

**EFFECT OF AQUEOUS AND HYDROALCOHOLIC EXTRACTS OF *Hyptis suaveolens* (L) POIT. (LAMIACEAE) ON SEEDLINGS OF *Zea mays* L. (POACEAE), *Phaseolus vulgaris* L. (FABACEAE) AND *Solanum lycopersicum* L. (SOLANACEAE), THREE CULTIVATED SPECIES**

**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. The results showed that extracts of *Hyptis suaveolens* leaves inhibit 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 total bioherbicide.

**Key words** : Weeds, biological control, allelochemicals, total bioherbicides

**Comment [AS1]:** Mention that this experiment was carried out either in soil or pot.

**Comment [AS2]:** Remove it

**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.

Comment [AS3]: Also add recent reference ?

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). 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 (Ahton 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.

Comment [AS4]: Add importance of bioherbicides as they are better than synthetic herbicides.

Comment [AS5]: How is it possible to destroy weeds in a crop plants when its extract is also harmful to main crop.

## 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).

Comment [AS6]: Also write the chemical analysis of the extracts

Comment [AS7]: Mention the protocol have you followed

Table 1 : Solution preparation summary

Organ	Quantity of substance + volume of solvent	Designation

	Distilled water	CT
		(CT = Control treatment)
	0.5 kg paste + 2 litres distilled water	T1
Fresh leaves	0.5 kg paste + 2 litres distilled water + 10% alcohol	T2
	0.5 kg paste + 2 litres distilled water + 20% alcohol	T3
	0.5kg of powder	T4
	0.5 kg powder + 2 litres distilled water	T5
Dry leaves (powder)	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.

Comment [AS8]: Mention when and why 2 trials were carried out

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

Filtered *Hyptis suaveolens* leaf extract (fresh or dried) was 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. 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.

Comment [AS9]: Why you have sprayed two plants after 7 days and Solanum after 10 days.

### 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 was assessed and observations were also made on the colour of the leaves and the appearance of the seedlings.

Comment [AS10]: Above you mention 10 days but 14 days Justify ?

Comment [AS11]: Mention color change and appearance help you to identification in mortality rate

### 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.

Comment [AS12]: Give reference

## 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.

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 ;  $\alpha = 0.05$ )

### 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 TM. 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).

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 ;  $\alpha = 0.05$ )

### 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 TM. 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 TM represent class 4 with a value of 0%.

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 ;  $\alpha = 0.05$ )

## 3.2. Discussion

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* 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 annuus* (Asteraceae) showed that extracts from its roots and leaves reduced seed germination, seedling development and weed dry weight (Batlang and Shushu, 2007).

#### 4. CONCLUSION

Comment [AS13]: Start it with general discussion

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.

For sustainable and environmentally friendly weed management, the use of allelopathic activities is one of the new options for the development of new biodegradable natural herbicides. However, in-depth research is needed to further confirm the herbicidal potential of *H. suaveolens* under pre-emergence and post-emergence weed conditions.

**Comment [AS14]:** There was no need to write lengthy conclusion. Write it short.

## CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

## REFERENCES

1. Marnotte, P., (1995). Utilisation des herbicides : contraintes et perspectives. Agriculture et Développement, 7 : 12-21.
2. Pell, M., Stenberg, B. and L., Torstensson (1998). Potential denitrification and nitrification tests for evaluation of pesticide effects in soil. Ambio. 27 : 24–28.
3. Konan, Y., Akanvou, L., N'Cho, S., Arouna, A., Eddy, B. and C. K. Kouakou (2014). Analyse de l'efficacité technique des riziculteurs face à l'infestation des cultures par les espèces parasites *Striga* en Côte d'Ivoire. Revue Ivoirienne des Sciences et Technologies. 23 : 212 - 223.
4. Kouadio, Y. P., Tiébré, M-S., Kouassi, R. H., Kassi, N. J. and N'Guessan K. E., (2014). Limites du désherbage chimique en bananeraies industrielles de Dabou au Sud de la Côte d'Ivoire. American Journal of Scientific Research. 101 : 64-74.

5. Dianda, W. M. (2016). Etude de l'efficacité et de la sélectivité du VEZIR 240 SL (Imazéthapyr 240g/l) contre les adventices du niébé et ses effets sur les propriétés agrochimiques du sol. Master thesis (unpublished). Option : Agronomie. Institut du Développement Rural, Université Polytechnique de Bobo-Dioulasso, Bobo-Dioulasso.
6. Islam, A. M. and H., Kato-Noguchi (2013). Allelopathic prospective of *Ricinus communis* and *Jatropha curcas* for bio-control of weeds. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, 63(8) : 731-739.
7. Ahoton, L.E., Alavo, T.B.C., Ahomadegbe, M.A., Ahanhanzo, C., and C., Agbangla (2010). Domestication du gros baume (*Hyptis suaveolens* (L.) Poit.) : Techniques de production et potentiels insectes ravageurs au sud du Bénin. *International Journal of Biological and Chemical Sciences* 4, 2010, 3 p.
8. Mishra, B. K. and B. K., Verma (1992). Flora of Allahabad district, Uttar Pradesh, India. Singh B and Singh MP Publishers, 530 p.
9. Friedman, J. (1995). Allelopathy, Autotoxicity, and germination. *In* Seed development and germination. CRC Press, Florida. 872 p
10. Dhima, K.V., Vasilakoglou, I.B. and A. S., Lithourgidis (2006). Allelopathic potential of winter cereal cover crop mulches on grass weed suppression and sugarbeet development. *Crop Science*, 46 :1682-1691.
11. Kouamé, S. (2022). Contribution à la valorisation agronomique des effets allélopathique de *Tithonia diversifolia* (Hemsl) A. Gray par l'usage des extraits hydro alcooliques comme bioherbicides. Master thesis, (unpublished), UFR Sciences biologiques, Université Peleforo Gon Coulibaly, Korhogo.
12. Arslan, M., Uremis, I., and A., Uludag (2005). Determining bio-herbicidal potential of rapeseed, radish and turnip extracts on germination inhibition of cutleaf ground-cherry (*Physalis angulata* L.) seeds. *Journal of Agronomy*, 4 :134-137.
13. Uremis, I., Arslan, M. and A. Uludag (2005). Allelopathic effects of some brassica species on germination and growth of cutleaf ground-cherry (*Physalis angulata* L.) seeds. *Journal of Biological Sciences*, 5 : 661-665.
14. Turk, M A. and A. M., Tawaha (2003). Allelopathic effect of black mustard (*Brassica nigra* L.) on germination and growth of wild oat (*Avena fatua* L.). *Crop protection* 22 : 673-677.
15. Malcolm, P.J., Holford, P., McGlasson, W.B. and S. Newman (2003). Temperature and seed weight affect the germination of peach rootstock seeds and the growth of rootstock seedlings. *Scientia Horticulturae*, 98 : 247-256.

16. Benmedour, T. (2010). Étude du pouvoir allélopathique de l'Harmel (*Peganum harmala*), le laurier rose (*Nerium oleander*) et l'ailante (*Ailanthus altissima* (Mill.) sur la germination de quelques mauvaises herbes des céréales. Thesis, (unpublished). Magister bio-physiologie végétale, Université Ferhat Abbas-Setif.
17. Zeghada, F. Z. (2009). Activité allélopathique et Analyse phytochimique. Master Thesis, Département de Biologie, Université d'Oran1, Oran (Algérie).
18. Batlang U. and Shushu D. D. (2007). Allelopathic activity of sunflower (*Helianthus annuus* L.) on growth and nodulation of bambara groundnut (*Vigna subterranea* (L) Verdc.). *Journal of Agronomy*, 6(4): 541-54.

UNDER PEER REVIEW