# EFFECT OF WATER EXTRACTS OF SEAWEEDS ON THE GROWTH OF SEEDLING ROOTS OF BUCKWHEAT (*FAGOPYRUM ESCULENTUM* MOENCH)

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**ABSTRACT**

Effect of water extracts of red algae Grateloupia divaricata, Chondrus pinnulatus, Ahnteltiopsis flabelliformis, Neorhodomela larix, Tichocarpus crinitus, of brown algae Stephanocystis crassipes, Coccophora langsdorfii, Sphaerotrichia divaricata, Saccharina japonica, Sargassum pallidum, Chorda filum, and green algae Ulva fenestrata and Codium fragile on the growth of seedling roots of buckwheat (Fagopyrum esculentum varieties Izumrud) was screened. It was showed that low and ultra-low (from $10^{-3}$ to $10^{-14}$ g of dried seaweeds per mL of distillated water) concentration of aqueous seaweed extracts may stimulate roots elongation of the buckwheat seedlings. The most perspective for use as biostimulants are red algae Ahnfeltiopsis flabelliformis, Neorhodomela larix, and brown alga Stephanocystis crassipes. Extracts of these algae in a studied range of concentrations increased growth of the seedling roots in maximum by 16, 20 and 15 % over control, respectively. The extracts of the other examined algae showed a weak or no stimulatory effect on the growth of seedling roots. The dose-effect curves of the majority of tested seaweed extracts were bi- or polymodal.

**Key words:** *seaweed extracts, Japan (East) Sea, growth of seedling roots of buckwheat, ultra-low concentrations, polymodality of dose-effect curves*

## 1. INTRODUCTION

The marine algae are one of the most important marine resources in the world and widely are used as human food, animal feed and raw material for many industries. More than 15 million metric tons of seaweeds are produced annually [1], a considerable portion of which is used as biofertilizers in agriculture and horticulture. The beneficial effects of seaweed products on the cultured plants are well documented. They improve seeds germination, seedlings development, increase plant tolerance to environmental stresses [2, 3], and enhance plant growth and yield [4–7]. More over seaweeds are used as soil amendment [8], in pests control [4] and plant disease management [9].

At present one of the most promising applications of seaweeds is their use as plant biostimulants. This influence is explained by content of plant growth-promoting substances such as cytokinins, auxins, gibberellins, abscisic acid, ethylene, polyamines and betaines in algal extracts [10–14]. It was shown that seaweed extracts may be active at low concentrations (less than 10 mg of seaweeds per mL (mgSW $mL^{-1}$)) [7, 15–17]. For example, aqueous extracts of *Sargassum johnstonii* at concentration from 0.1 to 0.8% (w/v) that is equivalent 1–8 mgSW $mL^{-1}$ increased rooting of *Vigna mundo* hypocotyl cuttings in 2.4–3.2 times, and enhanced vegetative growth (plant height, shoot length, root length, and number of branches) and reproductive parameters (flower number, fruit number, and fresh weight) of tomato [7]. Germination percentage of *Plantago lanceolata*, *Trifolium repens* and *Avena strigosa* seeds rose significantly when incubated with 0.05 % solution of *Laminaria digitata* [15]. The effect of the 20 % (0.2 mgSW $mL^{-1}$) extracts of *Sargassum wightii* gave an 11 % increase in seed germination, a 63 % enhance in number of lateral roots and 46 % increase in shoots length of *Triticum aestivun* in compare to control [16].

In recent years the beneficial effect of the ultra-low doses of bioactive compounds on the different kinds of organisms particularly on the growth of seedlings of agricultural plants was detected [18–21]. For example steroid glycosides from starfish *Asteropsis carinifera* increase growth of corn stem in the concentrations $10^{-12}$ and $10^{-8}$ ug $mL^{-1}$ [21]. However, there are not investigations of action of ultra-low concentrations (less than 0.01 mgSW $mL^{-1}$) of seaweed extracts. Due to presence of large number of bioactive compounds in seaweeds extracts [6, 22] some of which may be effective in ultra-low doses the beneficial influence of low and ultra-low concentrations of seaweed extracts on the vascular plants may be expected. So the first aim of present study was to test this presumption.

The second aim was to find the most perspective seaweeds for use as plant growth biostimulants at the Far East of Russia. We initiate this work because Russian coast of Pacific Ocean is rich by algal resources but in spite of high promising of application of seaweed resources in agri- and horticulture, they actually do not extensive used in Russia.

## 2. MATERIALS AND METHODS

*Seaweeds collection*

Five species of red algae (*Ahnfeltiopsis flabelliformis* Harvey) Masuda, *Grateloupia divaricata* Okamura, *Neorhodomela larix* (Turner) Masuda, *Chondrus pinnulatus* (Harvey) Okamura, *Tichocarpus crinitus* (S.G.Gmelin) Ruprecht), five species of brown seaweeds (*Stephanocystis crassipes* (Mertens ex Turner) Draisma, Ballesteros, F. Rousseau & T. Thibaut, *Coccophora langsdorfii* (Turner) Greville, *Sphaerotrichia divaricata* (C. Agardh) Kylin, *Sargassum pallidum* (Turner) C. Agardh and *Chorda filum* (Linnaeus) Stackhouse) and two green algae (*Ulva fenestrata* Postels et Ruprecht and *Codium fragile* (Suringar) Hariot) were collected in the Ussuryskii Bay near the Lazurnaya Bay (43$^o$09' N and 132$^o$06' E) and *Saccharina japonica* (Areschoug) C.E. Lane, C. Mayes, Druehl & G.W. Saunders (brown algae) was collected in Amursky Bay near the Kungasnii Cape (43$^o$08' N and 131$^o$53' E) at depth 0.5–1 meter at mid of October 2011. Algae were washed with seawater and hard brush to remove macroscopic epiphytes and sand particles, and then with tap water to remove adhering salt. Samples were air-dried (26 °C) during 2–4 days followed by thermostat dry at 60 °C for 12h.

*Preparation of seaweed liquid extracts (SLE)*

Dried seaweeds were hand crushed and powdered with coffee-grinder. Algae were heated with sterile distilled water in a ratio 1 : 100 (w/v) at 60 $^o$C for 45 min. Then the extracts were filtered through a filter paper and stored at 4 °C for further experimental studies. The filtrates were $10^{-2}$ g of dried seaweeds per milliliter (gSW mL$^{-1}$) extracts. Different concentrations of SLE were prepared by diluting of these extracts with distilled water.

*Buckwheat assay*

The seeds of buckwheat *Fagopyrum esculentum* Moench variety Izumrud were harvested in 2011. Seeds were germinated in rolls of filter paper [21]. Dry seeds (twenty seeds per strip) were spread on strips of filter paper (12 cm width and 42 cm length) previously moistened with test solution. Then the strips were rolled and placed into beakers with a small amount of test solution (100 mL). Three rolls with seeds were incubated at each concentration of algal extracts for 3 days in a thermostat at 26–27 °C (in total 60 seeds per the concentration). The control seedlings were grown in distilled water. Kinetin was used as positive control.

The length of the main root of the seedling was measured after incubation. The results were expressed as percentage of the control. Data were analyzed using Statistica 7.0 software (StatSoft). The data were tested for normality (Lillieform tests) and homogeneity of variances using the Leven test. Student t-test was used for analysis of significant differences of root length between control group and under different concentration of extracts. P-values less than 0.05 were considered statistically significant.

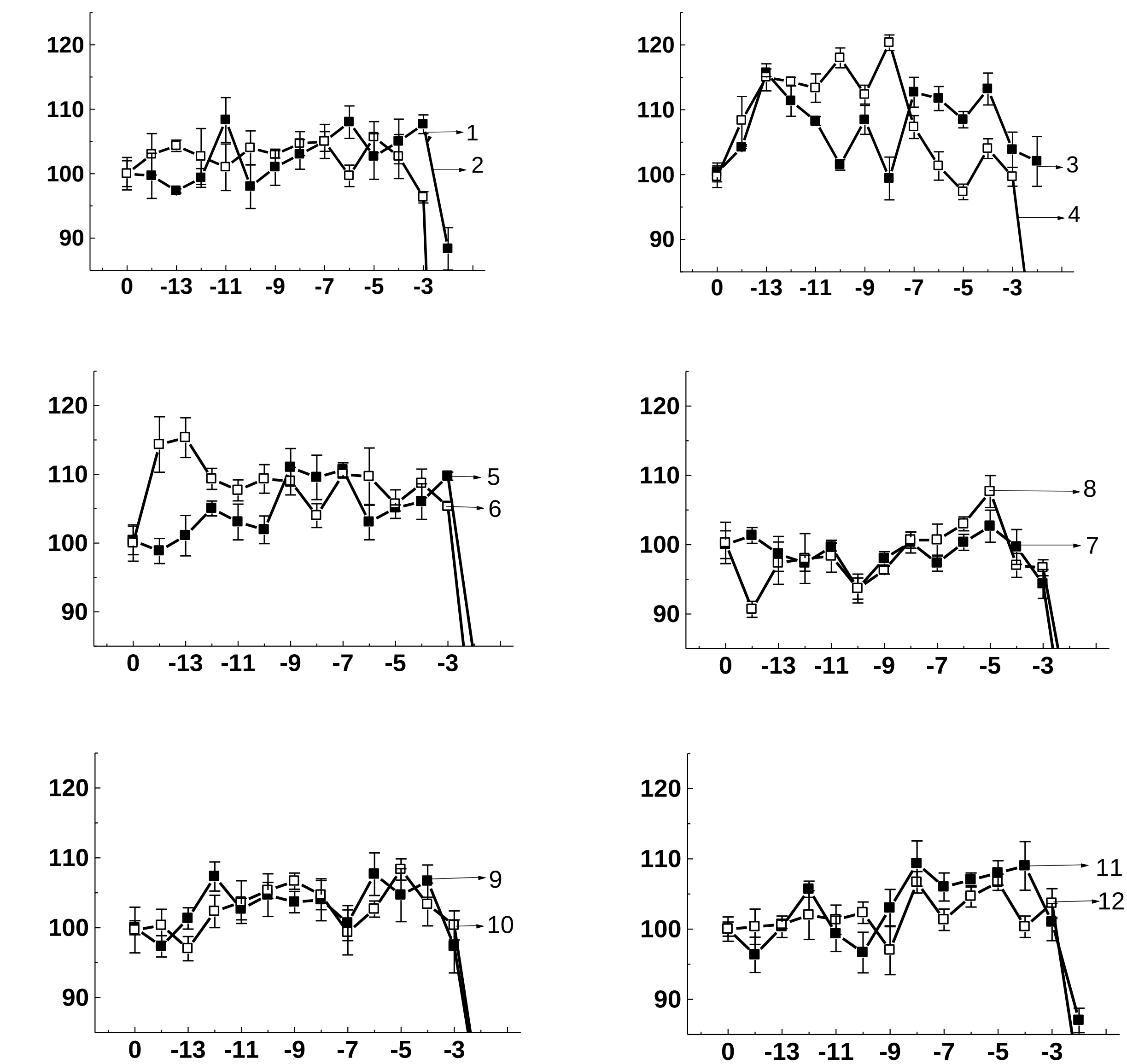
## 3. RESULTS

The extracts of all examined seaweeds significantly affected growth of seedling roots of buckwheat (Fig.). The highest acceleration of the roots growth was detected in the extracts of red algae *Ahnfeltiopsis flabelliformis* (**3**) (maximum of 16 % in compare to the control group), *Neorhodomela larix* (**4**) (of 20 %) and *Tichocarpus crinitus* (**5**) (of 11 %), and brown alga *Stephanocystis crassipes* (**6**) (of 15 %). The extracts of *Chondrus pinnulatus* (**2**), *Grateloupia divaricata* (**1**) (Rhodophyta), *Sphaerotrichia divaricota* (**8**), *Saccharina japonica* (**9**), *Sargassum pallidum* (**10**), *Chorda filum* (**11**) (Phaeophyceae) and both green algae (*Ulva fenestrate* (**12**) and *Codium fragile* (**13**)) showed the weak stimulatory effect on the growth of seedling roots. After 3 days incubation roots grown in extracts of these species were only of 5–9 % longer in compare with control group. Extracts of *Coccophora langsdorfii* (**7**) were not effective or inhibited root growth in almost whole concentration range. It should be noted that acceleration of the root growth reached 14 % over control when seeds were cultured with kinetin (**14**) at concentration of $10^{-9}$–$10^{-11}$ M.

Influence of seaweed extracts concentration in a range from $10^{-3}$ to $10^{-14}$ g of seaweed per one milliliter (gSW mL$^{-1}$) on root length was depended on algal species (Fig.). The highest stimulatory effect of *A. flabelliformis* (**3**) extract was detected at the concentrations of $10^{-13}$, $10^{-9}$, and in a range of $10^{-4}$–$10^{-7}$ gSW mL$^{-1}$. Extract of *N. larix* (**4**) had maximal effect at concentration $10^{-8}$–$10^{-13}$ gSW mL$^{-1}$, the higher concentration showed lower stimulation of root growth. While the maximal effect of extract of *T. crinitus* (**5**) was detected at $10^{-7}$–$10^{-9}$ and at $10^{-3}$ gSW mL$^{-1}$. The dose-effect curves of the most extracts was bimodal or polymodal (for *A. flabelliformis* (**3**), *S. crassipes* (**6**)). The extracts from both studied green algae (*U. fenestrate* (**12**) and *C. fragile* (**13**)) were not effective at concentration in a range of $10^{-14}$–$10^{-10}$ gSW $mL^{-1}$, at higher concentrations the dose-effect curves was bimodal. The maximal acceleration of the root growth was detected at $10^{-8}$ and $10^{-4}$–$10^{-5}$ gSW $mL^{-1}$.

## 4. DISCUSSION

Our results are evidently showed that low and ultra-low (less than $10^{-3}$ gSw $mL^{-1}$) concentration of aqueous seaweed extracts may stimulate roots elongation of the buckwheat seedlings. The most perspective for use as biostimulants are red algae *Ahnfeltiopsis flabelliformis*, *Neorhodomela larix*, and brown alga *Stephanocystis crassipes.* The stimulative effect exhibited by the extracts of these species was compared to those found at higher doses of seaweed extracts in other studies. For example, root of seedlings of *Vigna mundo* soaked with 10 % (0.1 gSW $mL^{-1}$) water extracts of *Sargassum myriocystum* was 1.8 times longer in compare with control [23]. Soaking of *Triticum aestivum* seeds in 5–10 % (0.05 – 0.1 mgSW $mL^{-1}$) extracts of *S. wightii* resulted in 40% increase in root length over control [16].



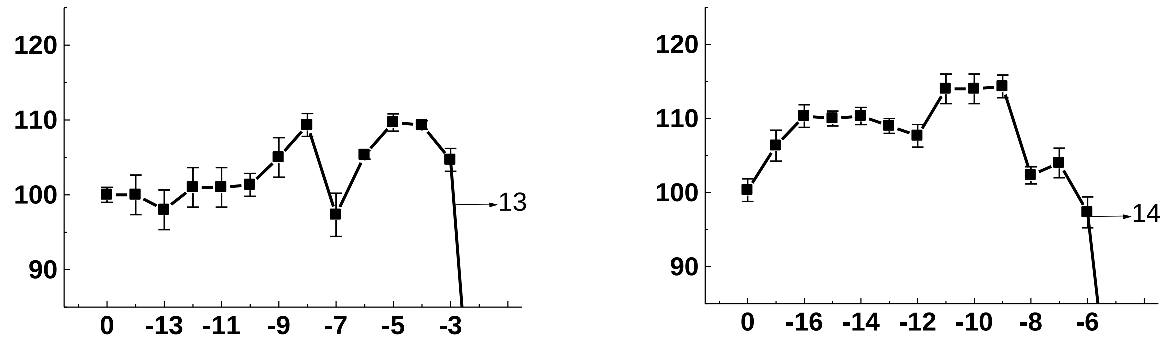



*Figure. Effect of water extracts of red algae (Grateloupia divaricata – **1**, Chondrus pinnulatus – **2** , Ahnteltiopsis flabelliformis – **3**, Neorhodomela larix, – **4**, Tichocarpus crinitus – **5**), of brown algae (Stephanocystis crassipes – **6**, Coccophora langsdorfii – **7**, Sphaerotrichia divaricata – **8**, Saccharina japonica – **9**, Sargassum pallidum – **10**, Chorda filum – **11**), green algae (Ulva fenestrate – **12** and Codium fragile – **13**) and kinetin – **14** on the length of root of buckwheat seedlings. On X-axis – lg concentration (gSW $mL^{-1}$) of seaweed extracts and lg molar concentration of kinetin; on Y-axis – length of roots, % of control.*

It should be noted that influence of ultra-low concentrations (less than $10^{-4}$ gSW $mL^{-1}$) of seaweed extracts on seedling roots development was studied for the first time. At present it is extensively speculated that beneficial effect of seaweed extracts on germination and growth of various land plants may be due to the presence of plant growth-promoting substances/hormones in the extracts [13–15]. Marine algae consist cytokinins, gibberellins, auxins, auxin-like and other growth-promoting compounds [11, 12]. Various cytokinins were identified in Chlorophyta, Phaeophyta and Rhodophyta [2, 3, 11, 12]. The total concentration of cytokinins in algae is usually very low. For example, the highest concentration of these substances among 11 Brazil red seaweeds detected in *Hypnea musciformis* does not exceed 454 pmol $g^{-1}$ of dry weight of alga [12]. In algal extracts cytokinin content may reach 66 μg $g^{-1}$ of dry weight of the extract [2]. Auxins also have been identified in numerals seaweeds. According to Yokoya et al. [12] their concentration in different species of Rhodophyta varies from less than 10 to 945 pmol $g^{-1}$ of dry weight of alga. The concentration of IAA in brown alga *Laminaria japonica* (=*Saccharina japonica*) is in a range of 90-95 μg $kg^{-1}$ of fresh weight [24], in *Undaria pinnatifida* it consists 8.47 μg $kg^{-1}$ of fresh weight [25], whereas red seaweed *Porphyra perforata* contains only 100 pg $g^{-1}$ of fresh weight (or 100 ng $kg^{-1}$) of IAA [26]. In algal extracts concentration of IAA may reach 50 mg $g^{-1}$ of dw of the extract [27].
We found that extracts of various algae have a different effectiveness. A possible explanation for this difference is variety in composition and content of plant growth regulators in extracts of the different seaweeds. Unfortunately, at this stage we can not compare concentration and composition of growth-promoting compounds in the tested macroalgae because to date there are not sufficient data in the scientific literature.
In the present study the dose-effect curves of different algal extracts were bi- or polymodal. These data are concordant with results of numerous researches studying effect of ultra-low doses of bioactive compounds on organisms [18, 20, 21, 28, 29]. For example steroid glycosides from starfish *Asteropsis carinifera* increased growth of corn stem in the concentrations $10^{-12}$ and $10^{-8}$ ug $mL^{-1}$, whereas it had not effect at $10^{-10}$ ug $mL^{-1}$ [21]. The polymodality of reaction of growth of roots of mono- and dicotyledons on the action of derivatives of triethanolamine in the concentration range from $10^{-3}$ to $10^{-15}$ was observed by Makarova et al. [18]. Burlakova et al. [30] generalizing action of ultra-low doses (ULDs) of different kinds of bioactive compounds concluded that polymodality of the dose-effect curves when maximums of activity observed at the specified concentration ranges alternate with "death zones" (range of concentrations with low or without activity) is a common feature of ULDs effects. The physiological and biochemical mechanisms of action of the ULDs of the bioactive compounds on different levels of the biological systems still are largely unknown. Nevertheless, it was shown that bioactive substances in concentration range from $10^{-9}$ to $10^{-17}$ specifically links with ligands or receptors located on the cell membrane forming new strongly-ordered complexes. That leads to alteration of the structure of cell membranes and may result in alteration of the functional state of the cell [29]. In this aspect polymodality of dose-effect curve can be explained by exchange of mechanisms of action of the active substance at different concentrations [30]. Also there are speculations explained polymodality of ULD effect by existence of some active centers with different affinity to the substrate in the enzyme. Polymodality of the dose-effect curves may be observed in the case if these centers are substrate inhibited systems [31]. There are attempts to explain effect of ULDs by modification of structure of the water that is conformation of stable water clusters under dissolving of the bioactive compounds (for review see [30].

These clusters may determine the catalytic reactions in biological systems as well as stimulation of the biological activity of very diluted solutions [32].

It should be noted that all algal extracts in concentration higher $10^{-3}$ gSW $mL^{-1}$ inhibited development of seedling roots. This result is concordant to finding of other researchers. It was shown that dilute extracts are more effective than the concentrated ones [17, 33]. For example, seeds soaked in 0.5 and 1% of *Sargassum wightii* hot water extract (1 : 20 w/v) showed faster germination in compare with seeds that were soaked at higher concentration (2%) [17]. An increase of root length, number of lateral roots, shoot length and number of branches of wheat *Triticum aestivum* were also found to be highest for the 20 % *S. wightii* liquid extract treatment (that are equivalent to 0.2 mgSW $mL^{-1}$), and declining at higher concentration [16]. Similary, germination of *Vigna mundo* was inhibited by *Sargassum myriocystum* extracts in concentration more than 10 % that equivalent to 10 mgSW $mL^{-1}$ [23]. Decrease in rooting of *V. mundo* seedlings was observed at concentration of aqueous extracts of *Sargassum johnstonii* more than 0.8% (8 mg $mL^{-1}$) with no rooting at 10% (100 mg $mL^{-1}$) [7]. Researchers suggested that initiation and development of roots probably requires low concentration of the active compounds and therefore a decline in rooting is observed at higher seaweed extract concentration [7]. Such a response is also common with hormones often promoting physiological processes at low concentrations and inhibiting at high concentrations [10].

In conclusion, the present study showed that algal extracts stimulate roots development of buckwheat seedlings in concentration less than $10^{-3}$ gSW $mL^{-1}$. In the most cases the dose-effect curves were bi- or polymodal. This data may be basis for new ways of application of seaweeds as biostimulators in agriculture. However, the investigations of mechanisms of action of ultra-low doses of seaweed extracts on the vascular plants are required. The most perspective to improve seeds germination as well as yield of crop plants are red algae *Ahnfeltiopsis flabelliformis*, *Neorhodomela larix*, and brown alga *Stephanocystis crassipes.*

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