

Cynanchum Acutum L: Phytochemical Screening, Allelopathic and Cyto/Genotoxicity Effects in the Plant Model *Arachis Hypogaea*

Bachir Hamidi ^{1,2*}, Djilani Ghemam Amara ^{1,3}, Zaid Alia^{1,3}, Ahmed Elkhalfa Chemsas ^{3,4},
Chafika.Rezkallah ^{5,6}, Messoudi Mohammed ^{1,3}, Mokded Rabhi ⁷

¹Laboratory of Biology, Environment and Health, Department of Biology,
Faculty of Life and Natural Sciences, University of El Oued, 39000, Algeria.

²Department of Cellular and Molecular Biology, Faculty of Natural Science and Life,
El Oued University, El Oued 39000, Algeria.

³Department of Biology, Faculty of Natural Science and Life, El Oued University, El Oued 39000, Algeria.

⁴Laboratory of Biodiversity and Application of Biotechnology in Agriculture,
El Oued University, El Oued 39000, Algeria.

⁵Laboratory of Applied Chemistry and Environment, Faculty of Exact Sciences University of Eloued, Algeria.

⁶Department of Biology Faculty of Exact Science and Nature, University of Tebessa, Algeria.

⁷Laboratory of Extemophile Plants, Biotechnology Centre of Borj-Cedria, Hammam - Lif,
Tunisia Environment and Health.

⁸Department of Plant Production and Protection, College of Agriculture and veterinary Medicine, Qassim University,
Buraydah, Saudi Arabia.

*Corresponding Author Email: hamidi-bachir@univ-eloued.dz

Abstract

The plant *Cynanchum acutum L.* growing in the Algerian desert is a weed a climber, but it has been used in popular medicine. This plant is rich in biologically active compounds, so it can be valued and exploited to produce specific biopesticides. In this regard, the work aims to assess the allelopathic effect of the aqueous extract of the air part of *Cynanchum ActumL* through phytochemical screening, where the results showed that the plant contained (coumarins, saponins polyphenols, flavonoids, alkaloids, terpenes, tannins, quinones, aldehydes, and cardioglycoside). Four levels of concentration were tested (control, 15mg/ml, 30mg/ml and 45mg/ml) where the extract affected negatively on all indicators of germination and initial development of *Arachis hypogaea L.* And their value decreased linearly until it disappeared at a concentration of 45 mg/ml, and the percentage of inhibition linearly increased until it reached 100% at concentration 45 mg/ml with statistically significant differences recorded. This confirms the existence of an inhibition process that was confirmed by the microscopic study to detect the cytotoxicity of the seeds of *Arachis hypogaea L.* Treatment with an aqueous extract with a concentration that inhibits root growth 50%, which was extracted from the root growth equation, which showed a decrease in the mitotic index with the observation of chromosomal abnormalities such as (the beginning of the formation of 2 micronucleus at interphase, micronucleus at interphase, binucleated cells at interphase, disturbed at metaphase, stickiness at metaphase, oblique at metaphase, fragments at metaphase, bridge at anaphase, and binucleated cells diagonal).

Keywords: *Cynanchum Acutum L*; Weed; Biopesticides; Allelopathic; Cytotoxicity; *Arachis Hypogaea L.*

INTRODUCTION

The huge losses caused by the presence of weeds in agricultural crops have been greatly underestimated in the past. It has recently been estimated that total losses to United States agricultural producers approach four billion dollars annually. Unless the underlying causes of these losses and the places where they occur are identified, their significance cannot be fully

assessed. When plants are grown in competition with each other, many environmental factors may be altered which will negatively affect the plants' growth processes. The most important of these factors are: light intensity, soil moisture, and soil nutrients. It is very difficult under conditions of actual competition to determine what role each factor plays in crop injury. There is rarely a single factor involved, but there is often a complex interplay of factors. Under normal conditions of plant growth(Shadbolt and Holm, 1956).

To reduce weed competition, farmers remove weeds and use herbicides, a wide variety of crop spacing, population densities, and establishment methods (such as direct seeding or transplanting) (Weaver, 1984).

The toxicity and pollution caused by synthetic pesticides have made them detrimental to both human health and the environment. As a result, there is a growing need for alternative solutions, and biopesticides have emerged as potential substitutes. Biopesticides are sourced from readily available materials, they are easily biodegradable, and they operate through various mechanisms of action. Moreover, they are more cost-effective and pose lower risks of toxicity to humans and non-target organisms. However, biopesticides still face hurdles in terms of formulation, registration, commercialization, as well as general acceptance and adoption(Lengai and Muthomi, 2018).

C. acutum L, a member of the Asclepiadaceae family, is a medicinal herb that grows as a climbing plant. It is primarily found along the edges of canals in cultivation areas near the River Nile and Mediterranean regions(AM, 2011).This herb has a long history of traditional use in Egypt and other parts of the world for treating various ailments, including skin diseases, pimples(Sayed et al., 2003),diabetes(Fawzy et al., 2008),skin ulcers(Atta et al., 2005),and bacterial infections(Dehghani et al., 2012). Recent studies have demonstrated its potential as an anticancer agent, showing effects against colon cancer cell lines (HCT-116) and hepatocellular carcinoma cell lines (HepG2)(Moustafa et al., 2014). Additionally, research by Estakhr et al. (2012) has highlighted the anti-inflammatory properties of this plant(Estakhr et al., 2012). Studies conducted on *C. acutum* have identified the existence of numerous natural compounds through phytochemical investigations(Youssef et al., 2019).

1. MATERIALS AND METHODS

1.1. Plant Material:

In March 2022, plant material (the aerial part) of *Cynanchum acutum L* was collected from the Hassi Khalifa, Oued Souf region, located in the southeastern part of the Algerian Sahara.

1.2. Phytochemical Screening:

Secondary metabolites (Polyphenols, flavonoides, tannins, alkaloids, cardioglycoside, coumarins, terpenes, saponins and quinones) were detected by the methods described in(Rajesh et al., 2014) and(Chelladurai and Chinnachamy, 2018).

1.3. Preparation of concentrations of aqueous extract:

The plant is dried under shade and later crushed. Aqueous extracts are prepared by soaking 45 g of dried and crushed plants in 1000 ml of distilled water at room temperature, in a dark environment, for 24 hours. After filtration, we obtained the mother aqueous extract at a concentration of 45 mg/ml. Then, dilutions of the mother aqueous extract are made to produce aqueous extracts with concentrations of 30 mg/ml and 15 mg/ml.

1.4. Cultivation Technique:

Arachis hypogaea L seeds, from the local cultivar, were used as a model plant to evaluate the allelopathic potential of three concentrations of an aqueous extract of the aerial part of *Cynanchum acutum* L (0, 15, 30, and 45 mg/ml). Distilled water was used as a control. The experiment followed a completely randomized design, consisting of three repetitions. Each repeat corresponds to a plastic box (10×10×5cm) containing 20 seeds. Seeds were placed on filter paper moistened with 50 mL of each concentration. The filter paper was kept moist and to prevent accumulation of the active substances, the filter papers were changed every 2 days. Experiments were carried out under specified temperature conditions (about 30 °C) and in dark conditions.

1.5. Macroscopic Analyses:

The calculated parameters were as follows:

-The germination percentage (GP %): is calculated using the formula

$$GP = (n * 100) / N$$

n: represents the number of germinated seeds and

N: the total number of seeds.(Aghamir et al., 2016).

- Inhibitive percentage (IP):

$$IP (\%) = [(C - E) / C] \times 100$$

C: The number of germinated seeds in sterilized water (control).

E:The number of germinated seeds at different concentrations of the aqueous extract of *C. acutum* .(Chung et al., 2001).

-Promotion index (PI):

$P.I = [nd_2 \times (1.00)] + [nd_4 \times (0.75)] + [nd_6 \times (0.5)] + [nd_8 \times (0.25)]$ where *n* is the number of seeds germinated at day *d*; Where a higher value of PI indicates a faster germination process(Laouedj et al., 2020).

-Germination stress tolerance index (GSI %):

$GSI\% = [P.I \text{ of seeds treated with the aqueous plant extract (stressed)} / P.I \text{ control seeds}] \times 100$ (Laouedj et al., 2020).

-Plant height stress tolerance index (PHSI %):

$PHSI\% = (\text{plant height of the aqueous extract-treated plant} / \text{the height of the control plant}) \times 100$ (Saima et al., 2018).

-Root length stress tolerance index (RLSI %):

$RLSI\% = (\text{The root length of the plant treated with the aqueous extract} / \text{The root length of the control plant}) \times 100$ (Saima et al., 2018).

-Dry matter stress tolerance index (DMSI %):

$DMSI\% = \text{Dry matter of the plant treated with the aqueous extract} / \text{Dry matter of the control plant} \times 100$ (Ahmad et al., 2015).

-Seed vigor (SV): [vigor index (I) and vigor index (II)]:

VI (I) = [seedling length (Root + Shoot) (cm) × The germination percentage %](Janmohammadi et al., 2008)

VI (II) = [seedling dry weight (Root + Shoot) × The germination percentage %](Aghamir et al., 2016).

-The tissue water content (TWC): It is calculated as follows:

(TWC %) = [(Fresh weight – Dry weight)/ fresh weight] × 100

1.6. Recovery Test :

To assess the impact of the aqueous extract on germination, we conducted a replanting experiment with seeds that did not initially germinate. These seeds had previously undergone treatment with the aqueous extract of the plant, which may have caused a delay or inhibition of germination. In order to evaluate their recovery potential, we thoroughly washed the seeds with distilled water and then replanted them. The treatment process for these seeds was the same as that used for the control seeds in the initial stage, involving the use of distilled water. We observed and recorded the extent to which the seeds regained their ability to germinate.

1.7. Microscopic Analyses:

In order to examine the cytotoxicity and mechanisms of action of the aqueous extract of *C. acutum*, seed extracts of *Arachis hypogaea L* were subjected to the same conditions described in section 2.4. The seeds were exposed to a concentration of the extract that inhibited 50% of root growth, which was determined based on the obtained germination percentage (GP%) curve (Luber et al., 2015). After a 48-hour exposure period, the roots were carefully removed. These excised roots were then collected and preserved in a solution of ethanol and acetic acid (3:1 ratio, respectively). Thereafter, they were stored at -20 °C for a minimum of 24 hours. Slides were prepared using the crushing technique. Schiff's reagent was used to stain nuclei and chromosomes, allowing observation in the dark at room temperature for approximately 1.5 hours. In addition, cells were stained with a 2% acetocarmine solution. Each slide consisted of two treated slides, and approximately one thousand treated meristem cells were evaluated. Various stages of mitotic division, possible nuclear chromosomal alterations, and nuclear alterations have been observed and documented. Mitotic index (MI), chromosome frequency (CA) and nuclear alterations (NA) were compared based on these observations (Aragão et al., 2015).

1.8. Statistical analyses:

All data measurements were expressed as mean ± standard deviation (SD) from three replicates. The statistical study was performed using SPSS Statistics for Windows, employing one-way analysis of variance (ANOVA), followed by Duncan's multiple range tests; $p < 0.05$

2. RESULTS**2.1. Chemical screening:**

The phytochemical screening of *C. acutum L* (the aerial part) was positive as shown in Tables 1.

Table 1: Phytochemical screening of the aerial parts of *C. acutum L*

Secondary metabolites	Resultats
Polyphenols	+
Flavonoids	+
Alcaloids	+
Terpenes	+
Tannins	+
Saponins	+
Quinones	+
Aldehydes	+
Cardioglycoside	+
Coumarins	+

The positive signs (+) indicate the detection of chemical classes within the analyzed plant material.

2.2. Macroscopic Analyses:

2.2.1 Evaluation of the First Cultivate :

The aqueous extract obtained from the aerial part of the *C. acutum L* plant had a detrimental impact on the *A. hypogaea L* plant. This negative effect became more pronounced as the concentration of the extract increased, adversely affecting the germination percentage, as well as the length, root and shoot growth, and dry and fresh weight of the plants. So its effect was as follows:

-The germination percentage showed a decline in response to different concentrations of *C. acutum L* extract. In the control group, the germination percentage was recorded at $96.67 \pm 5.77^*$, but it significantly decreased to $20 \pm 8.66^{***\%}$ at a concentration of 30mg/ml. At a concentration of 45mg/ml, germination was completely inhibited. The inhibition percentage, which is inversely related to the germination percentage, exhibited an upward trend with increasing concentrations of *C. acutum L*. At the control level, the inhibition coefficient was $0 \pm 5.98^*\%$, while it rose to $79.30 \pm 8.96^{***\%}$ at a concentration of 30mg/ml, and reached 100% at a concentration of 45mg/ml.

Similarly, the Promotion index exhibited a significant decrease in response to different concentrations of *C. acutum L* extract. At the control level, the Promotion index was measured at $17.58 \pm 0.8^*$, but it notably dropped to $3.83 \pm 1.8^{***}$ at 30mg/ml and completely ceased at a concentration of 45mg/ml. Similarly, the Germination stress tolerance index, which was $100 \pm 4.47^*\%$ at the control, decreased to $21.8 \pm 10.28^{***\%}$ at 30mg/ml and ceased at 45mg/ml.

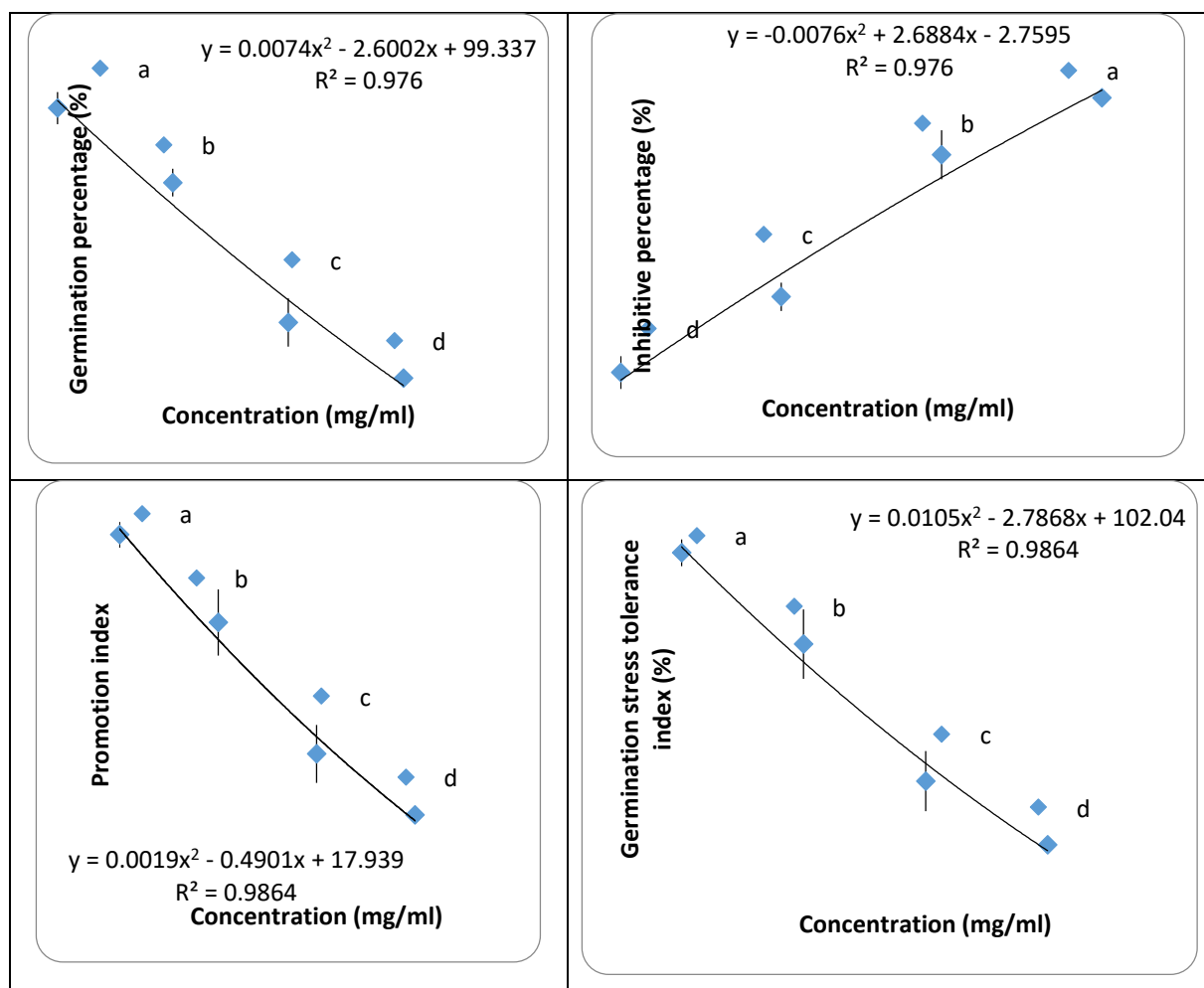
As for the shoot and root, their length is almost non-existent (non-existent increase) at concentration 45mg/ml, and they increase regularly with decreasing concentration of the aqueous extract of *C. acutum L*, as their length is $1.8 \pm 0.26^*$ cm and $4.33 \pm 0.29^*$ cm, their fresh weight is $0.2 \pm 0.008^*$ g and $0.25 \pm 0.015^*$ g, and their dry weight is $0.0363 \pm 0.002^*$ g and $0.0275 \pm 0.001^*$ g, respectively, in the control.

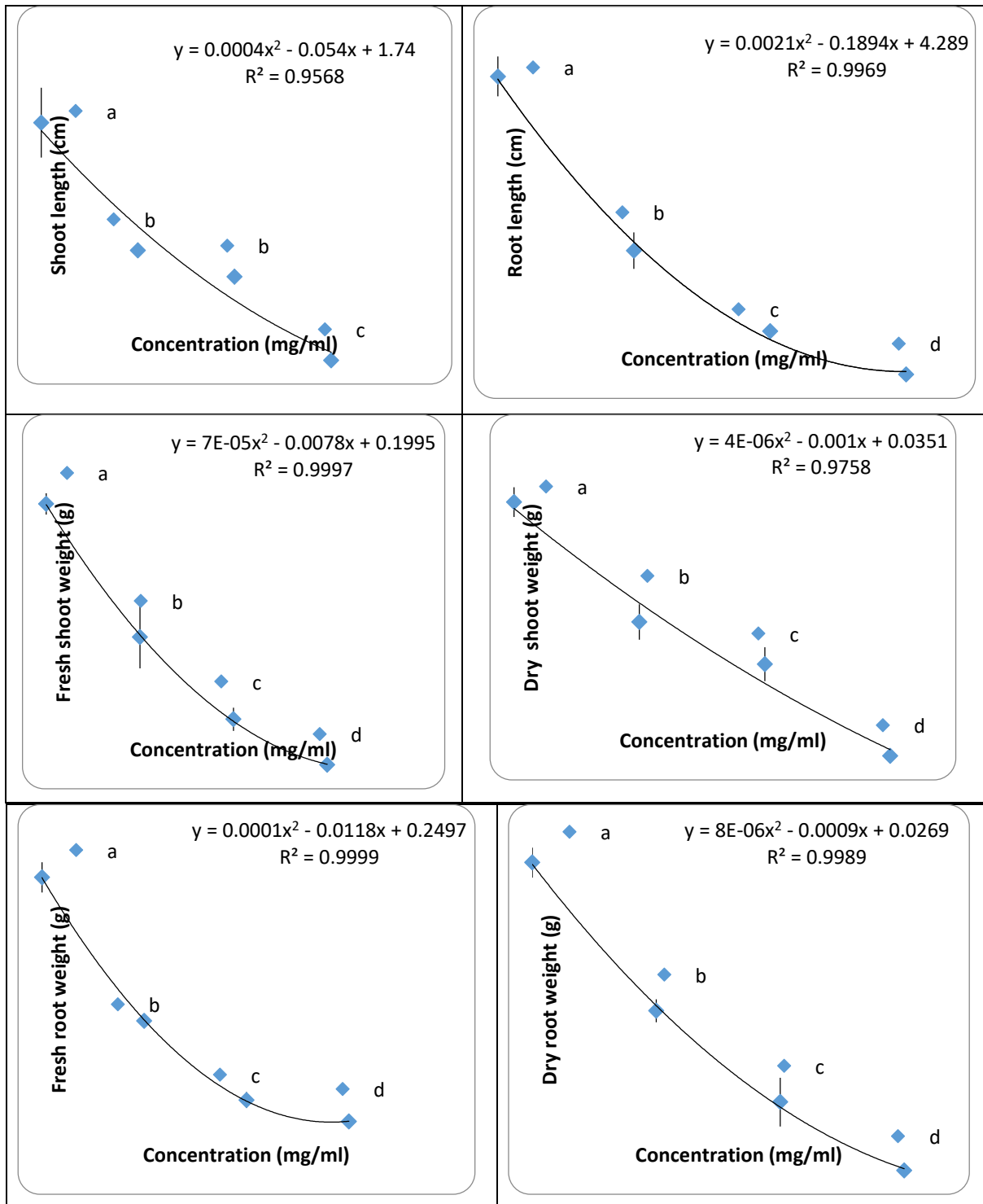
Moreover, the Plant height stress tolerance index, Root length stress tolerance index, and Dry matter stress tolerance index, which were initially $100 \pm 8.98^*\%$, $100 \pm 6.66^*\%$, and $100 \pm 3.86^*\%$ in the control group, decreased to $42.93 \pm 4.70^{***\%}$, $41.53 \pm 6.1^{***\%}$, and $51.88 \pm 2.43^{***\%}$ at a concentration of 30mg/ml, respectively. These indices became non-existent at a concentration of 45mg/ml.

The vigor index (I) and vigor index (II), measured at $595 \pm 86.75^*$ (cm. %) and $6176 \pm 587.08^*$ (mg. %), respectively, in the control group, exhibited a linear decline with increasing concentration. They reached $25.67 \pm 11.84^{***}$ (cm. %) and $373.33 \pm 167.6^{***}$ (mg. %) at 30mg/ml, respectively, and were absent at a concentration of 45mg/ml. Furthermore, the tissue water content in the plants was initially $85.81 \pm 0.51^*$ %, which gradually decreased with increasing concentration of the extract. At 30mg/ml, it measured $67.35 \pm 3.77^{**}$ %, and it was non-existent at a concentration of 45mg/ml. And the curves show that.

3.2.1 Evaluation of the Re-Cultivate:

When taking all the seeds that did not germinate in different concentrations of the aqueous extract of *C. acutum L*, then washing them well with distilled water, then replanting them as the control plants were sown (through distilled water), we did not see germination or seeds.





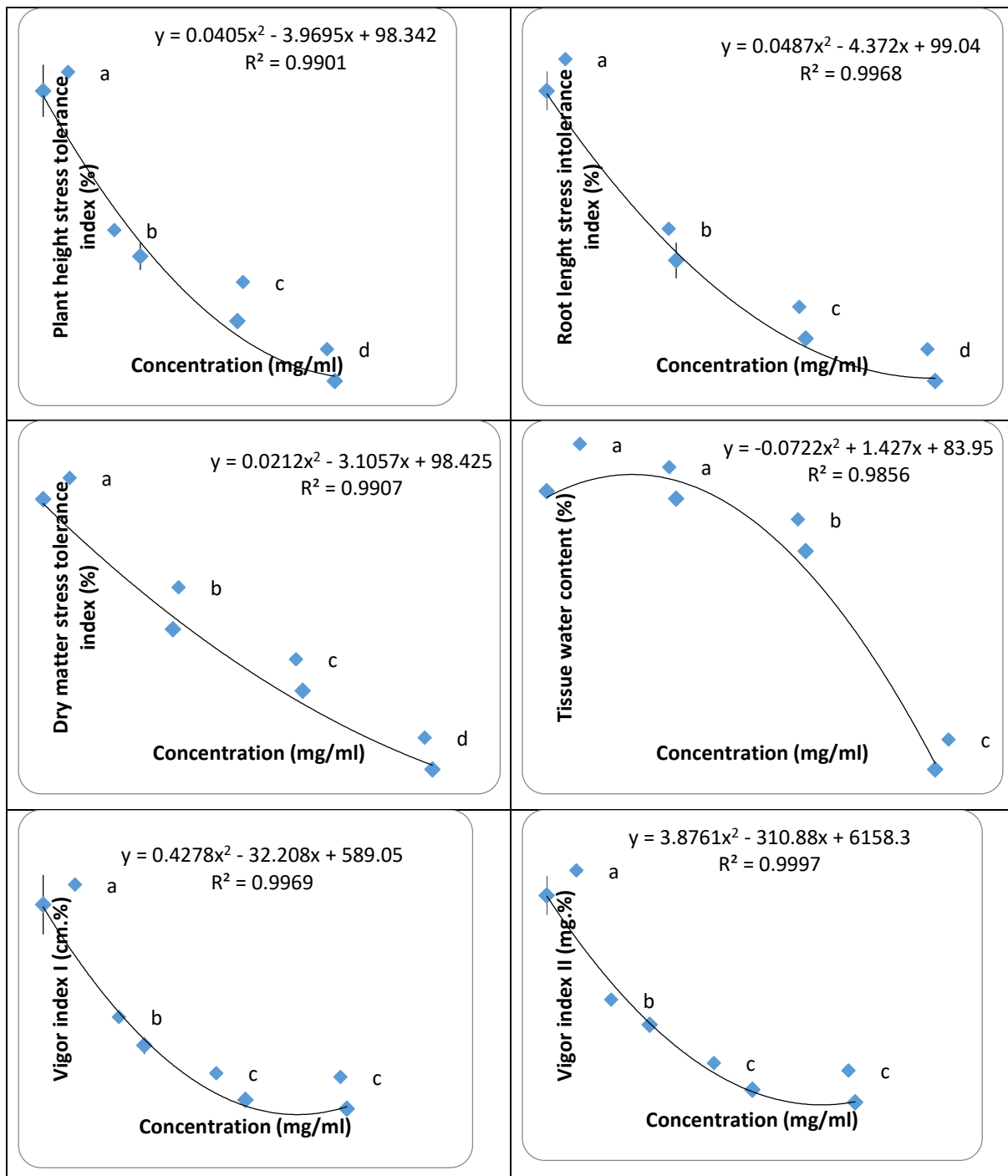


Figure 1: Effects of *C. acutum* L extracts on the early development and the calculated parameters of *Arachis hypogaea* L plantlets

2.3 Microscopic analyses:

Comparing the microscopic examination of the meristematic zone in the roots of *A. hypogaea* L seeds treated with an aqueous extract of *C. acutum* L at a concentration of 17, which inhibits root growth by 50%, to the meristematic zone of *A. hypogaea* L seeds treated with distilled water (control), under a magnification of 1000X, reveals noticeable distinctions.

The optical microscope observations demonstrate a significant reduction in the mitotic index and a decline in the percentage of mitotic phases (metaphase, anaphase and telophase) within the roots of *A. hypogaea L* seeds treated with the aqueous extract of *C. acutum L* when compared to the meristematic zone of the roots treated with distilled water. Moreover, the examination reveals the presence of chromosomal abnormalities during the cell cycle of the seeds treated with the extract, while no such chromosomal deviations were observed in the control group (micronucleus at interphase stage, binucleated cells at interphase stage, disturbed at metaphase, stickiness, Oblique at metaphase, chromosomal breaks, chromosomal bridge, and binucleated cells diagonal.). As the following pictures show:

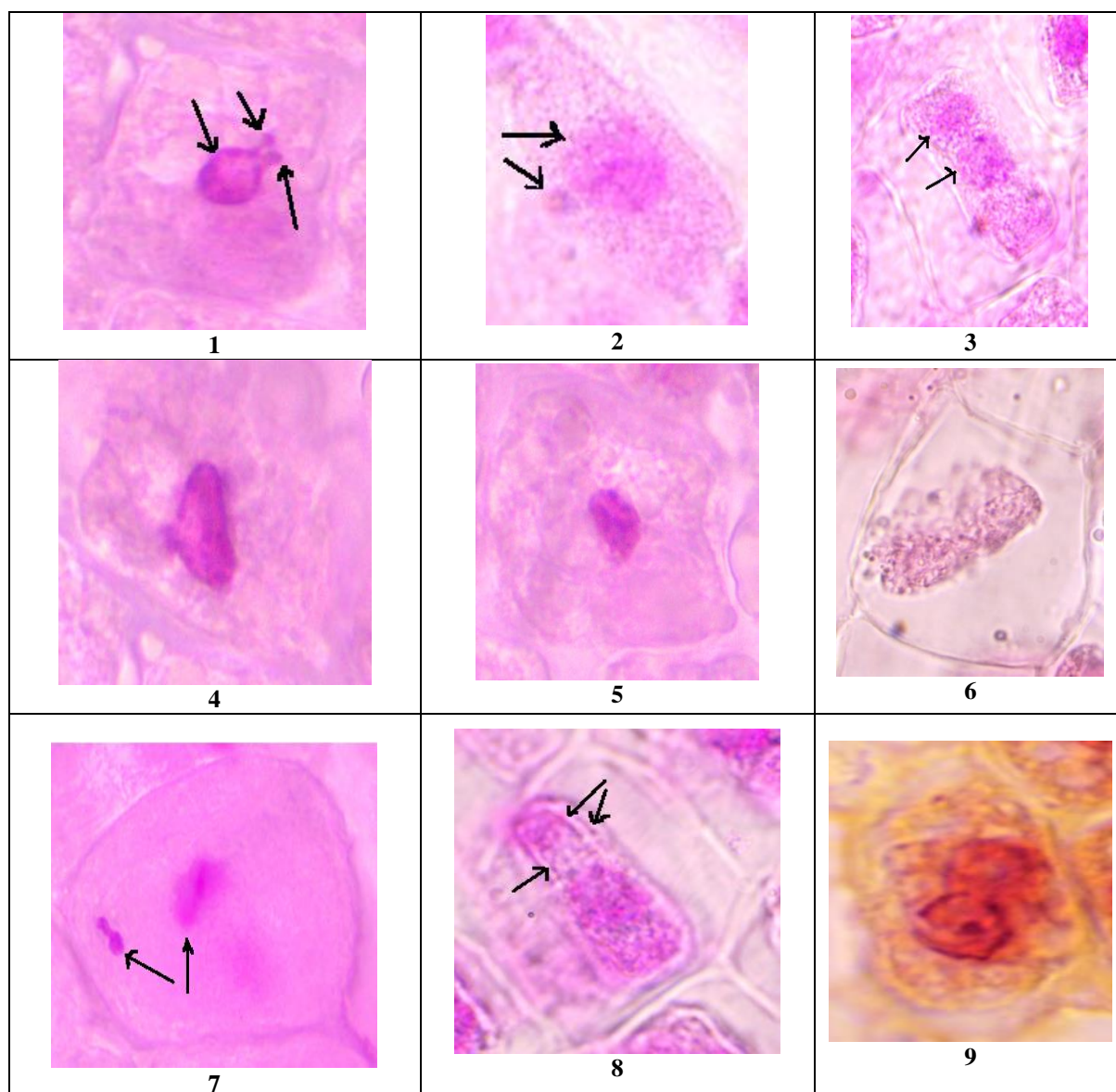


Figure 2: Types of mitotic abnormalities resulting from treatments of *A. hypogaea L* a root tips with aqueous extract of *C. acutum L*. (X = 1000). (1) The beginning of the formation of 2 micronucleus at interphase stage, (2) micronucleus at interphasestage, (3) binucleated cells at interphase stage, (4) disturbed at metaphase stage, (5) stickiness at metaphase stage, (6) oblique at metaphase stage, (7) fragments at metaphase stage, (8) bridge at anaphase stage, and (9) binucleated cells diagonal.

3. DISCUSSION

The results of the phytochemical screening of the aerial part of *C. acutum L.* growing in the Algerian Sahara presented in Table 1 are consistent with previous studies. The presence of polyphenol content among the aerial parts of *C. acutum* was 0.375 g/100 g and flavonoids 0.313 g/100 g (Abu Ziada et al., 2016). The milky latex of *C. acutum L.* contains polyphenols, flavonoids, tannins and alkaloids (Soliman et al., 2022). It also contains coumarin, scopoletin and terpene (Abdelhameed et al., 2021). Also of the aerial parts of *C. acutum L.* several compounds have been isolated including the simple coumarins scopoletin and scoparone (El-Demerdash et al., 2009). The genus *Cynanchum L.* contain saponins and carbohydrates (Zhao et al., 2004).

-Furthermore, the findings indicated that the aqueous extract derived from *C. acutum L.* possesses allelopathic properties that affect the germination and growth of crops. As the concentration of the aqueous extract increased, the germination percentage, root length, and shoot length of corn crops exhibited a decline (Golzardi et al., 2014). The germination percentage experienced a significant decline at the highest concentrations of aqueous extract, which can be attributed to the detrimental effect on the germination process (Moyer and Huang, 1997; Singh et al., 2003). The findings indicated that the aqueous extract obtained from *C. acutum L.* exhibited varying allelopathic effects on the germination and growth of wheat seedlings. As the concentration of *C. acutum L.* residue increased, the germination percentage, shoot and root length, and seedling weight of wheat showed a decline (Faridmarandi et al., 2014). Many studies indicated the phenolic compounds are responsible for the retardatory effect on plant growth and thus their presence causes significant damage to plant growth, and since we revealed that the aqueous extract of *C. acutum L.* contains phenols (Qasem and Foy, 2001; Sisodia and Siddiqui, 2009), it is an inhibitor.

The assessment of cytogenotoxic effects of a specific compound or extract is conducted using meristematic cells located in the root tip of the selected plant model (Andrade-Vieira et al., 2014). The treatments of *V. faba* root tips with crude latex resulted in various types of mitotic abnormalities, including micronucleus formation during the interphase stage, disturbance during metaphase, stickiness at metaphase, ring formation at metaphase, oblique alignment at metaphase, and star-like arrangements at metaphase and a clear observation was made that the application of 3% crude latex of *C. acutum L.* resulted in the cessation of the metaphase stage and the termination of both the anaphase and telophase stages. (Soliman et al., 2022). The extracts of *C. acutum L.* contain saponins that possess detergent-like characteristics and have the ability to bind to cell membranes. As a result, the normal functioning of cells is impacted, potentially resulting in cell death (Carvalho et al., 2019). The bioactivity profiles of 3 alkaloids (1–3), 12 coumarins (4–15), 2 phenylpropanoic acid derivatives (16 and 17), and 14 flavonoids (18–31) derived from 11 species within the Meliaceae and Rutaceae families were assessed. All compounds underwent testing in the wheat coleoptile bioassay, and those exhibiting the highest activities were further tested on the standard target species (STS) including *Lepidium sativum* (cress), *Lactuca sativa* (lettuce), *Lycopersicon esculentum* (tomato), and *Allium cepa* (onion). The majority of the isolated compounds demonstrated phytotoxic activity, with graveoline (3), psoralen (8), and flavone (18) displaying the most notable levels of bioactivity, similar to that of the commercial herbicide (Nebo et al., 2014). The furanocoumarins belonging to the coumarin class have garnered significant interest because of their phytotoxic properties. These compounds, when activated by light, can penetrate the DNA double helix and attach themselves to the pyrimidine bases (cytosine and thymine), impeding

DNA transcription and repair processes and sometimes resulting in cell degeneration. Additionally, allelochemicals such as saponins, tannins, and flavonoids are frequently mentioned as contributors to both direct and indirect effects. These compounds, due to their water solubility, can be naturally released into the environment (Carvalho et al., 2019); Since the plant *C. acutum L.* is rich in alkaloids, polyphenols, flavonoids, coumarins, tannins and saponins, this justifies the allelopathic property of the aqueous extract of *C. acutum L.* When the seeds treated with the aqueous extract of *C. acutum L.* were washed with distilled water, replanted and treated as the control group was treated, neither seeds germinated, which indicates final inhibition of these seeds.

CONCLUSION

The study was conducted to evaluate the plant *C. acutum L.* and the possibility of producing a specific herbicide. By studying the phytochemical screening and the effect of its aqueous extract on germination and primary growth of *A. hypogaea L.* Where phytochemical screening have shown that the plant *C. acutum L.* is rich in biologically active compounds (coumarins, saponins polyphenols, flavonoids, alkaloids, terpenes, tannins, quinones, aldehydes, and cardioglycoside), and it has an inhibitory effect. Where the germination of *A. hypogaea L.* is inhibited and the germination and initial growth of *A. hypogaea L.* linear decrease in germination, root and shoot length, and their dry and fresh weight with increasing the concentration of the aqueous extract of *C. acutum L.*, and the percentage of inhibition increased linearly with increasing the concentration of the aqueous extract of *C. acutum L.* As confirmed by the microscopic study of cytotoxicity. The lack of cleavage index and the emergence of chromosomal abnormalities in the treated seeds of *A. hypogaea L.*, compared with the seeds of *A. hypogaea L.* The control. Thus, the study concluded that *C. acutum L.* may be used to produce a herbicide for its inhibitory ability due to its allelopathic effect. But it is not used in *A. hypogaea L.* fields. *A. hypogaea L.* residues of *C. acutum L.* should also be avoided as a vegetable fertilizer for the soil in *A. hypogaea L.* fields.

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