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Trial of the use of aqueous extracts based on *Artemisia annua L* in the protection of soilless tomato crop (*Solanum lycopersicum L.* 1753) in Daloa (Central-Western Côte d'Ivoire)

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Abstract

Tomato (*Solanum lycopercicum L*) is one of the most cultivated crops in Côte d'Ivoire. However, its production is confronted with enormous constraints, especially the damage caused by pathogens and vectors of certain diseases. Faced with this damage, most growers resort to chemical products that are harmful to the environment, the user and consumers. Therefore, it becomes necessary to use the extract of Artemisia annua as a biopesticide in the control of diseases and pests of tomato crop in the field. The trial consisted in setting up an above ground tomato crop and then formulating a biopesticide based on *Artemisia annua L* at different doses (100% pure filtrate; 75% diluted filtrate; 50% and 25%) and applying it on the crop. It appears from this test that the biological extract based on *Artemisia annua L* , proved to be an insecticide or an insect repellent and biostimulator. Moreover, the dose of 100% of the pure extract of *Artemisia annua L* was the best on the agromorphological parameters and on the pathogenicity parameters.

Keywords: Tomato; *Artemisia annua L*; Biopesticide; Pathogen

1. Introduction

Agriculture is one of the main sectors of activity that contributes to the socio-economic development of populations. Among agricultural crops, tomato (*Solanum lycopersicum L.*) is one of the most produced vegetable speculations in the world both in the field and in vegetable gardens [1]. According to FAOstat, the world production of tomato from 1961 to 2018 has experienced a strong growth, it has increased from 27.6 million tons to 182.3 million tons. In Côte d'Ivoire, its production in 2018 is 44078 million tons [2]. In addition to its economic importance, the food importance and the many therapeutic virtues it contains make it the first most cultivated vegetable-fruit in the world [3]. However, the development of tomato cultivation in our tropical regions is faced with enormous difficulties. During its vegetative cycle, the tomato is continuously attacked by numerous pests and diseases causing damage to the different organs of the plant and at various stages of their growth and development. Thus, the high pest pressure, the influence of abiotic factors and rudimentary agricultural practices strongly reduce tomato production [4]. Of all these constraints, high pest pressure has been identified as the major constraint due to the crop losses inflicted on vegetable farmers [5]. Thus, to fight against these constraints, most growers resort to synthetic chemical products (insecticides, fungicides, etc.), while these products have a negative effect on the environment and human health. In order to reduce the negative impacts induced by the use of these products, it is important to turn to another form of control, namely biological control, which is more respectful of the environment and human health. It is from this context that was drawn the present topic which has for general objective to contribute to the optimization of the production of tomato by the use of aqueous extract of *Artemisia*

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annua L in the fight against the diseases and the pests of tomato in plantation. More specifically, the aim was to evaluate the damage caused by insect pests and pathogens of tomato and then to evaluate the bio-stimulatory effect of the aqueous extract of *Artemisia annua L* on the growth parameters of tomato in plantation.

2. Material and methods

2.1. Study site

This study was conducted at the University Jean Lorougnon Guédé (UJLoG) of Daloa (between 6°53 North latitude and 6°27 West longitude) in Côte d'Ivoire (Figure 1).

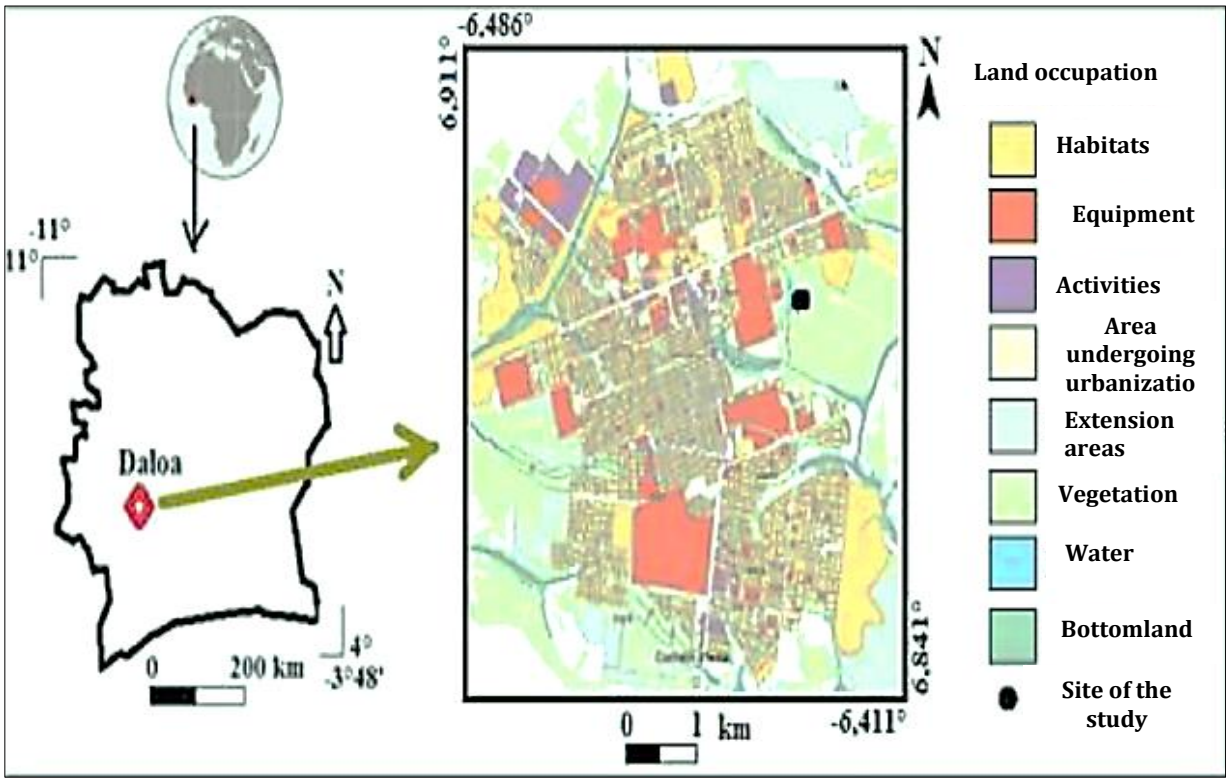


Figure 1 Location of the study site (Yobouet, 2021)

2.2. Plant material

The plant material consisted of hybrid tomato seeds of the F1 variety COBRA 26 and fresh leaves of *Artemisia annua L* provided by the Laboratory of Improvement of Agricultural Production of the University Jean Lorougnon Guédé Daloa.

2.3. Technical and chemical equipment

The technical equipment used in this study consisted of: a daba, a walker, a rake, an artisanal carbonizer, culture bags, a tape measure, a caliper, a graduated ruler, alveoli, a blender, a precision balance and a graduated cylinder. The chemical material used was banko D, a synthetic systemic fungicide.

2.4. Methods

2.4.1. Preparation of the culture substrate

Substrate formulation

The formulated substrate was a mixture of 50% soil and 50% carbonized rice husks, supplemented with chicken droppings.

Carbonization of rice husks

Carbonization of rice husks was done in three main steps and can take 4-8 h [6]:

- Step 1: First, it was a matter of making fire with a pile of wood. Then covered it with a carbonizer and then packed around the carbonizer the rice husks to be carbonized and let the combustion take place (Figure 2 A).
- Step 2: Secondly, ensure that the flame is activated by adding wood as needed (Figure 2 B).
- Step 3: At the end of combustion, the fire was extinguished by spraying water and allowed to cool for 20-30 minutes before collecting the charred rice husks in bags (Figure 2 C).

Figure 2 Carbonization process (JPG file, magnification 3456 x 4608)

2.4.2. Setting up the nursery

Construction of the greenhouse

The greenhouse shelter was constructed using tree trunks surrounded by mosquito netting. The top was covered with a transparent plastic to avoid the leaching of seeds by rainwater and to protect the plants from the sun (Figure 3).

Figure 3 Greenhouse (JPG File Magnification 3456 x 4608)

Cell filling, seeding and maintenance

Prior to planting, two 72-hollow honeycomb trays were filled with a mixture of 50% soil and 50% charred rice husks. Then, a tomato seed was sown in the hollows of the cells containing the substrate followed by a watering. After sowing, the seedlings were placed under the shade at a distance of 10 cm. Maintenance consisted of regular watering of the nursery for 21 days with additional chicken droppings. One week after germination the shade was gradually reduced to allow the seedlings to adapt to the climatic conditions of the study area.

2.4.3. Setting up of the plantation

It consisted in clearing with a walker and weeding with a daba of an area of 195 m2 in order to obtain a totally bare surface. Then a black plastic was placed on the ground to prevent the plot from becoming weedy.

In addition, growing media were made from black polyethylene bags filled with 50% soil and 50% carbonized rice husks and then supplemented with chicken droppings (Figure 4). The substrates were labeled according to the different treatments to be applied.

Figure 4 Filling the culture bags (JPG file, magnification 3456 x 4608)

2.4.4. Preparation of the aqueous extract of Artemisia annua L

The fresh leaves (200 g) of *Artemisia annua L* were used to prepare the aqueous extract. After rinsing with tap water, they were ground with a blender. The resulting grind was macerated in 4 liters of water and then stored at room temperature (25°C). After 24 hours of fermentation, the resulting solution was filtered through a white cloth (Figure 5) [7]. Then, 40 ml of liquid soap was added to the obtained filtrate. One liter (1 L) of the obtained solution was taken as undiluted stock solution (100%). On the other hand, the other 3 liters were each diluted to 75%; 50% and 25% respectively.

a b C

A: Mixer containing fresh leaves of *Artemisia annua L;* B: Filtration of the aqueous extract; C: Solution of Artemisia in jars

Figure 5 Preparation of *Artemisia annua L* solution (JPG file, magnification 3456 x 4608)

2.4.5. Treatments

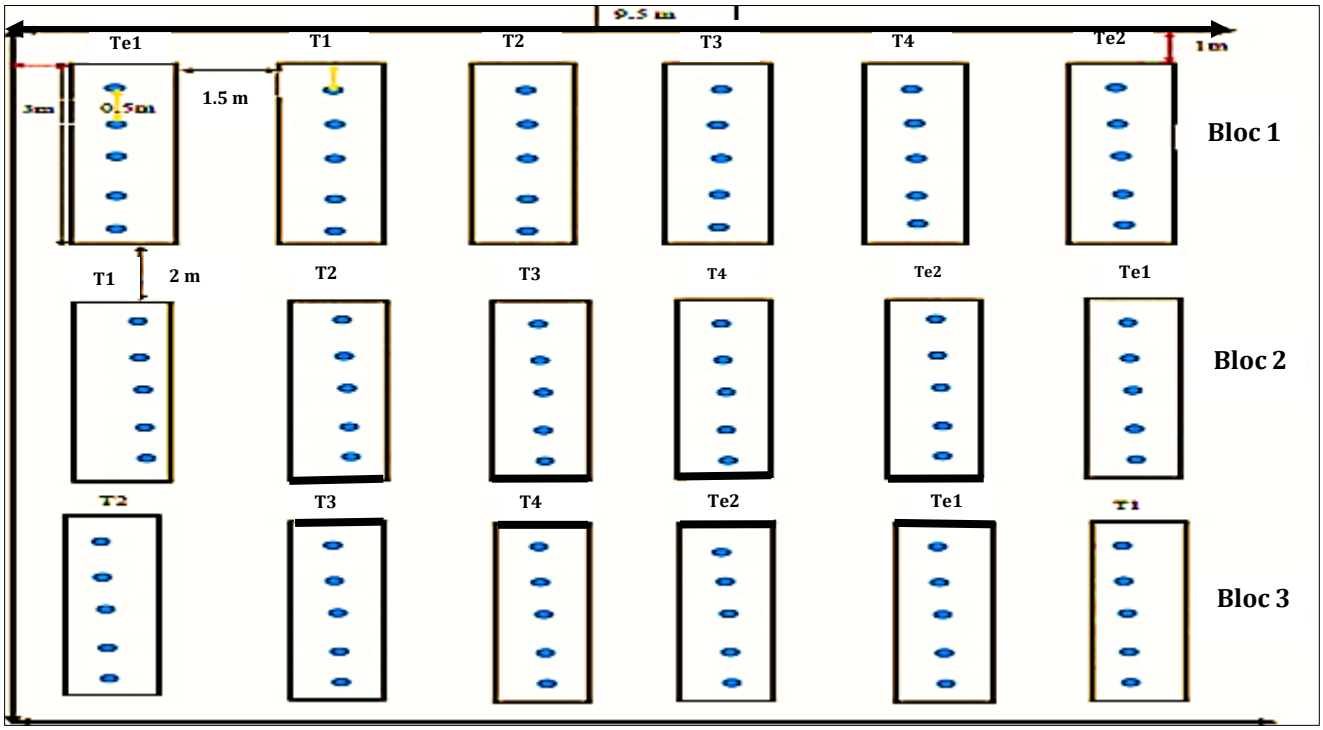
During the experiment, the treatments used were:

- Control 1: no treatment
- Control 2: banko D
  - T1: Undiluted *Artemisia annua L* extract;

- T2: Extract of *Artemisia annua L* diluted to 75%;
- T3: Extract of *Artemisia annua L* diluted to 50%;
- T4: Extract of *Artemisia annua L* diluted to 25 %.

2.4.6. Experimental device

The design used was a completely randomized Fischer block with 3 replicates and 6 treatments per sub-block. The sub-blocks were 2 m apart and composed of 6 elementary plots 1.5 m apart. Each elementary plot had 5 tomato plants with a row spacing of 0.5 m (Figure 6).



- Control 1 (Te1): No treatment; T2: Artemisia annua extract diluted to 75%; Control 2 (Te2): banko D;- T1: Undiluted *Artemisia annua L* extract; T3: Artemisia annua extract diluted to 50%; T4: Artemisia annua extract diluted to 25%

Figure 6 Diagram of the experimental set-up

2.4.7. Transplantation

The tomato plants, vigorous with 4 to 5 leaves and 16 cm high, were transplanted in the different substrates 21 days after sowing. Maintenance consisted of regular weeding and watering of the plantation.

2.5. Maintenance and application of treatments

The maintenance consisted among other things of weeding, replacement of dead plants and watering as well as the application of treatments on the tomato plants. In order to ensure the growth of the plants, the watering was limited to one watering every two days, preferably in the evening and to avoid watering after a strong rainfall. In addition, weeding consisted of removing all weeds that appeared on the surface of the substrates by hand. Dead plants were systematically replaced after observation. In addition, to protect the crop against pests, the plants were treated once a week with aqueous extracts of *Artemisia annua L* and banko D on the aerial parts until their fructification. Banko D, a synthetic systemic fungicide, was used three times during the crop cycle at the manufacturer's recommended rate (2 L/ha).

2.6. Data collection

Daily morning and evening surveys were conducted on the plot. The purpose was to observe and record insect damage and symptoms of certain diseases due to pathogens. These were then identified using literature reviews and crop data sheets related to tomato pests and diseases. In addition, agro morphological parameters such as height of main stem, diameter at collar were measured using tape measure and caliper respectively. The number of leaves and twigs were recorded by counting.

2.7. Statistical analysis

For the analysis, two software programs were used namely Microsoft Excel 2016 for the classification of numerical data and STATISTICA Software version 7.1, used for the analysis of variance tests.

3. Results

3.1. Symptom observed on tomato plants

3.1.1. Before the application of the treatments

Before the application of the different treatments, the surveys carried out on the experimental plot allowed to observe whitish spots on the tomato leaves (Figure 7).

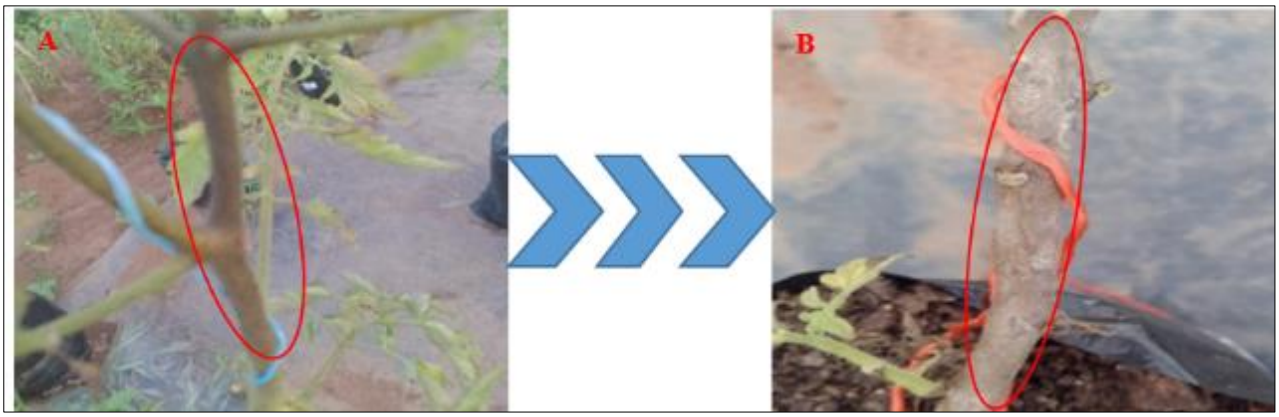
Figure 7 Whitish spots on the leaflets of the tomato leaf (JPG File Magnification 3456 x 4608)

3.1.2. During the application of the treatments

Figures 8 and 9 represent the symptoms observed on the tomato plants during the application of the treatments. These are the brown spots on the leaves and stems. The analysis of figures 8 and 9 shows that the development of brown spots on the leaves surrounded by a yellow halo and then those on the stems of the plants caused the drying of the leaves and stems (Figure 8 and 9).

A: Brown spots surrounded by a yellow halo on the leaves; B: Desiccated tomato leaves

Figure 8 Symptoms observed on tomato leaves (JPG File Magnification 3456 x 4608)



A: Brown spot on the stem; B: Desiccated tomato stem

Figure 9 Symptom of stem browning (JPG File Magnification 3456 x 4608)

3.2. Effect of treatments on the observed symptoms

3.2.1. Effect of treatments on whitish spots

Table 1 Effect of treatments on whitish spots observed on tomato plants

Treatments	Incidence (p c)
Control 1	93.33 ± 5.77^{c}
Control 2	76.00 ± 1.73^{b}
T1	33.00 ± 5.19^{a}
T2	71.33 ± 2.3^{b}
T3	66.36 ± 1.15^{b}
T4	49.00 ± 6.92^{ab}
P-value	0.0001

In the same column, numbers with the same letters are statistically identical. Control 1: No treatment; Control 2: Banco D; T1: Pure filtrate; T2: Filtrate diluted to 75%; T3: Filtrate diluted to 50%; T4: Filtrate diluted to 25%

Table 1 shows the incidence of whitish spots on tomato plants according to treatments. The analysis of Table 1 reveals hat the highest incidence is observed in the Control 1 treatment (93.33%). The same is true for Control 2 (76%). On the other hand, treatment T1 had the lowest incidence (33%) compared to treatments T2, T3 and T4 respectively 71.33%, 66.36% and 49%. However, statistical analysis revealed a highly significant difference between treatments with probability P = 0.0001. Therefore, it is appropriate to say that undiluted *Artemisia annua L* extract reduces the presence of whitish spots on the leaves of tomato plants.

3.2.2. Effect of treatments on leaf browning

The results of tomato leaf rustling according to treatments are recorded in Table 2 below. Observation of Table 2 shows that Control 2 (73.33%) and T4 (73.33%) treatments induced the same effects. The same is true for Control 1, T1, T2 and T3 treatments, whose respective values of 80.94%, 78.33%, 80% and 80.33 are statistically identical. The statistical analysis revealed that there was no significant influence between treatments (P = 0.186). From this analysis, it was found that there was no significant difference between the treatments with *Artemisia annua L* extract and the chemical treatment (banko D). However, *Artemisia annua L* extract diluted to 25% and banko D better protected tomato plants from leaf browning.

Table 2 Incidence of tomato leaf browning according to treatments

Treatments	Incidence (p c)
Témoin 1	80.94 ± 4.33[a]
Témoin 2	73.33 ± 8.85[a]
T1	78.33 ± 10.94 [a]
T2	80.00 ± 0.00 [a]
T3	80.33 ± 9.42 [a]
T4	73.33 ± 9.85 [a]
P-value	0.186

In the same column, numbers with the same letters are statistically identical. Control 1: No treatment; Control 2: Banco D; T1: Pure filtrate; T2: Filtrate diluted to 75%; T3: Filtrate diluted to 50%; T4: Filtrate diluted to 25%

3.2.3. Effect of treatments on stem browning

Table 3 represents the variation of the means of the incidence of stem browning according to the treatments. A very low incidence of stem browning is observed in Control 2, T1, T3 and T4 treatments respectively 0%; 0.33%; 0.33% and 0.66%. In contrast, Control 1 (2.66%) and T2 (2.33%) had the highest incidence. However, statistical analysis of the variance of the means revealed no significant difference between the different treatments applied (P = 0.775). Therefore, treatments with *Artemisia annua L* extract and banko D fungicide are effective in protecting tomato plants from stem browning.

Table 3 Variation in the means of stem browning incidence by Treatments

Treatments	Incidence (p c)
Control 1	2.66 ± 0.47[a]
Control 2	0.00 ± 0.00[a]
T1	0.33 ± 0.23[a]
T2	2.33 ± 0.47[a]
T3	0.33 ± 0.23[a]
T4	0.66 ± 0.47
P-value	0.775

In the same column, numbers with the same letters are statistically identical. Control 1: No treatment; Control 2: Banco D; T1: Pure filtrate; T2: Filtrate diluted to 75%; T3: Filtrate diluted to 50%; T4: Filtrate diluted to 25%

3.3. Effect of the treatments on the agro morphological parameters of tomato

The results of the data on the number of leaves, height and diameter at the neck of the plants are recorded in Table IV. The highest leaf numbers were observed in treatment T1 (15.60 leaves) and control 2 (15.33 leaves). On the other hand, the lowest numbers of leaves are recorded with the control 1 (11.66 leaves) also with the treatments based on extract of *Artemisia annua L* T2, T3 and T4 respectively 10.13; 11.93; 12.40 leaves. The analysis of these data allows us to say that there is a significant difference between the treatments and the controls with a probability (P = 0.22). Concerning the height, the controls Control 1 and Control 2 presented respectively 65.86 cm and 67.80 cm contrary to the biological treatments T1, T2, T3 and T4 which presented respectively statistically identical values 68.40; 68.40; 70.20 and 68.40 cm. However, no significant difference (P = 0.558) was observed between the treatments with *Artemisia annua L* extract and the controls. With the diameter at the crown, the treatments Control 1, Control 2, T2, T4 and T3 induced similar means respectively 8.65; 8.72; 8.87; 8.97 and 8.98 mm against 9.37 mm for the T1 treatment. However, there was no significant difference between treatments (P = 0.120), so it should be said that, treatments (biological and chemical) induced leaf production.

Table 4 Variation of the means of the number of leaves, height and diameter at the neck of the plants according to the treatments

Treatments	Nfe	Ht	Dc
Control 1	11.66 ± 5.19^{ab}	65.86 ± 5.95^{a}	8.87 ± 0.67^{a}
Control 2	15.33 ± 5.08^{a}	67.80 ± 2.21^{a}	8.65 ± 0.77^{a}
T1	15.60 ± 6.98^{ab}	68.40 ± 6.70^{a}	9.37 ± 0.68^{a}
T2	10.13 ± 4.73b	68.40 ± 6.70^{a}	8.72 ± 0.71^{a}
T3	11.93 ± 4.55^{ab}	70.20 ± 5.68^{a}	8.98 ± 0.83^{a}
T4	12.40 ± 8.31^{ab}	68.40 ± 4.74^{a}	8.97 ± 0.92^{a}
P-value	0.022	0.558	0.120

In the same column, numbers with the same letters are statistically identical. Dc: Diameter at the neck; Ht: Height of the stem; Nfe: Number of leaves; Control 1: Without treatment; Control 2: Banco D; T1: Pure filtrate; T2: 75% diluted filtrate; T3: 50% diluted filtrate; T4: 25% diluted filtrate

4. Discussion

4.1. Effect of treatments on symptoms observed on tomato plants

The results of the post-treatment diagnosis of tomato plants revealed the presence of three symptoms, namely: (whitish spots, black spots and browning of the stem). Studies conducted by [8] showed that these whitish spots on tomato leaves are characteristic of the tomato leaf miner (*Tuta absoluta*) which has been identified as one of the major pests of tomato crop in Africa. The study showed that undiluted *Artemisia annua L* extract reduced the presence of whitish spots on the leaves of tomato plants as it recorded the lowest incidence in contrast to the controls (Control 1 and Control 2) and other treatments. Indeed, these observed differences in incidence could be due to the repellent or insecticidal effect of *Artemisia annua L* on the pest Tuta absoluta. This corroborates the studies of [9], who worked on the valorization of aromatic plants in the integrated management of the main insect pests of Grande Morelle in South Benin: case of *Ocimum gratissimum* and *O. basilicum*. He demonstrated that aromatic plants, including *Artemisia annua L* , are known for their strong scents and their repellent, insect repellent and insecticidal effects against many animals such as arthropod pests. In addition, these properties would also be due to the terpene molecules contained in the plant *Artemisia annua L* . This argument is in agreement with that of [10] whose work has shown that the insecticidal activity of essential oils extracted from certain plants is related to their terpene richness. On the other hand, the vegetable extracts of medicinal plants are endowed with an antimicrobial activity which depends mainly on their chemical compositions and also on the nature of the solvents of extraction. Also [11] have shown that plant extracts have a very broad spectrum of action since they inhibit the growth of bacteria and fungi. In addition, according to [12] *Artemisia annua L* is a medicinal plant with antifungal activity because it has in its chemical composition flavonoids, steroids and terpenes that are responsible for this fungal activity. To this effect, symptoms such as black spots on the leaves and browning of the stem would be caused respectively by *Alternaria solania*, pathogen of alternaria and *Didymella lycopersici* [13]. On the other hand, the results related to the effect of aqueous extracts of *Artemisia annua L* on these fungal diseases, showed that the treatments compared did not have a significant effect on these pathogens responsible for black spots on the leaves and browning of tomato plants. This could be explained by the effect of secondary metabolites contained in the tested plant. Therefore, according to [14], the biological activity of a natural plant is directly related and correlated to its chemical composition. In addition, this inactivity could also be explained by the concentration range, because it has been shown that the inhibitory activity of the extracts of a plant is related to its concentration in the medium. In addition, the inefficiency observed with the chemical treatment (banko D) would be due to a resistance phenomenon developed by the agents responsible for the brown spots on the leaves and stems of the tomato plants.

4.2. Effect of different treatments on some agromorphological parameters

The results from the agromorphological parameters (number of leaves, height and crown diameter) of the plants revealed a significant difference between the treatments concerning the number of leaves. Contrary to the height and diameter at the collar, the statistical analysis did not reveal any significant difference between the treatments. In this regard, treatment T1 (undiluted Artemisia extract) and Control 2 (banko D) recorded the highest number of leaves compared to the other treatments used. This difference obtained could be explained by the biostimulant effect of the extracts on tomato leaves. Also, the increase in the number of leaves could be explained by the action of *Artemisia annua L* which stimulated the physiological activity of tomato plants, modulating by enzymatic or hormonal way and inducing

the production of metabolite. In addition, the high number of leaves on the plants treated with the biological extract (*Artemisia annua L*) could have a greater photosynthetic activity whose consequence would be the production of more photosynthetic assimilates [10].

5. Conclusion

The study on the use of aqueous extracts of *Artemisia annua L* is one of the first to have been conducted to control pests and diseases of tomato crop in the city of Daloa. Indeed, the aqueous extract based on *Artemisia annua L* revealed an insecticidal or insect repellent effect against *Tuta absoluta* and a biostimulant by inducing the increase of the number of leaves. The results also showed the absence of some viral and bacterial symptoms on tomato plants. However, the undiluted extract of *Artemisia annua L* allows to better protect the tomato crop against pathogens responsible for whitish spots, browning of leaves and stems observable on tomato plants. Moreover, these results will allow tomato producers to move towards an organic market gardening, preserving the health of consumers and protecting the environment. In perspective, it would be desirable to continue this study in order to determine the quantity of plant extract of *Artemisia annua L* to bring to the tomato culture to ensure its protection against the pathogens responsible for the browning of the leaves and the stems of tomato until the harvest. Also, to extract the active principle to formulate biopesticides based on *Artemisia annua*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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