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DEVELOPMENT OF A PROTOCOL FOR THE APPLICATION OF COMMERCIAL BIO-STIMULANT MANUFACTURED FROM *Kappaphycus alvarezii* IN SELECTED VEGETABLE CROPS

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Yield and quality

ABSTRACT

The field study was conducted to develop a protocol for application of commercially manufactured bio-stimulant (Brand name: AquaSap) from seaweed *Kappaphycus alvarezii*. Efficacy of the bio-stimulant was tested at 5% through foliar application in selected important vegetable crops. 3 to 4 applications were applied based on the crop cycle of the plant. Total 27 vegetable crops were studied during 2012 to 2015 and observed their response towards bio-stimulant applied in terms of general health of the plant, growth, yield and quality of the vegetable produce. 11% to 52% of yield increases were observed with improved quality in all 27 crops studied. Therefore seaweed bio-stimulants will have enormous potential to organic vegetable production in future.

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1 Introduction

Seaweed personifies not only as an alternative to conventional chemical fertilizers but also chronically used in agriculture, horticulture, cookies, ice-cream and jelly mix (Sumkiman et al., 2014). Further, it was well reported that seaweed extract contain nutrient of major and minor element, vital amino acid, essential vitamins and plant growth regulators which stimulate the growth and quality yield of crops. Application of seaweed liquid extract stimulate different aspects of plant like good health, development of root system, absorption of mineral, enlargement of shoot, increased rate of photosynthesis and crop yield (Sridhar & Rengasamy, 2010). Seaweed liquid extract have newly gained importance as foliar spray for lots of crops including various variety of grasses, flowers, cereals, vegetables and spices (Pramanick et al. 2013 & 2014). Further, Zodape (2001) tried various modes of seaweed extract application such as a foliar spray, application to soil and soaking of seeds before sowing and reported that extract not only enhances the germination of seeds but also increases uptake of plant nutrients and gives resistance to frost and fungal diseases.

The aqueous extracts of the alga *Codium fragile* was effective in increasing root length and it is 18.0% longer than the control in soybeans (Anisimov & Chaikina, 2014). Furthermore, Pise & Sabale (2010) treated fenugreek with 50% of seaweed and reported improvement in the concentration of carbohydrate, proteins, free amino acids, polyphenols and nitrogen content while comparing with control plants. Similarly, yield and nutrient content value were found higher in banana when treated with 5% of bio-stimulant (AQUASAP) of *Kappaphycus alvarezii* (Karthikeyan & Shanmugam, 2014). Vegetables are herbaceous plants and produce large amount of biomass within short period (Chatterjee & Thirumdasu, 2014). Vegetables are very essential to human health as they are rich in dietary fibre and source of essential vitamins, minerals, trace elements, vitamins and antioxidants.

In India, vegetable production was around 146.55 million tons from an area of 8.5 million hectare during 2010-2011. The 4 major vegetables viz. potato (28.9%), tomato (11.3%), onion (10.3%) and brinjal (8.1%) contribute 58.6% of total vegetable production. Other important vegetables are cabbage (5.4%), cauliflower (4.6%), okra (3.9%), peas (2.4%) and okra contribute 73% of total world production (Vanitha et al., 2013). The bio-stimulant manure from red seaweed *K. alvarezii* is well-off in potash with other primary nutrients like N, P, K and secondary nutrients like Cu, Zn, Fe, Mo, Mn, etc., in addition and to significant amount of plant growth regulators (Zodape et al., 2009; Prasad et al., 2010; Karthikeyan & Shanmugam, 2014). The present investigation describes the dosage and application protocol of bio-stimulant manufactured from *K. alvarezii* (AQUASAP is brand name of AquAgri) on some selected 27 vegetables crops for yield and quality improvement.

2 Materials and Methods

The trial was carried out at R&D plot of AquAgri Processing Private Limited and in the farmers' field in Manamadurai, Sivagangai Dt., Tamil Nadu, India. (Latitude is 9º42´56´´N and longitude 78º28´2´´E). The annual normal rainfall received by the district is 850 mm. The experiment trial was conducted in 8 plots with 6 m x 4m for each vegetable crop studied. The healthy seeds were selected and sowed carefully into the field and the trial crops were irrigated periodically and chemical fertilizers were applied to crops as per the recommendation of National Horticulture Board, India. Bio-stimulant (Aquasap) was collected from the stock of AquAgri Processing Private Limited and 5% solution was prepared and used.

2.1 Application protocol of bio-stimulant Aquasap for vegetable crops

Bio-stimulant (Brand name: AquaSap) manufactured from *K. alvarezii* was applied to the crops tested in the present investigation through foliar application. Three doses viz. vegetative, pre-flowering and post flowering stages were given to short-term plants whereas four doses were applied to long-term crops. Table 2-6 shows the application protocol for 27 crops tested in this study. The physico-chemical and nutritive value of the bio-stimulant (AquaSap) has been given in table 1.

2.1.1 Tomato (*Solanum lycopersicum* L.)

The trial on tomato (Co3 hybrid) was conducted in June 2012 (Table 2.) The seeds were sowed in nursery beds, then nursery plants were collected after 25th day of sowing and their roots were dipped at 0.7% of bio-stimulant Aquasap for 10min before transplantation. The first spray was given on 10th day of transplantation, second and third doses were sprayed on 25-30d (pre-flowering stage) and on 45-50d (flowering stage) respectively and last dose was applied at first picking stage (Table 3).

2.1.2 Lady's finger (Okra) (*Abelmoschus esculentus (L)* Moench) and Brinjal (*Solanum melongeana* L.)

The experiment of lady's finger (var. US 7902) and brinjal Co2 hybrid was also conducted in 2012 (Table 2). The okra seeds were soaked in 1% of bio-stimulant for 10 min. and the soaked seeds were sowed into the field directly. Treated seeds were also sowed in nursery beds and nursery plants (35d old) were collected, treated their roots with 0.7% of bio-stimulant for 10 min before transplantation. The application of bio-stimulant through foliar was given at the vegetative stage (15-20d), second spray at flowering stage (35-40d) and final spray was at first fruits picking stage (50-55d) (Table 3).

Table 1 Physico-chemical properties and Nutritive status of Aquasap bio-stimulant from seaweed *K. alvarezii.*

Parameters	Units	Results	Parameters	Units	Results
Physical properties of AquaSap			**Nutritive Value (Amino Acid)**		
			Alanine	g/100g	0.014
Organic Matter (%)	gm/100g	0.65	Arginine	g/100g	0.0003
Specific Gravity	-	1.14	Aspartic acid	g/100g	0.0019
Electrical Conductivity	dSm^{-1}	63.3	Cystine	g/100g	0.0017
pH (1% solution)	-	6.68	Glycine	g/100g	0.065
Moisture content (%)	gm/100g	94.82	Histidine	g/100g	0.0007
Total Ash (%)	gm/100g	4.53	Isoleucine	g/100g	0.0022
Macro and Micro Nutrient contents AquaSap			Leucine	g/100g	0.0022
			Lysine	g/100g	0.019
Parameters	**Units**	**Results**	Tryptophan	g/100g	0.007
Nitrogen (N)	gm/100g	0.007	Methionine	g/100g	0.0007
Phosphorous (P)	mg/kg	3.57	Phenylalanine	g/100g	0.0028
Potash (K)	gm/100g	1.50	Proline	g/100g	0.053
Sodium (Na)	gm/100g	0.26	Serine	g/100g	0.0013
Calcium (Ca)	gm/100g	0.03	Threonine	g/100g	0.0006
Silica (Si)	gm/100g	0.02	Tyrosine	g/100g	0.0016
Chlorine (Cl)	gm/100g	2.15	Valine	g/100g	0.0026
Magnesium (Mg)	mg/kg	0.04	Glutamic acid	g/100g	0.0022
Iron (Fe)	gm/100g	16.95	**Nutritive Value (Vitamins)**		
Sulphur (S)	gm/100g	0.03	Vitamin - A	IU/100g	3363.44
Boron as (B)	mg/kg	768	Vitamin – E	IU/100g	0.21
Copper (Cu)	mg/kg	1.1	Vitamin – C	mg/100g	22.52
Zinc (Zn)	mg/kg	2.15	Vitamin – B1	mg/100g	0.007
Manganese (Mn)	mg/kg	5.93	Vitamin –B5	mg/100g	301.1
Cobalt (Co)	mg/kg	0.92	Vitamin – B6	mg/100g	3170.2

2.1.3 Chillies *(Capsicum annuum L. Var. annuum*)

Hybrid chilli (US 612) was selected for present study and its seeds were sowed directly into field. The seaweed bio-stimulant was applied at vegetative stage (40-45d), at flowering stage (90-100d) and last dose was given at first fruits picking stage (125-130d). In the case of transplanted plant, nurseries were created (40d old) and treated their roots at 0.7% of bio-stimulant for 10min before transplantation. During growing period, first dose of bio-stimulant was given at 20-25th day of transplantation and second and last applications were given at 60-65th day (i.e. flowering stage) and at 80-85th day of transplantation (Table 3) respectively.

2.1.4 Capsicum (*Capsicum annuum L*)

Trial on capsicum (var. Arka Mohini) was carried out in January 2014 (Table 2). The bio-stimulant AquaSap was applied at vegetative (30-35d), flowering stage (60-65d) and fruits picking stage (90-95d). But in the case of transplants raised from 40d old nurseries whose roots were treated with 0.7% of bio-stimulant for 10min before transplantation, first dose was given at 20-25d (vegetative stage), 60-65d (flowering stage) and at 80-85d (first fruits picking stage) day of transplantation (Table 3).

2.1.5 Variety of Gourds

The experimental study on nine varieties of gourds, i.e., Ash gourd (var. MAH-1), Pumpkin (Arka Chandan), Snake gourd (Covai -951), Ridge gourd (US 66), Bottle gourd (WARAD MGH-4), Bitter gourd (US 475), Cucumber (local variety), Watermelon (Ankur Kashish) and Chow chow (Green Fruits) (Table 2) was conducted during 2012 to 2014. The seeds were soaked in 1% of bio-stimulant for 30 min. and the seeds were sowed in the study field. The application of bio-stimulant was given at vegetative stage (20-25d), flowering stage (60-65d) and first fruits picking stage (80-85d). In the case of chow chow, mature fruits were planted in the field, and bio-stimulant was first applied at vegetative phase of 25-30 days of plantation, pre-flowering phase (3rd month) and final dose was given at flowering phase (5th month) (Table 3). In the cucumber, first spray was done at germination phase (10-15th day), followed by second spray at 35-40th day (vegetative stage) and final dose was applied at flowering initiation stage (65-70d) (Table 4).

2.1.6 Cole crops, Root and Tuber vegetables

2.1.6.1 Potato (*Solanum tuberosum* L.)

The potato (var. Kufri Jyoti) trial was conducted in August 2013 (Table 2). Bio-stimulant AquaSap was applied at plant establishment stage (20-25d) vegetative phase (50-55d), early root development stage (80-85d) and last dosage was given at maturity stage (100-105d) (Table 5).

2.1.6.2 Cabbage (*Brassica oleracea var. capitata* L)

Maharani- F1, a hybrid variety of cabbage was taken for trial in January 2013 (Table 2). 40 days old nursery plant was created and root treatment was given at 0.7% of bio-stimulant for 10min before transplantation. Field application of bio-stimulant was applied at plant establishment stage (10-15d), second dose was sprayed at head initiation stage (35- 40d) and last spray was sprayed at head development phase (70-75d) (Table 5).

2.1.6.3 Cauliflower (*Brassica oleracea var. botrytis*)

In January 2013, cauliflower (var. Shobha F1) was taken for trial (Table 2). The root of nursery plants (35d) raised was treated with 0.7% of bio-stimulant for 10min and transplanted. During crop cycle, first spray of bio-stimulant aquasap was given at plant establishment stage (10-15d), the second dose at curd initiation stage (25-30d) and last dose was given at curd development stage (45-50d) (Table 5).

2.1.6.4 Beetroot (*Beta vulgaris* L) and Carrot (*Daucus carota* L)

The studies on beetroot (Vally Queen) as well as carrot (Pusa Kesar) were conducted in 2013 and bio-stimulant Aquasap was applied at vegetative stage (25-30d), early root development stage (55- 60d) and root maturity stage (80 -85d) (Table 5).

2.1.6.5 Radish (*Raphanus sativus* L) and Knol-Khol (*Brassica caulorapa*)

Radish (Roshni) and knol-khol (Early White) trial was undertaken in January 2013. The bio-stimulant was given at 10-15th (vegetative stage), 25-30th (early root development stage) and at 40 - 45th day of sowing (root maturity stage) (Table 5).

2.1.7 Other vegetable crops

2.1.7.1 Lima Bean (*Phaseolus lunatus* L) and Dolichos Bean (*Lab lab purpureus* var. *typicus*)

The experiment on Lima (Co2) and Dolichos (Ankur Goldy) beans were conducted during 2012. The seeds were soaked in 1% of bio-stimulant for 10min then sowed into the field. During crop period, three spray of bio-stimulant were given viz. at vegetative phase (20-25d), flowering stage (40-45d), pod formation stage (60-65d) and last spray was given at first picking stage (80-85th day of sowing) (Table 6).

2.1.7.2 Soybean (*Glycine max* (L.) Marr.)

The experiment on soybean (JSS 355) was conducted in July, 2012. The seeds were soaked in 1% of bio-stimulant for 10min and then the seeds were carefully sowed in the field. The crop was applied with bio-stimulant Aquasap for four times viz. at 20-25d, 40-45d, 60-65d and at 80-85th day of sowing (Table 6).

2.1.7.3 Moringa (*Moringa oleifera* L.)

The efficacy trial of AquaSap on drumstick (PKM-1) was conducted in 2012 (Table 2). The seeds were soaked in 1% of bio-stimulant for 10min and during crop period bio-stimulant aquasap was applied at nurseries stage (25-30th), pre-flowering phase (3rd month), flowering phase (4th month), and at fruits development stage (5th month of plantation) (Table 6).

2.1.7.4 Small Onion (*Allium cepa var. aggregatum*)

Trial on small onion (var. Co-ON-5) was conducted in June 2013. The seaweed bio-stimulant at 5% was sprayed as foliar application at establishment stage (10-15d), vegetative stage (25-30d), bulb formation stage (40-45d) and bulb development stage (60-65d) as shown in Table 6.

2.1.7.5 Bellary Onion (*Allium cepa var. cepa*)

Effect of bio-stimulant AquaSap on Bellary onion (var. Prema-178) was studied in June 2013. The application of 5% bio-stimulant was given on 10-15th (sowing establishment stage), 35-40th (vegetative stage), 60-65th (bulb formation stage) 75-80th (bulb development stage) day of sowing (Table 6).

3 Results and Discussion

All vegetable crops investigated in the present study responded well at 5% dose of bio-stimulant Aquasap (from seaweed of *K. alvarezii*). Highest yield was found in moringa with 52.83% over control followed by lady's finger, chillies, cabbage, garden lab lab, bellary onion, small onion, ash gourd, and snake gourd with 45.84%, 37.30%, 36.74%, 33.03%, 32.53%, 30.74%, 30.15%, and 30.02% respectively with improved quality (Table 2). Improved yield and quality in crops applied with seaweed liquid fertilizers have been well documented (Khan et al., 2009). Zodape et al. (2008) had observed that okra yielded 20.94% more over control with application of 2.5% extract of *K. alvarezii* and in tomato the increment when treated with 5% extract was 60.89% Zodape et al., (2011). The yield of brinjal with *Eucheuma* seaweed powder increased marginally higher to 41.1% (Eswaran et al., 2005). Similar kind of result with eggplant was reported when it treated with 2% extract *of Ascophyllum nodosum* (Bozorgi, 2012). Babu & Rengasamy (2012) observed that when chillies were treated with 1% and 2% of SLF of *K. alvarezii*, it

increased the crop yield to 23% and 15% respectively when compare to control.

The high yield was also observed in ash gourds with 30.15% over control followed by pumpkin, snake gourd, ridge gourd, bottle gourd, bitter gourd, cucumber, watermelon and chow chow with 28.56%, 30.02%, 11.98%, 28.03%, 26.64%, 24.19%, 25.89%, and 17.34% respectively (Table 2). Ahmed & Shalaby (2012) recommend that liquid extract of *E. intestinelis* (green alga), *G. pectinutum* (red alga) or commercial seaweed liquid extract (Algreen) in addition to manure is suitable product for better vegetative growth and yield of cucumber plants. The seaweed extract of *Ascophyllum nodosum* (3g/l) applied on watermelon plant, increased the fresh weight, fruits diameter and peel thickness than control plant (Abdel-Mawgoud et al., 2010).

Higher crop yields were observed in cabbage (36.74%), cauliflower (29.61%), beetroot (28.84%), knol-khol (28.80%), radish (26.08%), potato (23.90%) and carrot (14.21%) when compared to control plants (Table 2). The seaweed extracts powder Alga 600 and Seaforce-2 when applied on potato; it increased the dry tuber weight to 14.67% when compared with control (Sarhan, 2011). Abetz & Young (1983) observed that the yield and size of cauliflower increased when treated with *A. nodosum* extract.

The yield of treated plants in moringa, dolichos bean, bellary onion, small onion, lima bean and soybean were 52.83%, 33.03%, 32.53%, 30.74%, 25.38% and 22.10% respectively (Table 2). Soybean treated with extract of *Kappaphycus* at 15% and 12.5% showed highest grain yield of 57% and 46% respectively compared to the control and maximum straw yield was also found with treatment of 15% extract (Rathore et al., 2009). Similar kind of result in onion bulb (22.0%) when treated with *Eucheuma* seaweed powder (Eswaran et al., 2005) and high yield with improved quality of onion was found when treated with extract of *A. nodosum* (Dogra & Mandradia, 2012).

Table 2 Effect of bio-stimulant from seaweed *K. alvarezii* on yield of some vegetable crops.

Cultivar name	Variety name	Plantation type	Date of plantation	Yield increase over control (%)
Tomato	Co3 Hybrid	Seeds	03.06.12	20.94
Lady's finger	US 7902 Hybrid	Seeds	22.05.12	45.84
Brinjal	Co2 Hybrid	Seeds	03.06.12	24.53
Chillies	US 612 Hybrid	Seeds	01.01.14	37.30
Capsicum	ARKA MOHINI	Seeds	01.01.14	29.28
Ash gourd	MAH-1 Hybrid	Seeds	12.12.14	30.15
Pumpkin	ARKA CHANDAN	Seeds	12.12.14	28.56
Snake gourd	COVAI 951 F1 Hybrid	Seeds	20.07.12	30.02
Ridge gourd	US 66 Hybrid	Seeds	20.07.12	11.98
Bottle gourd	WARAD MGH-4	Seeds	20.07.12	28.03
Bitter gourd	US 475 Hybrid	Seeds	20.07.12	26.64
Cucumber	Local	Seeds	25.12.13	24.19
Watermelon	ANKUR KASHISH Hybrid	Seeds	25.12.13	25.89
Chow-chow	Green fruits	Fully matured fruits	15.08.13	17.34
Potato	KUFRI JYOTI	Seeds	15.08.13	23.90
Cabbage	MAHARANI- F1	Seeds	09.01.13	36.74
Cauliflower	SHOBHA -F1	Seeds	09.01.13	29.61
Beetroot	VALLY QUEEN	Seeds	18.01.13	28.84
Carrot	PUSA KESAR	Seeds	15.08.13	14.21
Radish	ROSHNI	Seeds	18.01.13	26.08
Lima Bean	Co2	Seeds	17.07.12	25.38
Doli Chos Bean	ANKUR GOLDY	Seeds	17.07.12	33.03
Soybean	JSS 355	Seeds	17.07.12	22.10
Moringa	PKM-1	Seeds	02.05.12	52.83
Small onion	CO-ON-5	Seeds	15.06.13	30.74
Bellary onion	PREMA - 178	Seeds	15.06.13	32.53
Knol-khol	EARLY WHITE	Seeds	18.01.13	28.80

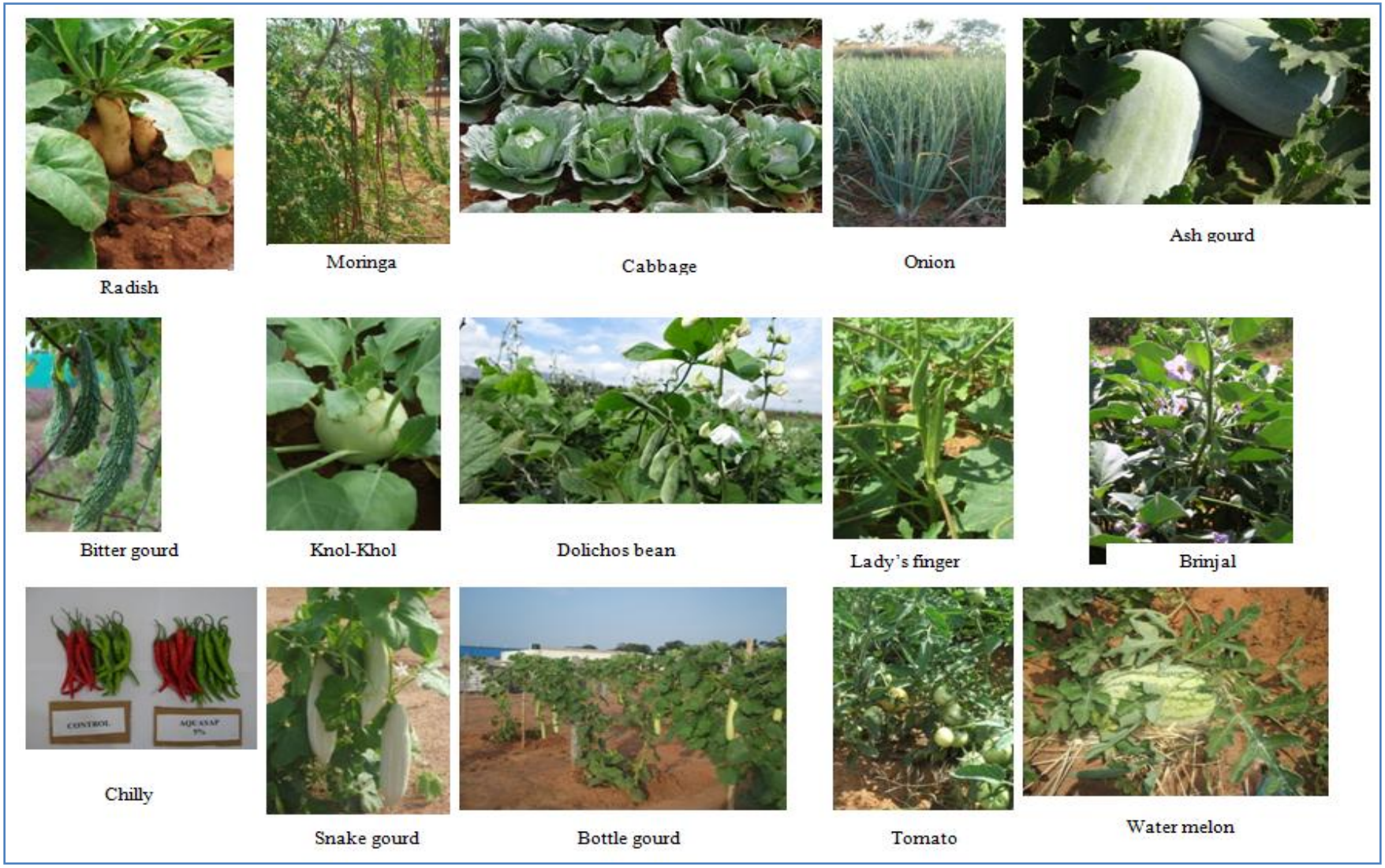

Figure 1 Some of the vegetable crops studied in the present investigation with their vegetable yield

Table 3 Dosage and application protocol of seaweed bio-stimulant used for home garden vegetable crops.

Crop name	Root dip	I Dose	II Dose	III Dose	TBS
Tomato [b]	Root of nurseries was dipped in 0.7% of bio-stimulant for 10min before transplantation.	10th day (After transplantation)	25-30th day (Pre-flowering stage)	45-50th day (Flowering phase)	21 (1+5+5+5+5)
Lady's finger [c] **(Okra)**	-	15-20th day (Germination stage)	35-40th day (Flowering stage)	50-55th day (First fruits picking stage)	16 (1+5+5+5)
Brinjal	Root of nurseries was dipped in 0.7% of bio-stimulant for 10min before transplantation.	15-20th day (Germination stage)	35-40th day (Flowering stage)	50-55th day (First fruits picking stage)	16 (1+5+5+5)
Chillies	Sowing	40-45th day (Vegetative stage)	95-100th day (Flowering stage)	125-130th day (Fruits picking stage)	15 (5+5+5)
	Transplantation**:** Root of nurseries was dipped in 0.7% of bio-stimulant for 10min before transplantation.	20-25th day (Days after transplantation)	60-65th day (Flowering stage)	80-85th day (First fruits picking stage)	16 (1+5+5+5)
Capsicum (Sweet pepper / Bell pepper)	Sowing	30-35th day (Vegetative stage)	60-65th day (Flowering stage)	90-95th day (Fruits picking stage)	15 (5+5+5)
	Transplantation**:** Root of nurseries is dipped in 0.7% of bio-stimulant for 10min before transplantation.	20-25th day (Days after Transplantation)	60-65th day (Flowering stage)	80-85th day (First fruits picking stage)	16 (1+5+5+5)

[a] Recommended dosage of bio-stimulant: 5%; [b] IV dose at 75-80th day (Picking phase); [c] Seed treatment; Seeds were soaked for 10min in 1% of bio-stimulant before sowing, TBS - Total bio-stimulant required Per acre (L).

3.1 Effect on plant disease control

Twenty seven vegetable crops studied in the present investigation looked healthy and generally free from disease as compared to their control plants. Extract of seaweed have been reported to increase resistance of plant against pest and diseases, increase plant growth and quality yield (Jolivet et al., 1991; Verkleij, 1992; Pardee et al., 2004; Hong et al., 2007; Jeyaraj et al., 2008). Similarly, Sultana et al. (2011) had reported that number of liquid seaweed extract found to control root rotting fungi like *Rhizoctonia solani*, *Macrophomina phaseolina*, *Fusarium* species and root kot nematode (*Meloidogyne* spp.) on a variety of crops. The resistance to frost and fungal disease were reported when seaweed extract was applied to some crops (Zodape, 2001). Ara et al. (1996) had observed that extract of *Sargassum* spp. controlled the root rot disease in sunflower plant. Seaweed fertilizer was found to boost the resistibility adjacent to disease and in addition to reduce the insect attack (Zahid, 1999). Dogra & Mandradia (2012) had found that extract of *A. nodosum* significantly reduced the downy mildew severity over control in onion plant and it had also been reported that seaweed extract of *Asparagopsis taxiformis* found to act against phytopathogens (Manilal et al., 2009). Lynn (1972) had observed that seaweed extract of *A. nodosum* protected *Capsicum annuum* and sweet pepper from stress to frost, microbial diseases and insect attack and increased the shelf life of fruits and better seed germination.

3.2 Effect of seed Treatment

Seed and root treatment had improved the viability of plantlets and grow vigorously as compared to control plants in the present study and it is in agreement with the literatures reports. The introductory soaking of *Triticum aestivun* seeds in 20% extracts of *Sargassum wightii* for 24 hrs gave an 11% increase in seed germination, a 63% enhance in number of lateral roots and 46% increase in shoot length in comparing to control (Kumar & Sahoo, 2011). 100% seed germination was observed in lowest concentration of SLF in black gram (Venkataraman Kumar et al., 1993) and SLF promote the seed germination as well as yield of the vegetable crops (Narasimha Rao & Reshmi Chatterjee, 2014). Treatment at 0.05% of concentrated extract of *Laminaria digitata* on *Plantago lanceolata*, *Trifolium repens* and *Avena strigosa* had given higher germination percentage (Thorsen et al., 2010).

Table 4 Dosage and application protocol of seaweed bio-stimulant used for gourds vegetable crops.

Crop name	Seed treatment	I Dose	II Dose	III Dose	TBS
Ash gourd	Seeds were soaked for 30min in 1% of bio-stimulant and incubated it for 6 days for before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)
Pumpkin	Seeds were soaked for 30min in 1% of bio-stimulant and incubate it for 6 days for before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)
Snake gourd	Seeds were soaked for 30min in 1% of bio-stimulant before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)
Ridge gourd / Ribbed gourd	Seeds were soaked for 30min in 1% of bio-stimulant before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)
Bottle gourd	Seeds were soaked for 30min in 1% of bio-stimulant before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)
Bitter gourd	Seeds were soaked for 30min in 1% of bio-stimulant before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)
Cucumber	Seeds were soaked for 30min in 1% of bio-stimulant before sowing	10th day (Germination phase)	35-40th day (Vegetative stage)	65-70th day (Flowering initiation to first picking stage)	16 (1+5+5+5)
Watermelon	Seeds were soaked for 30min in 1% of bio-stimulant before sowing	20-25th day (Vegetative stage)	60-65th day (Flowering stage)	80-85th day (Picking stage)	16 (1+5+5+5)

[a] Recommended dosage of bio-stimulant 5%, TBS - Total bio-stimulant per acre (Lit),

Table 5 Dosage and application protocol of seaweed bio-stimulant used for cole, root and tuber vegetable crops.

Crop name	I Dose	II Dose	III Dose	TBS
Chow chow (Chayote)	25 - 30th day (Vegetative phase)	3rd month (Pre-flowering phase)	5th month (Flowering phase)	15 (5+5+5)
Potato [b]	20-25th day (Plant establishment stage)	50-55th day (Vegetative phase)	80-85th day (Early root development Phase)	20 (5+5+5+5)
Cabbage [c]	10-15th day (Plant establishment stage)	35-40th day (Head initiation stage)	70-75th day (Head development phase)	16 (1+5+5+5)
Cauliflower [c]	10-15th day (Plant establishment stage)	25-30th day (Curd initiation stage)	45-50th day (Curd development phase)	16 (1+5+5+5)
Beetroot	25-30th day (Vegetative stage)	55-60th day (Early root development stage)	80-85th day (Maturity stage)	16 (1+5+5+5)
Carrot	25-30th day (Vegetative stage)	55-60th day (Early root development stage)	80-85th day (Maturity stage)	15 (5+5+5)
Radish	10-15th day (Vegetative phase)	25-30th day (Early root development stage)	40-45th day (Maturity stage)	15 (5+5+5)
Knol-khol	10-15th day (Vegetative phase)	25-30th day (Early root development stage)	40-45th day (Maturity stage)	15 (5+5+5)

[a] Recommended dosage of bio-stimulant 5%, [b] IV Dose at 100-105th day (Root maturity stage), [c] Root dip: Transplantation: Root of nurseries was dipped in 0.7% of bio-stimulant for 10min before transplantation, TBS - Total bio-stimulant per acre (Lit).

3.3 Effect of foliar application

Seaweed extract applied as foliar application found to significantly enhance the yield, growth and quality of crops (Pramanick et al., 2013). Seaweed liquid extract have gained importance to different range of crops like cereals, grasses, vegetables, species and flowers when applied through foliar application (Crouch & Van Staden, 1992). Seaweed extract is important to find out the organic sources for seed and foliar treatments for effective maintenance of vigour and viability (Dwivedi et al., 2014). The maximum yield of tomato (Zodape et al., 2011) and banana (Karthikeyan & Shanmugam, 2014) had been observed when using foliar application of *K. alvarezii* extract. Similar kind of result was observed by Pramanick et al., (2013) that the foliar application of seaweed sap improved the nutrient uptake capacity of crops.

In the present investigation, it was also observed that emerging of first flower appeared in all treated plants at least 5-10d earlier than control and similar kind of observation had been recorded in the literature. Dwivedi et al., (2014) reported that seaweed extracts not only increase the vegetative growth of the plant but it also triggers the early flowering, fruiting in crops and ultimately on seed yields. Seaweed extracts are ecologically safe, non-polluting, non-toxic, and harmless to human beings, animals and birds (Dhargalkar & Pereira, 2005). In addition to reducing the cost of inorganic fertilizers, application of seaweed bio-stimulants improves soil health, enhances the yield and quality of produce in organic vegetables production thereby increasing the domestic and international market (Chatterjee & Thirumdasu, 2014).

Conclusions

It can be concluded from the present study that 27 vegetable crops tested had responded well to bio-stimulant (Aquasap) manufactured from seaweed *K. alvarezii*. The average yield increased from 11.98% to 45.84% with much improved vegetable quality. Therefore, the protocol used in this study will be useful to the farmers to produce organic vegetables.

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Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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Table 6 Dosage and application protocol of seaweed bio-stimulant used for other vegetable crops.

Crop name	I Dose	II Dose	III Dose	IV Dose	TBS
Lima bean [b]	20-25th day (Vegetative phase)	40-45th day (Flowering stage)	60-65th day (Pod formation stage)	80-85th day (First picking stage)	21 (1+5+5+5+5)
Dolichos beans[b]	20-25th day (Vegetative phase)	40-45th day (Flowering stage)	60-65th day (Pod formation stage)	80-85th day (First picking stage)	21 (1+5+5+5+5)
Soybean [b]	20-25th day (Vegetative phase)	40-45th day (Flowering stage)	60-65th day (Pod formation stage)	80-85th day (First picking stage)	21 (1+5+5+5+5)
Moringa [b]	25-30th day (Nurseries stage)	3rd month (Pre-flowering phase)	4th month (Flowering phase)	5th month (Fruits development stage)	21 (1+5+5+5+5)
Small onion	10-15th day (Sowing to establishment stage)	25-30th day (Vegetable stage)	40-45th day (Bulb formation stage)	60-65th day (Bulb development stage)	20 (5+5+5+5)
Bellary onion	10-15th day (Sowing to establishment stage)	35-40th day (Vegetable stage)	60-65th day (Bulb formation stage)	75-80th day (Bulb development stage)	20 (5+5+5+5)

[a] Recommended dosage of bio-stimulant 5%, [b] Seed treatment - Seeds were soaked for 10min in 1% of bio-stimulant before sowing, TBS - Total bio-stimulant per acre (Lit).

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