Allelopathic effect of extracts of *Salicornia neei* Lag. In the germination of lettuce seeds (*Lactuca sativa* L.)

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Camila Cristina de França Ferreira
Master's student of the Graduate Program in Development of Environmental Processes – UNICAP, 50050-900 Recife-Pe, Brazil
E-mail: camila2021602082@unicap.br
ORCID: https://orcid.org/0000-0001-8168-8262

Josimar Gurgel Fernandes
Researcher at the Agronomic Institute of Pernambuco (IPA)
Av. General San Martin, 1371
Bongi, Recife, PE, CEP 50761-000
E-mail: josimar.gurgel@ipa.br
ORCID: https://orcid.org/0000-0002-0704-0668

Galba Maria Campos-Takaki
Professor of the ICAM-TECH School.
Center for Research in Environmental Sciences and Biotechnology Catholic University of Pernambuco – UNICAP, 50050-900 Recife-Pe, Brazil
E-mail: galba.takaki@unicap.br
ORCID: https://orcid.org/0000-0002-0519-0849

Rosileide Fontenele da Silva Andrade
Professor of the School of Health and Life Sciences Center for Research in Environmental Sciences and Biotechnology Catholic University of Pernambuco – UNICAP, 50050-900 Recife-Pe, Brazil
E-mail:rosileide.andrdade@unicap.br
ORCID: https://orcid.org/0000-0001-8526-554X

ABSTRACT
Salicornia neei is a halophyte plant that has a high interest in food, pharmaceuticals, and health maintenance. In this work, the objective was to evaluate the allelopathic effect of extracts prepared with the root, stem, and leaves of Salicornia neei applied to smooth lettuce (*Lactuca sativa* L.) seeds. For this, the extracts were prepared using 1g (root, stem, and leaf) macerated and solubilized in water. Then, the smooth lettuce seeds were soaked in the extracts for 30 and 60 minutes. The influence of the extracts on root growth (CR), germination percentage (PG) and germination index (GI) were evaluated. The results showed that the number of lettuce seeds germinated (after soaking for 30 min and 60 min) in all extracts of S. neei (root, stem, and leaf) was higher than the number of germinated seeds in the control treatment. In addition, the maximum germination percentage (PG) was obtained after treatment with seeds soaked in the extracts of S.neei leaf (soaking for 30min) and extract of S.neei root (soaking for 30min), both with PG of 130%. On the other hand, the maximum germination index (GI%) occurred after soaking treatment (for 60min) with S.neei leaf extract.In addition, the extract with the greatest biostimulant potential in root growth was the extract of *Salicornia neei* leaves (soaked for 60min). Therefore, the investigations carried out in this study show that the seed of smooth lettuce (*Lactuca sativa* var. capitata) soaked in leaf and root extract for 30 min were the ones that most promoted germination in smooth lettuce (*Lactuca sativa* var. capitate).

Keywords: Halophyte, Vegetable, Halophyte extracts, Biostimulant.

1 INTRODUCTION

One of the most important genera of halophytes in the world today is Salicornia sp., commonly called sea asparagus, which grows in a saline area and also enables sustainable cultivation for exploitation as a promising source of bioactive compounds. In this context, *Salicornia neei* has been known for its richness in minerals, dietary fibers and various bioactive substances (phytosterols and

phenolic compounds), which has enabled its use in food, agriculture and pharmacology, also due to its chemical composition and its biological activity (ISCA et al., 2014; OLIVEIRA and SAPATA, 2018, PENTEADO et al. 2022). It is a halophyte of the coastal region, and is present mainly in the restinga areas, and this species of halophyte plant develops in soils with high interstitial salinity. Research related to *Salicornia neei* points out that in adulthood, this halophyte is highly tolerant to salinity (THOMAZI et al., 2013; REIS et al., 2020).

Studies with *Salicornia neei* have highlighted the high nutritional and chemical quality of seeds and sprouts, as well as the potential for various uses for humans (BERTIN et al., 2014; TIMM et al., 2015). *Salicornia neei* is shown to be a complete food due to high amounts of crude protein (21.5-24%) and beta-carotene (15.96mg – 100mg), being a biochemical composition comparable to leafy vegetables (LU et al. 2010, DONCATO and COSTA, 2022).

Halophytes can be used in the recovery of areas degraded by salts. The coastal halophyte *Salicornia neei* Lag., formerly called ambiguous Sarcocornia (Michx.) M.A. Alonso & M.B. Crespo (COSTA et al., 2018; COSTA et al., 2019), grows in coastal soils, including flooded by seawater, where soil salinity can vary from 16 to 55 dS.m-1, and even hypersaline (FREITAS; COSTA, 2014).

Seed germination is considered a stage of considerable importance for the life cycle of plants, encompassing a wide variety of events that may be associated with the development of a structure with reproductive potential (BEWLEY et al., 2013; From VITIS et al.2014).

Therefore, the perpetuation of plant species is evidenced through studies on seed germination. In this context, biological events are essential, as well as soil fertility for the germination of several species to occur, and thus, it will be possible to obtain information regarding the conditions in which seeds can germinate (ALBRECHT; PENAGOS, 2012; Mehalaine et al., 2023).

The beginning of the germination process is called soaking in which there is a rapid and high water uptake, totally rehydrating the tissues. By definition, germination incorporates those events that begin with the entry of water into the quiescent dry seed and end with the elongation of the embryonic axis (BEWLEY et al., 2013).

In turn, lettuce (Lactuca sativa) is an herbaceous plant, annual, being considered the leafy vegetable of greater importance in the diet of the Brazilian population, assuring to this species the expressive economic importance (YURI et al. 2002.) Because it is a vegetable adapted to planting throughout the year and because it presents a relatively easy cultivation, it represents an important source of income, since its fast cycle allows the realization of several plantings per year (UFMS, 2009).

The literature has long described that some species of seaweed have always been recognized as excellent fertilizers and natural biostimulants for plants (MOREIRA et al. 2005). The use of biostimulant products is an alternative technique that has presented favorable results for crop growth (CASTRO and VIEIRA, 2001; Mórgor et al., 2008).

Biostimulants refer to the mixture of plant regulators with other compounds of a different biochemical nature. Biostimulant products or plant extracts have been gaining more and more space in agriculture. These compounds have the ability to stimulate plant responses to diseases and abiotic stresses (ZODAPE, 2001; STADNIK, 2003). Thus, products that exhibit biostimulant action can contribute to plant development and influence its productivity (CASTRO, 2006).

The application of natural compounds that seek stimulating effect in olerícola species has occurred aiming at higher productivity and quality.

There are researches on the effects of different forms, sources and doses of these compounds on the growth and production of vegetables (PEREIRA and MELLO, 2002).

In the present work the effect of soaking of different extracts of *Salicornia neei* on the germination of smooth lettuce (Lactuca sativa) seeds was evaluated.

2 MATERIALS AND METHODS

2.1 BOTANICAL MATERIAL

Salicornia neei with 90 days of growth was collected in a greenhouse at the headquarters of the Agronomic Institute of Pernambuco (IPA), Recife, Pernambuco (Figure 1).

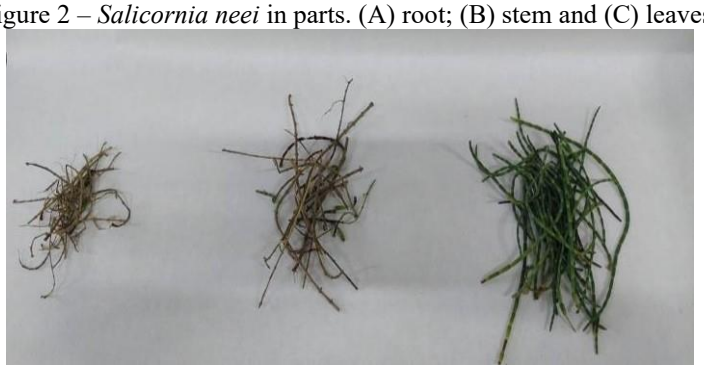
Figure 1 – Floração from *Salicornia neei* (A)/ (B) and Morphology from *Salicornia neei* (C)

Source: Own authorship (2023)

2.2 COLLECTION AND SANITIZATION

The halophyte plant *Salicornia neei* was collected, then washed in running water and distilled water, and then separated into parts (root, stem and leaves) that were cut into pieces of 8 to 10cm (Figure 2).

Figure 2 – *Salicornia neei* in parts. (A) root; (B) stem and (C) leaves.

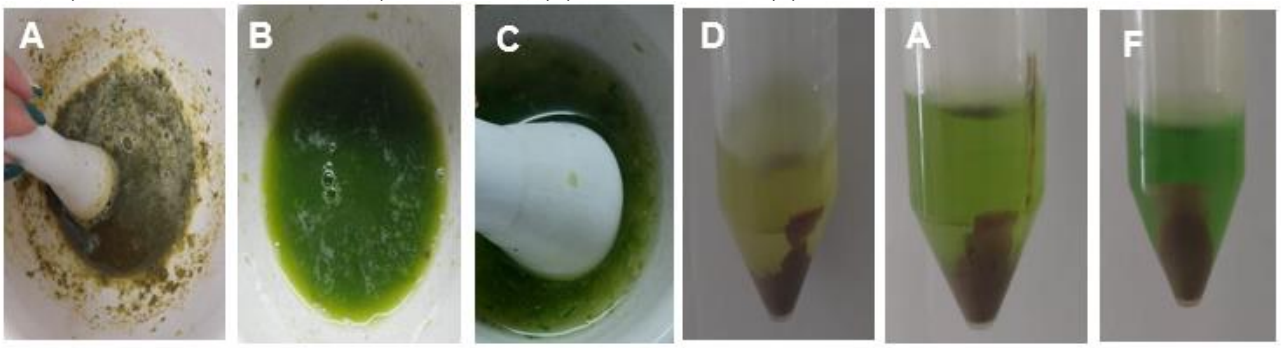


Source: Own authorship (2023)

2.3 PREPARATION OF *SALICORNIA NEEI* EXTRACTS

The extracts of *Salicornia neei* were prepared from 3g of each part of the plant (root, stem and leaves) and then were crushed and macerated (Figure 3) with the addition of 25mL of sterile water for root, stem and leaves. Then, the extracts were centrifuged at 6000rpm for 10 min., and the supernatant was separated from the pellet (plant biomass) (Figure 3). The non-use of organic solvents is related to the fact that they have inhibitory action in relation to germination.

Figure 3 – Maceration process to obtain the extracts of Salicornia neei: (A) Maceration of the root; (B) maceration of the stem; C) maceration of the leaves; D) Root extract; (E) Stem extract and (F) Leaf extract.



Source: Own authorship (2023)

2.4 APPLICATION OF *SALICORNIA NEEI* EXTRACTS IN LETTUCE SEEDS (*LACTUCA SATIVA* L.)

The extracts obtained from *Salicornia neei* were used in the induction tests of lettuce seed germination evaluating the breakdown of dormancy through the presence of chemical alleles in the

extracts of *Salicornia neei*. The seeds used were previously disinfested, by immersion in the solution of sodium hypochlorite at 1%, for three min., and then washed in sterile water (BRASIL, 2009).

The treatments were composed of seeds soaked in root, stem and leaf extracts for thirty and sixty minutes, and in sterile distilled water for the same period of time as control. After the time of soaking in the extracts of root, stem and leaf treatments, the seeds were placed in gerbox type boxes and followed for a period of 7 days. For all treatments we used 30 seeds per plot, which were distributed in a gerbox with dimensions of 11 cm x 11 cm x 3 cm. The boxes were previously disinfested with 70% alcohol and placed in UV light for fifteen minutes before being used.

2.5 EVALUATION OF THE POTENTIAL OF *SALICORNIA NEEI* EXTRACTS IN THE GERMINATION OF LETTUCE SEEDS (*LACTUCA SATIVA* L.)

To evaluate the effect of the concentrations of this extract on the physiological performance of seeds and on the antioxidant metabolism of seedlings, the following tests were performed:

Germination index (%): conducted from those sown in gerbox-type boxes, on two sheets of filter paper moistened with sterile water, in the proportion of 2.5 times the mass of the paper (Figure 7). The evaluations were performed seven days after sowing and the results were expressed as a percentage of normal seedlings (Brasil, 2009).

First germination count (%): performed 24 hours after sowing, together with the germination test. The results were expressed as a percentage of normal seedlings (Brasil, 2009). The germinated seeds were recorded daily for 7 days and the appearance of 2 mm or more of radical was considered as germination.

Root Length (%): performed 7 days after twinning. The results were expressed as percentage of normal seedlings (Brasil, 2009).

To obtain the results, the following equations were used:

- Seed germination (%) = $\frac{\text{number of germinated seeds in the sample x 100}}{\text{Number of twinned seeds in the control}}$
- Root length (%) = $\frac{\text{Sample root length x 100}}{\text{Control root length}}$
- Germination Index (GI%) = Seed germination (%) x Root length (%) 100%

3 RESULTS AND DISCUSSION

3.1 POTENTIAL OF *SALICORNIA NEEI* EXTRACTS IN GERMINATION PERCENTAGE (PG%) OF *LACTUCA SATIVA* L.

In the present research, the number of lettuce seeds germinated after soaking (for 30 min and 60 min) in all extracts of S. neei (root, stem and leaf) was higher than the number of seeds germinated in the control treatment (sterile water 30min and 60min) as shown in Table 1.

The aqueous extracts of *Salicornia neei* caused an increase in germination percentage (PG). The results differ from the studies of Ferreira & Aquila (2000), because the allelopathic effects act significantly increasing the period necessary for the seeds to germinate.

The treatments with leaf extract (soaking 30min and 60min) contributed to an increase in the number of germinated seeds (30 seeds) when compared to the control treatment with water (on average 34 germinated seeds). The same effect was obtained with the stem extract 60 min (30 germinated seeds) and the stem extract 30 min and 60 min (30 germinated seeds).

Table 1- Effect of treatments with extracts of *Salicornia neei* in lettuce seeds *Lactuca sativa* L. evaluated by the number of germinated seeds

Imbibition treatments	**Number of Sprouted Seeds**
Sterile Water 30 min (control)	23
Sterile Water 60 min (control)	24
Root Extract 30 min	30
Root Extract 60 min	30
Stem Extract 30 min	26
Stem Extract 60 min	30
Leaf Extract 30 min	30
Extract Sheet 60 min	30

In addition, the germination percentage (PG%) after treatment with *Salicornia neei* extracts showed a significant effect on seed germination (Table 2). The highest percentage of germination was observed in seeds soaked with extracts of S.neei leaf 30min and S.neei root 30min (both 130%).

The differences in the allelopathic responses of compounds from different organs of the same plant was recorded with Artemisia absinthium where the aerial parts had a greater allelopathic effect in relation to the other parts of the plant. (DELACHIAVE et al. 1999). The amount and variety of allelochemicals and their release by plant organs vary from species to species. For example, PIRES et al. (2001), working with Leucaena leucocephala (Lam.) de Wit., found that by increasing the concentrations of the aqueous extract of this species, the greater the toxic effect on the germination of seeds of picão-preto and caruru. A similar effect was verified by PERIOTTO et al. (2004), where they

demonstrated that the extracts of Andira humilis stems and leaves in the highest concentration (16%) significantly inhibited the percentage of germination of lettuce seeds, and in lower concentrations (4, 8 and 12%) the inhibitory effect was not verified.

Table 2 - Germination percentage (PG%) after treatments with extracts of *Salicornia neei* in seeds of *Lactuca sativa* L.

Imbibition treatments	**Germination Percentage (PG%)**
Root Extract 30 min	130%
Root Extract 60 min	125%
Stem Extract 30 min	113%
Stem Extract 60 min	125%
Leaf Extract 30 min	130%
Extract Sheet 60 min	125%

The pH of the extracts of *Salicornia neei* were respectively 6.71 for the extract S.neei root; 6.41 for the extract S.neei stem and 6.81 for the extract S. neei leaves . Information available in the literature emphasizes that pH effects on germination and seedling development are presented in extremely acidic or alkaline media, and the use of a pH in the range of 6.0 to 7.5 is recommended for laboratory experiments (FILHO; ROBERTS; RODRIGUES, 1997; SON; Dutra, 2007; Kerbauy, 2004).

Larcher (2000) points out that pH values in the range between 6.0 – 7.5 favor biochemical processes and plant nutrition. Thus, considering the pH value of the aqueous extracts *Salicornia neei*, it can be concluded that this parameter is outside the range that could affect the germination or initial development of the species under study.

In research conducted by FURINI et al. (2020), to evaluate the allelopathic and genotoxic potential of the aqueous extract of Santa Maria Herb (a plant of the Amaranthaceae family) on lettuce seeds (*Lactuca sativa* L.), they have demonstrated efficacy in the action of chemical compounds derived from the secondary metabolism of plants. The resistance or tolerance to chemical compounds can act triggering allelopathic or cytotoxic effects and may be specific according to the species, some more sensitive than others, as is the case of lettuce.

Studies conducted with plants of the Amaranthaceae family demonstrate examples of allelopathy like those found in the study, as shown in Table 3.

Table 3 – Examples of Allelopathies on cultivated plants

Weed Plant	Culture	Effects on Culture
Amaranthus palmiere	Onion and carrot	Plant residue stimulates the growth and fresh weight of seedlings.
Amaranthus retroflexus (giant caruru)	Corn and soybeans	Aqueous extract stimulates the growth of hypocotyl in soybean and choleopium in corn

Source: Pires and Oliveira (2011)

The allelopathic effect, according to MILLER (2019), is classified into two types: autotoxicity characterized as an intraspecific mechanism of allelopathy, which occurs when a plant species releases a specific chemical that inhibits or slows the germination or growth of the species itself, and heterotoxicity that occurs when the released substance has a phytotoxic effect affecting the germination and growth of plants of another species. For the treatments analyzed, the allelopathic effect positively stimulated the number of germinated seeds and, consequently, the germination percentage.

3.2 EFFECT OF *SALICORNIA NEEI* EXTRACTS ON ROOT GROWTH

Two treatments were performed with the extracts (soaking for 30 minutes and soaking for 60 minutes) in direct contact with the extracts of S. neei. According to Figure 4 it was possible to detect the significant difference in the effect of Salicornia sp. extracts on the root length of lettuce seeds (after 60 min of imbibition) (Figures 4A, 4B and 4C) when compared to the control treatment (Figure 4D).

As in the treatments with the extracts of *Salicornia neei* mentioned there was immersion of the seeds in the extract, it was verified a clear demonstration of a biostimulatory effect of root growth, provided by the extract in direct contact with the seeds. Such results are presumed to be due to substances contained in said extracts and which may have acted as leaf growth stimulants.

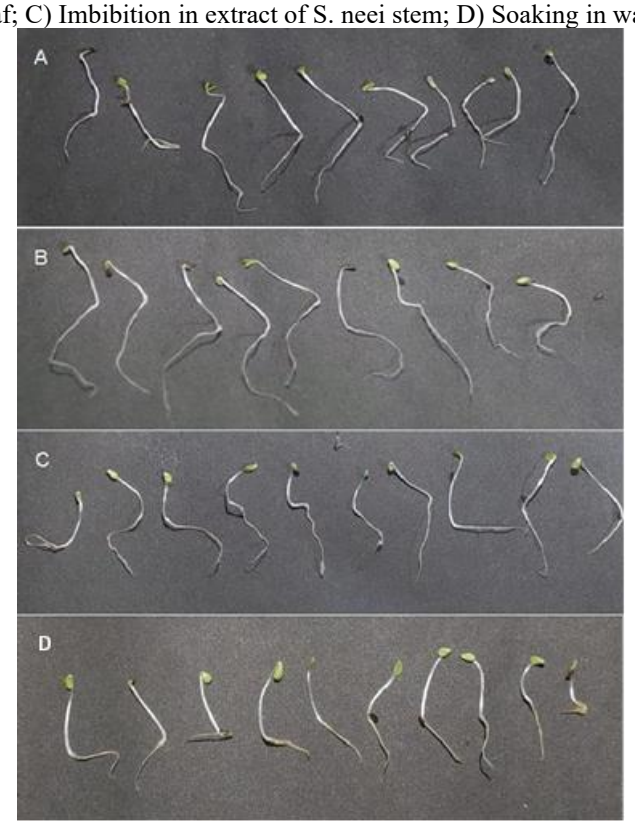
The aqueous extracts of *Salicornia neei* altered the growth of lettuce seedlings, resulting in a stimulus in the root length (CR). Similar CR data for radish and lettuce were obtained by Ribeiro et al. (2009) under the influence of extracts of leaves, roots and sheaths of Crinum americanum. Similar symptoms were also observed by Gatti et al. (2004), Periotto et al. (2004) and Maraschin-Silva & Aquila (2006).

The initial growth of seedlings is more sensitive than germination, because for each seed, the phenomenon is discrete, germinating or not. In general, the roots are more sensitive to the substances present in the extracts when compared to the other structures of the seedlings. This is due to the fact

that the roots are in direct and prolonged contact with the extract (allelochemicals) in relation to the other structures of the seedlings, and/or to a reflection of the distinct physiology between the structures (AQUILA et al., 1999; CHON et al., 2000; FERREIRA & AQUILA, 2000; CHUNG et al., 2001).

In this aspect, the results obtained lead to the assumption that in a lettuce production system, the greater number of leaves will provide greater photosynthesis and consequently greater size and volume of the plant. Consequently, plants with such attributes, when placed in packaging for marketing, will present greater volume in single packages, greater weight and yield for processed products (LIMBERGER, 2012).

Figure 4 – Effect of Salicornia neei extracts on lettuce seeds for 60 minutes: A) Imbibition in S. neei root extract; B) Soaked in extract of S. neei leaf; C) Imbibition in extract of S. neei stem; D) Soaking in water.



The longest root average for all treatments was observed in the treatment with S.neei leaf extract 60min (2.86cm), followed by root extract as shown in Table 3.

Table 3- Effect of *Salicornia neei* Extracts on the average root length

Samples/ Imbibition/ Time (min)	**Average length of lettuce seed root (cm)**
Sterile Water/30	0,90
Sterile Water/60	1,30
Root Extract/30	1,90
Root Extract/60	2,70
Stem Extract/30	1,83
Stem Extract/60	2,42
Leaf Extract/ 30	1,77
Leaf Extract/ 60	2,86

According to Da Silva (2012) the release of allelochemicals is related to the survival capacity of the plant, where these substances can inhibit or stimulate the development of adjacent plants, reducing competition for production factors such as water, light and nutrients.

On the germination of L. sativa all treatments with extracts of *Salicornia neei* stimulated the root length of lettuce seedlings, without, causing significant effects on length. These differences can be attributed to the presence of substances with allelopathic potential as growth stimulators.

3.3 GERMINATION INDEX (GI%) AFTER TREATMENTS WITH EXTRACTS OF *SALICORNIA NEEI* IN SMOOTH LETTUCE SEEDS

The seeds showed expressive growth and high Germination Index (GI) with growth stimulation in the presence of *Salicornia neei* extracts.

The best germination index (GI) for all treatments was observed in the treatment with S.neei leaf extract 60min (274%), followed by root extract 30 min as shown in Table 4.

Table 4 – Germination index (GI%) after treatments with extracts of *Salicornia neei* in smooth lettuce seeds

Imbibition treatments	**Germination Index (GI%)**
Root Extract 30 min	274
Root Extract 60 min	253
Stem Extract 30 min	221
Stem Extract 60 min	258
Leaf Extract 30 min	241
Extract Sheet 60 min	275

The classification of the Germination Index (GI), of BELO. (2011), presented results of "potentiation in germination" for all treated seeds (Table 5).

Table 5 – Classification from the Germination Index (GI)

Germination Index (GI)	**Classification**
< 30	Very Phytotoxic
30 - 60	Phytotoxic
60 - 80	Moderately Phytotoxic
80 - 100	Non-Phytotoxic
> 100	Potentiates germination

Source: Belo, 2011.

According to FERREIRA & ÁQUILA (2000), germination is less sensitive to allelochemicals than seedling growth, however, the experimental quantification is much simpler, because for each seed the phenomenon is discrete, germinates or does not germinate. In this context, we can verify that lettuce seeds were sensitive to the extracts, suffering allelopathic effect.

Allelochemicals can act differently depending on the environment and the stage of the life cycle in which the target plant is, since both reflect different physiological states. These effects can also be varied when considering in which organ of the plant they are acting (SILVA, 2006).

However, based on the results obtained, it is suggested that allelopathic compounds were present in the extracts and caused such effects. It is noteworthy that the results obtained in the laboratory for allelopathy may not be confirmed under natural conditions, given the simultaneous occurrence of biotic and abiotic factors that may interfere in the final results.

4 CONCLUSION

The investigations carried out with *Salicornia neei* leaf extracts with the imbibition of 60 min demonstrated maximum germination index. In addition, significant germination percentage results were obtained after treatment with seeds soaked in extracts of S.neei leaf (soaking for 30min) and extract of S.neei root (soaking for 30min). Therefore, the data show that the extracts of S. neei after soaking treatment, can induce the germination of smooth lettuce seeds, and can be used as a bioinoculant in agriculture with quality and in a sustainable way.

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