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The Effect of Allelochemical of *Parthenium hysterophorus* L., *Eucalyptus creba* F. Muell., and *Casuarina cunninghamiana* Miq. on the Germination and Seedling Growth of *Lepidium sativum* L.

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Abstract. Allelochemicals are widely known to have antagonistic effects on the environment by inhibiting the physiological activities of other plants. However, this is the whole characteristic of allelochemicals since their effects are not only limited to inhibiting. In low concentration, allelochemicals could have a promoting effect on other plants especially on the germination and seedling stage. This research was conducted to examine the allelochemical activity of *Parthenium hysterophorus* L., *Eucalyptus creba* F. Muell., and *Casuarina cunninghana* Miq. on *Lepidium sativum* L. with the sandwich method. The leaves of parthenium, eucalyptus, and casuarina were dried and layered between two 5 mL agar in multi well plates and the sterilized seeds of *Lepidium sativum* L. were set to germinate on the agar. The results showed that the leaves of parthenium, eucalyptus, and casuarina caused lower germination rate compared to the control treatment but led to the greater shoot and root length of *Lepidium sativum*.

Keywords: allelopathy; casuarina; eucalyptus; parthenium weed; seedling

INTRODUCTION

In a broad definition, allelopathy is the effect of the release of chemical compounds from one plant to the neighboring plants (Rice, 1984). However, the definition that is largely used limits the allelopathy to only the antagonist effect of the chemical substance that is released to the environment and could inhibit the germination and growth of other organisms (Allaby, 2012). Allelopathic compounds are secondary metabolites, or the compounds that do not have a role in the basic metabolic activity of the plant and only appear sporadically (Rice, 1984).

Allelopathy is the biochemical (allelochemical) that is released from the plant's leaves, root, bark, or other parts by root exudation, leaching, volatilization or residue decomposition (Ferguson & Rathinasabapathi, 2003). In nature, the allelopathy activity is affected by its biosynthesis, mode of release, mode of action, detoxification, and joint action of allelochemical, the growth media condition, and the growth condition of both receiver and donor plant (Inderjit & Duke, 2003; Kobayashi, 2004)

Parthenium hysterophorus L. (hereafter referred as parthenium), *Eucalyptus crebra* F. Muell (hereafter referred as eucalyptus), and *Casuarina cunninghamiana* Miq (hereafter referred as casuarina) are the plants which have specific and unique use of their allelopathic compounds. Parthenium is a pantropical noxious weed that invests the cropping, grazing, and environmental area which allelopathic character poses harm to human health, animal husbandry, crop production, and biodiversity (Bajwa et al., 2018; Evans, 1997). Eucalyptus and casuarina are important tree species cultivated for the essential oil, timber, and land reclamation (Ben Ghnaya et al., 2016; Kaur et al., 2011). The vegetation of both eucalyptus and casuarina shows very poor understory development, hypothetically because the litter of the trees contain allelochemicals that inhibit the growth of understory plants (Batish et al., 2001). Because of the vital role of these three plants, it is essential to understand their impact on the environment, especially the allelochemical character.

The effects of the allelopathy will be tested on the common cress (*Lepidium sativum* L.) seeds. It is hypothesized that the allelopathic compounds of the three plants have a negative effect on the germination,

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and the reduced germination will also reduce the seedling growth (root length and shoot length) of the common cress (the seedling growth positively corresponds to germination).

METHODS

This experiment was conducted in September 2018 in Plant Science Laboratory, The University of Queensland, Gatton campus. The experiment was conducted in randomized block design with pre-prepared multiwell plates. The method used in this experiment was adopted from the sandwich method for allelopathic effect of leaf litter (Fujii et al., 2004). For the treatment, leaves of parthenium, eucalyptus, and casuarina, each of a weight 50 mg, were dried and layered between two 5 mL of agar in the multi well plates. Each plate contained six wells; three wells on the left-hand side contained replications of control variables (agar water only) and three wells on the right-hand side contained the treatment variables (agar and leaf layer) with the same concentration.

It was very important to do this experiment in a sterile condition. The bench, the hood, and forceps, needed to be sterilized with ethanol 70% before starting the process. As for the common cress seeds, they were sterilized by soaking them in natrium hypochlorite 2% for 20 minutes to prevent contamination. After being soaked in the sodium hypochlorite, the seeds were rinsed and strained thoroughly using distilled water and from this step on, the process had to be done under the hood. Five seeds needed to be sown in each well by using forceps dipped into the ethanol then dried with a paper towel to sterilize it. The agar needed to be covered all the time and had to be opened as little as possible and the seed sowing on the agar had to be done as quickly as possible. After sowing, each plate needed to be sealed with parafilm to prevent moisture loss and labelled with the information of treatment, species, and group number to be collected in the incubation. The seeds incubated for 1 week under the day-night cycle of 12 hours at the temperature of 25^0 C.

The results then were analysed with ANOVA and descriptive statistics by using SPSS software, And Tukey test was used for the data that shows a significant difference. The data collected from these experiments were the number of germinated seed, the number of contaminated seed, the shoot length, and the root length.

RESULTS AND DISCUSSION

The contamination rate in this experiment was regrettably high (Figure 1) which affected the germination rate and eventually the shoot and root length.

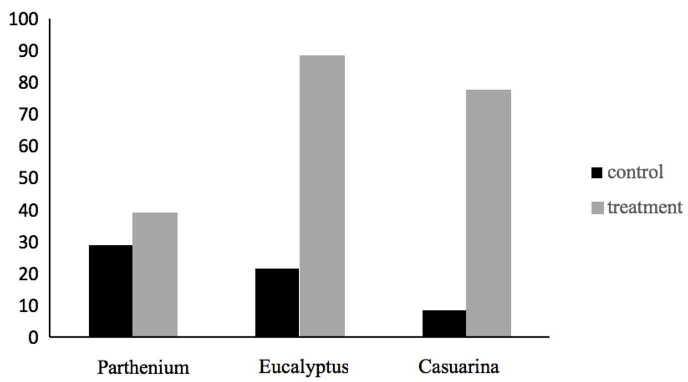

Figure 1. *Contamination rate during the germination stage of the common cress seed (%)*

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There was significant difference between the germination of the control variable and treatment variable (Figure 2) for parthenium (p value: 0.000), eucalyptus (p value: 0.000), and casuarina (p value: 0.023) with the control variable of each species having a higher germination rate.

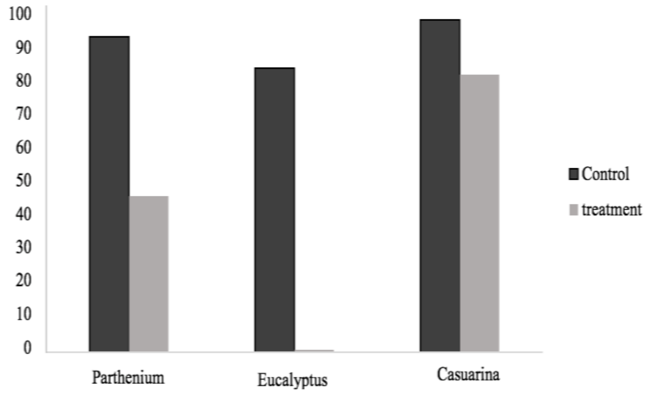

Figure 2. *Germination rate of the common cress seed (%)*

The average shoot length had greater results in the treatment variable compared to the control variable (Figure 3). There was a significant difference between them in the parthenium species (p value: 0.013), eucalyptus (p value: 0.000), and casuarina (p value: 0.000).

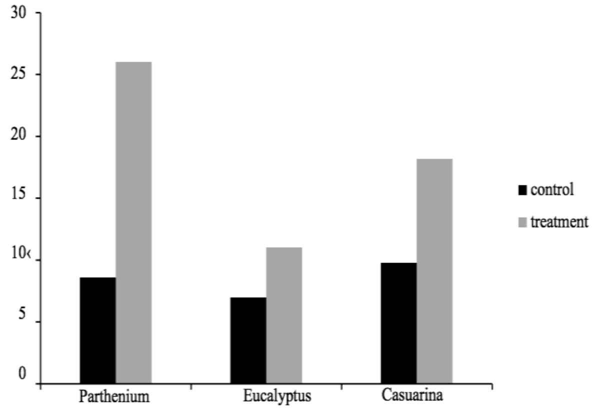

Figure 3. *Mean Shoot Length of the common cress seed (cm)*

The results of the root length showed that the seeds sown with the allelopathic compounds had greater root length compared to the ones without allelopathy from each species (fig. 4). The significant difference of the root in the control and treatment variable was found in the parthenium treatment (p value: 0.004), eucalyptus (p value: 0.000), but not in the casuarina species (p value: 0.106, > 0.05).

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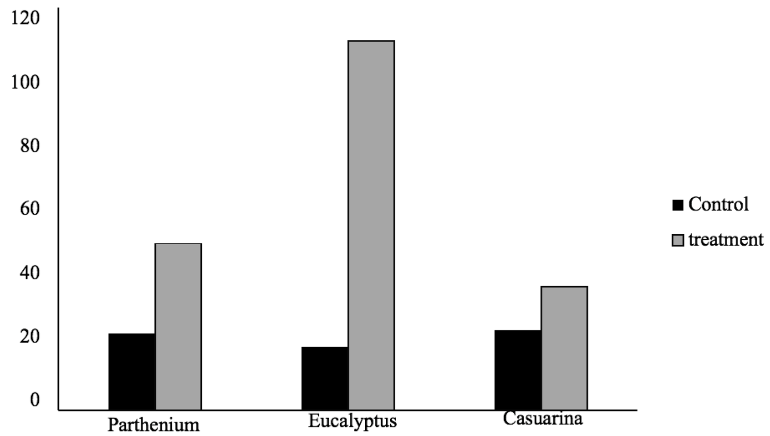

Figure 4. *Mean root Length of the common cress seed (cm)*

Parthenium

The allelopathic compounds found in parthenium are primarily the phenolics derivatives (including but not limited to ferulic, chlorogenic, anisic acid, vanillic, caffeic) and sesquiterpene lactose (parthenin, damsin, coronopolin) that secreted from stem, leaves, seeds, and pollen (Evans, 1997; Khaket et al., 2012; Singh et al., 2003). Even though there are many chemicals that acts as allelopathy, the most important allelopathy in parthenium is the parthenin (Belz et al., 2007).

The result of the experiments shows that the germination rate is not proportionally related to the root and shoot length in the treatment of allelopathic leaves of all species. Under the allelopathic treatment, the germination rate is significantly suppressed, however, the shoot and root length of the seed is greater. Allelopathic compounds can affect the germination and seedling growth separately, and the germinated seedling can grow better in the allelopathic agar compared than the plain agar for several reasons.

First, the allelopathy compounds in this experiment did not negatively affect the growth of the common cress seedling because the dosage is not the effective dose to cause growth failure. The study conducted by Belz et al (2007) found that species have different sensitivity to leaf extract of the parthenium. Experiment conducted on two different plants show that the effective dose of parthenin will cause phytotoxicity on germination of billygoat weed (*Ageratum conyzoides* L.) at the effective dose of 24.8 mg/mlb while it needs 95.5 mg/mlb to cause phytotoxicity on lettuce (*Lactuca sativa* L.) (Belz et al., 2007). (Abdul Raoof & Siddiqui, 2013) conducted a research on the effect of parthenin on the cytomorphology of broad bean (*Vicia faba* L.) and found that parthenin can cause abnormality in the mitotic cell division that cause chromosomal distortion, and hence, inhibitory of growth. When the leaf litter is incorporated into the growth media, parthenin is released and affects cellular activity of the root tip of the plants (Abdul Raoof & Siddiqui, 2013). Unfortunately, there is no sufficient literature on the effective dosage of parthenin that will cause phytotoxicity on common cress so it is hard to know how the allelopathy influences on this species.

Second, at low dose, parthenin treatments could stimulate root growth (Belz, 2008; Belz et al., 2007). Parthenin has the biphasic dose/response relationship where it has stimulatory effect in low dose and inhibition in higher dose (Belz, 2008). Besides, parthenium weed is rich in N, P, K, Ca, Mg, Zn, Cu, and chlorophyll content that have the potential role as compost (Khaket et al., 2012; Kishor et al., 2010; Patel, 2011). Parthenium weed is suitable for composting, although the high level of parthenin and

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phenolic may inhibit early growth and development of other plants (Patel, 2011).

Third, the leaf used in this experiment could not contain the most optimum allelopathic compound possible. The effectiveness of the parthenin fluctuates throughout its life cycle. Parthenin is produced during the entire life cycle, but reaches maximum value during the generative stage (Belz, 2008).

Eucalyptus and Casuarina

The most dominant allelochemical found in eucalyptus is the monoterpenoids class and in casuarina species is the phenolics, both affect the understory vegetation (Batish et al., 2001; Kaur et al., 2011). In this experiment, the leaf litter from eucalyptus and casuarina decrease the germination of the common cress seedling respectively by 76% and 16% compared to the control treatment. It is in line with the conclusion from the study by Kaur et al. (2011) of which allelopathic effect of the leaf litter from the eucalyptus species has antagonist effect on the energy metabolism and photosynthetic process, hence reducing the successfulness of germination. The literature specifically on Casuarina crebra is insufficient, however, the allelopathic compound of the plant of the same genus (*Casuarina equisetifolia* L.) is proven to inhibit the germination, seedling growth and dry weight of the tested plants (*Medicago sativa* and *Ageratum conyzoides*) with the most effective allelopathic compound extracted from leave fresh needle leave, followed by fresh and decaying litter respectively (Batish et al., 2001).

In this research, the germination of common cress seed is most inhibited when sown in the eucalyptus agar, and the surviving seed grows into strong seedling (shoot length 11 mm, root length: 110 mm). The data on root length and shoot length of eucalyptus and casuarina shows that the seedlings have better performance in the allelopathic agar compared to the plain agar with significant difference, except for the root length of casuarina treatment. The possible reason for this, as found in the parthenium, is the allelopathy of eucalyptus on the low dose also able to increase the seedling growth. The study conducted by El-Darier (2002) found that 1% concentration of eucalyptus leaf-litter water has a 232% better effect on the shoot length of broad bean (*Vicia faba* L.) than the 5% concentration. In Maize (*Zea Mays* L.) the difference of the effect between these two concentrations is 132%. The polyphenols released from eucalyptus such as humic, fulvic, and cinnamic can promote the metabolic process and seed growth if occurred in the threshold concentration (El-Darier, 2002). Furthermore, the concentration of the allelopathic compounds in the eucalyptus and casuarina litter are not exactly in the harmful level for the surviving seedling, and it is possible that the germinated seed is vigorous enough to grow in that concentration. The eucalyptus and casuarina litter can also provide the organic matter for the growth of the seedling and the growing environment free from fungi and microbe infection, because the essential oil of the eucalyptus has the insecticidal, antimicrobial, and fungicidal characters (Kaur et al., 2011).

CONCLUSION

This experiment accepts the first hypothesis, but rejects the second one. In the conclusion, the allelopathy of parthenium, eucalyptus, and casuarina inhibits the germination of the common cress but the seedling growth shows better performance in allelopathic- leaf agar compared to the plain agar. There are abundant factors that affect the allelochemical activity in the plants and the effect is not always inhibitory, but also stimulatory. It is crucial to maintain the clean condition of the experiment as well as the feasibility of the seed, either to avoid the contamination and to achieve reliable number of samples for statistical analysis. Furthermore, it is important to explore the other options of the experimental designs to examine the allelopathic effect, such as the

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plant box method or other laboratory experiments, the field trials to get the more nature-like condition, as well as the experiments to examine the allelochemicals in the cellular level.

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