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Phytochemical Screening, Allelopathic and Bioherbicidal Potentialities of Euphorbia Guyoniana Boiss. and Reut. Leaf Extract

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Abstract. Euphorbia guyoniana Boiss. & Reut. (Euphorbiaceae) is well known for the chemical diversity of its phytoconstituents, and toxicological properties that have been found in literature. However, research articles about their allelopathic characteristics are rarely reported. The current research study was conducted to explore phytochemical screening, allelopathic effects and their potential uses as natural herbicide of Euphorbia guyoniana Leaf extract, harvested from Oued Metlili, Algerian Sahara, on germination and seedling growth of four adventitious species (Bromus rubens L., Phalaris minor Retz., Plantago lagopus L., Ammi visnaga L.) and one cultivated species (Triticum durum L.) in laboratory conditions. The analyses of crude plant extract confirmed the flavonoids, anthocyanins, phenols and tannins, steroids, saponins, free quinone, sterols and triterpenes as well as carohydrates in the extract. Our results show that the leaf extract of Euphorbia guyoniana had significant allelopathic and bioherbicidal activity on germination and seedling growth of adventitious species and stimulatory activity on cultivated species at lower concentrations.

Keywords. Allelopathy, Bioherbicide, Euphorbia guyoniana, Inhibition, Adventitious species, Algerian Sahara.

1. Introduction

Allelopathy term definesas the positive or negative, direct or indirect effects of one plant species on anotherplant may inhibit germination, emergence and seedling growth, by production of allelochemicals from leaves, flowers, seeds, stems and roots of living or decomposing plant materials (Rice, 1984; Weston, 1996; Babula et *al*., 2009; Monem et *al*., 2012).These allelochemicals may also serve as agrochemical agents and have the potential to be used as bioherbicides (Duke, 2003).

Allelopathy is therefore considered as a promising technique for biological control to minimize the reliance on synthetic herbicides for controlling weeds (Bhadoria, 2011; About et *al*., 2019).The use of a natural herbicide can reduce adverse impacts on the environment because the synthetic herbicides are often toxic and cause environmental pollution as well as develop herbicide-resistant weed biotypes (Batish et *al*., 2007; Weih et *al*., 2008; Heap, 2013).Several published results confirm that Some medicinal plants have inhibitory effects on some adventitious species and their allelochemicals inhibit weed growth (Lin et *al*., 2003, 2004).

Euphorbia guyoniana Boiss. & Reut. (locally named *Lebina*) is an indigenous Saharan species growing in sandy and desert habitats, belongs to the large family of Euphorbiaceae. The Euphorbia genus is the most representative of the family (Quezel and Santa, 1963; Ozenda, 1991).This genus'splants are known for their rich content on secondary metabolites. This species is used in Algerian traditional medicine against the venomous bites of scorpions and is known as a wart remover (Bellakhdar, 1997). Moreover, several studies have shown that *Euphorbia guyoniana* has several biological activities including antioxidant and antimicrobial activities (Bouaziz et *al*., 2009; Benmeddour, 2016), toxicity effect (Kemassi et *al*., 2013), allelopathic effect (Salhi et *al*., 2013), insecticidal effect (Kemassi et *al*., 2015; 2019).

The current research was undertaken to explore allelopathic effects and their potential uses as natural herbicide of *Euphorbia guyoniana* Boiss. & Reut. Leaf extract on germination and seedling growth of four weeds (*Bromus rubens* L., *Phalaris minor* Retz., *Plantago lagopus* L., *Ammi visnaga* L.) and one cultivated species (*Triticum durum* L.) in laboratory conditions.

2. Material and Methods

2.1. Collection of Sample

Fresh plant material of *Euphorbia guyoniana* Boiss. & Reut. (Leaves) was collected during the vegetative stage from Oued Metlili (Ghardaïa Region- Algerian Sahara) with the geographic coordinates of 32°.19'91.51 "N and 3°.77'17.23 "E.

2.2. Extraction and Bioassay Procedure

The collected plant materials of *Euphorbia guyoniana* Boiss. & Reut. were washed with tap water, then distilled water and were air-dried, then ground to a fine powder and stored in glass jars until use. The plant extract was obtained using reflux extraction. One hundred grams of plant was mixed with a hydro-methanolic solution (2/3 methanol +1/3 distilled water). The mixture was heated at a temperature of 60°C with a heating flask for six hours. A filtration was then carried out using filter paper. The collected filtrate undergoes a treatment under reduced pressure in a rotary evaporator in order to eliminate the methanol. The recovered extract was stored until its use for biological testing (Kemassi et *al*., 2019).

The seeds were washed with distilled water, then surface sterilized with 2% sodium hypochlorite for 3 minutes. 15 seeds of each of the weed (*Bromus rubens* L., *Phalaris minor* L., *Plantago lagopus* L., *Ammi visnaga* L. (LAM) and crop species (*Triticum durum* Desf.) were placed in sterilized Petri dishes of 9 cm diameter lined with two layers of sterilized filter paper and treated on the first day with 3 ml of plant extract, the control was irrigated with 3 ml of distilled water and 3ml herbicide for synthetic herbicide (GLYFONUT 36 SL) and were kept moist throughout the study period using distilled water. Different concentrations were prepared: 50%, 40%, 30%, 20%, 10%, 5%, 2.5% and 1% to evaluate the inhibitory effect of the leaf extract on the germination and seedling growth of adventitious species and a cultivated species. For each experiment considered, five repetitions are carried out. Germination monitoring was performed for 10 days under controlled conditions in laboratory, constantly recording the number of germinated seeds in each batch. The shoot (SL) and root length (RL) of corresponding species were measured (morpho-metric study) and the inhibitory potential of each extract was then examined (Cherif *et al*., 2016).

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2.3. Qualitative Phytochemical Analysis

The leaf extract was screened by using standard methods (Harbone, 1973) for the detection of the following constituents:

2.4. Alkaloids

Approximately three milliliters of extract were added to 3 ml of HCl (1%) and heated for20 min. The mixture was then cooled, and 1 ml of Mayer's reagent was added drop by drop. The formation of a greenish colored or cream precipitate indicates the presence of alkaloids (Lerato et *al*., 2017).

2.5. Flavonoids

3ml of plant extract was treated with 1 ml of NaOH (10%). The formation of an intense yellow color was an indication of the presence of flavonoids (Lerato et *al*., 2017).

2.6. Phenols and Tannins

About 2 ml of crude extract was added to solution of FeCl3 (5%). The presence of tannins and phenols was revealed by a black or blue-green color (Lerato et *al*., 2017).

2.7. Coumarins

2 ml of plant extract was added to 3 ml of NaOH (10%). The formation of a yellow color indicated the presence of coumarins (Lerato et *al*., 2017).

2.8. Anthocyanin

Approximately 2 ml of the prepared plant extract was added to 2 ml of HCl (2N) and ammonia. The appearance of a pink red coloration that turned blue violet indicated the presence of anthocyanin (Lerato et *al*., 2017).

2.9. Saponins

The detection of saponins was performed by adding About 3 ml of plant extract to 3 mL of distilled water and vigorous lyshaken. The formation of a stable persistent froth was taken as a positive test for saponins (Lerato et *al*., 2017).

2.10. Steroids

5 ml of chloroform and 5 ml of H2O4 were added to 500 µl of the prepared plant extract. The presence of steroids was indicated by a color change from violet to blue or green or a ring of blue/green or if the upper layer turned red and the sulphuric layer was yellow with a green fluorescence (Lerato et al., 2017).

2.11. Sterols and Triterpenes

The detection of sterols and triterpenes was evidenced by the Libermann method. We evaporate 1 ml of the plant extract in a Petrie dish, the dry residue is dissolved by acetic acid, and then we added 0.5 ml of sulphuric acid. The appearance of a red or purple ring at the interphase, turning blue then green, indicates a positive reaction (Trease et Evans., 1987).

2.12. Free Quinone

1 g of dry plant material was ground and mixed with 15–30 mL of petroleum ether. After shaking and resting for 24 hours, the extract was filtered and concentrated by steam rota. The presence of free quinine was confirmed by adding a few drops of NaOH (10%), when the aqueous phase turns yellow, red or purple (Dohou, 2003).

2.13. Carbohydrates

A mixture of Fehling solutions A and B with equal volumes was added to plant extract. A red colored precipitate indicated the presence of reducing sugars (Jaradat et *al*., 2015).

3. Quantitative Phytochemical Analysis

3.1. Estimation of Total Phenolic Content (tpc)

The concentration of TPC in the plant extractwas determined using spectrophotometric Method, according to the colorimetric method, based on using Folin-Ciocalteu reagent. To 200 µL of plant extract, 1 mL of Folin-Ciocalteu reagent (diluted 10 times with distilled water) and 800µL of Na_2CO_3 (7.5%) were added. The samples were incubated for 30 min at 50ºC and then cooled. Gallic acid served as control to construct calibration curve for calculation of phenolic content. Then absorbance of solution was noted at 765 nm (Cliffe et *al*., 1994).

3.2. Estimation of Total Flavonoid Content (tfc)

Total flavonoid content was measured according to the Aluminium chloride colorimetric method.25 µL of the plant extract was added to 300 µL of $NaNO_2$ and 300 µL of $AlCl_3$ (10%) in a test tube and left for five min. Then 100 µL of NaOH (2%) were added. Finally the absorbance of this mixture was noted at 510 nm. A calibration curve of standard quercetin solution was prepared to calculate TFC (Kim et *al.*, 2003).

4. Studied Parameters

4.1. Inhibition Rate (I.R.)

According to Côme (1970), this parameter explains the ability of a substance or preparation to inhibit seed germination. It is the ratio of the difference between the number of seeds sown and germinated to the total number of seeds sown (Ben khettou, 2010).

$$I.R. = \frac{(Number\ of\ seeds\ sown - Number\ of\ germinated\ seed)}{Number\ of\ seeds\ sown} . 100\% \qquad (1)$$

4.2. Herbicide Efficiency

The herbicidal efficacy of the tested extracts is examined according to the scale of the Commission of Biological Tests of the French Society of Phytiatrics and Phytopharmacy (CBT). The scale is as follows:

- 95 to 100%: Very good efficiency.
- 80 to 95%: Good efficiency.
- 60 to 80%: Average efficiency.
- 40 to 60%: Low efficiency.
- < 40% : Efficiency without practical application.

4.3. Germination Speed (Tm)

According to Côme (1970), it is the percentage of germinated seeds or the germination rate after a certain time after sowing. The average time required for germination (time required to germinate 50% of the seeds).

$$T_m = \frac{(N_1T_1 + N_2T_2 + \ldots\ldots + N_nT_n)}{N_1 + N_2 + \ldots\ldots + N_n} = \left({}^{1}/_{C_v}\right) . 100\% \qquad (2)$$

So:

N_1 : number of germinated seeds at time T_1.

N_2 : number of germinated seeds at time T_2.

N_n : number of germinated seeds at time T_n.

4.4. Efficiency Concentration (EC)

The 50% efficacy concentration (EC_{50}) is the amount of a material that can induce a 50% success rate in the treated population. This is the amount that causes half (50%) of the treated sample to die (Côme, 1970). Also an EC_{90} generates a success rate of 90%. EC_{50} and EC_{90} are estimated using the Probit method (Kemassi, 2014).

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4.5. Morpho Metric Measurements

Morpho-metric measurements were made in each Petri dish, and measurements of the root length (RL) and the shoot length (SL) of the germinated seeds were made using millimetric paper.

4.6. Statistical Analysis

The results obtained from the various experimental tests were statistically interpreted with the "XLSTAT version 2014" software**.** Analysis of variances (ANOVA) and the differences between the parameters were evaluated by Fisher LSD test. Difference were considered when P=0,05.

5. Results and Discussion

5.1. Qualitative Phytochemical Analysis

The results of the phytochemical screening tests of leaf extract of *Euphorbia guyoniana* Boiss. & Reut. are presented in Table 1. Analysis confirmed the presence of flavonoids, Anthocyanins, Phenols and tannins, steroids, saponins, free quinone, sterols and triterpenes, carohydrates in the extract. Alkaloids and coumarins were absent.

The presence of several active compounds in the plant's leaves was an interesting aspect of this study. These results are in agreement with those obtained by Amar et *al*. (2012) and Boudiar et *al*.(2010), Phytochemical tests revealed the presence of sterols, triterpenes, flavones, carotenoids, alkaloids and anthocyanins in the plant.

5.2. Quantitative Phytochemical Analysis

The total phenolic content (TPC) of the plant extract was measured in comparison to standard Gallic acid and expressed as mgGA/100g of dry plant sample. In contrast, the total flavonoid Content (TFC) was measured in comparison to the standard Quercetine and expressed as mgGA/100g of dry plant Sample. The TPC was 2423.80 mg gallic acid equivalent/100g of dry plant sample. The TFC was 34.88mg of Quercetin equivalent/100g of dry plant sample (table 2).

The plant of *Euphorbia guyoniana* has been subjected to many chemical studies. We reported the isolation from the aerial parts of two new polyester diterpenes, named guyonianin A and B, a rare class of bicyclic diterpenes (Ahmed et *al*., 2006).The isolated compounds **1** and **2** were identified as novel jatrophane diterpenes esterified with acetic, benzoic and isobutanoic acids (Rédei et *al*., 2015). One novel alkaloid, 1,5-diphenyl-3-styryl-2-pyrazoline 1, in addition with six known flavonoids namely, kaempferol, kaempferol 3-*O*-glucoside, kaempferol 3-rutinoside, quercetin, quercetin 3-*O*-glucoside, and rutin (Boudiar et *al*.,2010). Two novel jatrophane diterpenes named guyonianin G (**1**) and H (2), The isolated compounds were identified as14 oxojatropha-6(17),11-diene pentaester derivatives acylated with acetic, isobutanoic and benzoic acids (Kúsz et *al*.,2016).

5.3. Inhibition Rate (%)

The effects of leaf extract of *Euphorbia guyoniana* on the percent inhibition of germination on adventitious species and cultivated species were determined (Figure 1, 2, 3, 4, 5). In general, a high inhibitory effect on germination of all adventitious species (*Bromus rubens* L., *Phalaris minor* L., *Plantago lagopus* L., *Ammi visnaga* L. (LAM) was observed. low inhibitory effect was reported with cultivated species (*Triticum durum* Desf.).The results showed that the pure leaf extract and diluted to 50%, 40%, 30% 20% and 10% has very good effectiveness for the inhibiting adventitious seeds germination (≥95%). While the rest concentrations (5%, 2.5% and 1%), the rate of inhibition varied between 86.66% and 6.66%.The highest tolerance to the allelopathic effect of leaf extract was observed only with *Triticum durum*, its inhibition percentage is ≤ 37,77% (Efficiency without practical interest).

There are significant allelopathic and bioherbidal effects on germination of adventitious species by various concentrations of the extract compared to controls, which are in conformity with the presence of several compounds (flavonoids, Anthocyanins, Phenols and tannins, steroids, saponins, free quinone, sterole and triterpenes, Carohydrates) in the leaf extract of *Euphoria guyoniana* Boiss. & Reut. These results are in agreement with those obtained by (Salhi et *al*. 2013). Several research

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studies have indicated that the presence of allelochemicals could be the source of complete or partial inhibition of germination and reduction of seedling growth (Rice, 1974; Einhelling, 1995; Salhi, 2011; Cherif, 2020). These allelochemicals have the potential to be employed as bioherbicides (Inderjit, 2003). Different plant species' allelochemicals affect different Physiological processes impede nutrient and ion absorption by changing plasma membrane permeability and have an effect on enzymes responsible for phytohormone production (Rice, 1979).

5.4. Germination Speed (Tm)

In the present experiment, it was observed that the germination speed of adventitious species and cultivated species seeds treated with leaf extract of *Euphoria guyoniana* was slower than that reported for the control lot. We noticed the absence of germination in the lots treated with the leaf extract from the concentration of 10% (*Phalaris minor*), 20% (*Bromus rubens*, *Ammi visnaga* and 30% (*Plantago lagopus*). For other concentrations, the germination speed varies between 6.4 and 9.1 seeds per day.
Phenolic compounds may play a role in controlling the activity of plant hormones. The synthesis or functioning of several growth-related enzymes is also sometimes disrupted (Sato et *al*., 1982). Suppression of the degradation of indole acetic acid (IAA) by various phenols (Lee et *al*., 1982).

5.5. Efficiency Concentration (CE50, CE90)

Table (3) shows the EC_{50} and EC_{90} values calculated for leaf extract of *Euphoria guyoniana* it was found that the leaf extract was more harmful to adventitious species (*Bromus rubens*, *Phalaris minor*, *Plantago lagopus*, *Ammi visnaga*) than the cultivated species (*Triticum durum*).
The EC_{50} of the leaf extract were very important in the order of 0,00116mg/mL, 0.00061mg/mL, 0.00764mg/mL and 0.00639mg/mL on *Bromus rubens*, *Phalaris minor*, *Plantago lagopus* and *Ammi visnaga* respectively. On the other hand, the recorded EC_{90} for the same extract were 0,00820mg/mL, 0,00596mg/mL, 0,002538mg/mL, 0,02291mg/mL on *Bromus rubens*, *Phalaris minor*, *Plantago lagopus* and *Ammi visnaga* respectively. The EC_{50} and EC_{90} values estimated of the extract on cultivated species *Triticum durum* were 0,06371mg/mL; 0,20010mg/mL respectively.
The variance in the efficiency concentrations' values (EC_{50} and EC_{90}) are probably due to the difference in the morphologic characteristic, biology, structure and seed size of the test plant.

5.6. Seedling Growth

The inhibitory effect of leaf extract of *Euphoria guyoniana* was clearly demonstrated on root and shoot growth of adventitious species (*Bromus rubens*, *Phalaris minor*, *Plantago lagopus*, *Ammi visnaga* (table 4; 5; 6; 7 respectively) and the cultivated species *Triticum durum* (table 8).
These results showed that the seedling growth was highly affected compared with control. In comparison to the shoot growth of adventitious species root growth was more sensitive to the leaf extract of *Euphoria guyoniana*. This inhibitory activity on the root and shoot growth increased with increasing the concentration. In contrast, stimulatory activity was observed in root growth of cultivated species, with stimulation at the lower concentrations and inhibition at the higher concentrations.
The inhibitory effect on the seedling growth of adventitious species is an indicator of phytotoxicity. The reduction in root and shoot growth may be attributed to reduced cell division of seedlings, because the phenolic allelochemicals inhibit the cell division and alter the ultrastructure of cells (Li et *al*., 2010). Previous studies have demonstrated that allelopathic plants affect the germination and seedling growth of many species (Hussain et *al*., 2011; Scavo et *al*., 2018, Qasem, 2002). Certain chemicals released from foliage parts, such as allelochemicals, amino acids, carbohydrates, and/or phytohormones, may be responsible for the inhibitory or stimulatory effects (Tukey, 1969). The allelochemicals induce the gross morphological effects on seed germination, root elongation and coleoptile, root and shoot development (Dey et *al*., 2012).In addition, cell division could be inhibited and cell ultrastructure altered by phenolic allelochemicals (Gomaa et *al*., 2014).

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Conclusion

This research study focuses on the phytochemical analyses, allelopathic and bioherbicidal power of leaf extract of *Euphoria guyoniana* harvested from the north-eastern region of the Algerian Sahara, against four adventitious species *Bromus rubens*, *Phalaris minor*, *Plantago lagopus*, *Ammi visnaga* and one cultivated species *Triticum durum*. The phytochemical analyses showed the presence of several compounds (flavonoids, anthocyanins, phenols and tannins, steroids, saponins, free quinone, sterols and triterpenes, carohydrates). These findings confirm the strong allelopathic and bioherbicidal effect. Our results show that the leaf extract of *Euphoria guyoniana* had significant allelopathic and bioherbicidal activity on adventitious species and stimulatory activity on cultivated species at lower concentration. We can conclude that *Euphoria guyoniana* is a promising plant, it is possible to use it as a natural herbicide to control adventitious species.

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