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Evaluation Of The Allelopathic Effect Of Cassia Absus L. Seeds On The Germination And Growth Of Two Target Weeds And Two Cultivated Pea Varieties

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Abstract
The persistence of synthetic molecules can severely compromise the soil fertility of agricultural fields. The discovery of a natural herbicide could reduce these harmful environmental impacts. To identify natural plant-based products with herbicidal properties, the allelopathic potential of Cassia abssus L. was studied using an aqueous extract of its seeds at different concentrations (3, 4.5, and 6 g/L) during the germination of target cultivated and weed plants belonging to different genera and varieties of legumes. We tested these extracts on two weed species and two varieties of garden pea (Merveille de Kelvedon and Douce Provence) at a temperature of 25°C ±1. The inhibitory effect of these extracts has little impact on the germination of all the target plants studied. However, it is much more pronounced on elongation, which is more marked in the radicle than in the aerial parts, and on fresh biomass production. Our results suggest that Cassia absus seeds could become an invasive species if introduced into Algeria.
Keywords: *Cassia absus, Aqueous extract, allelopathic potential.*

INTRODUCTION

Weeds have always been a significant obstacle to agricultural activities. Competition for water, minerals, and light directly affects the growth and yield of cultivated plants. Production losses of the world's main crops due to pests (insects, microorganisms) and weeds are estimated at 35%. In Africa, and in Senegal in particular, weed pressure can reach up to 56%. This figure can rise to 70% without adequate monitoring (Doré et al., 2004; Popa et al., 2008).

Faced with this alarming situation in the country's agricultural systems, recent decades have seen national and international organizations promote the use of herbicides to modernize and intensify agricultural production. Dependence on herbicides is primarily linked to their diversity, their effectiveness against almost all weed species, and their ease of use (Valantin-Morison et al., 2008).

Biological control offers an alternative approach to pests, diseases, and weeds in agriculture (Mason and Spanner, 2006). However, the application of biological weed control has often proven difficult in practice (Müller-Schärer et al., 2020).

Allelopathy is considered a promising technique for biological control. It is a set of direct or indirect, positive or negative biochemical interactions between plants (Macías et al., 2007). However, its weed control is controversial. Several studies have shown that a crop's ability to suppress weeds varies considerably from one variety to another. This difference is partly explained by the ability of these crops to secrete chemicals that affect weed growth, namely allelopathy (Sánchez-Moreiras et al., 2004).

Some plants have been used empirically for millennia for their therapeutic properties. Among these, Cassia abssus, whose active molecules are currently the subject of research, has, to our knowledge, no study on the allelopathic properties of this plant. However, one could expect significant allelopathic properties in this plant, these two properties deriving from the activity of secondary metabolites. Furthermore, the introduction of a

plant requires prior knowledge of its allelopathic properties, indeed, to prevent it from becoming a weed or an invasive species. This work is situated within this perspective.

MATERIALS AND METHODS

To study the allelopathic properties of Cassia absus we carried out bioassays which consisted of testing the effects of aqueous extracts of Cassia absus on the germination and growth (length, biomass production) of plants belonging to different genera, species and varieties.

Plant Material

We tested the allelopathic properties of the aqueous extract of Cassia absus seeds on different genera, species, and varieties. These include: Pisum sativum variety Merveille de Kelvedon, Pisum sativum variety Douce Provence, Hedysarum coronarium and Hedysarum carnosum.

Taxonomic Classification

Table 1 presents the classification of the plants used in the study of the allelopathic power of the aqueous extract of Cassia absus seeds.

Table 1: Taxonomic classification of the studied plant.

	Allelopathic plant	**Target Plants**			
Vernacular name	**Pig's senna**	**Garden pea**	**Green pea**	**Sulla**	**Sulla**
Kingdom	Plantae	Plantae	Plantae	Plantae	Plantae
Phylum	Tracheophyta	Tracheophyta	Tracheophyta	Tracheophyta	Tracheophyta
Class	Magnoliopsida	Magnoliopsida	Magnoliopsida	Magnoliopsida	Magnoliopsida
Order	Fabales	Fabales	Fabales	Fabales	Fabales
Family	Caesalpiniaceae	Fabaceae	Fabaceae	Fabaceae	Fabaceae
Genus	Cassia	Pisum	Pisum	Hedysarum	Hedysarum
Species	Cassia absus	Pisum sativum	Pisum sativum	Hedysarum coronarium	Hedysarum carnosum
Variety	-	Merveille de Kelvedon	Douce Provence	-	-
Synonym	Chamaecrista absus	Lathyrus oleraceus	Lathyrus oleraceus	Sulla coronaria	Sulla carnosa

Plant Description

Cassia absus

An annual herbaceous plant reaching 60 (–100) cm in height, branching upwards, covered with long, stiff glandular hairs all over, with a lemon scent. Leaves alternate, paripinnate, 3–7 cm long, with 2 pairs of leaflets; stipules linear, up to 8 mm long; petiole without a large gland, but rachis with a gland between each pair of leaflets; leaflets nearly sessile, elliptic, up to 4.5 cm x 3 cm, wider in the upper pair, apex obtuse. Inflorescence: a terminal or axillary raceme, up to 13 cm long, with 4–6 flowers. Cassia absus is morphologically variable, which is hardly surprising given the extent of its distribution.

Pisum sativum

Glabrous, glaucous, creeping or climbing plant, leaves with 1-3 pairs of large, oval or oblong leaflets with amplexicaul stipules, toothed at the base, with a branched tendril, flowers large, 5-3 mm, in groups of 1-3 on an articulated peduncle, calyx with lanceolate acuminate teeth, standard with 2 bumps at its base, wings adherent to the keel, frog style folded lengthwise, laterally compressed at the apex, linear oblong pod, obliquely truncate at the apex.

Pisum sativum variety Merveille de Kelvedon

This variety is an early, hardy, dwarf shelling pea with wrinkled seeds needing support.

Pisum sativum variety Douce Provence

Douce Provence variety is also a dwarf, early-maturing shelling pea variety, but with smooth, round seeds and a very compact texture. It is often appreciated for its mild, high-quality flavor.

Hedysarum coronarium

A robust plant with appressed pubescence, with spreading, ascending stems 10-80 cm tall. Leaves with 7-11 oval, more or less rounded leaflets, some reaching 3 cm by 2 cm, hairy on the underside and margins. Ornamental flowers of a bright purplish hue, in dense, oblong racemes. Calyx with segments longer than the tube. Corolla 10-15 mm long with a standard extending beyond the keel. Pod small with 2-1 segments 1-3 mm long. Glabrous and spiny.

Hedysarum carnosum

A hairless, somewhat plow-like plant with an erect main stem and spreading lateral stems 10-30 cm long. Leaves with 5-7 oblong leaflets, emarginate and mucronulate at the apex. Pink flowers 10-12 mm in diameter in a loose raceme. Calyx with segments shorter than the tube. Pods pendulous, almost straight, with 2-5 more or less spiny segments.

Preparation of Aqueous Seed Extract of Cassia absus

Three solutions of aqueous extract of Cassia absus of increasing concentrations (0.3; 0.45; 0.6g/100ml) are prepared according to the following procedure: the sieved powder of Cassia absus seeds is decocted in distilled water at 100°C for 2h, then the extract is filtered through filter paper and it is kept at a temperature of 4°C.

Allelopathic Potential of the Aqueous Seed Extract of Cassia absus

The allelopathic properties of the aqueous extract of Cassia absus seeds on the germination and growth parameters (length and biomass production) of the target plants were tested.

Germination Parameter

Dormantness Breaking

The seeds of wild plants exhibit seed coat dormancy, which we broke by scarification. Water pretreatment, or hydropriming, is commonly used to accelerate and homogenize germination. We pretreated the scarified seeds of wild plants by immersing them in water for 24 hours. These seeds were then dried at room temperature until ready for germination.

Germination

The cultivated seeds were washed thoroughly under running water and then rinsed with distilled water. Spontaneous or cultivated seeds were then placed in groups of 15 in a Petri dish lined with a double layer of filter paper moistened with distilled water (control) or with solutions of the aqueous extract of Cassia absus at different concentrations (0.3, 0.45, and 0.6g/100ml). The soaking volumes were identical for both treatments. To maintain optimal hydration, the seeds were re-soaked daily. Germination took place in the dark at 25°C for 7 days.

Germination Rate

Seed germination was monitored for 7 days, during which the cumulative germination rate and the cumulative percentage of germination (% compared to the control) were calculated. Germinated seeds were counted daily, and the maximum number of germinated seeds in the control (water) was considered to be 100%. The germination rate in the presence of the extract is estimated relative to 100% of the control.

Growth Parameter

Length Growth

The growth of the radicle and aerial parts is estimated by measuring their length. Daily observations provide an indication of any potential germination delay induced by the aqueous extract.

Biomass Production

After 7 days of germination, and depending on different concentrations of the aqueous extract of Cassia absus, the fresh and dry weights of the aerial and root parts of the target species are measured. Fresh samples are weighed in aluminum foil bags using a precision balance to determine the fresh mass. These same samples

are then placed in an oven for 24 hours at 80°C and weighed again to determine the dry mass. Quantitative results are expressed as mean + SEM.

RESULTS

Effect of the aqueous extract of Cassia absus seeds on the germination of target plants

Germination of Pisum sativum variety Merveille de Kelvedon

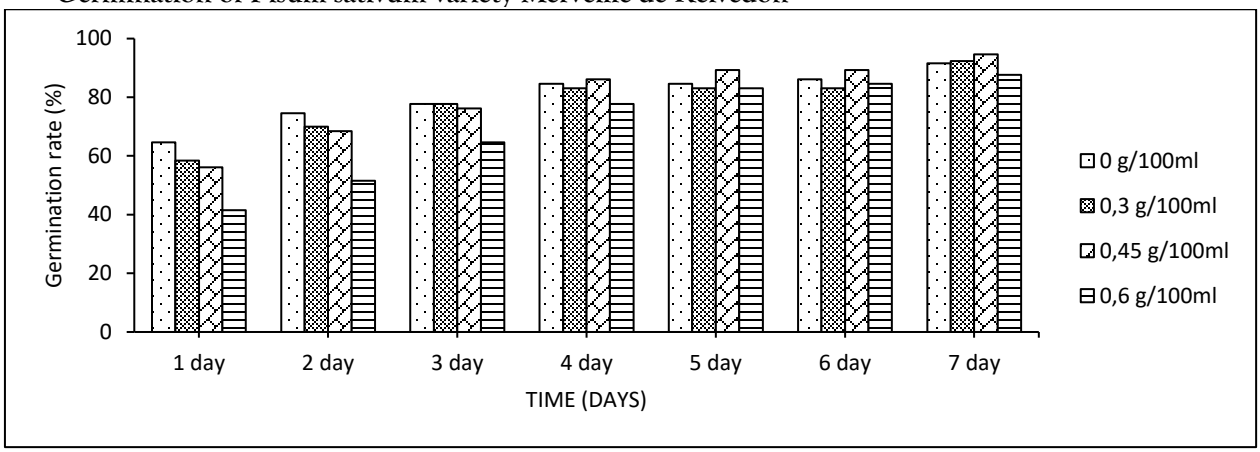

Figure 01: Cumulative germination rate of Pisum sativum variety "Merveille de Kelvedon" as a function of time and different concentrations of aqueous extract of Cassia abssus seeds.

The seeds of Pisum sativum variety Merveille de Kelvedon used exhibit good germination rates (90% of the seeds germinated after 7 days). The aqueous extract of Cassia absus delayed seed germination, particularly at a concentration of (0.6g/100ml), but without affecting the final cumulative germination rate, which was equivalent to that of the control.

Germination of Pisum sativum variety Douce Provence

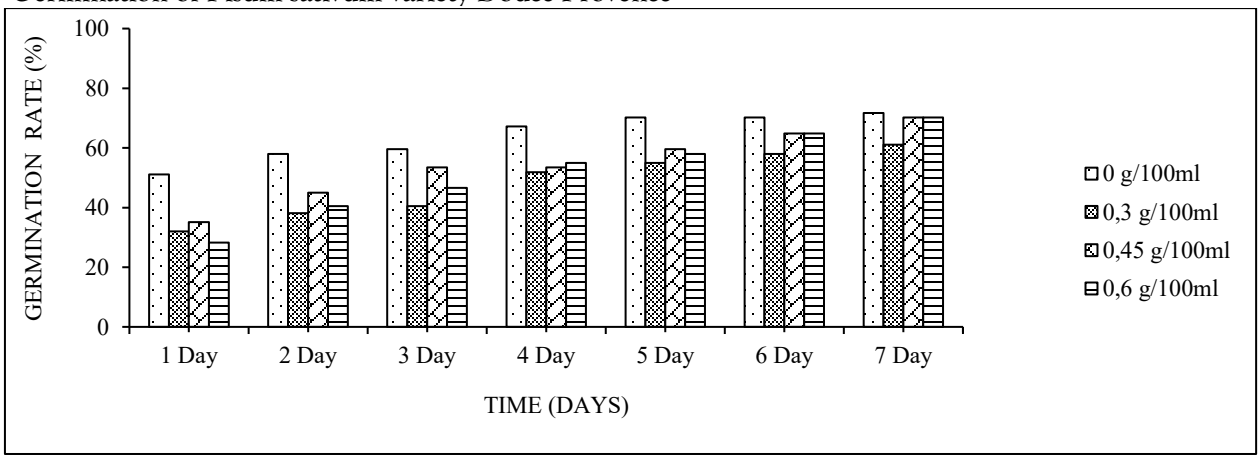

Figure 02: Cumulative germination rate of Pisum sativum variety Douce Provence as a function of time and different concentrations of aqueous extract of Cassia abssus seeds.

The seeds of the Douce Provence variety of peas used had a lower germination rate than those of Merveille de Kelvedon variety; approximately 75% of the seeds germinated after 7 days. The aqueous extract delayed seed germination at all concentrations used. By the seventh day, the cumulative germination rates across the different concentrations were almost equivalent to those of the control.

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Germination of Hedysarum coronarium

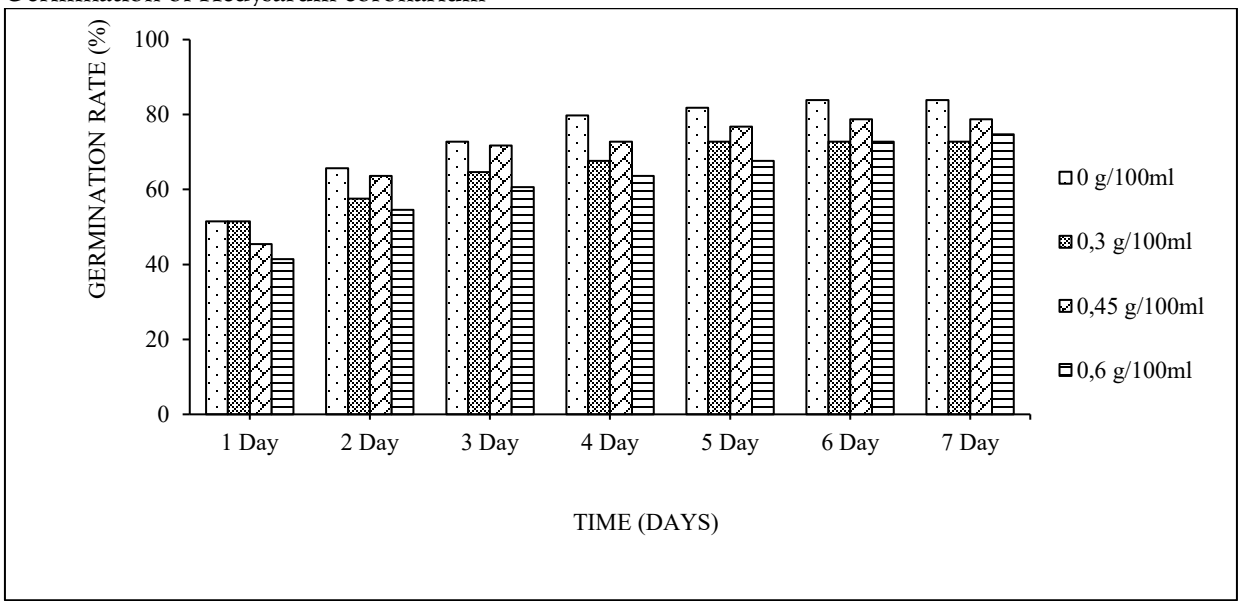

Figure 03: Cumulative germination rate of Hedysarum coronarium as a function of time and different concentrations of aqueous extract of Cassia absus seeds.

The cumulative germination rate of Hedysarum coronarium is only slightly influenced by the aqueous extract of Cassia absus. Germination is only slightly delayed compared to the control.

Germination of Hedysarum carnosum

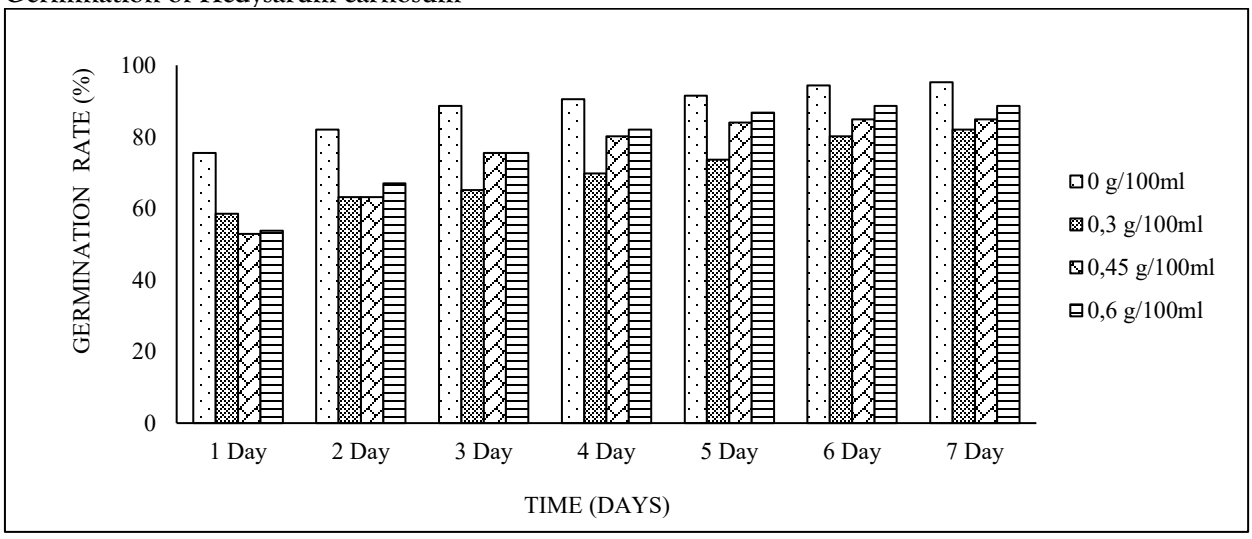

Figure 04: Cumulative germination rate of Hedysarum carnosum as a function of time and different concentrations of aqueous extract of Cassia absus seeds.

The germination rate of the control was approximately 90%, indicating a higher germination rate than that of Hedysarum coronarium. The aqueous extract of Cassia absus appeared to have little effect on the germination of this species, unlike the effect observed for Hedysarum coronarium. The aqueous extract of Cassia absus had almost no effect on the germination rate of the plant seeds used; the inhibition of germination by the extract was weak compared to the control. Nevertheless, there was a moderate delay in germination in almost all tests.

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Effect of the aqueous extract of Cassia absus seeds on the growth of target plants Pisum sativum varieties Merveille de Kelvedon and Douce Provence

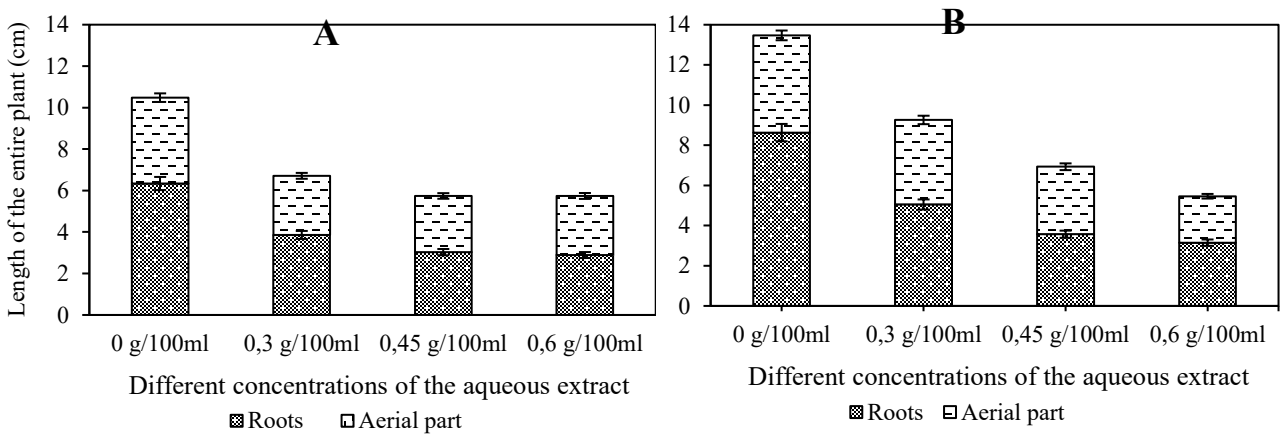

Figure 05: Effect of different concentrations of aqueous extract of Cassia absus seeds on the longitudinal growth of Pisum sativum varieties Merveille de Kelvedon (A) and Douce Provence (B)**.**

The aqueous extract of Cassia absus exerts an inhibitory effect on the longitudinal growth of pea seedlings (Fig. 05A). This effect is more pronounced with higher concentrations of the aqueous extract and results from the inhibition of the radicle and aerial parts, which represent approximately 57% and 40% of the control, respectively.

The effects induced by different concentrations of the extract in the whole plant follow a similar pattern to that observed in the pea variety Merveille de Kelvedon, but the inhibition is more pronounced for Douce Provence (54% of the control) than for Merveille de Kelvedon (40% of the control). Growth inhibition affects both the aerial parts and the radicle, which is the most affected, 62% of the control is observed at the concentration 0.6g/ml.

Hedysarum coronarium and Hedysarum carnosum

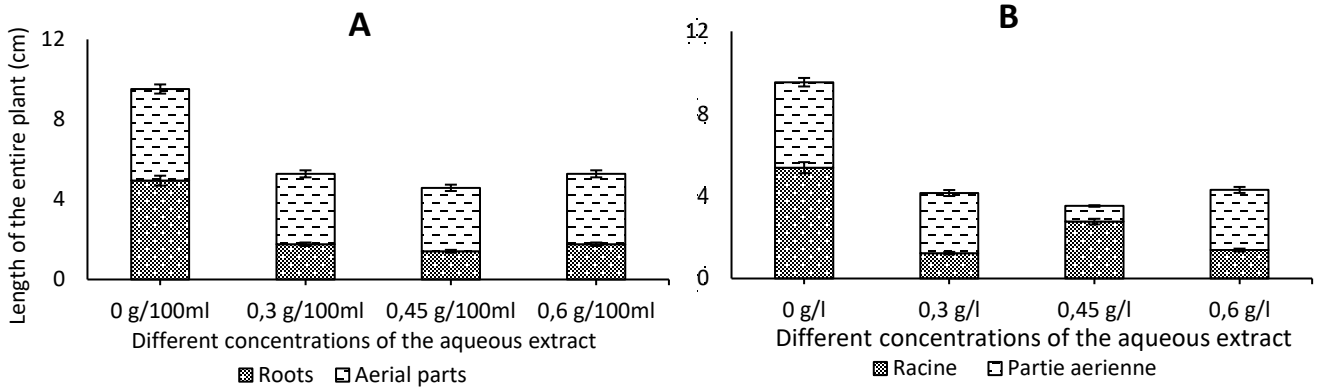

Figure 06: Effect of different concentrations of aqueous extract of Cassia absus seeds on the longitudinal growth of Hedysarum coronarium (A) and Hedysarum carnosum (B) seedlings.

The aqueous extract of Cassia abssus exhibits a depressive effect on the growth of Hedysarum coronarium seedlings, with growth inhibition of approximately 50% at 0.6 g/ml. The aerial parts of Hedysarum coronarium were less inhibited by the aqueous extracts of Cassia absus than the radicle. The observed reduction was approximately 63% and 20% of the control at the 0.6 g/ml concentration of Cassia absus

extract. In Hedysarum carnosum, the effects induced by increasing concentrations on plant elongation follow a similar pattern and are of the same order of magnitude as those observed in Hedysarum coronarium; indeed, at 0.6 g/ml, growth inhibition is approximately 43% of the control.

In the case of the radicle, a significant inhibition of elongation is observed. However, for the aerial parts, a slightly greater inhibition was observed than that reported for Hedysarum coronarium.
Indeed, after 7 days of germination, the inhibition of radicle growth was 70% of the control for the 0.6 g/ml concentration, and 30% of the control for the aerial parts at the same concentration.
The trials conducted confirmed the presence of inhibitory effects on seedling elongation, particularly more pronounced in the radicle than in the aerial parts.

Effect of Cassia absus seeds extract on the fresh biomass of target plants Pisum sativum varieties Merveille de Kelvedon and Douce Provence

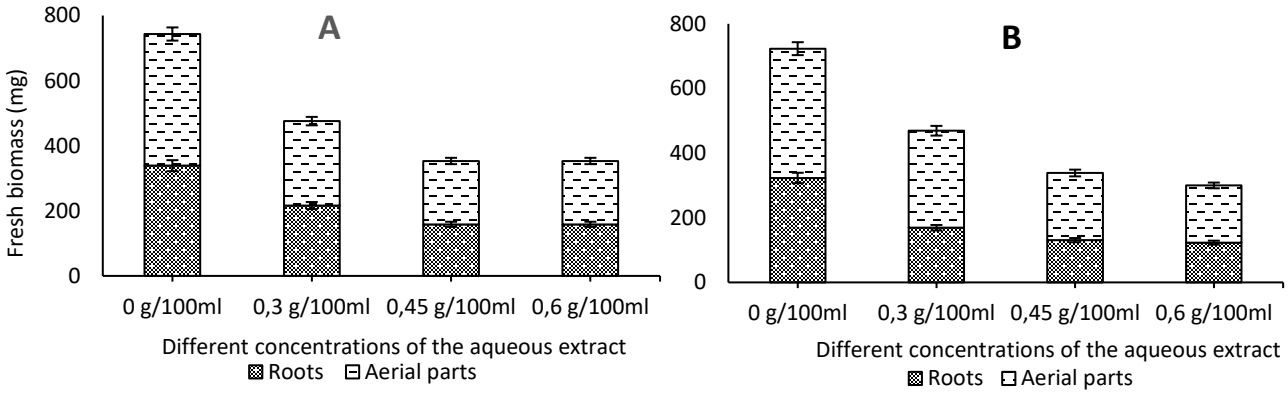

Figure 07: Effect of different concentrations of aqueous e[illegible] fresh biomass (mg) of Pisum sativum varieties Merveille de Kelvedon (A) and Douce Provence (B).
The aqueous extract of Cassia absus seeds induces a remarkable reduction in the fresh biomass of whole plants (Fig. 07 A, B). At a concentration of 0.6 g/ml, a nearly identical reduction of approximately 60% is observed in both the radicle and aerial parts.

Hedysarum coronarium and Hedysarum carnosum

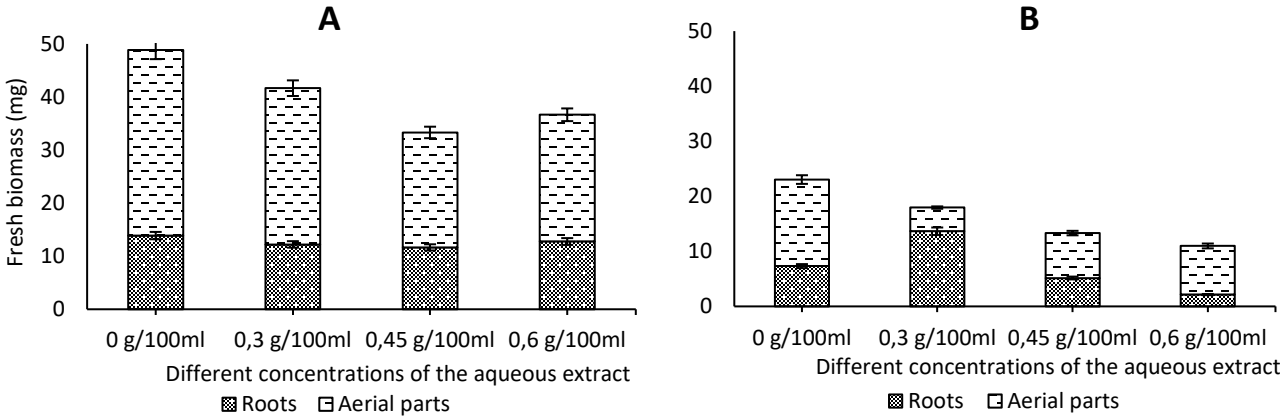

Figure 08 : Effet de différentes concentrations de l´extrait aqueux des graines de Cassia absus sur la production de biomasse fraiche en (mg) de Hedysarum coronarium (A) Hedysarum carnosum (B).

The aqueous extract of Cassia absus seeds induces a remarkable reduction in the fresh biomass of entire plants of Hedysarum coronarium and Hedysarum carnosum (Fig. 08 A, B). For the concentration of 0.6 g/ml, a nearly similar reduction is observed in both the radicle and the aerial parts, which is about 65%.

Effect of Cassia absus seed extract on the dry biomass of target plants

Pisum sativum varieties Merveille de Kelvedon and Douce Provence

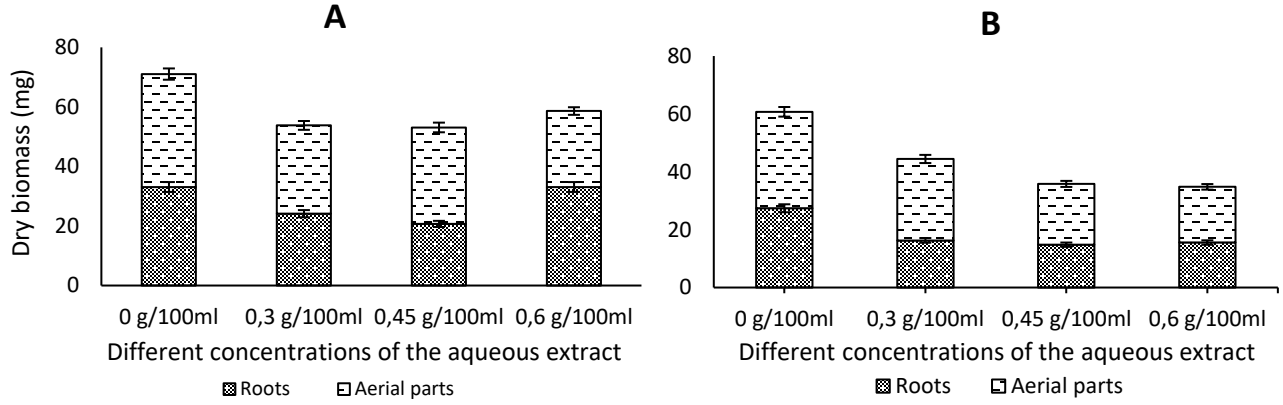

Figure 9: Effect of different concentrations of aqueous extract of Cassia absus seeds on the dry biomass production (mg) of Pisum sativum varieties Merveille de Kelvedon (A) and Douce Provence (B).

The aqueous extract of Cassia absus seeds induces a remarkable reduction in the dry biomass of the entire plants of Pisum sativum varieties Merveille de Kelvedon (A) and Douce Provence (Fig. 09 A, B). It is noted that at the concentration of 0.6 g/ml, there is an almost similar reduction for the radicle and the aerial parts, which is about 40%.

Hedysarum coronarium and Hedysarum carnosum

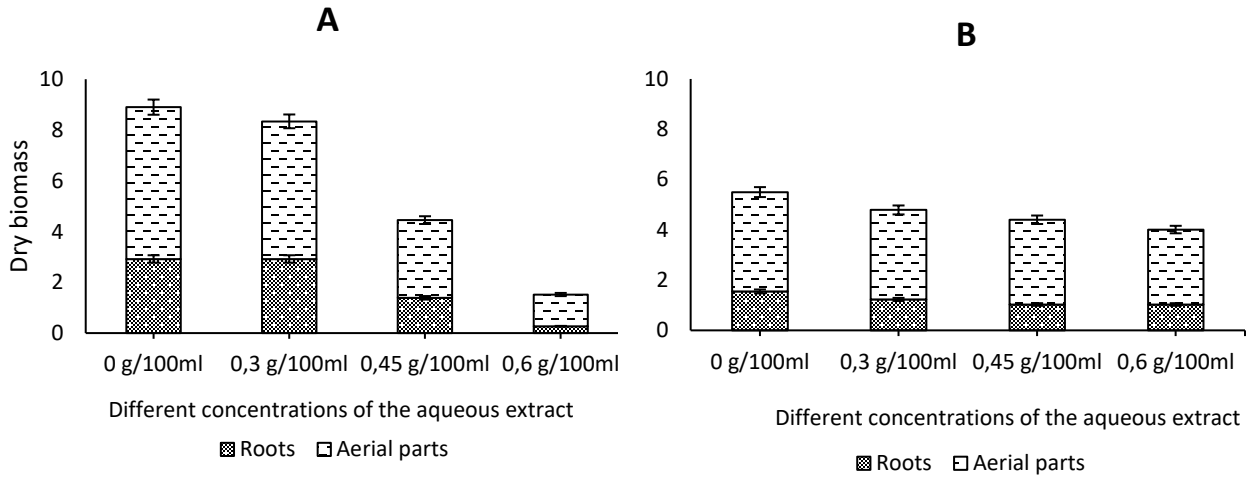

Figure 10: Effect of different concentrations of the aqueous extract of Cassia absus seeds on the production of dry biomass in (mg) of Hedysarum coronarium (A) Hedysarum carnosum (B).

DISCUSSION

This work focused on evaluating the allelopathic properties of Cassia absus seeds. We specifically used the aqueous extract, which is a better representation of natural conditions (Bouton, 2005). Using different concentration gradients of the aqueous extract (0, 0.3, 0.45, and 0.6 g/100 ml), we tested it on target plants belonging to different cultivated and wild genera and varieties.

Effect of the aqueous extract of Cassia absus L.

Germination

Our results showed that the aqueous extract of Cassia absus had little effect on the germination of the seeds of the target plants used, manifesting as a slight decrease in the germination rate with an increase in the latency period. These effects cannot be attributed to the osmotic effects of the aqueous extract of Cassia absus, as the osmotic potential of the extract remains constant across the different concentrations used.

The allelopathic compounds, which are released in small quantities, may mimic the action of plant hormones, particularly those also derived from the shikimate pathway, such as gibberellins, known to promote germination. This explains the possible stimulatory effects observed (Bouton, 2005).

Length Growth

The results recorded showed that the aqueous extract of Cassia absus sheaths exerts an inhibitory effect on seedling length growth, which is more pronounced on the radicle than on the aerial parts. Thus, the aqueous extract of Cassia absus sheaths contains allelopathic substances that appear to inhibit cell multiplication at the meristem level or in the cell elongation zone, primarily at the radicle. The extent of this inhibition varies with the target species used. Sorgoleone inhibits root elongation in Lactuca sativa but not in Zea mays L. (Gonzalez et al., 1997).

Biomass Production

Our results showed that the aqueous extract exerted a primarily inhibitory effect on biomass production, mainly affecting the radicle. The biomass reduction observed under the effect of the aqueous extract varies with the genera and varieties used.

Among the allelopathic substances that act on cell elongation, we can mention phenolic compounds; coumarin inhibits mitosis in onion roots. A single compound can have multiple sites of action: ferrulic acid, for example, acts on mitochondrial respiration as well as on chlorophyll synthesis and growth hormone activity; it also inhibits potassium uptake by plants. However, this is not the case in our experiment because we used an aqueous extract. The difference in results observed between the two extractions (aqueous and ethanolic) confirms the significant allelopathic activity of the phenolic compounds, primarily cinnamic acids (Bouton, 2005). The allelopathic activity of Cassia absus could be due to the alkaloids chaksine and isochaksine. These aqueous extracts of the aerial parts of Cassia absus have allelopathic effects on the formation of root nodules in peanuts and mung beans; this would be consistent with the antimicrobial activity (Van der Maesen, 2008).

Evaluation of Allelopathic Potency

The aqueous extract of Cassia absus exerted a marked inhibitory effect on the germination and growth of lettuce, which is commonly used for its allelopathic sensitivity (Macias et al., 2007).

Furthermore, the aqueous extract of Cassia absus seeds exerted an even stronger inhibitory effect due to its potent allelopathic effect. Thus, Cassia absus could become an invasive species if introduced into Algeria. It is therefore necessary to study its allelopathic potency under real-world field conditions to determine whether or not to introduce it into agricultural systems and to leverage its allelopathic properties by applying it to weeds. Indeed, the production of allelopathic compounds varies according to morpho-physiological characteristics, climatic factors, and edaphic factors (Evano and Chabanne, 2001); thus, allelopathy is overestimated in the laboratory (Wardle et al.,1998).

CONCLUSION

The seeds of Cassia abssus L., and in particular its aqueous extract, possess allelopathic properties manifested by inhibitory effects that primarily affect elongation and biomass production. This inhibition, which is always more pronounced at the radicle level, varies in intensity depending on the genera and target varieties used. According to our results, the aqueous extract of Cassia abssus appears to have a high allelopathic potency. However, it cannot be used as a biological herbicide because it has no significant effect on germination. Furthermore, although its growth-inhibiting effect is exerted, it cannot be used as a post-emergence herbicide because it lacks selectivity.

Cassia abssus seeds are widely marketed worldwide for their purported therapeutic properties. However, this plant is often confused with Nigella sativa, whose therapeutic properties and chemical composition have been and continue to be extensively studied. A thorough study of the chemical composition of this plant is urgently needed to avoid the risks associated with its confusion with Nigella sativa.

Moreover, it would be worthwhile to continue the study of the allelopathic properties of Cassia abssus using extracts obtained with organic solvents capable of extracting compounds known to possess significant allelopathic properties, such as phenols. The aqueous extract we used exhibited high allelopathic potency, likely related to the activity of water-soluble alkaloids (chaksine and isochaksine).

Given the economic importance of Cassia abssus, only a precise understanding of its allelopathic properties would allow us to decide on its introduction in Algeria. Indeed, it must not pose risks to agriculture and biodiversity by becoming a weed or an invasive species.

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