138-143.

ISSN 2278-7763

I .J Crouch & J.Van Staden (1992).Effect of seaweed concentrate on the establishment and Response of tomatoes to seaweed based nutrient sprays, Proc Fla State Hort Soc, 97 **:** 151-157.

K.Gandhiappan & P.Perumal (2001).Growth promoting effect of seaweed liquid fertilizers (*Enteromorph intentinalis*) on the same crop plant (*Sesamum indicum), Seaweed Res Utiln,* 23 (1and2): 23-25.

A.Hamblin (1991).Sustainable agricultural systems: what are the appropriate measures for soil structure, *Aust J Soil Res,* 29:709-715.

S. D, Hankins & H. P, Hockey (1990).The effect of liquid seaweed extract from *Ascophyllum nodosum* (Fucales, Phaeophyta) on the two spotted red spider mite *Tetranychus utricae*, *Hydrobiologic*a, 204 (205): 555 – 559.

R,. Kavipriya P. K, Dhanalakshmi, S. Jayashree & N. Thangaraju (2011).Seaweed extract as a biostimulant for legume crop, green gram. *J Ecobiotechnol*, 3(8):16 -19.

M .Munda & F. Gubensk, (1975).The amino acid composition of some common marine algae from Iceland, *Bot Mar,* 19 : 85-92.

N.Pise & A. B, Sabale (2010).Effect of seaweed concentrates on the growth and biochemical constituents of *Trigonella foenum – graecum* L, J Phytol, 2(4): 50-56.

W.A, Strik ,M .S Novak and J. Van Staden (2003).Cytokinins in macroalgae. *Plt. Growth Regul* 41: 13 - 24.

S.Sylvia, M, Baluswami, M. D Vijaya Parathasarathy & V. Krishnamurthy(2005).Effect of liquid seaweed fertilizers extracted from *Gracilaria edulis* (Gmel.) Silva, *Sargassum wightii* Greville and *Ulva lactuca* Linn. On the growth and yield of *Abelmoschus esculentus* (L) Moench, *Indian Hydrobiol* 7: 69-88.

W. D Temple & A, Bomke (1989). Effects of kelp (*Macrocystis integrifolia* and *Ecklonia maxima*) foliar applications on bean crop yield, *Plant soil* 117:85-92.

ISSN 2278-7763

V, Veeragurunathan, V ,Meenakshi sundaram and C. Balachandar (2011). Comparative studies on fertilizing efficiency of LSF from three seaweeds on the growth of *Capsicum annum*. *Seaweed Res Utiln* 33(1&2): 143-149.

V, Venkataraman Kumar R, Mohan R,Murugeswari and M. Muthusamy (1993).Effect of crude and commercial seaweed extracts on seed germination and seedling growth in green gram and black gram, *Seaweed Res Utiln* 16(1&2): 23-27.

T, Yamamoto Y, Qtsuka, M. Okazaki & K I, Okamato (1979).The distribution of chemical elements in algae, *In* : Marine algae in pharmaceutical science, Hoppe, H.A., Levering, T. and Tanaka, Y.(eds), Walter de Gruyte, Berlin, 569-607.

X, Zhang and R, E Schmidt (1997).The impact of growth regulators on the α – tocopherol status in water – stressed *Poa pratensis* L. *Int. Turfgrass Res.J* 8 : 1364 - 1373.

X. Zhang, and E. H. Ervin (2004). Cytokinin containing seaweed and humic acid extracts associated with creeping bent grass leaf cytokinins and drought resistance. *Crop Sci.*, 44: -10.

S T, Zodape S Mukherjee, M P, Reddy & D.R, Chaudhary (2009).Effect of *Kappaphycus alvarezii* extract on grain quality, yield and some yield components of wheat (*Triticum aestivum* L.). *Int J Plant Prod*, 3: 97 - 101.

S.T. Zodape, S.Mukhopadhyay, K.Eswaran, M.P.Reddy and J.Chikara (2010). Enhanced yield and nutritional quality in green gram (*Phaseolus radiata*) treated with seaweed (*Kappapyucus alvarezii*) extract. *J.Sci. and Ind.Res.*, 70**:** 215-219.

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Table 1.Total ionic chromatogram (GC – MS) of *Caulerpa racemosa* obtained with 70eVusing an Elite – 5 MS capillary column with He gas as the carrier.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	9.54	8 – Heptadecane	$C_{17}H_{34}$	238	0.98
2.	9.76	Heptadecane	$C_{17}H_{36}$	240	1.57
3.	11.22	3,7,5,11,15-Tetramethyl- 2- hexadecane -1- ol	$C_{20}H_{40}O$	296	7.57
4.	11.32	2-Pentadecanone, 6,10,14 -trimethyl-	$C_{18}H_{36}O$	268	1.60
5.	12.78	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	15.57
6.	13.01	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	4.37
7.	14.42	Phytol	$C_{20}H_{40}O$	296	13.65
8.	14.87	8 - hexadecenal, 14 - methyl - (Z)-	$C_{17}H_{32}O$	252	1.25
9.	16.36	Hexadecanoic acid, 2 - hydroxyl-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	1.30
10.	17.72	4,8,12,16 - Tetramethylheptadecan - 4 -olide	$C_{21}H_{40}O_2$	324	0.51
11.	19.14	Hexadecanoic acid, 1- (hydroxymethyl)-1-2-ethanediyl ester	$C_{35}H_{68}O_5$	568	0.94
12.	19.69	Palmitoyl chloride	$C_{16}H_{31}ClO$	274	2.36
13.	22.49	Heptacosane	$C_{27}H_{56}$	380	0.76
14.	24.03	Squalene	$C_{30}H_{50}$	410	0.54
15.	27.29	Stigmastan -6, 22-dien, 3,5- dedihydro-	$C_{29}H_{46}$	394	6.35
16.	27.56	Stigmastan- 3-ol,5-chloro-, acetate, (3á, 5á)-	$C_{31}H_{53}ClO_2$	492	8.48
17.	31.32	β -Sitosterol	$C_{29}H_{50}O$	414	28.57
18.	34.17	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412	3.64

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Table 2. Biological activity of Phytocomponents identified in *Caulerpa racemosa*

No.	Name of the compound	Molecular Formula	Compound Nature	Activity
1.	8 – Heptadecane	$C_{17}H_{34}$	Organic compound	Substitute of alkanes (Methae, ethane, hexane, pentane, hepta decane and octadecane)
2.	Heptadecane	$C_{17}H_{36}$	Organic compound	Substitute of alkanes (Methae, ethane, hexane, pentane, hepta decane and octadecane)
3.	3,7,5,11,15-Tetramethyl- 2-hexadecane -1- ol	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial
4.	2-Pentadecanone, 6,10,14 -trimethyl-	$C_{18}H_{36}O$	Terpene compound	Antimicrobial
5.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, Antimicrobial, Nematicide, Pesticide, 5-Alpha reductase inhibitor
6.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	Palmitic acid ester	Antioxidant, Antimicrobial, Nematicide, Pesticide, 5-Alpha reductase inhibitor
7.	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial, Antifungal, Anti insecticidal, Miticide , Chlorophyll constituents, Precursor for synthetic forms of Vit E & Vit K1.
8.	8 - hexadecenal, 14 - methyl - (Z)-	$C_{17}H_{32}O$	Ester compound	No activity reported
9.	Hexadecanoic acid, 2 - hydroxyl-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	Fatty acid ester	No activity reported
10.	4,8,12,16 - Tetramethylheptadecan - 4 -	$C_{21}H_{40}O_2$	-	-

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	olide			
11.	Hexadecanoic acid, 1-(hydroxymethyl)-1-2-ethanediyl ester	$C_{35}H_{68}O_5$	Fatty acid ester	No activity reported
12.	Palmitoyl chloride	$C_{16}H_{31}ClO$	Chloride compound	Palmitic acid preparation
13.	Heptacosane	$C_{27}H_{56}$	Hydrocarbon	No activity reported
14.	Squalene	$C_{30}H_{50}$	Triterpene	Antimicrobial, Antioxidant, Chemo preventive, Pesticide
15.	Stigmastan -6, 22-dien, 3,5-dedihydro-	$C_{29}H_{46}$	Triterpene and Steriod compound	Antimicrobial
16.	Stigmastan- 3-ol,5-chloro-, acetate, (3á, 5á)-	$C_{31}H_{53}ClO_2$	Hydroxyl compound	No activity reported
17.	β -Sitosterol	$C_{29}H_{50}O$	Vitamin compound	Vitamin E, Antioxidant
18.	Stigmast-4-en-3-one	$C_{29}H_{48}O$	Fucosterol, Steriod compound	Antimicrobial

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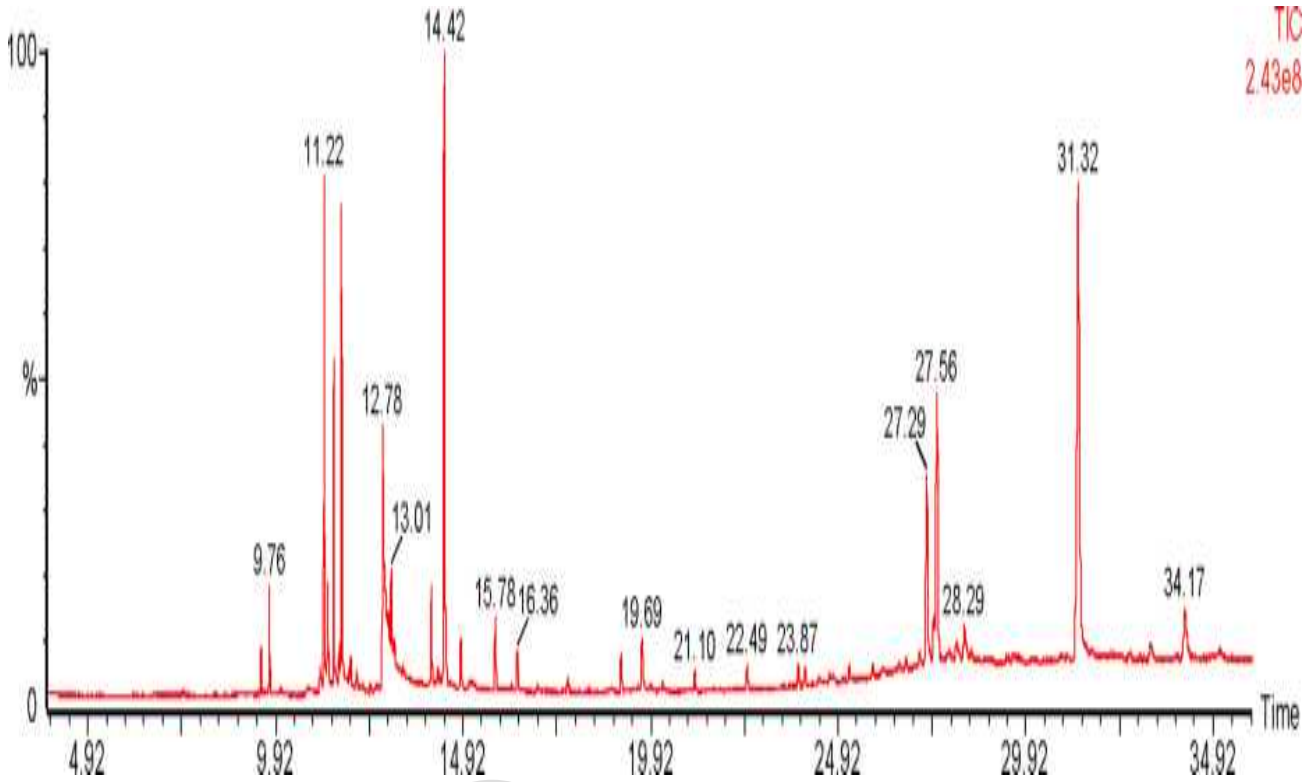

Fig.1 Components identified in the *Caulerpa racemosa* seaweed extract using GC- MS

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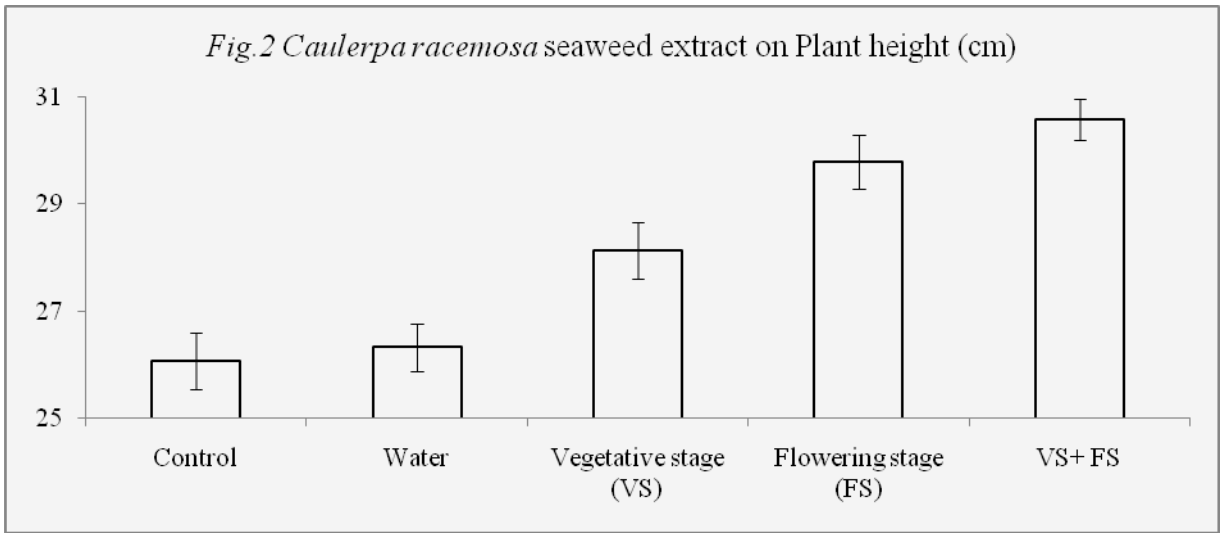

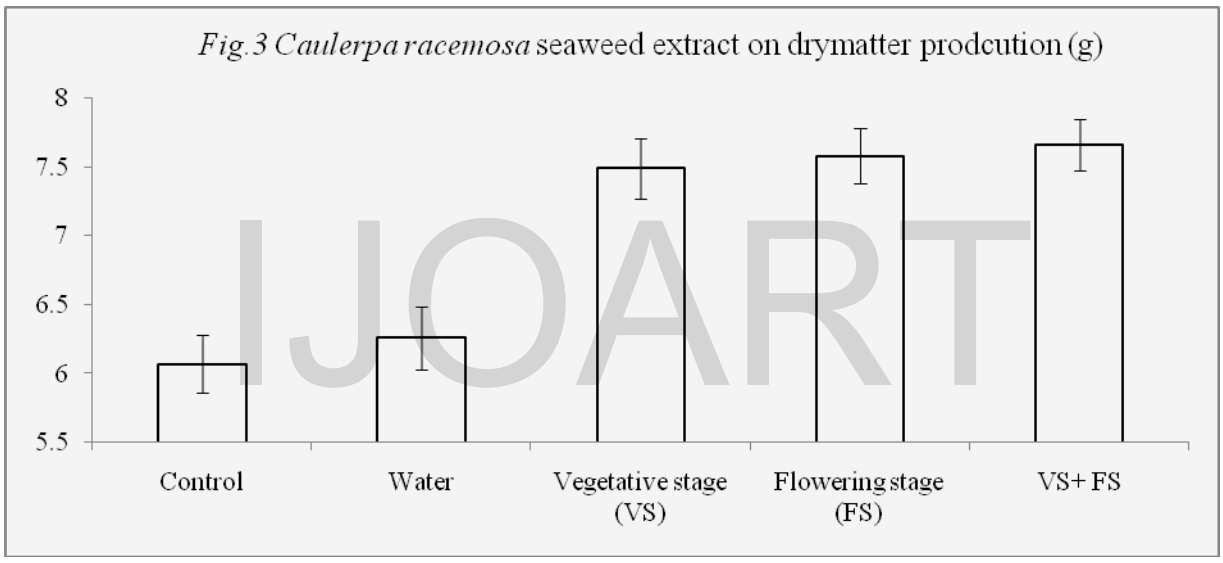

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Fig 4. Caulerpa racemosa seaweed extract on Leaf Area Index and Crop Growth Rate (g $m^{-2}d^{-1}$)

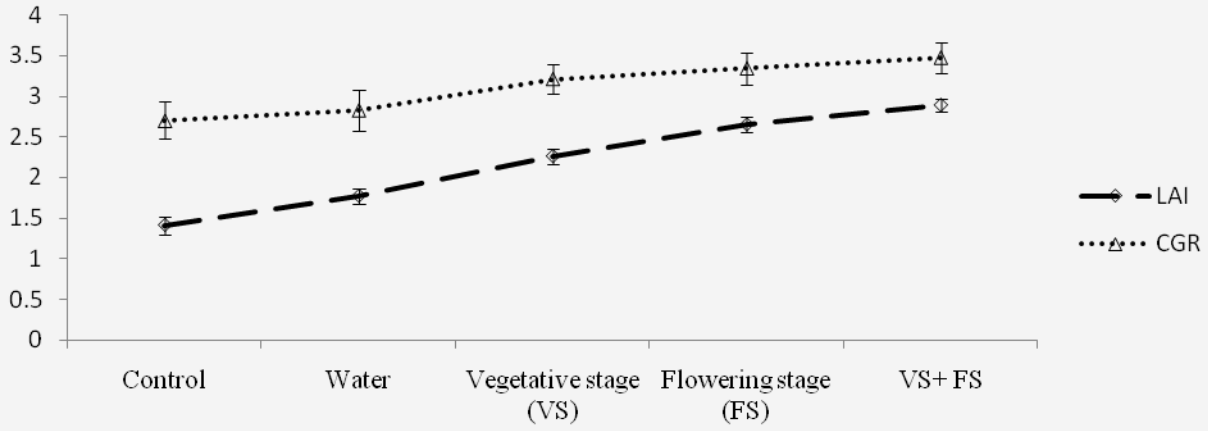


Fig.5. Caulerpa racemosa seaweed extract on Total Chlorophyll content

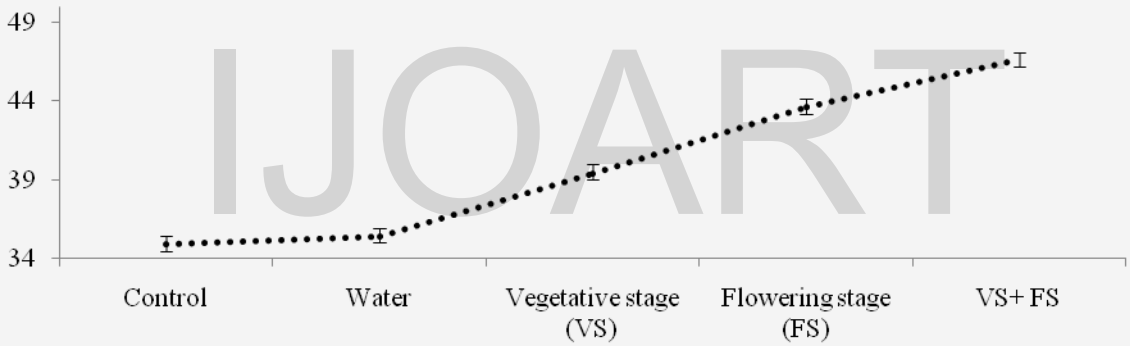
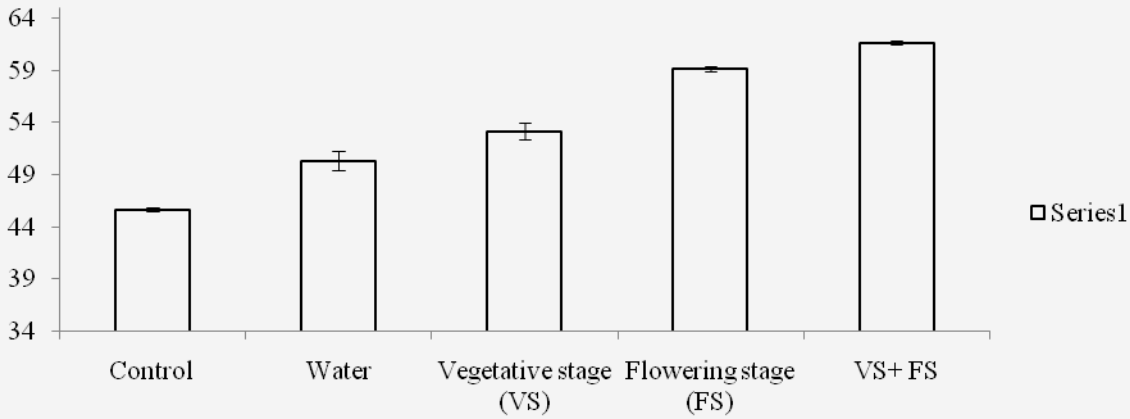


Fig.6 Caulerpa racemosa seaweed extract on Number of pods plant -1



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