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Evaluation of brown seaweed (*Padina pavonica*) as biostimulant of plant growth and development

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An innovative horticulture nutrient and biodegradable support is described in this paper for replacing plastic culture pots. This support is prepared with *Luffa aegyptica*, plant having a water holding capacity higher than that of the regular soil and that is also biodegradable. Brown seaweed *Padina pavonica* was incorporated as an organic fertilizer of plant growth. Chemical analysis of the aqueous extract of this alga showed the presence of macronutrients such as nitrogen (N), phosphorus (P) and potassium (K) necessary for development and growth of plants. Agar-agar was added as a solidifying agent. A medium containing only soil and another containing soil with chemical fertilizer served as controls. Sunflower seeds grown in medium supplemented with brown seaweed; (*P. pavonica* + agar (4% or 6%) + *L. aegyptica* have a growth rate (length and diameter of the stem, number of leaves) that is slower than the plants grown in a medium with a comparable amount of the soil with chemical fertilizer. However, the plants in the soil and others in the soil with chemical fertilizer and the media (seaweed + *L. aegyptica* + agar 4%) have not completed their growth while the plants grown in the media (seaweed + *L. aegyptica* + agar 6%) continued to grow. A biodegradability test showed that a piece of support (seaweed + agar 1.5% + *L. aegyptica*) presented a degradation rate higher than the support with only Luffa and agar 1.5%, while a piece of plastic had not degraded. The results of our study have shown that this support has helped to extend the duration of growth and enhanced the quality of the plants. Ultimately, the fabricated support presented fertilizer properties, water retention and biodegradability and could serve in horticulture as an alternative to plastic pots and chemical fertilizer.

Key words: Brown seaweed, *Padina pavonica*, Biostimulant, Luffa, water retention, biodegradability.

INTRODUCTION

Compared to organic fertilizers, chemical fertilizers are not sufficient to procure alone all the minerals and nutrients required by the plant: nitrogen, phosphorus, potassium, magnesium and other trace elements. Indeed, chemical fertilizers are not compatible with organic farming and have adverse effects on health and the environment. In addition to their high cost, they alter the quality, fertility, structure and humus of the soil. Modern agriculture is looking for new biotechnological advances that would allow a reduction in the use of chemical inputs without affecting crop yield or the farmer's income. In recent years, the use of natural seaweed as fertilizer has allowed for substitution in place of conventional synthetic fertilizer (Hong et al., 2007).

The agricultural sector is by far the largest user of water in the world. Referring to the consumption of water

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by evapo-transpiration, for 80 to 90% of the water is used in agriculture. Unfortunately, the efficiency of water use is very low and does not exceed 45% with more than 50% of losses, so it is more in agriculture than in other sectors that one can achieve substantial water savings. To meet these challenges, we must dramatically improve the use of water resources and technology and irrigation management. Moreover, the culture pots commonly used in horticulture are composed of non-biodegradable plastic. As a result, the plant with its clod of earth must be removed from the plastic pot before being planted in the ground or into a larger pot. The pot containing the plant initially is thrown by the consumer and thus contributes to environmental pollution.

Today, there are pots for growing plants made from biodegradable materials. The advantage of using such materials is that the plant can be buried with its support as it degrades naturally. Among the materials used in the manufacture of biodegradable pots are cellulose fibers, peat, starch or mixtures thereof. One of the major drawbacks of this type of materials is the non-existence of nutrients for the plant. On the contrary, the degradation of a device for packaging made of this material involves microorganisms in the soil whose activity requires nitrogen consumption. The plant is then deprived of part of the nitrogen present in its environment. The issue of chemical fertilizers, water shortage and non-biodegradable plastic pots is then at the heart of the debate and the cause of the ecological impacts threatening the health sector, agriculture and environment.

The main objective of this study is to producing a horticultural biodegradable nutrient support that does not require a large consumption of water, with a relatively low cost of production. The materials for the manufacture of this support are: The brown alga *Padina pavonica* as biofertilizer, lignocellulosic fiber (*Luffa aegyptica*) as water retention agents and the Agar-agar as a plasticizer. This support according to this composition will be fully biodegradable. The composition of this material has not been documented in any scientific publications to date. In Lebanon, the coastal marine environment that contains at least 243 algal species (Lakkis and Novel-Lakkis, 2000) is sadly untapped. In the literature, *P. pavonica* has already been studied for cytotoxic activity (Ktari and Guyot, 1999) for antibacterial activity (Chbani et al., 2011) and for antioxidant properties (Mawlawi et al., 2012) but has never been an object of research up to date as fertilizer. This has guided the choice of this seaweed in our experiments.

MATERIALS AND METHODS

Collection of seaweed: Biofertilizer agent

The brown alga *P. pavonica* belonging to the family Dictyotaceae was freshly collected manually from the coastal area of the Mediterranean, El Mina (34° 26'N-35° 50'E) in Tripoli, Lebanon during March 2011. It was washed with seawater to remove all the unwanted impurities such as adhering sand particles and epiphytes. Then, the seaweed was transported in polythene bags just moistened in the laboratory (Cabioc'h et al., 2006).

Preparation of seaweed liquid extracts (SLE) for physico-chemical analyses

The seaweed were thoroughly washed using tap water and ultrapure water to remove sea salts on the surface of samples. After that, fresh algae were cut into small pieces and boiled with ultrapure water under stirring for one hour on a hot plate. Then, the extract was filtered (Kumar and Sahoo, 2011).

To study and compare the composition of minerals and nutrients of *P. pavonica* liquid extract to other algae liquid extract used in other research, a dosage of the primary nutrients (N, P, K), secondary nutrients (Ca, Mg, S), trace elements (Cu, Zn, Fe, Co, Mn, Cl, Na), alkalinity (HCO_3^-) and undesirable elements (Ni, Pb, Cr Al, Cd) were analyzed by the method described by Normalization French Association (AFNOR). The pH of the SLE was directly measured using a pH meter ORION type. Each assay was performed twice.

Preparation of the water retention agent

The mature fruit of the species *L. aegyptica* (belonging to the family Cucurbitaceae) used in small pieces as a water retention agent in this study.

Agar-agar: Solidifier agent

The Agar-agar solidifier agent is extracted from red algae (Rhodophyceae) belonging to the families of Gelidiaceae. The Agar used was Fluka ® type (Cat. No.05040, Sigma-Aldrich Chemie GmbH) was prepared according to two different dilutions (4% and 6%) in water to compare the growth of sunflower plants.

Manufacture of horticultural biofertilizer and biodegradable support

Brown seaweed *P. pavonica* previously were dried at room temperature away from light and then ground in the form of fragments of 1 to 2 mm^2. The support was composed with algae *P. pavonica* (5%), crushed Luffa (3%) in pieces and Agar (92%) diluted with water. The hot mixture was then poured into molds and allowed to cool and solidify at room temperature. Finally, after cooling, the support was removed from the molds (Figure 1).

Germination and growth of sunflower seeds were conducted on the two supports, one containing only the soil and the other containing soil with chemical fertilizer were used as controls.

Experiment

Eight white sunflower seeds, Vilmorin type, with uniform shape, size, color and weight were incubated in water at 30°C for 48 h before inoculation. Every two sunflower seeds treated were sown at a depth of 1 cm in each support: (seaweed + *L. aegyptica* + Agar 4%), (seaweed + *L. aegyptica* + Agar 6%), (soil 100%) and (soil + chemical fertilizer). Then the 4 supports were placed in mini-greenhouse at 22 to 28°C, 70 to 85% relative humidity, 600 to 1,000 µmol photons m^{-2} s^{-1} light intensity and 12 h photoperiod during the period of observation. The seeds were watered regularly. The root system, the length of the stem, the stem diameter and number of

Figure 1. Biofertilizer support: a: Support with seaweed (*Padina pavonica*) + Lupha + Agar (4%). b: Support with seaweed (*Padina pavonica*) + Lupha + Agar (6%).

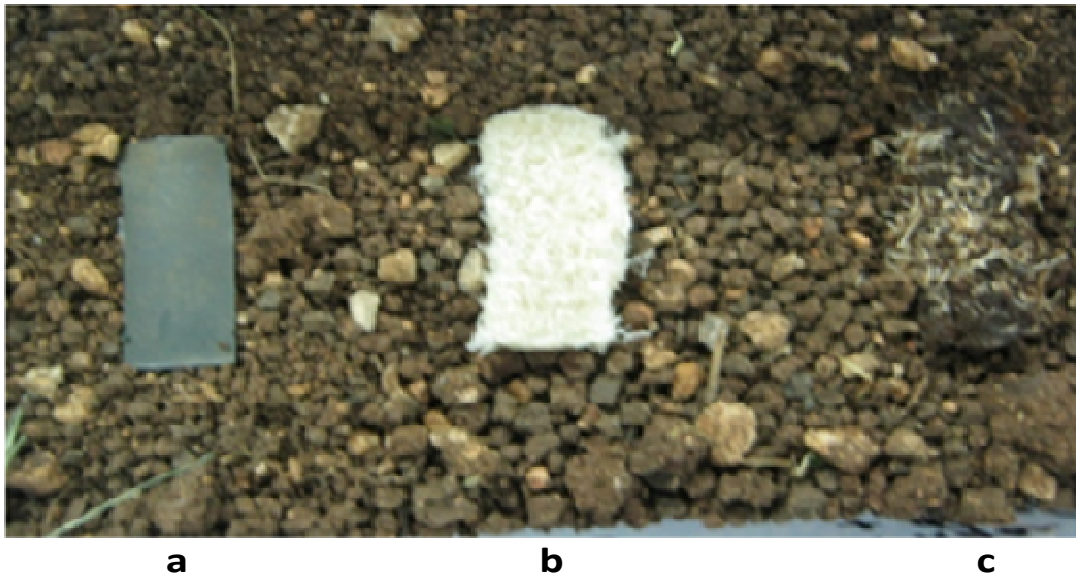

Figure 2. Biodegradability test of 3 fragments: a: Plastic fragment, b: Fragment with Luffa aegyptica + Agar and c: fragment of biofertilizer support with Luffa aegyptica + seaweed + Agar.

leaves were monitored by foot slide with an interval of 2 days within 18 days and after transfer in the soil. All samples were realized in triplicate.

Biodegradability test of nutritional support

A fragment (50 mm long, 20 mm wide and 2 mm thick) of each supports; plastic pots (Figure 2a), control support (*L. aegyptica* + Agar) (Figure 2b) and nutritional support (seaweed + *L. aegyptica* + Agar) (Figure 2c) was buried in 15 mm underground for 4 weeks. The soil was watered twice a week.

Testing of water holding capacity of nutritional support

To determine the volume of water retained in the nutritional support compared to that of the soil, a test of water-holding capacity of these materials has done achieved. Two supports (seaweed + *L. aegyptica* + Agar 6%) and (100% soil), were dried at 30°C and were weighed. Each support was then immersed in a water bath at room temperature until saturation and was weighed. The water holding capacity of each support is the measure of the volume of water retained per 100 g of support.

RESULTS

Chemical constituents

The chemical constituents of *P. pavonica* seaweed extract were analyzed and presented in Table 1. The

Table 1. Chemical constituents of *Padina pavonica* seaweed liquid extract.

Chemical constituents	(mg/L)
Nitrogen	10.90
Phosphorus	9.26
Potassium	160.13
Calcium	110.22
Magnésium	1.20
Sulphur	235.00
Zinc	-
Copper	-
Sodium	73.40
Chloride	85.09
Iron	-
Manganese	0.22
Cobalt	-
Carbonate HCO_3^-	207.40
Nickel	-
Plomb	-
Chrome	-
Aluminium	-
Cadmium	-

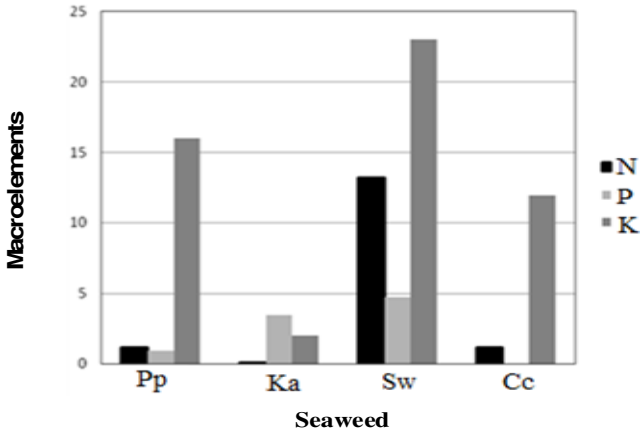

Figure 3. The macronutrients (N, P and K) measured in seaweeds: *Padina pavonica* (Pp), *Kappaphycus alvarezzii* (Ka), *Sargassum wightii* (Sw) and *Caulerpa chemnitzia* (C.c).

color of the SLE of *P. pavonica* is brown and the pH was 7.22. Figures 3 and 4 represent the comparison of macronutrients (N, P,K) and micronutrients (Ca, Mg, SO_4^{2-}) of the brown seaweed *Sargassum wightii* (Sw), the green seaweed *Caulerpa chemnitzia* (Cc) (Sivasankar et al., 2006), the red seaweed *Kappaphycus alvarezzii* (Ka) (Rathore et al., 2009) and the brown alga *P. pavonica* (Pp) liquid extract. For the macronutrients N, P and K, the liquid extract of Pp is rich in potassium and has nitrogen content lowest after Ka. P_2O_5 is the lowest (this element is not detected in the liquid extract of the

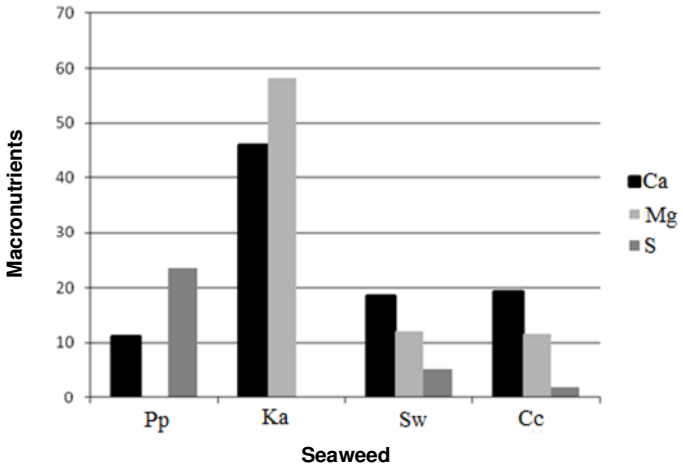

Figure 4. The micronutrients (Ca, Mg and SO_4^{2-}) measured in seaweeds: *Padina pavonica* (Pp), *Kappaphycus alvarezzii* (Ka), *Sargassum wightii* (Sw) and *Caulerpa chemnitzia* (C.c).

green alga (Cc). The amount of potassium K is lower than that of the brown seaweed (Sw) but higher than that of the red seaweed Ka and the green seaweed (Cc). For micronutrients elements Ca, Mg, SO_4^{2-}, the liquid extract of Pp is rich in sulfate S_2O4^- has the lowest levels of calcium.

Plant growth

The growth of the stem of the sunflower plant is lower in the support with seaweed + *L. aegyptica* + agar (no significant difference between the agar at 4 and 6%) and is higher in the soil and in the soil with chemical fertilizer (Figures 5 and 6). The diameter of the stem is lower (flexible stem) into the soil and into support with seaweed + *L. aegyptica* + agar 4% and higher (rigid stem) in support with seaweed + *L. aegyptica* + agar 6% and in soil with chemical fertilizer (Figure 7). The growth of leaves is highest in the soil with chemical fertilizer followed by the support with seaweed + *L. aegyptica* + agar (6%) (Figure 8). Figure 9 represents the roots of plants grown in the support with seaweed+ *L. aegyptica* + agar 6% who are visible, thick and crossed. The roots also have white color characteristic of a good development. No root system is observed in the support with seaweed + Luuffa aegyptica + agar 4%. The roots of the plants grown in soil and in soil with chemical fertilizer are also visible, thin, long and pale. After 20th day growth, the sunflower plants began to yellow in the media with chemical fertilizer (Figure 10a), in support with seaweed + *L. aegyptica* + agar (4%) (Figure 10b) and in the soil (Figure 10c). Only the plant grown in the medium with seaweed; *P. pavonica* + *L. aegyptica* + agar (6%) has a good growth (Figure 10d). 66 days after transfer in the soil (Figure 11a) all plants have faded. Only the sunflower plant grown in the support with seaweed *P. pavonica*

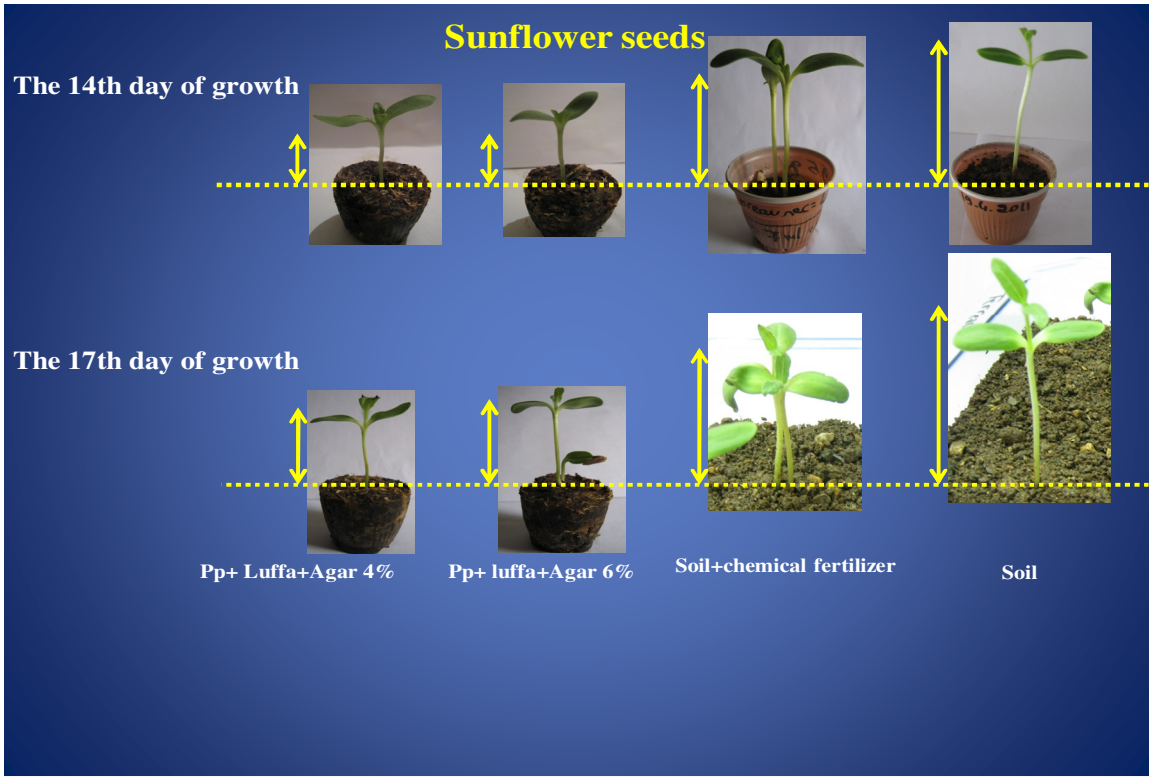

Figure 5. The sunflower plants in the 14th and 17th days of growth.

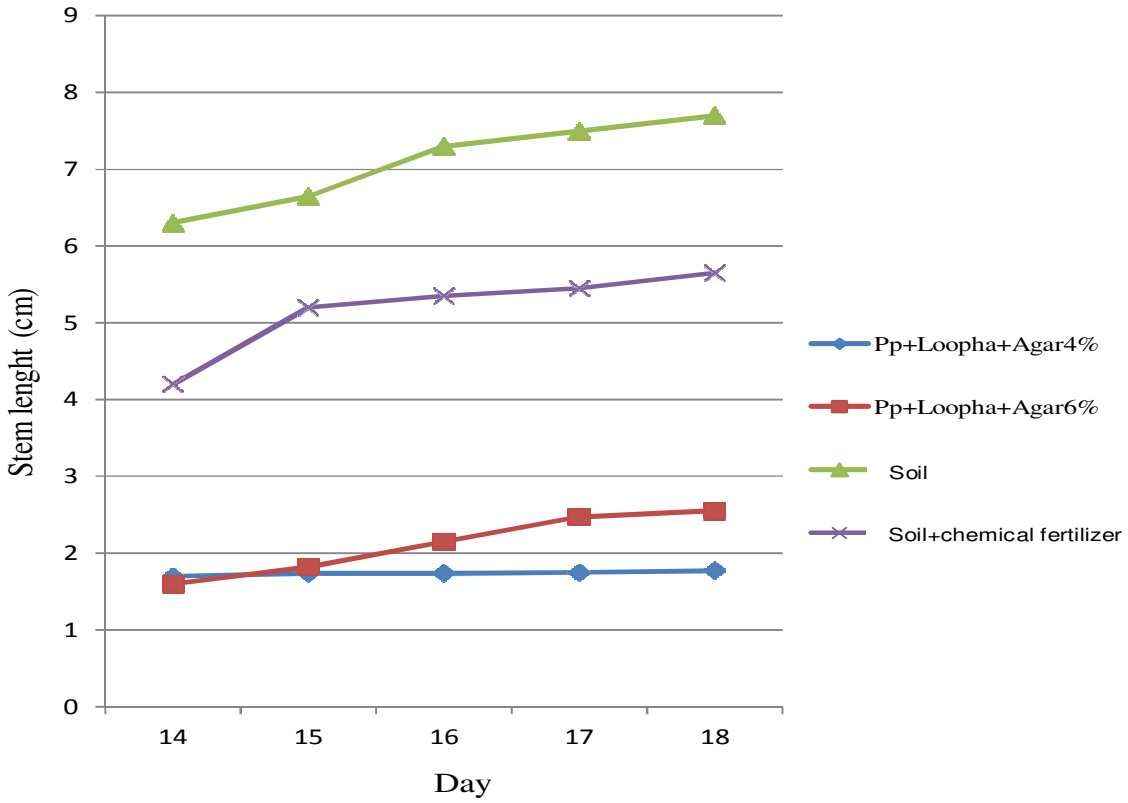

Figure 6. Variation in the length of the stem of sunflower plants over time.

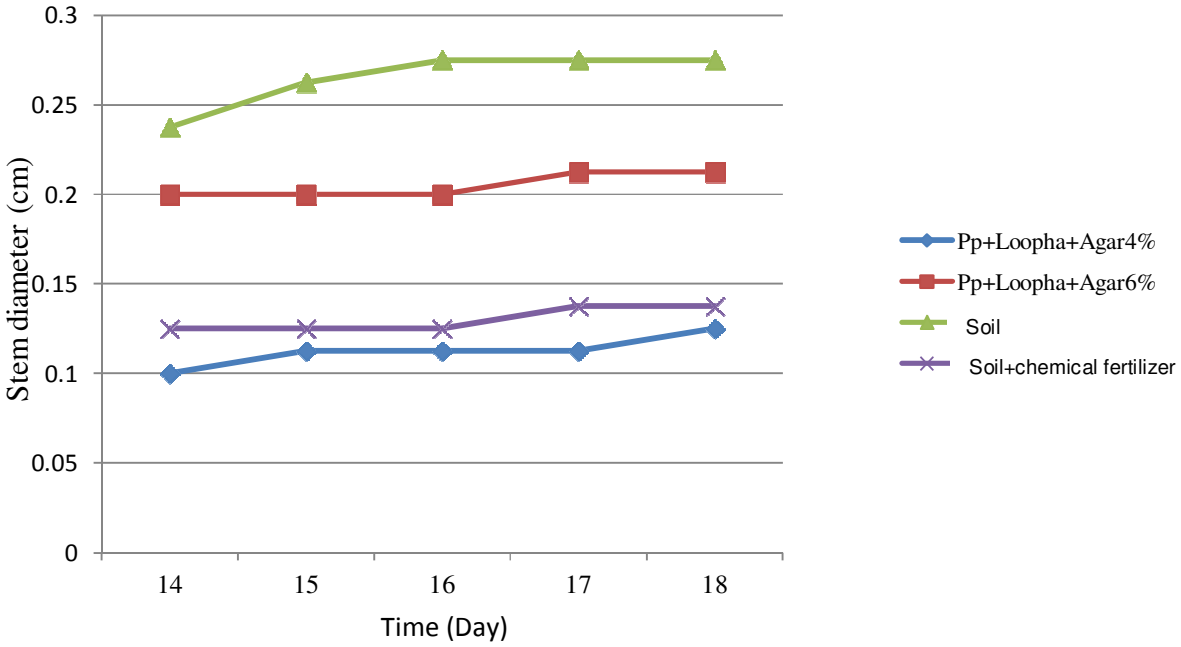

Figure 7. Variation in the diameter of the stem of sunflower plants over time.

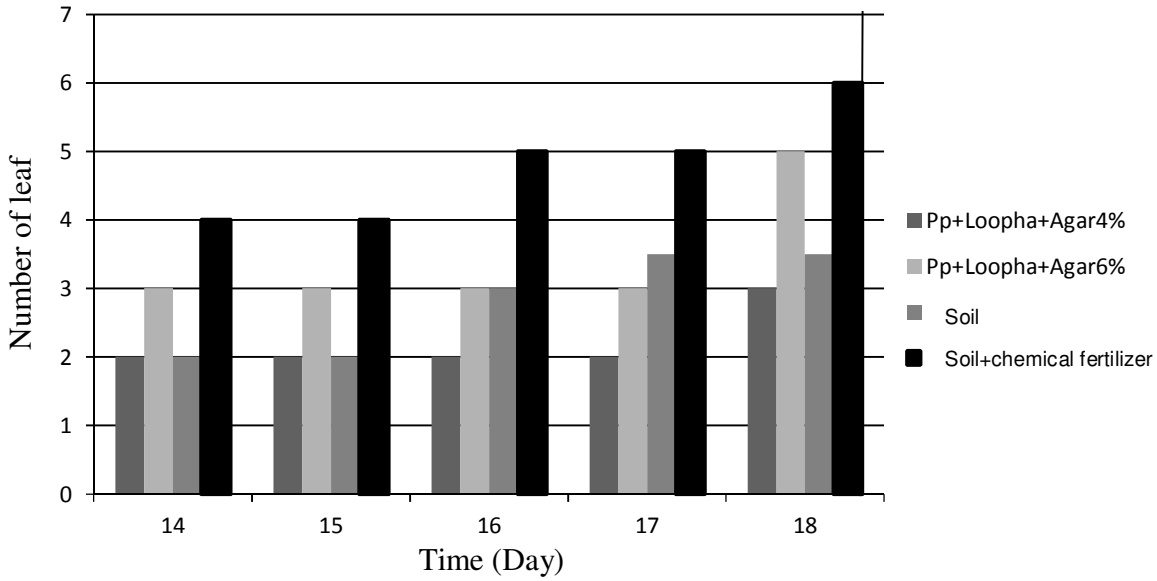

Figure 8. Variation in number of leaves of sunflower plants during 18 days.

+ *L. aegyptica* + agar 6% continued to grow in the soil (Figure 11b).

Degradation and water holding capacity

After 4 weeks, degradation fragments of the support containing the seaweed + *L. aegyptica* + Agar (Figure 12c) is higher than that of fragments containing only fiber *L. aegyptica* and agar (Figure 12b). As expected, no degradation of the plastic fragment is observed (Figure 12a). The support (Seaweed + Luffa + Agar) has a water holding capacity which is three times that of the support with the soil (Table 2).

DISCUSSION

P. pavonica nutrients (N, P, K, Ca, S) composition is not negligible compared to other brown seaweed; *S. wightii*,

Figure 9. The root system in the four media at the end of the eighteenth day of growth.

a green seaweed; *C. chemnitzia* (Sivasankar et al., 2006) and a red seaweed; *K. alvarezzii* (Rathore et al., 2009). The results of the measurement of growth parameters (stem length, stem diameter and number of leaf) during the 18 days of culture did not yield expected results cited in literature. Indeed, the growth of sunflower plants was lower in media containing *P. pavonica* + Luffa + Agar compared to the growth of plants in the soil with chemical fertilizer and in the soil only. The root system after 18 days of growth was more developed in the media containing *P. pavonica* + Luffa + Agar (6%). The plants grown in soil with chemical fertilizer and medium with seaweed and Agar (4%) began to fade after the 20th day of growth up until they started to wilt. The plant in the medium with seaweed + Luffa + Agar (6%) continued to grow.

This implies that, since the algae was added to the medium in mashed form without being submitted to a decomposition process, such as transformation into a powder form, and because of its ability to retain water and mineral elements and slowly release them to the plant (capacity of water storage) on a longer period of time. The algae can retain nutrients and release them slowly to the plants. It contains important nutrients such as nitrogen (N), phosphorus (P) and potassium (K), calcium (Ca) and sulfate (SO_4^{2-}) that will become available to the plants after decomposition (Khan et al., 2009). The decomposition of the algae is taken in charge by micro-organisms that degrade the algae into carbon dioxide, water and nutritious substances for the algae (mineralization). Seaweed also tend to enrich the medium with hormones such as cytokinins (Durand et al., 2003), auxins (Stirk et al., 2003), gibberellins (Wildgoose et al., 1978) and betaines (Blunden and Gordon, 1986; Whapham et al., 1993). They contain in addition, precursors of compounds elicitors that promote germination, growth and maintenance of plant health (Kloareg et al., 1996). Our results correlate with those in the literature.

Indeed, several scientific studies (Blunden, 1991; Crouch and Van Staden, 1994; Moore, 2004; Norrie and Keathley, 2006; Hong et al., 2007; Khan et al., 2009; Kumar and Sahoo, 2011) have demonstrated the action of seaweed extracts in stimulating plant growth. These extracts are known to enhance the growth of vegetables, fruits and other crops (Blunden, 1991; Crouch and van Staden, 1994; Washington et al., 1999).

In addition, when applied to seeds, added to the soil or sprayed on crops and vegetative stages of flowering, seaweed extracts can stimulate seed germination (Hong et al., 2007), growth (Moore, 2004; Khan et al., 2009) and yield of various crops (Arthur et al., 2003; Norrie and

Figure 10. Aspect of plant grown in different medias and transferred in the soil at the 20th day a: Plant grown in soil with chemical fertilizer, b: Plant grown in support with seaweed + Luffa + Agar (4%), c: Plant grown in soil, d: Plant grown in support with seaweed + Luffa + Agar (6%).

Keathley, 2006; Masny et al., 2004; Ei-Zeiny, 2007; Kumar and Sahoo, 2011). The observation of good color and good root and good leaf development in support with seaweed and Agar (6%), compared to those observed in the soil and the soil with chemical fertilizer, could be explained by an efficient uptake of nutrients at these levels. This is consistent with Mugnai et al. (2007) who argues that bio-stimulants, even those containing varying

Figure 11. (a): Sunflower plant after 66th day growth in the soil, (b): in support with *Padina pavonica* + Luffa + agar (6%) and transferred in soil after 18 days.

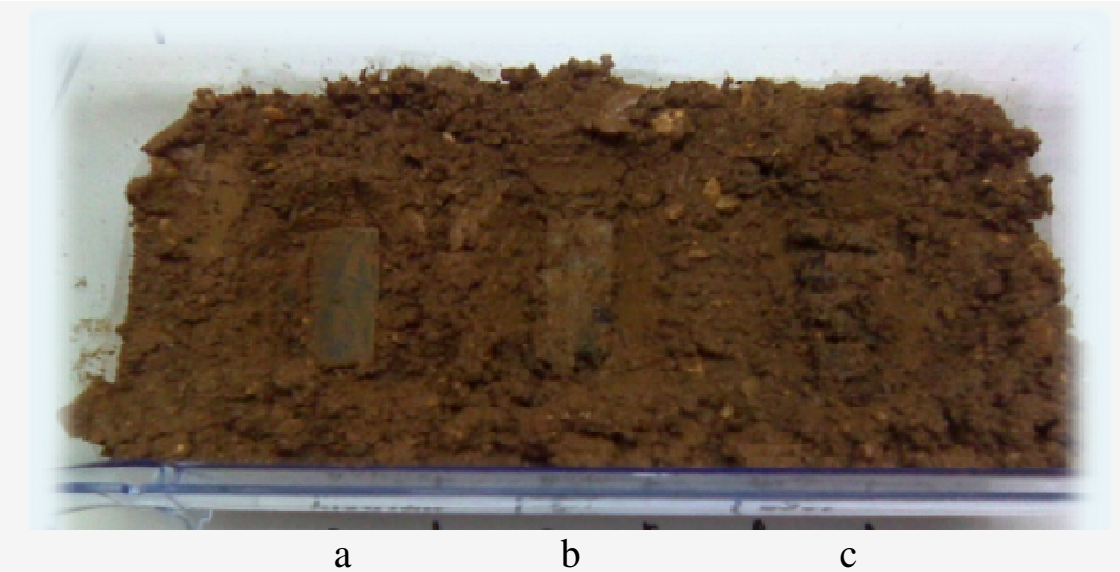

Figure 12. Results of degradation test after 4 weeks. (a): Plastic fragment. (b): Fragment of support with Luffa + agar. (c) Fragment of support with seaweed (*Padina pavonica*) + Luffa + Agar.

levels of mineral fertilizers, cannot provide all the essential nutrients in amounts that plants need (Schmidt et al., 2003), but one of their main functions is to increase the absorption of minerals by the plant, thus improving nutrient use efficiency at the roots (Vernierie et al., 2005) and leaves (Mancuso et al., 2006).

Table 2. Results of water holding capacity test of support with seaweed+ Luffa+ Agar and of soil sample.

Parameter	Weight of the dry sample (g)	Weight of Saturated sample (g)	Amount of water (g) retained by 100 g of sample
Biofertilizer support	15	66.8	445.33
Soil	96	143.3	149.27

According to Metting et al. (1990), Jeannin et al. (1991) applications of seaweed extract reduced the shock of transplanting in souci seedlings, cabbage (Aldworth and van Staden, 1987) and tomato (Crouch and van Staden, 1992) by increasing the size and strength of the root (Khan et al., 2009). Products made from seaweed stimulate growth and root development, this stimulatory effect was more pronounced when the extracts were applied to an early growth stage of corn, and the response was similar to that of auxin, an important hormone promoter of root growth (Jeannin et al., 1991). Other authors (Ouhssine et al., 2007) have shown that the use of waste as a fertilizer for algae resulted in a major growth of maize root growth allowing an important and also that of the aerial part which is found by the number of leaves, diameter and stem length and yield.

Increased growth of plants, development of deep roots, improved training of lateral roots (Atzmon and van Staden, 1994) and the increase of the total root system were also reported by Slavik (2005). Similarly, treatment with algae extracts has improved both the ratio root/shoot and biomass accumulation in tomato seedlings by stimulating root growth (Crouch and van Staden, 1992). The biostimulants in general are able to affect root development by improving both the formation of lateral roots (Atzmon and van Staden, 1994; Vernieri et al., 2005) and the increase of the total root system (Thompson, 2004; Slavik, 2005; Mancuso et al., 2006). Improved root systems could be influenced by endogenous auxins and other compounds in the extracts (Crouch and Van Staden, 1992).

The seaweed extracts improve the absorption of nutrients through the roots causing additional and strong overall growth of the plant (Crouch et al., 1990). In addition, abiotic stresses such as drought, salinity and temperature extremes can affect the performance of most crops (Wang et al., 2003) and limit agricultural production worldwide. Collectively, studies suggest that the products generate algal tolerance to abiotic stress in plants and bioactive substances derived from algae confer tolerance to stress and improve plant performance (Khan et al., 2009).

The total absence of undesirable elements in the analysis of the aqueous extract of *P. pavonica* such as nickel, plumb, chromium, aluminum and cadmium means that the algae will not contribute to soil pollution. This reflects the quality of sea water in which they were collected. After 4 weeks, the biodegradability of the substrate produced is verified by the observation of a degradation fragment containing the seaweed *P. pavonica* higher than that of fragments containing only fiber Luffa. No degradation of the plastic fragment is observed. These results confirm that the algae are effective in the degradation of the material in the ground. This contribution is particularly due to their nitrogen supply to soil microorganisms responsible for degradation.

The support achieved in this study presented a high capacity to retain water; this is due probably to the use of lignocellulosic fibers, contained in the mature fruit of the species *L. aegyptica*. The grinding of plant natural fibers gives advantageously calibrated granulation glitter that can be introduced into the composition of the carrier as an agent for water retention that is 100% natural and can thus be naturally degraded by microorganisms. The large capacity (345%) of the support to hold irrigation water would explain the first assumption that the water retained in the media could have a delayed effect on plant growth. This water allows the slow diffusion of the nutrients of the algae into the medium at a certain concentration.

In terms of costs, despite appearances, organic farming is as competitive as conventional farming. Biological agriculture almost certainly requires more employees and control because of the lack of use of pesticides. In addition, it is often limited in terms of area and is very costly in terms of certifications. But on the other hand, conventional farming has many negative externalities caused by the misuse of the land in excess pesticides, negative impacts on health, etc... Thus, several feasibility studies have shown that if these externalities were included in the calculations, organic production is at least as expensive as conventional production.

Conclusion

This study had as a main objective the production of a plant growing support which is both biodegradable and of low manufacturing cost. The constituent materials are seaweed (*P. pavonica*), vegetable fiber (Luffa) and Agar-agar. According to this composition, this material has been the subject of no scientific publications to date.

The results of our study have shown that on one hand, the support based on algae does not have a significant organic fertilizer short-term effect on growth of sunflower plants. But it does increase the duration of growth and increases the quality of the plants. Regarding the test of capacity to retain water produced, the media containing the Luffa has a holding capacity of water higher than the

soil and equivalent three and a half times its dry weight. This contributes very much to lower water expenses.

Similarly, the biodegradability test conducted revealed that the degradation of the media containing algae is higher than that of the substrate containing only Luffa. These results confirm that algae contribute to the degradation of the soil by supplying nitrogen for the microorganisms responsible for degradation.

This embodiment is particularly advantageous since it provides a growing medium that can be planted with the plant; it allows the overcoming of the non-biodegradable plastics constituting the pots in which plants are proposed to consumers. On the other hand, the cost of support base made of seaweed (*P. pavonica*), vegetable fiber (Luffa) and Agar-agar is very affordable compared to the cost of the plastic pot when you consider also that it is biodegradable and does not contribute thereby to the pollution of soil and the environment.

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