*Journal of Experimental Agriculture International*

***Volume 46, Issue 11, Page 598-604, 2024; Article no.JEAI.127014***
***ISSN: 2457-0591***
*(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)*

# Effect of Aqueous and Hydroalcoholic Extracts of *Hyptis suaveolens* (L.) Poit. (Lamiaceae) on Seedlings of Three Cultivated Species: *Zea mays* L. (Poaceae), *Phaseolus vulgaris* L. (Fabaceae), and *Solanum lycopersicum* L. (Solanaceae)

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***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: https://doi.org/10.9734/jeai/2024/v46i113081

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/127014

***Original Research Article***

***Received: 11/09/2024***
***Accepted: 21/11/2024***
***Published: 25/11/2024***

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***Cite as:*** *Yapi, Arnaud-Freddy, Hervé Cedessia Keassemon Kone, Arsène Gue, and Fred Alain Serges Kouame. 2024. "Effect of Aqueous and Hydroalcoholic Extracts of Hyptis Suaveolens (L.) Poit. (Lamiaceae) on Seedlings of Three Cultivated Species: Zea Mays L. (Poaceae), Phaseolus Vulgaris L. (Fabaceae), and Solanum Lycopersicum L. (Solanaceae)". Journal of Experimental Agriculture International 46 (11):598-604. https://doi.org/10.9734/jeai/2024/v46i113081.*

## ABSTRACT

Synthetic herbicides use in weed management has negative effects on human health and environment. This study was carried out to assess the allelopathic effects of *Hyptis suaveolens* on seedlings of *Solanum lycopersicum*, *Zea mays* and *Phaseolus vulgaris*. Aqueous and hydroalcoholic solvents at concentrations of 10% and 20% were used to prepare extracts from fresh and dried leaves of this species in foliar and root application in honeycomb plates. The results showed that extracts of *Hyptis suaveolens* leaves inhibite the development of seedlings of the target species in root application. Concerning the effect of the extracts on the development of *Zea mays* seedlings, the 20% hydroalcoholic extracts of dry and fresh leaves showed a better mortality rate. This rate varied between 61.67% and 71.08% depending on the species. For *Phaseolus vulgaris* seedlings, the 20% hydroalcoholic extract of fresh leaves showed a better mortality rate (72.43%). In the case of *Solanum lycopersicum* seedlings, the aqueous extract of dried leaves induced a better mortality rate (88.97%). It may be concluded that extracts of *Hyptis suaveolens* leaves could be sprayed as bioherbicide.


*Keywords: Weeds; biological control; allelochemicals; total bioherbicides.*

## 1. INTRODUCTION

The use of synthetic herbicides has long been beneficial for controlling weeds in agricultural production (Marnotte, 1995). However, the abusive and prolonged use of these synthetic herbicides is causing considerable environmental damage. This is reflected in the degradation of agricultural land (Pell et al., 1998), the development of herbicide-resistant weeds (Konan et al., 2014 ; Kouadio et al., 2014 ; Dianda, 2016), and the release of chemical residues into the environment and crop products.

In order to reduce the dangers associated with the use of these synthetic herbicides, researchers are studying plants with a view to developing environmentally-friendly biological herbicides. Several plant species synthesise molecules capable of inhibiting germination and growth of neighbouring plants. This phenomenon, known as allelopathy, has been demonstrated in numerous studies and offers promising prospects for weed management. The natural compounds present in certain species could be successfully used as bioherbicides (Islam and Kato-Noguchi, 2013 ; Zerouug, (2019)). In order to reduce the dangers linked to the use of these synthetic herbicides, several studies are carried out by researchers on plants with a view to developing biological herbicides that respect the environment (Page and Grume, 2014; Chouki and Rala, 2019; Babasidi and Benyazza, 2022).

Among plants in the Lamiaceae family, there is a potentially invasive weed called *Hyptis suaveolens* which inhibits the germination and growth of plants in its habitat (Ahoton et al., 2010). It is distributed in tropical and subtropical regions (Mishra and Verma, 1992). The present study was carried out to test the effects of aqueous and hydroalcoholic extracts of *Hyptis suaveolens* (Lamiaceae) leaves on post-emergence seedlings of three cultivated species. The aim of this study is to contribute to the agronomic development of *Hyptis suaveolens* through the use of its leaf extract as a bioherbicide.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of *Hyptis suaveolens* Extracts

To prepare extracts from dried *Hyptis suaveolens* leaves, the leaves of vigorous, healthy plants in the juvenile stage were harvested, dried in the shade for 10 days and then ground to a powder using a blender. The powder obtained was weighed and mixed with 10% and 20% aqueous or hydroalcoholic solvents, depending on the treatment. In the case of fresh leaves, a portion of the previously harvested leaves was washed and then ground using a blender. The obtained paste was weighed and mixed with aqueous and 10% and 20% hydroalcoholic solvents. The various obtained products were wrapped in cloth and placed under a press. The applied pressure enabled the extraction of substances contained in fresh and dry leaves of *H. suaveolens* (Table 1). The different substances obtained were used for the treatments.

**Table 1. Solution preparation summary**

| Organ | Quantity of substance + volume of solvent | Designation |
|---|---|---|
| | **Distilled water** | **CT (CT = Control treatment)** |
| Fresh leaves | 0.5 kg paste + 2 litres distilled water | T1 |
| | 0.5 kg paste + 2 litres distilled water + 10% alcohol | T2 |
| | 0.5 kg paste + 2 litres distilled water + 20% alcohol | T3 |
| Dry leaves (powder) | 0.5kg of powder | T4 |
| | 0.5 kg powder + 2 litres distilled water | T5 |
| | 0.5 kg powder + 2 litres distilled water + 10% alcohol | T6 |
| | 0.5 kg powder + 2 litres distilled water + 20% alcohol | T7 |

## 2.2 Sowing and Setting Up Nurseries

For each species (*Solanum lycopersicum, Phaseolus vulgaris* or *Zea mays*), seven (7) honeycomb plates were used. Three (3) replicates were carried out per treatment on a honeycomb plate. Each honeycomb plate was filled with potting soil that had been moistened beforehand. After placing one seed per tray, watering was carried out every day until one week after sowing for the bean and maize tests and ten (10) days after sowing for the tomato tests. Two (2) trials were carried out for each species. The two tests were carried out to see if the action of the extracts is root or foliar.

### 2.2.1 Evaluation of the effect of *Hyptis suaveolens* extracts as a foliar spray

Filtered *Hyptis suaveolens* leaf extract (fresh or dried) were placed in a sprayer. It was then applied solely to the leaves of seedlings of each species. In foliar spraying, the age of *Phaseolus vulgaris* and *Zea mays* seedlings was one week (7 days) after sowing, while that of *Solanum lycopersicum* seedlings was ten (10) days. At these times, the different plants of the species generally have at least 2 leaves.

For each seedling, 5 ml of the corresponding solution was sprayed onto the leaves of the seedlings. Observations on the colour of the leaves and the appearance of the seedlings were made over two (2) weeks while the seedlings were watered daily. The number of dead seedlings was also noted.

### 2.2.2 Evaluation of the effect of *Hyptis suaveolens* extracts as a root spray

The second trial was carried out on the roots. In this trial, the various *Hyptis suaveolens* extracts were applied to the roots of the seedlings. For each seedling, 5 ml of the corresponding solution was poured onto the soil at the foot of the seedlings. Before application, the age of *Phaseolus vulgaris* and *Zea mays* seedlings was one week (7 days) after sowing, while that of *Solanum lycopersicum* seedlings was 14 days after sowing. The mortality rate were assessed and observations were also made on the colour of the leaves and the appearance of the seedlings. The mortality rate was assessed by counting the number of dead plants.

## 2.3 Data Analysis

The data was recorded in Excel and then processed using STATISTICA 7.1 software. When statistically significant differences were observed with ANOVA at the 5% threshold, the Fischer test was used to divide the different averages into homogeneous groups.

# 3. RESULTS AND DISCUSSION

## 3.1 Results

### 3.1.1 Effect of extracts on maize, bean and tomato seedlings following foliar application

In maize seedlings, leaf yellowing was observed with treatments T5, T6, and T7, 48 hours after application. For the other treatments, no effect was observed on the seedlings. In the case of bean seedlings, yellowing and wrinkling of the leaves were observed with treatments T1, T2, T3, T5, T6 and T7. There was no effect with T4. These effects were also observed after 48 hours. With regard to tomato seedlings, extracts of dry and fresh leaves of *H. suaveolens* had no effect on the seedlings. Seven days after treatment, all the seedlings that had been stressed by the treatments resumed their growth.

### 3.1.2 Effect of extracts on maize, bean and tomato seedlings by root application

*3.1.2.1 Zea mays (Poaceae)*

After root application of the various extracts, yellowing of the seedling leaves was observed 48 hours after treatment. After 7 days, the leaves of the seedlings dried out. Mortality was observed in the second week after treatment. Mortality rates ranged from 0% (control) to 71.08% (T3). For treatments T7, T6 and T2, mortality rates were 61.67%, 36.12% and 35.15% respectively. Statistical analysis showed that there were significant differences between treatments (*P = 0.006*) at the 5% level (Table 2). Four classes stand out. Treatments T3 and T7 represent the class with the highest mortality rate, with values estimated at 71.08% and 61.67% respectively. Treatment T4 had the lowest mortality rate (3.57%). No mortality was observed in the control treatment.

*3.1.2.2 Phaseolus vulgaris (Fabaceae)*

48 hours after the treatments, wilting was observed on the leaves of the seedlings. Seven days later, yellowing and leaf drop were observed, followed by death of the seedlings in treatments T3, T6 and T7. Treatments T1, T5 and T2 had no effect at this time. Mortality was observed in these treatments from the second week onwards. No inhibitory effect was detected on seedlings in treatments T4 and CT. Statistical analysis at the 5% level revealed significant differences between treatments (P = 0.005). The Fisher test was used to divide the treatments into 5 distinct classes (Table 3).

*3.1.2.3 Solanum lycopersicum (Solanaceae)*

With the exception of treatment T4, leaf wilting was observed 48 hours after application of extracts of fresh and dried leaves of *H. suaveolens*. Observations made one week later revealed collar rot followed by plant death in all treatments except T4 and CT. Statistical analysis at the 5% level revealed significant differences between treatments (*P = 0.009*). The Fisher test revealed 5 classes at treatment level (Table 4). Treatment T5 represents class I, with a mortality value of 88.97%. Treatments T4 and CT represent class 4 with a value of 0%.

## 4. DISCUSSION

The different extracts had more or less harmful effects on the seedlings depending on the species and the part treated with the extracts. On contact with the seedlings, extracts of fresh *Hyptis suaveolens* leaves caused a yellowing effect on *Zea mays* leaves and wrinkling of *Phaseolus vulgaris* leaves after 3 to 4 days. After this period, the seedlings resumed normal growth. This shows that the extracts stressed the seedlings of both crops shortly after application. In the case of *Solanum lycopersicum* seedlings, the extracts had no effect on the seedlings. This short-lived inhibitory effect of the extracts on *Zea mays* and *Phaseolus vulgaris* seedlings could be due to the low dosage of extract applied to the leaves of the seedlings. According to Friedman (1995) and Dhima et al. (2006), allelopathy only occurs when the critical quantity of allelochemicals reaches the target plant or seed. In *Solanum lycopersicum* seedlings, the ineffectiveness of foliar spray extracts was observed by (Kouamé, 2022) with *Tithonia diversifolia* extracts.

Aqueous and hydroalcoholic extracts of dry and fresh leaves of *Hyptis suaveolens* applied as a root spray at a dose of 5 ml showed an effect on seedlings. These results show a herbicidal effect of *Hyptis suaveolens* leaf extracts on seedlings. The herbicidal effect can be seen directly in the weakening of the crown followed by the death of some *Solanum lycopersicum* and *Phaseolus vulgaris* seedlings. For *Zea mays* seedlings, yellowing and desiccation followed by the death of some seedlings were observed. High concentrations of *H. suaveolens* extract (20% hydroalcohol) showed the best mortality rates on *Zea mays* and *Phaseolus vulgaris* seedlings. In fact, the high concentrations of dried and fresh leaf extracts were sufficient to create a dysfunction in the metabolism of plants of both species. It could be said that the inhibitory effect increases with the concentration of the extracts. These results are similar to those obtained by Arslan et al. (2005), Uremis et al. (2005) and Turk and Tawaha (2003). In their work, these authors showed that inhibition increased with increasing extract concentration. Similarly, our results are in agreement with those of Friedman (1995) and Malcolm et al. (2003). Benmeddour (2010) showed that at doses of 3% and 5%, *Peganum harmala* and *Nerium oleander* extracts inhibit the development of *Silybum marianum* seedlings. Furthermore, in the post-emergence treatments carried out on *Solanum lycopersicum*

**Table 2. Mortality rate of *Zea mays* seedlings after *H. suaveolens* extracts application**

| Treatments | Mortality rate (%) |
|---|---|
| Fresh leaves + 20% hydroalcohol (T3) | 71,08 a |
| Dry leaves + 20% hydroalcohol (T7) | 61,67 a |
| Fresh leaves + 10% hydro alcohol (T6) | 36,12 b |
| Fresh leaves + 10% hydro alcohol (T2) | 35,15 b |
| Fresh leaves + distilled water (T1) | 22,33 b c |
| Dry leaves + distilled water (T5) | 20,85 b c |
| Dry leaves or powder (T4) | 3,57c |
| Distilled water (CT) | 0,0 c |

*(Means followed by the same letter are not significantly different, ANOVA, Tukey's HSD test ; α =0.05)*

**Table 3. Mortality rate of *Phaseolus vulgaris* seedlings after *H. suaveolens* extracts application**

| Treatments | Mortality rate (%) |
|---|---|
| Fresh leaves + 20% hydroalcohol (T3) | 72,43 a |
| Dry leaves + 20% hydroalcohol (T7) | 58,94 b |
| Dry leaves + 10% hydro alcohol (T6) | 17,72 c |
| Fresh leaves + 10% hydroalcohol (T2) | 12,01 cd |
| Dry leaves + distilled water (T5) | 10,84 cd |
| Fresh leaves + distilled water (T1) | 7,12 cd |
| Distilled water (CT) | 0,0 d |
| Dry leaves (T4) | 0,0 d |

*(Means followed by the same letter are not significantly different, ANOVA, Tukey's HSD test ; α =0.05)*

**Table 4. Mortality rate of *Solanum lycopersicum* seedlings after *H. suaveolens* extracts application**

| Treatments | Mortality rate (%) |
|---|---|
| Dry leaves + distilled water (T5) | 88,97 a |
| Fresh leaves + 10% hydroalcohol (T2) | 70,58 b |
| Dry leaves + 10% hydroalcohol (T6) | 58,73 c |
| Fresh leaves + distilled water (T1) | 48 c |
| Dry leaves + 20% alcohol (T7) | 44,12 cd |
| Fresh leaves + 20% alcohol (T3) | 35,26 cd |
| Dry leaves or powder (T4) | 0 d |
| Distilled water (CT) | 0 d |

*(Means followed by the same letter are not significantly different, ANOVA, Tukey's HSD test ; α =0.05)*

plants, the highest mortality rate was observed with the dry leaves of *Hyptis suaveolens*. These observations confirm the allelopathic nature of *Hyptis suaveolens*. In addition, these results could explain the invasive nature of *H. suaveolens* individuals under natural environmental conditions. These results corroborate those obtained by Zeghada (2009) ; according to his studies, natural substances produced by plants are capable of delaying or even inhibiting seed germination and seedling growth under favourable conditions. Studies on *Helianthus annus* (Asteraceae) showed that extracts from its roots and leaves reduced seed germination, seedling development and weed dry weight (Batlang and Shushu, 2007).

## 5. CONCLUSION

The study indicated that all extracts of the fresh and dried leaves of *Hyptis suaveolens* had a strong inhibitory effect on the development of *Zea mays, Phaseolus vulgaris* and *Solanum lycopersicum* seedlings. The 20% hydroalcoholic extracts of fresh and dried *Hyptis suaveolens* leaves were tested as better inhibitors of *Zea mays* seedling development. The solvent that conferred the greatest herbicidal efficacy was 20% hydroalcohol, for both fresh and dried leaves. The extract of fresh leaves diluted with 20% hydroalcohol was the best inhibitor of the development of *Phaseolus vulgaris* seedlings. The best solvent was 20% hydroalcohol and the best effect was obtained with fresh leaves. The aqueous extract of dry leaves was tested as the best inhibitor of post-emergence development of *Solanum lycopersicum* seedlings by root spraying. The herbicidal effect of the aqueous and aqueous-alcoholic extracts proves that *Hyptis suaveolens* possesses allelochemicals that are released into the environment. However, in-depth research is needed to further confirm the herbicidal potential of *H. suaveolens* under pre-emergence and post-emergence weed conditions.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

## ACKNOWLEDGEMENT

We thank the Biological Sciences training and research unit for hosting this research.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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