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# Piper nigrum allelopathy on the germination and initial growth of *Lactuca sativa* and *Panicum maximum*

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**Abstract**

The increased demand for food promotes greater use of agricultural inputs and pesticides used in pest control. Many of these products have negative environmental and public health impacts. Thus, there is a need for alternative products, with allelopathic capacity that reduce such impacts. The objective of this work was to verify the allelopathic potential of *Piper nigrum* L. extracts on the germination and initial growth of *Panicum maximum* Jacq. and *Lactuca sativa* seeds and seedlings. The experimental design used was completely randomized, using two plant organs (leaf and seed), three extractors (hexane, ethyl acetate and methanol) and five concentrations (0 mg/L, 200 mg/L, 400 mg/L, 600 mg/L and 800 mg/L). It was verified the influence of these extracts on germination speed index (IVG), germination percentage (G), mean germination time (TMG), allelopathy index (IA), shoot growth (CPA) and in the root growth (CR). The leaf and seed extracts, at all concentrations and extractors tested, did not show phytotoxicity on germination and initial growth of *Lactuca sativa*. For Panicum maximum, ethyl acetate and especially methanol seed extracts, at the highest concentrations tested, had the most significant negative effects on the weed, controlling seed germination and inhibiting the initial growth of seedlings of this species. Thus, it was possible to infer those extracts from *Piper nigrum* seeds have potential use in the control of the weed *Panicum maximum*.

**Keywords:** allelochemicals, lettuces, pepper

## Introduction

One of the main limitations to the increase of agricultural productivity is related to pest and disease control (Silva et al., 2017). The increased demand for food and technological advances popularized the use of pesticides and herbicides in agriculture. Hereupon, the Brazilian pesticide market has increased by 190% in the last decade at a growth rate greater than double that presented by the world market (Rigotto et al., 2014). Bessa et al. (2010) highlights the negative impacts of pesticides used in conventional agriculture, awakening studies that demonstrate the bioaccumulative damage in the environment, with monitoring studies, evaluation of the persistence of these products and the use in the environment. In Brazil, herbicides account for 45% of total pesticide consumption, which stands out the importance of this class of agricultural insums in our reality (Rigotto et al., 2014).

Thus, the knowledge of the harmful effects of the use of chemical pesticides, associated with consumers' concern about food quality, has encouraged studies on new techniques for control and management of agricultural pests, including the use of natural products, which are less aggressive to the environment (Tavares et al., 2009). In this context, studies on allelopathy are very relevant because they allow the discovery and identification of new substances with potential use in the control of weed plant species and consequent reduction in the use of conventional herbicides (Mignoni et al., 2018). Allelopathy is described as any direct or indirect, harmful or beneficial effect that one plant exerts on another, by the production of chemical compounds, or allelochemicals, released into the environment (Rice, 1992). Allelochemicals come from secondary metabolism, have a defense function, and show the ability to interfere with the conservation, growth of seedlings and vegetative


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vigor of adult plants. These compounds have aroused deep interest in the possibility of being used as a natural method of effective biological control and preservation of the environment and food from chemical contamination, becoming an appropriate practice for sustainable agriculture (Souza Filho, 2006). Several authors have evidenced the allelopathic potential of several species of the genus *Piper* on insects and fungi (Almeida et al., 2012; Borella et al., 2012; Pinheiro et al., 2016) and also on weeds (Lustosa et al., 2007). Research has revealed that some species of this genus, such as black pepper *(Piper nigrum* L.), contain phytotoxic compounds that are released into the environment through different parts of the plant (Cardoso et al., 2005). The objective of this work was to verify the allelopathic potential of leaf and seed extracts of *P. nigrum* L. on the germination and initial growth of coloniao grass *(Panicum maximum* Jacq.) and lettuce *(Lactuca sativa* L.). The hypothesis is that the application of *P. nigrum* extract has an allelopathic effect on the tested plants, affecting their development.

**Materials and Methods**

*Experimental design*

The work was carried out at the Laboratory of Seeds and Forest Ecophysiology of the Federal University of Espírito Santo, Brazil. Lettuce seeds *(L. sativa*), variety lisa and coloniao grass (*P. maximum*) variety mombasa were used as test species. The experimental design used was completely randomized for both species, with two plant organs (leaf and seed), three extractors (hexane, ethyl acetate and methanol) and five concentrations (0 mg/L, 200 mg/L, 400 mg/L, 600 mg/L and 800 mg/L), in an attempt to contemplate all the allelopathic potential of the plant.

*Germination and initial growth bioassays of P. maximum and L. sativa submitted to leaf and seed extracts of P. nigrum.*

Leaves and seeds of black pepper were collected from a commercial crop located in the municipality of São Mateus, northern Espírito Santo. After collection, the material was packed in paper bags and deposited in a greenhouse with forced ventilation, kept at a constant temperature of 40°C for 3 days. For the preparation of the extracts, after dehydration, the seeds and leaves were crushed separately in a blender for 20 minutes. The material obtained was submerged in different solvents with increasing polarity (hexane, ethyl acetate and methanol) and remained macerated for 7 days. After this period, the macerated was filtered on filter paper and the extracts obtained were placed to dry in a chapel with forced air flow. After drying the solvents, the dry material obtained was diluted in distilled water to obtain the following concentrations: 200 mg/L, 400 mg/L, 600 mg/L and 800 mg/L for each of the three extractors and for the two plant organs. In order to verify the action of the extracts on seed germination and the initial growth of lettuce and coloniao grass seedlings, distilled water was used as control (0 mg/L).

For the germination test, five replicates of 20 seeds for each species were sown in Petri dishes containing two sheets of sterilized germitest paper, moistened with 3 mL of extracts at concentrations of 0 mg/L, 200 mg/L, 400 mg/L, 600 mg/L and 800 mg/L. The plates were kept in a germination chamber B.O.D. under constant illumination at 20°C for lettuce and 25°C for coloniao grass (Tomaz et al., 2010) for seven days. The germination percentage (G) indicated by the number of germinated seeds, i.e., those with radicle protrusion greater than 2 mm (Brazil, 2009), germination speed index (IVG) (Maguire, 1962), mean germination time (TMG) (Lopes & Franke, 2011) and allelopathy index (IA) (Williamson & Richardson, 1988).

After seven days, the lengths of the radicle and shoot of the seedlings were measured and the average results expressed in centimeters.

*Determination of the water potential of the extracts*

The water potential of extracts may have an inhibiting effect on germination and seedling development and therefore may be confused with the allelopathic effect of extracts. To exclude the effect of the water potential on the germination and initial growth of the species, this parameter was evaluated by using a WP4 potentiometer and the results presented in Megapascal (MPa) (Delgado & Barbedo, 2012).

*Statistical analysis*

The means were submitted, using the ASSISTAT software version 7.7, to ANOVA and were compared by the Tukey test at the level of 5% significance.

**Results and Discussion**

*Water potential of leaf and seed extracts of P. nigrum L.*

The extracts used were evaluated for water potential (Table 1). It is known that water potentials above -0.4 Mpa and -0.8 Mpa, respectively, do not affect the germination of seeds of *L. sativa* (Bertagnolli et al., 2003) and *P. maximum* (Belido et al., 2016). The maximum value obtained was -0.31 Mpa (Table 1). Therefore, all the water potential of the extracts were outside the range of possible osmotic stress for the lettuce seeds and coloniao grass, so it is possible to infer that such parameter had no influence on germination and development of seedlings.

**Table 1.** Water potential of extracts of *Piper nigrum* leaves and seeds. (EEF: ethyl acetate leaf extract; EHF: hexane leaf extract; EMF: methanol leaf extract; EES: ethyl acetate seed extract; EHS: hexane seed extract; EMS: Methanol seed extract).

| Extract | Water potential (MPa) | T °C | Extract | Water potential (MPa) | T °C |
|---|---|---|---|---|---|
| EEF 200mg/L | -0.09 | 29.2 | EMS 200mg/L | -0.11 | 29.7 |
| EEF 400mg/L | -0.19 | 29.1 | EMS 400mg/L | -0.18 | 29.6 |
| EEF 600mg/L | -0.19 | 29 | EMS 600mg/L | -0.18 | 29.7 |
| EEF 800mg/L | -0.21 | 29.1 | EMS 800mg/L | -0.20 | 26.9 |
| EES 200mg/L | -0.19 | 29.8 | EHF 200mg/L | -0.16 | 29.1 |
| EES 400mg/L | -0.20 | 29.7 | EHF 400mg/L | -0.24 | 29.3 |
| EES 600mg/L | -0.20 | 29.7 | EHF 600mg/L | -0.24 | 29.2 |
| EES 800mg/L | -0.20 | 29.7 | EHF 800mg/L | -0.31 | 29.6 |
| EMF 200mg/L | -0.21 | 29.6 | EHS 200mg/L | -0.24 | 29.7 |
| EMF 400mg/L | -0.23 | 29.6 | EHS 400mg/L | -0.26 | 29.8 |
| EMF 600mg/L | -0.23 | 29.6 | EHS 600mg/L | -0.26 | 29.8 |
| EMF 800mg/L | -0.24 | 29.7 | EHS 800mg/L | -0.26 | 29.8 |

*Influence of P. nigrum extracts on the germination and initial growth of L. sativa*

Essentially, only the more concentrated leaf and seeds extracts *of P. nigrum* caused a reduction in the germination percentage (G) of lettuce seeds, promoting, at most, reductions close to 10% in this variable (Table 2). However, this lowering can't be considered a phytotoxic event as the G in all treatments still reached at least 80% (Stanisavljevic et al., 2011). Plant extracts may or may not compromise germination and initial growth depending on the target species and concentration used.

Qin et al. (2018) reinforced this idea by investigating the allelopathic effect of aqueous extracts *of Eucalyptus urophylla* leaves on several native tree species, observing stimulatory, inhibitatory and neutral effects. Pinheiro et al. (2016), when working with different species of the genus *Piper,* verified a significant inhibitory effect of *Piper aduncum* and *Piper collosum* on lettuce seed germination but did not verify, as observed in this study, a phytotoxic effect from P*iper medium* on these seeds, even at the highest concentrations used (70%). In addition, working with *P. nigrum* extracts of different polarities on the germination and growth of several cultivated plants, Yan et al. (2006) perceived a wide range of stimulation, inhibition and nontoxic results, which can indicate that different species of the genus *Piper* may not have phytotoxic effect on the germination of lettuce seeds, a fact observed in this study.

When analyzing the IVG, only the most concentrated methanol extracts, from both the leaf and seed, promoted a reduction in this parameter, but without this being reflected in the G of this species. The other extracts did not present significant effect on the IVG of the seeds or promoted their increase, favoring the species. In this respect, it is worth noting the beneficial effect of the hexane extracts that promoted a significant increase in the IVG of the seeds. Also, only the most concentrated foliar and seed methanol extracts and seed ethyl acetate negatively affected the TMG of lettuce seeds. This impairment in IVG and TMG was probably due to the greater presence of piperine and other piperamides in these extracts owing to their higher polarity and, consequently, greater extraction capacity of these secondary metabolites when compared to the hexane extract (Tiwari et al., 2020). Piperine is an amyctic alkaloid, abundant in black pepper, and has been described as capable of influencing plant development mainly during germination, having allelopathic effects (Pereira et al., 2016; Tiwari et al., 2020). It is noteworthy that the hexane extracts promoted a leaning to reduce the TMG, indicating a positive effect on the species. This was probably due to the low extraction capacity of alkaloids such as piperine and great potential of flavonoid extraction by this extractor, with known antioxidant potential, a fact also verified in other studies (França, 2015; Lucena et al., 2017).

The effect on IA also occurred with significant differences in relation to control. IA greater than 50% indicate significant allelopathic effect (Williamson & Richardson, 1988) however, this fact was not observed in this study, despite the difference when comparing to control. Furthermore, this isolated parameter may not faithfully reflect the observed situation, since it only demonstrates the degree of allelopathy exercised by the tested substance and should be observed in conjunction with other parameters (Costalonga & Batitucci, 2020).

**Table 2.** G (germination percentage), IA (Allelopathy Index), IVG (Germination Speed Index), TMG (Average germination time), CPA (Shoot growth) and CR (Radicle Growth) of lettuce seeds submitted to leaf extracts and *seeds of Piper nigrum* L. Means followed by the same capital letter on the line and small letter on the column do not show differences at the level of 5% by the Tukey test. EMF (methanol leaf extract), EHF (hexanic leaf extract), EEF (ethyl acetate leaf extract), EMS (methanol seed extract), EHS (hexanic seed extract), EES (ethyl acetate seed extract).

| (G) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L | (IA) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMF** | 0.97±0.02 aA | 0.92±0.00 bA | 0.92±0.00 bA | 0.92±0.02 bA | 0.95±0.02 aA | **EMF** | -4.30±0.00 aA | 1.08±0.00 aB | 1.08±0.00 aB | 5.38±0.70 aC | 5.38±0.66 aC |
| **EHF** | 0.97±0.02 aA | 0.96±0.00 aA | 0.96±0.00 aA | 0.95±0.02 aA | 0.96±0.00 aA | **EHF** | -4.30±0.00 aA | -3.23±0.00 bB | -3.23±0.00 bB | -2.15±0.02 bB | -3.23±0.00 bB |
| **EEF** | 0.97±0.02 aA | 0.96±0.01 aA | 0.96±0.01 aA | 0.92±0.01 bB | 0.92±0.01 bB | **EEF** | -4.30±0.00 aA | -3.23±0.00 bB | -3.23±0.01 bB | 1.08±0.03 bC | 1.08±0.02 cC |
| **EMS** | 0.97±0.02 aA | 0.92±0.00 bB | 0.91±0.02 bB | 0.83±0.02 cC | 0.95±0.02 bA | **EMS** | -4.30±0.00 aA | 1.08±0.00 aB | 2.15±0.50 aB | 1.75±0.70 bB | 2.15±0.00 cB |
| **EHS** | 0.97±0.02 aA | 0.92±0.00 bB | 0.89±0.02 bC | 0.95±0.02 aA | 0.87±0.02 cC | **EHS** | -4.30±0.00 aA | 1.08±0.00 aB | 4.30±0.80 cC | 1.35±0.80 bB | 6.45±0.80 aC |
| **EES** | 0.97±0.02 aA | 0.85±0.02 cC | 0.81±0.02 cD | 0.85±0.02 cC | 0.88±0.00 cB | **EES** | -4.30±0.00 aA | 8.60±0.80 cC | 12.9±0.80 dC | 8.60±0.80 aC | 5.38±0.20 aB |

| (IVG) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L | (TMG) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMF** | 0.60±0.02 aA | 0.55±0.02 bAB | 0.50±0.02 cAB | 0.48±0.01 bB | 0.43±0.01 cB | **EMF** | 1.69±0.13 aC | 1.66±0.15 aBC | 1.82±0.13 aB | 1.99±0.10 aAB | 2.09±0.20 aA |
| **EHF** | 0.60±0.02 aC | 0.65±0.01 aAB | 0.66±0.02 aAB | 0.68±0.03 aA | 0.65±0.03 bB | **EHF** | 1.69±0.13 aA | 1.43±0.22 bA | 1.53±0.13 bA | 1.37±0.28 bA | 1.56±0.25 cA |
| **EEF** | 0.60±0.02 aC | 0.66±0.02 aBC | 0.67±0.03 aBC | 0.76±0.03 aA | 0.79±0.04 aA | **EEF** | 1.69±0.13 aA | 1.53±0.20 abABC | 1.54±0.22 bAB | 1.24±0.21 bC | 1.28±0.23 cC |
| **EMS** | 0.60±0.02 aA | 0.57±0.03 bAB | 0.52±0.03 bcAB | 0.47±0.02 bB | 0.47±0.01 cB | **EMS** | 1.69±0.13 aB | 1.76±0.08 aB | 1.94±0.10 aAB | 2.13±0.07 aA | 2.13±0.07 aA |
| **EHS** | 0.60±0.02 aB | 0.69±0.04 aAB | 0.73±0.05 aA | 0.70±0.05 aAB | 0.68±0.05 abAB | **EHS** | 1.69±0.13 aA | 1.45±0.09 bA | 1.38±0.14 bA | 1.44±0.14 bA | 1.48±0.15 cA |
| **EES** | 0.60±0.02 aBC | 0.64±0.04 aAB | 0.64±0.05 abAB | 0.74±0.03 aA | 0.50±0.04 cC | **EES** | 1.69±0.13 aB | 1.56±0.10 abBC | 1.57±0.13 bBC | 1.35±0.06 bC | 1.99±0.15 bA |

| (CPA) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L | (CR) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMF** | 0.59±0.05 aA | 0.58±0.03 aA | 0.58±0.03 aA | 0.57±0.02 aA | 0.57±0.02 aA | **EMF** | 3.05±0.11 aA | 2.93±0.03 aA | 2.93±0.07 aA | 2.90±0.07 aA | 2.88±0.09 aA |
| **EHF** | 0.59±0.05 aA | 0.58±0.03 aA | 0.59±0.02 aA | 0.58±0.02 aA | 0.59±0.03 aA | **EHF** | 3.05±0.11 aA | 2.96±0.06 aA | 2.98±0.07 aA | 2.98±0.10 aA | 2.94±0.09 aA |
| **EEF** | 0.59±0.05 aA | 0.58±0.03 aA | 0.59±0.03 aA | 0.59±0.02 aA | 0.58±0.02 aA | **EEF** | 3.05±0.11 aA | 2.91±0.03 aA | 2.92±0.07 aA | 2.87±0.08 aA | 2.84±0.11 aA |
| **EMS** | 0.59±0.05 aA | 0.58±0.04 aA | 0.58±0.04 aA | 0.57±0.04 aA | 0.57±0.02 aA | **EMS** | 3.05±0.11 aA | 2.93±0.08 aA | 2.86±0.08 aA | 2.80±0.12 aA | 2.77±0.07 aA |
| **EHS** | 0.59±0.05 aA | 0.57±0.04 aA | 0.57±0.05 aA | 0.43±0.02 bB | 0.42±0.03 bB | **EHS** | 3.05±0.11 aA | 2.91±0.07 aA | 3.04±0.02 aA | 2.96±0.09 aA | 2.99±0.06 aA |
| **EES** | 0.59±0.05 aA | 0.57±0.05 aA | 0.57±0.04 aA | 0.47±0.01 bB | 0.43±0.02 bB | **EES** | 3.05±0.11 aA | 2.91±0.07 aA | 2.93±0.08 aA | 2.92±0.06 aA | 2.80±0.12 aA |

When analyzing the initial growth of lettuce seedlings, only the most concentrated EHS and EES extracts (600 and 800 mg/L) promoted a reduction, around 27%, in the CPA of lettuce seedlings when compared to the control (Table 2). There was no significant difference in CR in any of the extracts tested. This result on CR is interesting because the radicle tends to be the place where most of the components of these extracts are concentrated due to greater contact with it, thus being the most affected variable (Periotto et al., 2004).

However, in this study, no differences in roots were found. Similar results were found by Terço & Leone (2017), when working with *Piper aduncum* extract also on the CPA and CR of *L. sativa*, not observing major changes. This result reinforces the thesis that *P. nigrum* extracts do not present significant inhibitive effect on *L. sativa* seeds and seedlings. Thus, when considering the variables tested in Table 2, it is possible to infer that the leaf and seed extracts *of P. nigrum* did not compromise the germination of the seeds and the initial growth of the seedlings of this species. Therefore, these extracts present potential use in weed control due to the fact that it does not effectively affect the germination and initial growth of seeds and seedlings of *L. sativa* L.

*Influence of P. nigrum extracts on germination and initial growth of P. maximum*

Only the extracts EES 800 and EEF 800 reduced the IVG of grass seeds while leaf hexane extracts promoted an increase in this parameter (Table 3). Ethyl acetate and methanol are very efficient in extracting piperines from macerated material, unlike hexane that extracts, mainly, nonpolar compounds. This higher concentration of alkaloids caused by the affinity for ethyl acetate extraction may explain the reduction observed in IVG. The possible higher extraction of flavonoids by hexane may also indicate an explanation for the positive effect of these extracts on the germination speed of *P. maximum* seeds (França, 2015). Practically all extracts showed an inhibitory effect on the germination percentage (G) of grass seeds.

Again, the EES and EMS extracts, in their highest concentrations, inhibited the grass seeds with greater intensity reducing the G around 50%. The extracts EEF, EMS and EES, in their highest concentrations (600 and 800 mg/L), increased the TMG of the seeds while the hexane extracts promoted the reduction of this parameter favoring the germination of coloniao grass seedlings. This was probably due to the greater presence of the bioactive alkaloids such as piperine and other polar compounds in methanolic and ethyl acetate extracts when compared to the hexane extracts, evidencing this negative oscillation in G, IVG and TMG by these treatments. These results are in accordance with those of the literature where several studies have demonstrated an association between polar *Piper* extracts and negative effects on germination of various wild species and weeds (Herro & Callaway, 2003; Siddiqui, 2007; Pereira et al., 2016).

All extracts promoted the increase in IA, especially the most concentrated ones. The greatest negative effect, with levels higher than 50%, on IA was also in extracts EMS 600 and 800 and EES800, indicating that seed extracts, at the highest concentrations used, were more effective in inhibiting the germination of *P. maximum* seeds. All extracts negatively affected the CPA of grass seedlings, except for leaf hexane extracts that promoted an increase in the length of the aerial part. In this variable, once more, the extracts EMS800 and EMS600 were highlighted, which caused the most marked reduction in this parameter (89%) and seem to be in conformity with the effects of the chemical constitution of the secondary metabolites found in this plant (Pereira et al., 2016). As for CR, all extracts contributed negatively to seedling development. Again, the most concentrated seed methanol extract (EMS800) showed the most significant reduction in radicle length, decreasing its size by approximately 91% when compared to the control, results similar to those obtained by Siddiqui (2007) when exposing plants of the *genus Vigna* to extracts of *P. nigrum*. Thus, it is possible to infer that the more concentrated methanolic seed extracts compromise, in a very effective way, the initial growth of coloniao grass seedlings and have great potential for use in biological control of weeds, due to their allelopathic capability.

**Table 3.** G (germination percentage), IA (Allelopathy Index), IVG (Germination Speed Index), TMG (Average germination time), CPA (Shoot Growth) and CR (Radicle Growth) of coloniao grass seeds submitted to leaf and seed extracts of *Piper nigrum* L. Means followed by the same capital letter on the line and small letter on the column do not show differences at the level of 5% by the Tukey test. EMF (methanol leaf extract), EHF (hexanic leaf extract), EEF (ethyl acetate leaf extract), EMS (methanol seed extract), EHS (hexanic seed extract), EES (ethyl acetate seed extract).

| (G) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L | (IA) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMF** | 0.77±0.02 | 0.68±0.03 | 0.81±0.02 | 0.69±0.0 | 0.65±0.04 | **EMF** | 17.21±1.90 | 26.88±3.51 | 12.91±2.15 | 35.48±0.0 | 30.11±4.12 |
| | aA | bB | aA | bcB | aB | | aC | bB | dC | cA | cAB |
| **EHF** | 0.77±0.02 | 0.66±0.05 | 0.57±0.0 | 0.64±0.04 | 0.54±0.02 | **EHF** | 17.21±1.90 | 24.73±5.55 | 22.58±3.51 | 21.35±0.0 | 32.26±4.12 |
| | aA | bB | cC | cB | bC | | aC | bB | cBC | dBC | cA |
| **EEF** | 0.77±0.02 | 0.66±0.02 | 0.57±0.0 | 0.64±0.02 | 0.54±0.03 | **EEF** | 17.21±1.90 | 29.03±2.48 | 38.71±2.15 | 37.18±0.0 | 41.94±2.48 |
| | aA | bB | cC | cB | bC | | aC | bB | bA | cA | bA |
| **EMS** | 0.77±0.02 | 0.76±0.02 | 0.55±0.03 | 0.36±0.02 | 0.39±0.02 | **EMS** | 17.21±1.90 | 18.28±3.51 | 40.86±2.15 | 61.29±3.51 | 58.07±2.15 |
| | aA | aA | cdB | eC | cC | | aC | cC | abB | aA | aA |
| **EHS** | 0.77±0.02 | 0.69±0.02 | 0.67±0.02 | 0.71±0.02 | 0.49±0.03 | **EHS** | 17.21±1.90 | 25.81±2.15 | 27.96±2.15 | 23.66±2.15 | 47.36±2.15 |
| | aA | bB | bB | abB | bC | | aC | bB | cB | dB | bA |
| **EES** | 0.77±0.02 | 0.60±0.04 | 0.51±0.02 | 0.46±0.02 | 0.38±0.02 | **EES** | 17.21±1.90 | 35.48±3.51 | 45.16±4.12 | 50.54±2.48 | 59.14±2.48 |
| | aA | cA | dC | dC | cD | | aD | aD | aB | bB | aA |

| (IVG) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L | (TMG) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMF** | 0.25±0.02 | 0.25±0.01 | 0.26±0.02 | 0.27±0.0 | 0.25±0.02 | **EMF** | 4.07±0.3 | 3.99±0.2 | 3.99±0.3 | 3.82±0.0 | 4.10±0.3 |
| | aA | bcA | bA | cA | bA | | aA | bA | bA | bA | bA |
| **EHF** | 0.25±0.02 | 0.31±0.04 | 0.31±0.02 | 0.32±0.8 | 0.36±0.0 | **EHF** | 4.07±0.3 | 4.07±0.4 | 3.30±0.2 | 2.94±0.0 | 2.78±0.1 |
| | aA | aB | aB | abB | aC | | aA | abA | cB | cB | cB |
| **EEF** | 0.25±0.02 | 0.24±0.01 | 0.22±0.02 | 0.22±0.02 | 0.20±0.0 | **EEF** | 4.07±0.3 | 4.22±0.3 | 4.55±0.4 | 3.78±0.0 | 4.90±0.2 |
| | aA | bcAB | bcAB | dAB | cB | | aBC | abBC | aAB | bC | aA |
| **EMS** | 0.25±0.02 | 0.26±0.01 | 0.26±0.01 | 0.21±0.03 | 0.23±0.01 | **EMS** | 4.07 ±0.3 | 3.82±0.2 | 3.85±0.4 | 4.74±0.3 | 4.44±0.3 |
| | aAB | bA | bA | dB | bcAB | | aBC | bcC | bC | aA | abAB |
| **EHS** | 0.25±0.02 | 0.28±0.02 | 0.26±0.03 | 0.28±0.03 | 0.26±0.02 | **EHS** | 4.07±0.3 | 3.64±0.4 | 3.92±0.5 | 3.53±0.2 | 3.94±0.2 |
| | aA | aA | bA | bcA | bA | | aA | bcA | bA | bcA | bA |
| **EES** | 0.25±0.02 | 0.22±0.01 | 0.21±0.01 | 0.33±0.01 | 0.20±0.02 | **EES** | 4.07±0.3 | 4.59±0.1 | 4.78±0.2 | 3.02±0.1 | 5.02±0.3 |
| | aB | cBC | cC | aA | cC | | aBC | aAB | aA | cC | aA |

| (CPA) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L | (CR) | 0 mg/L | 200 mg/L | 400 mg/L | 600 mg/L | 800 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **EMF** | 2.40±0.11 | 0.74±0.04 | 0.63±0.05 | 0.57±0.06 | 0.50±0.04 | **EMF** | 3.31±0.1 | 1.63±0.1 | 1.42±0.1 | 1.40±0.1 | 1.38±0.1 |
| | aA | cdB | cBC | bcC | cC | | aA | cdB | cC | cC | cC |
| **EHF** | 2.40±0.11 | 2.90±0.13 | 2.85±0.15 | 2.79±0.09 | 2.99±0.08 | **EHF** | 3.31±0.1 | 2.66±0.1 | 2.96±0.1 | 1.91±0.1 | 3.06±0.1 |
| | aC | aAB | aAB | aB | aA | | aA | bC | aBC | bC | aB |
| **EEF** | 2.40±0.11 | 1.02±0.09 | 0.62±0.08 | 0.72±0.06 | 0.69±0.07 | **EEF** | 3.31±0.1 | 3.06±0.1 | 2.16±0.1 | 2.07±0.1 | 2.03±0.1 |
| | aA | bB | cC | bC | bC | | aA | aB | bC | aC | bC |
| **EMS** | 2.40±0.11 | 0.60±0.04 | 0.51±0.02 | 0.26±0.01 | 0.26±0.04 | **EMS** | 3.31±0.1 | 1.06±0.0 | 0.74±0.0 | 0.50±0.1 | 0.31±0.0 |
| | aA | dB | cB | dC | dC | | aA | eB | fC | eD | eE |
| **EHS** | 2.40±0.11 | 0.82±0.01 | 0.48±0.04 | 0.47±0.04 | 0.37±0.06 | **EHS** | 3.31±0.1 | 1.72±0.0 | 0.97±0.1 | 0.83±0.0 | 0.59±0.0 |
| | aA | cB | cC | cC | cdC | | aA | cB | eC | dC | dD |
| **EES** | 2.40±0.11 | 1.15±0.03 | 0.81±0.03 | 0.49±0.02 | 0.35±0.08 | **EES** | 3.31±0.1 | 1.50±0.0 | 1.19±0.1 | 0.97±0.0 | 0.55±0.0 |
| | aA | bB | bC | cD | cdD | | aA | dB | dD | dD | dE |

## Conclusions

The extracts tested showed no or little negative influence on germination and initial growth of *L. sativa* which indicate non-phytotoxic effect on this cultivated species. The hexane extracts, in general, did not alter or promote a positive effect on the variables tested in both species. The seed extracts, at the highest concentrations tested, showed the highest efficiency in the control of the species *P. maximum*. Among the extractors tested, methanol proved to be more efficient in controlling seed germination and inhibiting the initial growth of this species. Thus, methanolic extracts of *P. nigrum* seeds have potential for use in the control of weed species such as *P. maximum*.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.