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Allelopathic Effect of Three Wild Plants (*Azadirachta indica*, *Tithonia diversifolia* and *Thevetia peruviana*) on Tomato (*Lycopersicum esculentum* Mill.) Growth and Stimulation of Metabolites Involved in Plant Resistance

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Abstract

The aim of this study was to determine the allelopathic effects of *Azadirachta indica* oil and aqueous extracts of *Tithonia diversifolia* and *Thevetia peruviana* on the growth and stimulation of metabolites involved in tomato plant resistance. Randomized in blocks within a shaded area, the different treatments prepared at 10% and 15% (v/v and w/v) in water were subsequently applied on tomatoes seeds to monitor the effect on germination, and on tomatoes leaves to monitor the effect on growth and resilience of the plants. The result showed that in stressful conditions all the treatments significantly inhibit ($p < 0.05$) the germination capacity of the seeds from 21.22% to 92.61%, the germination rate from 39.82% to 92.76% and the germination viability of the seedlings from 64.67% to 100%. However, the negative allelopathic effect of the treatment was significantly reduced ($p < 0.05$) when used for germination initiation by botanical priming. In addition, while *T. diversifolia* at 10% promotes a better aerial and root growth in tomato plants, *T. peruviana* at 15% induces the activation of resistance mechanisms in tomato plants by increasing protein levels to 104.5%, phenol levels to 183.33% and peroxidase enzyme

activity to 586.15%. Therefore, allelopathic compound of wild plants would be a good alternative for growth promotion and resistance of tomato crops.

Keywords

Allelopathic, Wild Plants, Growth, Plant Resistance, Tomato

1. Introduction

Tomato is a very popular and widely grown vegetable in the world. Its production takes an important place in Cameroon's agricultural economy. Indeed, it accounts for 36% of the country's total vegetable production, potentially employs 1,645,200 people and takes place in all agro-ecological basins, in both peri-urban and rural areas in all seasons [1]. However, the production of this vegetable is limited by multiple abiotic and biotic constraints that affect yields. Pest pressure has been identified as the major constraint due to crop losses to market gardeners [2] [3]. To control these pests and thus improve production yields, producers systematically use synthetic pesticides [3]. However, the results of this control method remain controversial as studies have shown the adverse effect of chemical pesticides on human health and on the environment [3] [4]. Yet, among the new crop protection technologies, the use of effective and less toxic botanical insecticides would be an alternative to the use of synthetic pesticides in the control of insect pests [5] [6]. Therefore, plant extracts which are environmentally friendly [7] could be a good substitute. The aim of this study was to evaluate the benefit of an ecological approach of fighting pests on tomatoes cultures.

Among the candidate pesticide plants, *Azadirachta indica* A. Juss, *Tithonia diversifolia* (Hemsl.) A. Gray and *Thevetia peruviana* (Pers.) K. Schum can claim to have been the subject of numerous scientific publications confirming their extraordinary biological activity. Native to tropical America, *T. diversifolia* is an invasive and recolonizing plant species belonging to the Asteraceae family [8]. It is known for its fertilizing properties; studies have shown its positive impact on production yields [9] [10] [11]. Moreover, it is rich in secondary metabolites including alkaloids and phenolic compounds [12]. Like the latter, *A. indica* has been the subject of much scientific research. This plant originating from the Indian subcontinent and belonging to the family of Meliaceae is much known for its insecticidal properties [13] [14]. Indeed, it acts in the insect by provoking lack of appetite, disturbance of the hormonal cycle, and by preventing the normal development and the optimal growth [15] [16]. As for *T. peruviana*, it is an ornamental plant native to Central and South America and belonging to the Apocynaceae family [17]. All parts of this plant are considered a potential source of biologically active compounds with insecticidal [18], fungicidal [19] [20] and bactericidal [21] action. The understanding of the link between the raw extracts

of these three wild plants and the different stages of growth and development of tomato can help to elucidate the conditions of their use in agriculture.

Indeed, the positive allelopathic properties of wild plants have been demonstrated on seed germination [22], plant growth [23], pest control [18] [24] and microorganisms responsible for some plant diseases [20] [25]. Thus, the objective of this study was to determine the allelopathic effects of *A. indica* oil and aqueous extracts of *T. diversifolia* and *T. peruviana* on the growth and metabolites production involved in tomato plant resistance. The effect of the total biochemical constituents of these three wild plants will be determined on the germination capacity, germination rate, germination viability of seedlings, aerial and root growth of the plants, and induction of increased synthesis of phenols, proteins, peroxidases and polyphenol oxidases in tomato plants in a shade area.

2. Material and Methods

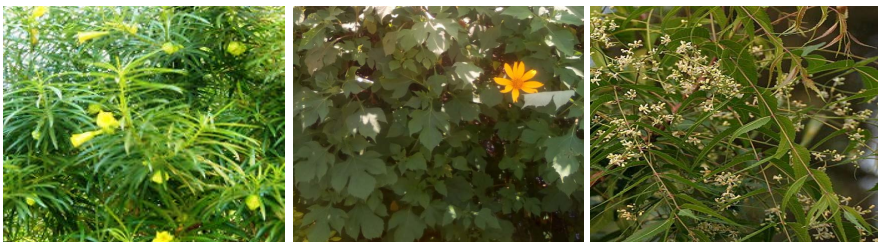
The study was carried out in the shade area of the local company (EcoAgriConsulting and Solution for Africa, Obili-Yaoundé, Cameroon) during September-December, 2018. The laboratory experimentation was conducted in the Biotechnology Centre of the University of Yaoundé I, Nkolbisson, Cameroon.

2.1. Plant Material

The tomato seeds (Rio master variety) were purchased at a local company (Royal Master, Cameroon). Fresh leaves of *Tithonia diversifolia* (773 m, 03˚51'28.4"N, 11˚30'00.3"E) and *Thevetia peruviana* (747 m, 03˚51'29.5"N, 11˚29'52.3"E) were collected at the University of Yaoundé I. *Azadirachta indica* oil, cold extracted from the kernel of the seeds, was purchased at a local market (Mokolo, Yaoundé) (**Figure 1**).

2.2. Preparation of Plants Extracts

Fresh leaves of *T. diversifolia* and *T. peruviana* were harvested, washed three times using tap water, and then cut into small chips of about 4 cm of length. Finally, there were macerated at concentrations of 0.1 and 0.15 kg/L of water (w/v) for six days. Following maceration, the solutions were filtered with fine mesh cloth sieve. The 10% and 15% solutions of *A. indica* oil were obtained by emulsifying 0.1 and 0.15 L/L of water (v/v) respectively.



(a) (b) (c)

Figure 1. *Thevetia peruviana* (a), *Tithonia diversifolia* (b) and *Azadirachta indica* (c) leaves and flowers.

2.3. Germination Test

Two seeds lots were primed for 1 h and 3 h into the different extracts by total immersion. After botanical priming, seeds were re-dried to near original weight under shade and then germinated in 120 × 120 mm Petri dishes (16 in each) containing three layers of absorbent paper soaked in 10 ml of distilled water. A seed not subjected to this pretreatment was germinated in the presence of 10 ml of each extract (stressful conditions). Three trials were done with ten Petri dishes per trial. The Petri dishes were incubated in the dark at room temperature and observed daily for six days. A seed was considered to have germinated when the radical pierced the coat according to [26]. Germination capacity, germination rate and germination viability were calculated according to the following equations as:

Germination capacity:

$$\mathrm{GC} = \left(ni/N\right) \times 100$$

where:

ni = *Cumulative number of seeds germinated at each observation i,*

N = *Total number of seeds germinated.*

Germination rate:

$$\mathrm{GR} = \left(N1 \times 1\right) + \left(N2 - N1\right)1/2 + \left(N3 - N2\right)1/3 + \cdots + \left(Nn - Nn - 1\right)1/n$$

where:

$N1$, $N2$, $N3$, $\cdots$, $Nn-1$, Nn = *percentage of seeds germinated on day* 1, 2, 3, $\cdots$, $n-1$ *and n.*

Germination viability:

$$\mathrm{GV} = \left(Np/Ng\right) \times 100$$

where:

Np = *Number of seeds that have formed a complete seedling after germination,*

Ng = *Number of germinated seeds.*

2.4. Evaluation of Agro-Morphological Growth Characters

The agro-morphological characters of plant growth were measured every two weeks, starting from the fourth week after transplanting, and stopped at the beginning of flowering. These parameters included stem diameter measured with a caliper, the number of leaves counted by hand, plant height taken with a ruler and leaf area determined using the following formula by [27]:

$$\mathrm{LA} = 0.5\left(L \times W\right)$$

where:

L = *length of leaf,*

W = *maximum width.*

At the beginning of flowering, three plants per treatment were collected and after washing the roots with water to remove all soil particles, the plants were

fractionated, and the fresh and dry root and aerial weights were measured. Dehydration took place at 80˚C in the incubator for 48 h.

2.5. Determination of the Total Phenolic Compounds Content

The extraction and quantitative measurement of the content of total phenolic compounds were carried out according to a modified protocol developed by [28]. Briefly, 1 g of fresh leaves was crushed at 4˚C in 10 ml of 80% methanol; the homogenate was agitated for 10 min, and then centrifuged at 4˚C three times at 10,000 g for 10 min. The recovered supernatants were mixed, and the pellets obtained were re-suspended three times in 5 ml of 80% methanol followed by a 5 min agitation. After the second centrifugation step at 4˚C, the supernatant was collected and mixed with the previously collected supernatant to constitute the phenolic extract. The concentration of phenolic compounds was determined by the method of [29] using the Folin-Ciocalteu reagent. Absorbance was measured at 725 nm. Total phenolic compound contents were expressed in mg equivalent of catechin per g of fresh weight (mg eq catechin/g FW).

2.6. Determination of the Content of Total Protein

The total native protein content extraction was performed as described by [30] with modification. Briefly, 1 g of fresh leaves was grounded in 10 ml of extraction buffer (Tris-HCl 10 mM pH 7.5, Triton X-100 2%, NaCl 3 M) at 4˚C, stirred for 10 min and kept on ice. After 30 min incubation at 4˚C, the sample was centrifuged at 10,000 g for 30 min at 4˚C. The pellet was submitted to a second round of extraction. Both supernatants were mixed with 0.5 volume of n-butanol and 1/10 of 3 M NaAc pH 4.5. Samples were kept on ice for 1h with agitation every 10 min, and centrifuged twice at 10,000 g for 15 min at 4˚C. The supernatants were mixed to form the total proteins extract and stored at 4˚C. The quantification of total proteins was performed according to the [31] method using BSA as the standard. The absorbance was measured at 595 nm using a UV-VIS 1605 Shimadzu spectrophotometer. The amount of total proteins was expressed as mg of BSA equivalent per g of fresh weight (mg eq BSA/g FW).

2.7. Determination of Enzyme Activities

The peroxidase specific activity (POX) expressed as enzyme per g of fresh matter using spectrophotometer at 470 nm (A_{470}/min/UE/g FW) of the total native proteins extract was determined by the method of [32]. Similarly, the polyphenol oxidase (PPO) specific activity expressed as enzyme per g of fresh weight at 330 nm (A_{330}/min/EU/g FW) of the total native proteins extract was described by [33] using catechol as a substrate.

2.8. Statistical Analysis

Data analysis was performed using Graphpad Prism 5.0. All results were expressed as means ± standard deviation and subjected to Analysis of Variance (ANOVA). Where significant differences were found, pairs of samples were

compared using Tukey's test at $p < 0.05$.

3. Results

3.1. Germination Performance of Seeds

Under stressful conditions, all extracts reduced the germination capacity, germination rate and germination viability of tomato seedlings (Table 1). The lowest value of germination capacity was obtained with *T. diversifolia* at 10%, Td1 (6.25%) which otherwise did not allow the formation of viable seedlings, as *T. diversifolia* at 15% (Td2) did and both concentrations of *A. indica* (Ai1 and Ai2). However, the negative effect of the extracts was reduced when used for germination initiation by botanical priming. *T. diversifolia* at 10% from the 1h botanical priming was the extract that shows the highest values for all the germination characteristics evaluated.

Table 1. Variation of germination index of treated and untreated seeds with different plants extracts.

	Stress condition	1 h botanical priming	3 h botanical priming
	Germination capacity, GC (%)		
C	84.58 ± 0.92^{f}	100.0 ± 0.00^{a}	90.25 ± 0.38^{de}
Td1	06.25 ± 0.00^{m}	100.0 ± 0.00^{a}	87.50 ± 0.00^{ef}
Td2	23.96 ± 1.04^{k}	81.25 ± 0.00^{g}	93.75 ± 0.00^{bc}
Tp1	66.63 ± 1.06^{i}	94.17 ± 0.42^{bc}	93.67 ± 0.08^{bc}
Tp2	37.50 ± 0.00^{j}	95.46 ± 0.91^{b}	86.75 ± 0.75^{f}
Ai1	18.75 ± 0.00^{l}	86.75 ± 0.75^{f}	91.62 ± 1.06^{cd}
Ai2	18.75 ± 0.00^{l}	76.33 ± 0.88^{h}	80.83 ± 0.41^{g}
	Germination rate, GR		
C	4.42 ± 0.35^{fgh}	6.16 ± 0.51^{ab}	4.79 ± 0.14^{efgh}
Td1	0.32 ± 0.12^{k}	6.34 ± 0.29^{a}	5.91 ± 0.29^{abc}
Td2	0.83 ± 0.14^{jk}	5.03 ± 0.09^{defg}	5.36 ± 0.05^{bcde}
Tp1	2.66 ± 0.12^{i}	6.21 ± 0.63^{ab}	5.05 ± 0.26^{cdefg}
Tp2	1.30 ± 0.26^{j}	5.81 ± 0.15^{abcd}	5.19 ± 0.12^{cdef}
Ai1	0.83 ± 0.12^{jk}	4.39 ± 0.28^{fgh}	3.95 ± 0.08^{h}
Ai2	0.65 ± 0.00^{jk}	5.46 ± 0.52^{bcde}	4.23 ± 0.25^{gh}
	Germination viability, GV (%)		
C	59.44 ± 0.96^{f}	82.37 ± 1.95^{b}	70.95 ± 0.83^{d}
Td1	00.00 ± 0.00^{l}	86.96 ± 0.90^{a}	65.08 ± 1.37^{e}
Td2	00.00 ± 0.00^{l}	76.61 ± 0.53^{c}	78.97 ± 1.78^{bc}
Tp1	21.00 ± 1.73^{j}	60.83 ± 1.44^{f}	72.70 ± 1.10^{d}
Tp2	20.00 ± 0.00^{j}	61.39 ± 1.92^{ef}	77.47 ± 0.95^{c}
Ai1	00.00 ± 0.00^{l}	51.11 ± 1.92^{g}	15.33 ± 1.77^{k}
Ai2	00.00 ± 0.00^{l}	42.57 ± 0.50^{h}	34.92 ± 1.37^{i}

Means with the same letter are not significantly different at $p < 0.05$. C: control; Td1 (Td2): *T. diversifolia* at 10% (15%); Tp1 (Tp2): *T. peruviana* at 10% (15%); Ai1 (Ai2): *A. indica* at 10% (15%).

3.2. Agro-Morphological Characteristics

The effect of foliar application of the treatments on root and aerial growth of tomato plants is presented in Table 2. Plants treated with *T. diversifolia* at 10% (Td1) showed the highest values for volume (17.83 ± 0.6 ml), fresh weight (21.18 ± 0.83 g/plant) and root dry weight (3.39 ± 0.11 g/plant), while those treated with *T. peruviana* at 10% (Tp1) showed the highest value for root length (45.33 ± 0.88 cm). Also, regardless of the aerial growth characteristic considered, *T. diversifolia* at 10% had the highest values.

3.3. Phenols and Proteins Contents

The total protein content in treated plants (5.16 to 6.81 mg eq BSA/g FW) was higher than that of untreated plants (3.33 ± 0.25 mg eq BSA/g FW) (Figure 2). However, increasing the concentration of *T. diversifolia* and *A. indica* reduced their eliciting capacity on protein synthesis by 20.54% and 17.82% respectively, while increasing the concentration of *T. peruviana* boosts its effect by 18.64%. Also, *T. diversifolia* at 15%, Td2 (0.23 ± 0.03 mg eq catechin/g FW) and *A. indica* at 15%, Ai2 (0.14 ± 0.02 mg eq catechin/g FW) did not significantly affect phenol levels in tomato plants compared to control (0.18 ± 0.004 mg eq catechin/g FW) (Figure 3). However, the highest value of phenol content was observed in plants treated with *T. peruviana* at 15%, Tp2 (0.51 ± 0.02 mg eq catechin/g FW) which increased this biochemical character to 183.33% compared to the control.

Table 2. Effect of treatments on agro-morphological characteristics of tomato plants.

	Root characteristics			
	RL (cm)	V (ml)	FRW (g)	DRW (g)
C	19.33 ± 0.33^{d}	7.50 ± 0.5^{d}	5.67 ± 0.58^{e}	1.1 ± 0.1^{d}
Td1	38.67 ± 0.88^{b}	17.83 ± 0.6^{a}	21.18 ± 0.83^{a}	3.39 ± 0.11^{a}
Td2	22.33 ± 0.88^{cd}	14.83 ± 0.44^{b}	15.01 ± 0.89^{bc}	2.39 ± 0.19^{bc}
Tp1	45.33 ± 0.88^{a}	15.50 ± 0.29^{ab}	14.31 ± 0.24^{c}	2.53 ± 0.13^{bc}
Tp2	20.50 ± 0.29^{cd}	11.67 ± 0.88^{c}	16.80 ± 0.28^{b}	2.9 ± 0.1^{ab}
Ai1	20.33 ± 0.88^{cd}	7.67 ± 0.17^{d}	6.84 ± 0.76^{e}	1.82 ± 0.08^{c}
Ai2	24.33 ± 0.67^{c}	09.83 ± 0.17^{cd}	10.09 ± 0.88^{d}	2.55 ± 0.05^{bc}
	Arial characteristics			
	Ø (cm)	H (cm)	NL	LA (cm^2)	AFW (g)	ADW (g)
C	0.53 ± 0.05^{c}	51 ± 1.68^{c}	11 ± 0.41^{d}	428.28 ± 7.31^{b}	74.18 ± 4.48^{de}	40.92 ± 0.72^{c}
Td1	0.88 ± 0.05^{a}	112.5 ± 1.44^{a}	20.75 ± 0.48^{a}	676.31 ± 9.1^{a}	132.09 ± 1.59^{a}	63.67 ± 2.37^{a}
Td2	0.88 ± 0.05^{a}	110.5 ± 0.5^{a}	20.5 ± 0.65^{a}	655.38 ± 7.21^{a}	108.75 ± 1.25^{b}	58.35 ± 0.95^{ab}
Tp1	0.88 ± 0.05^{a}	96 ± 0.41^{b}	20.5 ± 0.5^{a}	617.41 ± 32.89^{a}	91.58 ± 1.18^{c}	52.51 ± 2.01^{b}
Tp2	0.83 ± 0.05^{ab}	104 ± 2.45^{ab}	20.25 ± 0.63^{ab}	720.97 ± 12.51^{a}	80.25 ± 0.35^{cd}	53.31 ± 1.21^{b}
Ai1	0.7 ± 0.08^{b}	95 ± 3.86^{b}	16.5 ± 0.65^{c}	513.03 ± 33.6^{b}	60.95 ± 1.65^{e}	39.06 ± 1.39^{c}
Ai2	0.83 ± 0.09^{ab}	92.75 ± 4.82^{b}	18 ± 0.58^{bc}	497.94 ± 11.42^{b}	86.35 ± 1.65^{c}	51.11 ± 1.31^{b}

Means with the same letter within a column are not significantly different at $p < 0.05$. C: control; Td1 (Td2): *T. diversifolia* at 10% (15%); Tp1 (Tp2): *T. peruviana* at 10% (15%); Ai1 (Ai2): *A. indica* at 10% (15%), RL: root length, V: volume, FRW (DRW): fresh (dry) root weight, Ø: diameter, H: plant height, NL: number of leaves, AFW (ADW): aerial fresh (dry) weight.

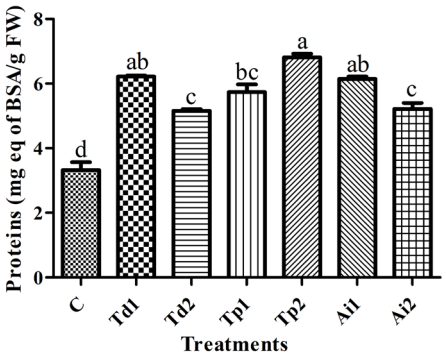


Figure 2. Variation of total native protein content in plant treated and untreated with different plants extracts. Means with the same letter are not significantly different at $p < 0.05$. C: control; Td1 (Td2): *T. diversifolia* at 10% (15%); Tp1 (Tp2): *T. peruviana* at 10% (15%); Ai1 (Ai2): *A. indica* at 10% (15%).

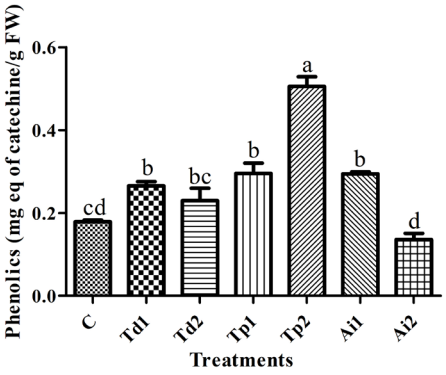


Figure 3. Variation of total phenolic content in plant treated and untreated with different plants extracts. Means with the same letter are not significantly different at $p < 0.05$. C: control; Td1 (Td2): *T. diversifolia* at 10% (15%); Tp1 (Tp2): *T. peruviana* at 10% (15%); Ai1 (Ai2): *A. indica* at 10% (15%).

3.4. Enzymatic Activities

The effects of foliar application of the treatments on the enzymatic activity of peroxidase (POX) and polyphenol oxidase (PPO) are shown in Figure 4 and Figure 5. With the exception of *A. indica* at 15%, Ai2 (POX activity = 1.18 ± 0.04 A_{470}/min/UE/g FW; PPO activity = 0.56 ± 0.03 A_{330}/min/EU/g FW), all extracts boost the POX (Figure 4) and PPO (Figure 5) activity of total proteins in tomato plants. The best effect is obtained with *T. diversifolia* at 10% (Td1) and *T. peruviana* at 15% (Tp2) for POX activity and with *A. indica* at 10% (Ai1) for PPO activity.

4. Discussion

The purpose of this study was to determine the allelopathic effects of *A. indica* oil and aqueous extracts of *T. diversifolia* and *T. peruviana* on the growth and induction of metabolites involved in tomato plant resistance. The results of the germination test showed that all extracts had a significant allelopathic effect ($p < 0.05$) on the germination performance of tomato seeds. Indeed, under stressful

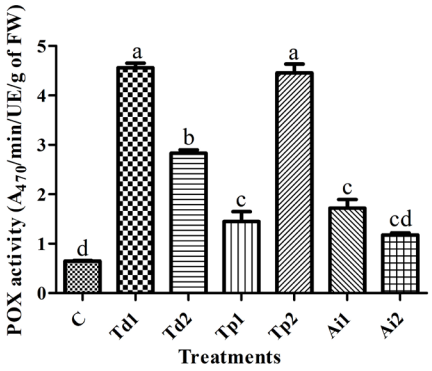


Figure 4. Variation of total peroxidases activities in plant treated and untreated with different plants extracts. Means with the same letter are not significantly different at $p < 0.05$. C: control; Td1 (Td2): *T. diversifolia* at 10% (15%); Tp1 (Tp2): *T. peruviana* at 10% (15%); Ai1 (Ai2): *A. indica* at 10% (15%).

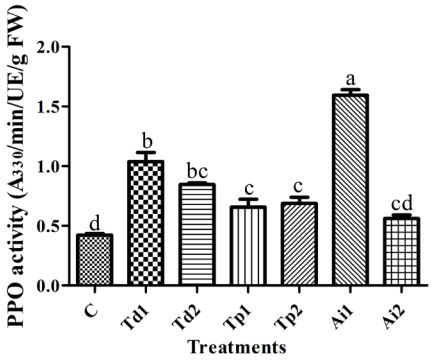


Figure 5. Variation of total polyphenol oxidases activities in plant treated and untreated with different plants extracts. Means with the same letter are not significantly different at $p < 0.05$. C: control; Td1 (Td2): *T. diversifolia* at 10% (15%); Tp1 (Tp2): *T. peruviana* at 10% (15%); Ai1 (Ai2): *A. indica* at 10% (15%).

condition, the extracts showed a phytotoxic effect evaluated from 21.22% to 92.61% on the germination capacity, from 39.82% to 92.76% on the germination speed and from 64.67% to 100% on the germination viability of the seedlings. Likewise, studies have shown the inhibitory effect of *T. diversifolia* on the germination of seeds of *Solanum melongena* L. [34], *Vigna inguiculata* L. [23] and *Oryza sativa* L. [35]. In addition, the phytotoxic effect of *T. peruviana* was observed on the germination of *Parthenium hysterophorus* L. [36] and those of *A. indica* were reported on several varieties of *Vigna unguiculata* L. [37]. Similarly, the negative allelopathic effects of crude extracts of other wild plants have been cited in numerous studies. For example, the phytotoxic effect of crude extracts of *Cakile maritima*, *Calligonum polygonoides*, *Senecio glaucus* and *Zygophyllum album* has been observed on seeds of *Echinochloa crus-galli* (L.) P. Beauv [38]. However, in the present study the use at 10% and 15% of *T. diversifolia*, *A. indica* and *T. peruviana* for germination initiation by botanical priming had a stimulating effect on the germination performance of tomato seeds compared to their use in stressful conditions. This ability of the extracts can be explained by

the fact that during re-dehydration (hardening), they induce the production and activation of the enzymes involved in the lifting of dormancy. In fact, according to [39], priming stimulates the activation of endo-β-mannase, which in turn triggers the production of ethylene, a hormone involved in the lifting of seed dormancy. Moreover, [40] suggested that this pre-germination operation allows an increase in the level of antioxidant enzymes, while [41] proposed instead an improvement and synchronization of DNA replication in all embryonic cells. According to [42] this pre-activation of the cell cycle is one of the mechanisms by which priming induces better germination performance. Thus, the results of this study show that the type of allelopathic effect exerted by *A. indica* oil and aqueous extracts from fresh leaves of *T. diversifolia* and *T. peruviana* on the germination performance of tomato seeds depends on the condition of application of the latter on the seeds (before or during germination).

Determination of the effect of extracts on the growth of tomato plants showed that all the growth variables analyzed are significantly affected ($p < 0.05$) by them. However, the overall analysis of the different growth parameters evaluated shows that *T. diversifolia* at 10% allowed the best root growth of tomato plants. Surely, it boosts root volume by 137.73%, root fresh weight by 273.54% and root dry weight by 208.18% compared to the control. In addition, this treatment also boosts stem diameter by 66.04%, plant height by 120.59%, leaf count by 88.64%, leaf area by 57.91%, fresh aerial weight by 78.07% and dry aerial weight by 55.6%. This positive allelopathic effect of *T. diversifolia* on tomato plant growth can be explained by the fact that this wild plant is an important provider of nutrients for phosphorus, potassium and nitrogen [9] [10] [11]. Indeed, many authors reported an increase in aerial and root growth of *Vigna sinensis* L. [43], *Amaranthus spinosus* L. [22], *Vigna unguculata* L. [23] [44] and *Zea mays* L. [45] due to *T. diversifolia* biomasses. While *T. diversifolia* has an inhibitory effect on tomato seed germination under stressful conditions, it does boost plant growth after seedling establishment. This suggests that the allelopathic effect of *T. diversifolia* observed depends on the stage of development of the tomato.

Determination of the effect of different extracts on the induction of the synthesis of biochemical resistance factors in tomato plants showed that *T. peruviana* at 15% is the extract with the most significant positive allelopathic effect ($p < 0.05$). In fact, it increases the protein rate to 104.5%, the phenol rate to 183.33% and the peroxidase activity of proteins in tomato plants to 586.15%. This over-expression of phenolic compounds and peroxidases due to *T. peruviana* at 0.15 kg/L (15%) may be correlated with increased resistance in tomato plants. Among the proteins induced during plant defense, peroxidases are well known and play a major role in reinforcing the physical barriers of the cell wall [46] and increasing phytoalexin synthesis [47]. As for phenolic compounds, they are recognized as constituent elements of plant defense mechanisms against pathogenic microorganisms. The mode of action of these pre-infectional molecules, whose content may increase with infection, might be related to their antimicrobial properties, their involvement in strengthening plant cell walls and

their ability to modulate and induce host defense reactions [48]. Thus, it appears from this study that the 0.15 kg/L extract of *T. peruviana* has a high elicitation power of tomato defense mechanisms, involving the increase of total proteins, total phenolic compounds and the peroxidase activity of total proteins. However, with the exception of *A. indica* at 10%, all extracts stimulated the polyphenol oxidase activity of total proteins in the range of 57.14% to 278.57% compared to the control. The involvement of this enzyme in plant defense had been demonstrated in numerous studies. As a telling example, [49] found that tomato plants over-expressing potato polyphenol oxidase showed increased resistance to *Pseudomonas synringae* pv. tomato, the causal agent of bacterial spot disease. Similarly, [50] showed that over-expression of this enzyme increased the resistance of transgenic poplars against herbivores. Thus, in this study, it appears that except for *A. indica* at 10%, all the extracts elicit the polyphenol oxidase biosynthesis pathway in tomato plants.

This study demonstrated that *T. diversifolia* crude extract at 0.1 kg/L (10%) of can be used by tomato growers to improve the germination performance of the seed (by botanical priming) and growth of tomato plants, while the crude extract at 0.15 kg/L (15%) of *T. peruviana* can be used for the induction of tomato plant resistance. However, it would be interesting before a possible use of these extracts in agriculture, to quantify the sustainability of their effects in the field and to elucidate the molecular mechanisms underlying their action in the different phases of tomato cultivation.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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