EFFECT OF SEAWEED LIQUID FERTILIZER (SLF) OF *CYSTOSEIRA INDICA* ON THE GERMINATION RATE OF CHICKPEA (*CICER ARIETINUM* L.)

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خلاصہ

چنا ایک سالانہ پیداوار والا پودا ہے جو بشمول پاکستان دنیا کے بیشتر معتدل اور نیم بنجر علاقوں میں پایا جاتا ہے چونکہ پاکستان کی ساحلی پٹی انواع و اقسام کی اشنو کا گھر ہے۔ یہ مطالعہ چنے کے دو اقسام (دیسی اور کابلی) کے انکرن کی شرح نمو پر سیسوسارا امدیکا مامی حُردحیات کے ارات کا مشاہدہ کرنے کے لئے کیا گیا۔ چنے کی دونوں اقسام کے لیئے 1%، 2%، 3%، 4% اور 5 % ارتکاز کا تجربہ کیا گیا۔ شرح نمو ابتدائی لمبائی، پلیمیول اور ریڈیکل روزانہ کی بنیاد پر جانچے گئے۔ نتائج سے پتا چلتا ہے کہ تمام ارتکاز میں پودوں کے بڑھنے کا عمل معمول سے پہلے شروع ہو گیا۔ دیسی چنے نے SLF کے کم ارتکاز 1% اور 2% پر اچھا نتیجہ دیا جبکہ کابلی چنے نے 4% پر امید افزاء نتائج دکھائے لیکن کنٹرول اور SLF کے درمیان فرق کم تھا۔ مطالعہ اور مشاہدے سے یہ نتیجہ احد کیا جا رہا ہے کہ حُردحیات سیسوسارا امدیکا کا سب بطور SLF چنے کی افزائش میں بہترین کردار ادا کر سکتا ہے۔

Abstract

Chickpea (*Cicer arietinum* L.) is an annual plant that is found in temperate and semiarid areas of the world including Pakistan. Since Pakistan has a coastal belt that is home to different types of seaweeds, this study was conducted to observe the effects of seaweed *Cystoseira indica* on the germination rate of Desi and Kabuli chickpeas. Five concentrations of seaweed liquid fertilizer (SLF), 1%, 2%, 3%, 4% and 5% were tested for both varieties of chickpeas. The germination percentage and initial lengths of plumules and radicles were observed daily. Results showed that germination started earlier than usual in all concentrations. Overall, Desi chickpeas responded well in lower concentrations of SLF (1% and 2%), whereas Kabuli chickpeas showed promising results in higher concentration (4%), and the difference between control and SLF was less. This study concludes that seaweed liquid extract of *Cystoseira indica* can be used for a better germination rate of chickpeas.
Keywords: Seaweed Liquid Fertilizer (SLF), *Cystoseira indica*, Chickpea, Legumes, Germination Rate.

Introduction

Chickpeas (*Cicer arietinum* L.) are annual legume plants that are majorly grown and consumed in the Middle East, India, Pakistan, Turkey, Australia, Mexico, Canada, the United States and many parts of Africa (Koul, *et al.*, 2022). Generally, they are divided into two main types; namely Kabuli and Desi types. Desi chickpeas are smaller in size, dark brown colour and possess a hard and wrinkled seed coat whereas Kabuli chickpeas are rather large in size and have a very thin layer of the seed coat. Chickpeas are not only known as a major source of protein (Portari, *et al.*, 2005), but also hold the ability to grow well in drought-hit areas, due to which they are one of the major leguminous plants that are easily grown in desert areas of Pakistan. To meet consumer's demand for the legume, it is necessary to increase the productivity of the plant; either by increasing the area of cultivation or by the use of different types of fertilizers.

The successful production of chickpeas is directly related to the rate of germination of seeds (Rich, *et al.*, 2022). The germination tests of viable seeds are carried out throughout the world (Ghaleb, *et al.*, 2022) and several chemical as well as organic forms of fertilizers have been successfully tested and used for better germination rates. Different studies have also shown that seaweeds liquid extracts show better results on the germination rate and crop yield of different plants due to greater amount of nutrients and plant growth hormones (Möller & Smith, 1998; Sivritepe, 2008; Rao & Chatterjee, 2014; Popescu, 2016). But according to our survey, studies on the effects of seaweed liquid fertilizers (SLFs) on the germination rate of chickpeas are scarce. Therefore the main objective of this research is to observe the effects of seaweed liquid fertilizer (SLF) obtained from brown seaweed *Cystoseira indica* on the germination rate of Desi and Kabuli chickpeas.

Materials and Methods

Sample Collection and Preparation

The brown seaweed *C. indica* was collected from Buleji, Karachi during the months of March-April 2022. Fresh samples were brought to the laboratory where they were washed thoroughly with tap water to remove sand particles and epiphytes, and then shade dried and stored at room temperature until further use. Some specimens were kept in 4% formalin for identification purpose.

To prepare a 100% stock solution, 60 g of powdered *C. indica* was boiled for one hour with 1200 mL of sterilized distilled water (Bhosle, *et al*., 1975). Five different concentrations *i.e.*1%, 2%, 3%, 4% and 5% were made from the stock solution.

Visual Observation Test for Seed Health: Desi and Kabuli Chickpea seeds were bought from a local market. Seed health was tested by Visual Observed Test as described by International Seed Testing Association (ISTA). Seeds that showed discolouration or any other abnormality in visual observation were not included in the experiment.

Germination Test: The whole experiment was carried out with five replicates. Five sterile petri plates were used per concentration *i.e.* control, 1%, 2%, 3%, 4% and 5%. Seeds of both varieties of chickpeas were soaked in tap water for an hour. In each petri plate, 10 visually healthy seeds were placed. 10 mL of each concentration of seaweed liquid extract was given to respective petri plates on every alternate day, while in control, sterilized distilled water was given following the modified method of (Sivasankari, *et al*., 2006). The same procedure was followed for both Desi as well as Kabuli chickpeas. The period of germination, germination rate and initial length of plumules and radicles were recorded on a daily basis (Fig. 1).

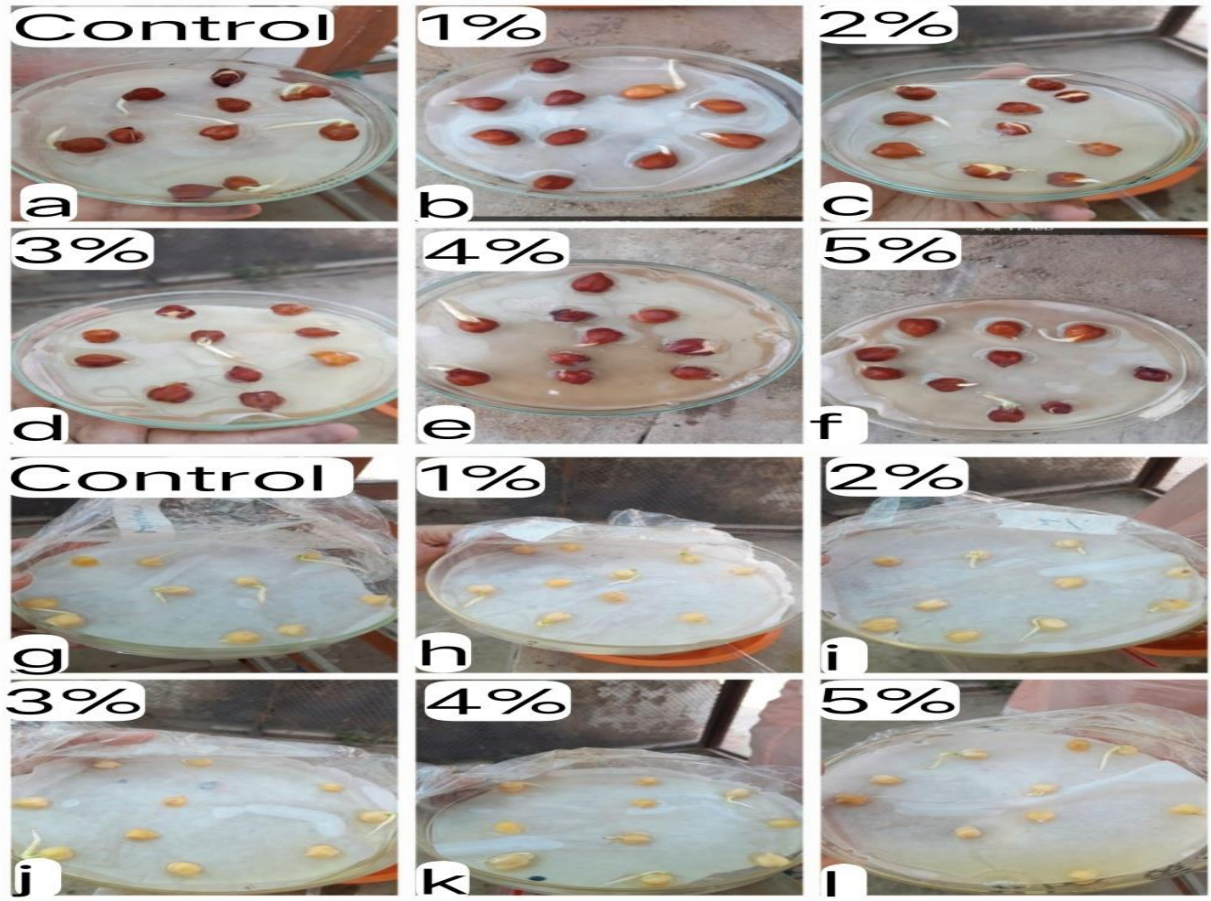

Fig. 1. Germination patterns of control and different concentrationsof SLF in Desi (a-f) and Kabuli (g-l) Chickpeas.

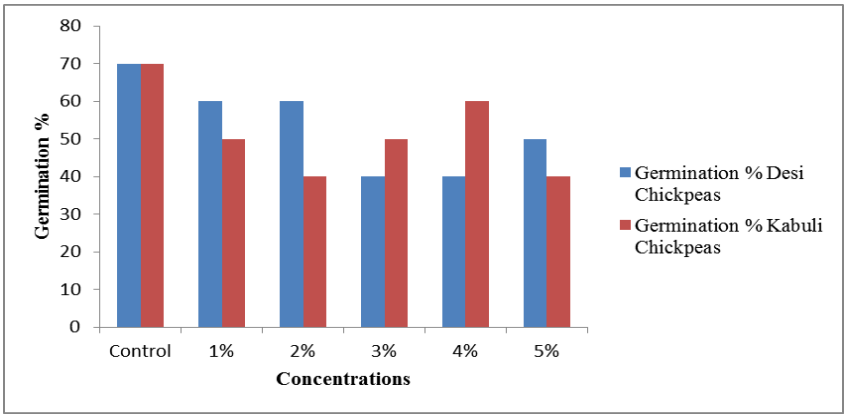

Fig. 2. Germination rate of Desi and Kabuli Chickpeas.

Table 1. Length of radicle and plumule in Desi Chickpeas.

Concentrations of SLF (%)	1st day		2nd day		3rd day		4th day		5th day	
	R (cm)	P (cm)	R (cm)	P (cm)	R (cm)	P (cm)	R (cm)	P (cm)	R (cm)	P (cm)
Control	-	-	0.2	-	*	-	*	-	*	-
	0.3	-	0.8	-	*	-	*	-	*	-
	0.5	-	1.1	-	1.4	0.2	1.6	0.4	2	0.6
	1.1	-	1.8	-	1.8	0.5	1.9	0.9	2.1	1.4
	1.2	-	1.8	-	1.8	0.3	1.9	0.6	2.1	1.6
	1.2	-	2.1	-	2.5	-	2.9	0.1	3.2	0.7
	1.6		2.3	-	2.9	0.1	3.6	0.5	4	1.5
1%	-	-	0.2	-	*	-	*	-	*	-
	0.1	-	0.2	-	0.2	-	0.2	-	0.2	-
	0.2	-	0.3	-	0.3	-	0.3	-	0.4	0.7
	0.2	-	0.6	-	0.7	-	0.8	0.2	1	0.7
	0.6	-	0.6	-	1.3	-	1.9	-	2.1	0.3
	0.8	-	1.6	-	2.9	0.3	4.1	0.9	5	1.6
2%	0.3	-	0.5	-	0.6	-	0.7	-	*	-
	0.3	-	0.5	-	0.7	-	0.8	-	0.9	-
	0.4	-	1.1	-	1.1	-	1.2	-	1.3	-
	0.6	-	1.1	-	1.2	-	1.2	0.1	2.6	0.6
	0.6	-	1.6	-	2.3	-	2.9	0.1	3.2	0.5
	1.3	-	1.8	-	2.9	0.5	3.7	1.1	5.5	2.2
3%	-	-	0.2	-	*	-	*	-	*	-
	-	-	0.2	-	*	-	*	-	*	-
	0.1		0.3	-	0.4	0.5	0.5	1.4	0.6	2.5
	1.6		1.3	-	2.4	-	3.6	0.2	4.9	0.5
4%	-	-	0.4	-	*	-	*	-	*	-
	-	-	0.6	-	*	-	*	-	*	-
	0.4	-	0.8	-	0.8	-	0.9	-	1	-
	0.5	-	1.8	-	1.8	0.1	1.9	0.5	2	0.8
5%	-	-	0.3	-	0.3	-	0.3	-	0.3	-
	0.1	-	0.4	-	0.4	-	0.4	-	0.5	-
	0.1	-	0.7	-	0.7	-	0.7	-	0.7	-
	0.8	-	1.2	-	1.2	-	1.2	-	1.2	-
	1	-	1.6	-	1.6	-	1.6	-	1.6	-

(*) indicates decomposition of germinated seed, (**R**) = radical, (**P**) = plumule, & (-) indicates that the radical & plumule did not emerge.

Table 2. Length of radicle and plumule of Kabuli Chickpeas.

Concentrations of SLF (%)	1st day		2nd day		3rd day		4th day		5th day	
	R (cm)	P (cm)	R (cm)	P (cm)	R (cm)	P (cm)	R (cm)	P (cm)	R (cm)	P (cm)
Control	-	-	0.2	-	*	-	*	-	*	-
	0.3	-	0.5	-	0.6	-	0.7	-	0.8	-
	0.6	-	1.9	-	1.7	-	1.6	-	1.2	-
	1	-	2.3	-	2.4	0.7	2.5	1.4	2.6	2.6
	1.7	-	2.6	-	2.7	0.1	2.8	0.7	2.9	1.1
	1.8	-	2.9	0.1	2.9	0.9	3	1.6	3.1	2.5
	2.1		3.1	0.3	3.2	1.2	3.3	2	3.4	2.9
1%	-	-	0.3	-	*	-	*	-	*	-
	-	-	1	-	*	-	*	-	*	-
	1	-	1.5	-	1.5	-	1.6	-	1.6	-
	2.1	-	2.2	-	2.3	0.5	2.3	0.8	2.4	1.6
	2.3	-	2.4	-	2.7	0.4	2.9	0.9	3	1.4
2%	0.5	-	1	-	*	-	*	-	*	-
	0.9	-	2	-	2	-	2	-	2	-
	1.2	-	2.3	-	2.3	0.3	2.3	0.9	2.3	1.3
	1.5	-	2.5	-	2.5	-	2.5	0.5	2.5	0.9
3%	0.1	-	0.1	-	*	-	*	-	*	-
	0.2	-	0.2	-	0.2	-	0.3	-	0.3	-
	0.2	-	0.5	-	*	-	*	-	*	-
	0.4	-	3	-	3.1	0.5	3.2	1.2	3.3	1.9
	2.1	-	3.3	-	3.4	0.2	3.5	0.6	3.6	1
4%	-	-	0.1	-	*	-	*	-	*	-
	0.3	-	0.5	-	0.5	-	0.5	-	0.5	-
	0.2	-	0.5	-	*	-	*	-	*	-
	1.5	-	2	-	2.1	0.3	2.2	0.8	2.4	1
	1.6	-	2.2	-	2.6	0.4	3.1	1	3.4	1.5
	1.8	-	2.9	-	3.5	0.5	4.2	1.2	4.5	1.6
5%	0.3	-	0.4	-	0.4	-	0.4	-	0.4	-
	0.5	-	0.5	-	0.5	-	0.5	-	0.5	-
	1.9	-	2.4	-	2.4	-	2.5	0.4	2.5	0.8
	2.2	-	2.9	-	2.9	0.1	2.9	0.5	2.9	0.9

(*) indicates decomposition of germinated seed, (**R)** = radical, (**P)** = plumule, & (-) shows that the plumule & radicle did not emerge

Results and Discussion

Chickpeas (*C. arietinum* L.) are known for their high nutritional values as they are rich in proteins, fibers and carbohydrates. They have very low amount of fat and sodium due to which they are one of the most highly consumed leguminous plants throughout the world (Koul, *et al.,* 2022). In this experiment, the effects of seaweed liquid fertilizer (SLF), prepared from brown algae *C. indica* were tested in different concentrations for better germination rates of Desi as well as Kabuli chickpeas. But very few studies are present that show the response of chickpeas to seaweed liquid fertilizers (SLFs). The germination rate of two varieties of chickpea was studied with five concentrations of 1%, 2%, 3%, 4% and 5% of SLF. As 10 imbibed seeds of Desi and Kabuli chickpeas were placed in each petri plate of respective concentrations, as well as in petri plates containing sterilized distilled water, which was used as control (Fig. 1). For each concentration and control, five replicates were used, and germination rate in term of percentage and initial lengths of plumules and radicles were recorded. This study was carried out for five days.

After two days of starting the experiment, radicles started to emerge in Desi as well as Kabuli chickpeas. On the second day after emergence, most seeds had germinated in both varieties. Among all concentrations used, Desi chickpeas showed the highest germination rate in 1 and 2 % of SLF, which was 60%, followed by 5% SLF concentration which showed 50% germination while 3% and 4% concentrations of SLF showed 40% germination rate (Fig. 2). In Desi chickpeas, the control plates showed maximum germination of seeds, which

was 70%. But it was also observed that after germinating, some seeds did not grow further and started to decompose. The plumule in such seeds did not emerge. Ramarajan, *et al*., (2012) showed that 1 and 2% SLF concentrations of *Sargassum wightii* and *Ulva lactuca* showed increased germination rate on soybeans, and suggested that these results could be due to enzyme activity triggered by growth-promoting hormones. The study of Kavipriya, *et al*., (2011) also showed that lower concentrations of green as well as brown seaweeds including *Sargassum plagiophyllum*, *Turbinaria conoides*, *Padina tetrastromatica*, *Dictyota dichotama*, *Ulva lactuca* and *Caulerpa scalpelliformis* can be used for improved germination rate of green gram (*Vigna radiata*).
Although many chemical fertilizers are used for better production of chickpeas. These fertilizers are not only responsible for causing environmental pollution but are also leave hazardous marks on health of living organisms. Moreover, chemical fertilizers can decrease the quality of food, increase the resistance of weeds also pathogens that are responsible for diseases, and can cause soil degradation over a longer period (Chandini, *et al.*, 2019, Pandya, & Mehta, 2023). Seaweed liquid fertilizers (SLFs) are known to show significant results as compared to chemical and other organic-based fertilizers (Ramya, *et al*., 2015; Hidangmayum & Sharma, 2017). A recent study by Huda, *et al*., (2023) shows that the use of SLF obtained from red seaweed (*Gracilaria tenuistipitata* var. *liui*) had a positive impact on the growth and yield of *Vigna radiata* (Mung bean).
Kabuli chickpeas showed a higher germination rate in 4% concentration of SLF, while 1% and 3% SLF concentrations showed 50% germination rate and 2% and 5% concentrations of SLF showed 40% germination rate (Fig. 2). The control of the Kabuli chickpea had a similar germination rate as that of the Desi chickpeas which was 70% (Fig. 2). It was slightly higher than the concentrations tested but it was also observed that in control, some seeds decomposed soon after germination, whereas in SLF concentrations, the ratio of seed decomposition was lower than in control, thus proving a better germination rate of SLF.

Along with the germination rate, initial lengths of radicles and plumules were also recorded on a daily basis. Although this experiment possessed five replicates for each concentration with ten seeds per replicate, for measuring radicles and plumules, ten seeds were observed for the respective concentration.

The results showed that in Desi chickpeas, radicles germinated two days after the treatment with SLF, while plumules emerged on the third day. The highest length of radicle and plumule was seen at 2% SLF. On the first day of observation, the length of the radicle was 1.3 cm, while the plumule measured 0.5 cm on the final day of the experiment, the maximum radicle and plumule lengths were measured as 5.5 cm and 2.2 cm respectively. In 1%, concentration of SLF, the length of the radicle when starting the experiment was measured at 0.8 cm, which reached 5 cm on the final day, and plumule length was measured 0.3 cm in the beginning and reached 1.6 cm. In the case of 3% concentration of SLF, the radicle length of 1.6 cm was recorded on the first day of observation and it reached 4.9 cm, while the plumule emerged 0.5 cm and reached up to 2.5 cm till ending the experiment. The concentration of 4% SLF had the radicle length of 0.5 cm in starting that reached 2 cm, while its plumule emerged 0.1 cm and reached 0.8 cm at the end of the experiment. At 5% concentration of SLF, the length of the radicle was recorded as 1 cm in the beginning, but it did not exceed beyond 1.6 cm, which is the lowest length in all concentrations and plumule in 5% did not emerge. The seeds after five days of observation started to decompose. The control of Desi chickpeas had the radicle length of 1.6 cm on the first day of germination, which reached a maximum length of 4 cm while the plumule emerged at 0.1 cm and reached a maximum length of 1.6 cm (Table 1).

This positive growth of Desi chickpea seeds in lower concentrations is supported by the study of Kalaivanan, *et al*., (2012) reported that lower concentrations of seaweed extract not only result in greater germination rate of black gram (*Vigna mungo*) but also improved overall root and shoot lengths of the plant. Pandya & Mehta, (2021) also reported that 1.5% and 2% concentrations of *Ulva lactuca* showed greater plant growth in black gram, and suggested that better growth could be due to the auxin like activity of polysaccharides and minerals. A study by Kurakula & Rai, (2021) has also proven that Desi chickpeas show increased germination and growth parameters when treated with 2% SLF solution of *Ascophyllum nodosum*.

In the case of Kabuli chickpeas, the maximum length of the radicle was observed in 4% concentration of SLF, which was 1.8 cm on the first day of germination and later on reached 4.5 cm. This is supported by the observations of Chitra & Sreeja (2013), which concluded that SLF obtained from *Gracilaria corticata* showed increased root and shoot lengths of green gram when treated with 4% of the extract whereas, plumule length for the same concentration was initially 0.5 cm, and reached the maximum length of 1.6 cm on the last day of the experiment. In the concentration of 5% SLF, the radicle quickly germinated to the length of 2.2 cm in the beginning, but did not grow beyond 2.9 cm, and was recorded as minimum radicle length in all tested concentrations. The plumule length in 5% SLF was initially 0.1 cm, but could not exceed 0.9 cm. At 1%, concentration of SLF, in the beginning, the length of the radicle was 2.3 cm which reached the maximum length of 3 cm on the final day of the experiment, whereas the plumule emerged 0.4 cm on the third day and reached 1.4 cm on the final day. 2% SLF concentration showed the radicle length of 1.5 cm that reached 2.5 cm on the final day of the experiment while the plumule length at the same concentration did not exceed 1.3 cm. In the case of 3% concentration of SLF obtained from *C. indica*, initially, the maximum length of the radicle was recorded as 2.1 cm which reached a maximum length of 3.6 cm while the plumule was 0.2 cm at the beginning

and reached a maximum length of 1 cm. However, for the same concentration, the largest plumule was measured 1.9 cm on the final day of the trial. The highest plumule length in the Kabuli chickpea was observed in the control, which was 2.9 cm and the maximum length of the radicle in the control was 3.4 cm (Table 2). Lower length of radicles was observed in the control of both varieties which indicates positive effects of SLF and is supported by investigations of Pasqualone, *et al*., (2021), it proved that controls of both varieties of chickpeas did not show as much growth as organic fertilizers, probably because of the higher amount of biologically active compounds present in fertilizers tested. Nerlekar, *et al*., (2021) also reported that freshwater plants; *Hydrilla verticillata* and *Eichhornia crassipes* increase the growth of radicles and plumules in maize and chickpeas when used in 30% and 50% concentrations.

This study showed promising results of SLF on the germination rate of Desi and Kabuli chickpeas as both varieties started germination earlier than their normal germination time. In both types of chickpeas, germination started two days after starting the experiment. The germination of seeds after two days itself shows the positive effects of seaweed extracts on chickpea seeds, as usually chickpeas are known to germinate after 7-15 days of sowing (Gaur, *et al*., 2012).

In general, Desi chickpeas outperformed Kabuli chickpeas in terms of germination rate. However, both types of chickpeas only responded well for about 5 days before the seeds in all concentrations began to disintegrate. The decomposition could be due to a decrease in the protein content of seeds, as proteins in chickpea seeds can decrease rapidly after 48 hours (Rehman, *et al*., 2008). Also, it was observed that the amount of extract in the petri plates and the light intensity both had an impact on the germination rate. Chickpeas did not display much germination when placed under laboratory conditions (in the absence of natural sunlight). It was also reported that light intensity plays an important role in germination (Islam, *et al*., 2019). Moreover, chickpeas began to decompose as the extract dose increased. This is similar to the study of Sivasankari, *et al*., (2006), which revealed that seeds of *Vigna sinensis* (black-eyed pea) showed optimum germination rate when treated with the low amount of SLF obtained from *Sargassum wightii* and *Caulerpa chemnitzia*, and started to decompose when treated with high dosage due to higher stress. Nevertheless, the decomposition could also be due to the presence of more nutrients than necessary for seeds to germinate (Jennings & Tulloch 1965).

Conclusion

Germination of seeds in a crop is an essential step as it leads to successful crop yield. Since overall results of this research show that both varieties of chickpeas showed a positive response to seaweed liquid fertilizer (SLF) obtained from seaweed *C. indica*, our study concludes that SLFs can be used as an organic fertilizer for increasing the germination rate of the plants studied. Although the use of SLFs will lead to better and quicker germination in chickpeas, it is suggested to sow the seeds in the soil after 4-5 days of germination, and SLF should be given in small quantities to avoid decomposition. Moreover, SLF has been proven to be beneficial for the germination and growth of agricultural plants but there is a lack of study on the use of SLF on chickpeas. This study is the first step towards understanding the effects of SLF on chickpeas, therefore there is still a need to study the effects of SLF on the further growth of both varieties with more replicates.

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