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Research Article

Efficacy of Aqueous Extract of the Seeds of *Annona muricata* L. in the Control of Late Blight (*Phytophthora infestans*) of Tomato (*Lycopersicon esculentum* Mill.) in the Field

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Abstract

Objective: An experiment was conducted to propose an agroecological alternative to synthetic fungicides for the control of late blight of tomato in field. The aim of this study was to evaluate the effect of aqueous extract of *Annona muricata* seeds (AqEAM) against late blight and yield parameters of tomato.

Methods: Two varieties (Rio Grande and Lindo F1) constituting the main plots and three treatments (aqueous extract of *A. muricata*, synthetic fungicide Mancozeb 800g/kg and control) represented sub-plots were used in a split-plot design with four replicates. The treatments were applied two weeks after transplanting and the dose of AqEAM used was 70g/L. Incidence, severity of late blight and agronomic parameters were evaluated.

Results: The results show that the application of AqEAM significantly reduced the incidence of late blight in the field by 55.84% and the severity by 80.35% compared to the control at 76 days after transplanting (DAT). Furthermore, no significant differences were observed between AqEAM and the synthetic fungicide on the incidence and severity of late blight. In terms of yield (t ha^{-1}), tomatoes treated with AqEAM showed a significant increase of 26.14% and 25.7% respectively compared to the control and the synthetic fungicide. The Lindo F1 variety yielded best (10.80t ha^{-1}) than Rio Grande variety (6.60t ha^{-1}).

Conclusion: These results suggest that aqueous seeds extract of Annona muricata is effective as the synthetic fungicide and would therefore be recommended as an alternative in biological control of late blight of tomato.

Keywords: *Annona muricata* extract, antifungal, *Lycopersicon esculentum*, *Phytophthora infestans*, yield parameters

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1 INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is an herbaceous plant of the *Solanaceae* family and originated from western coastal part of the South America continent[1]. Due to its high nutritional value, tomatoes are considered to be one of the most economically important vegetable crops globally[2]. It is rich in mineral elements, vitamins A, C, E and phenolic antioxidants[3]. Its consumption reduces the incidence of cardiovascular diseases, various cancers and degenerative diseases associated with old age[4,5]. With a global production of around 120 million tons per year, it is the second most consumed vegetable in the world after potatoes[6]. Tomatoes are highly susceptible to diseases caused by oomycetes, the most important of which is late blight of tomato caused by *Phytophthora infestans* (Mont.) de Bary. It is one of the most costly diseases worldwide[7]. Its damage in the field affects fruit quality and can lead to tomato yield losses of up to 80%[8]. Recently, it is observed changes in the population structure of *P. infestans* have led to the advent of new genotypes containing two mating types A1 and A2 that are more aggressive and resistant to synthetic fungicides[9]. Tomato cultivation therefore requires a significant input of synthetic fungicides to reduce field infection of plants by late blight while preserving yield. However, due to their toxicological properties, the abusive use of synthetic fungicides is becoming a real danger for humans and the environment. As soon as they are applied in agriculture, synthetic fungicides contaminate soils, affect biodiversity and are also washed into surface or groundwater during rainfall. Synthetic fungicides can also accumulate in food chains; a small concentration in water can lead to a high concentration in the tissues of consumers in general[10, 11].

Faced with this perilous situation, in Agroecology, the search for effective, less costly, non-polluting alternative methods is topical. The formulation of natural substances of plant origin that could have beneficial biostimulator effects in plant protection and production is a promising avenue. Plants with biodegradable pesticide effects synthesize compounds with antifungal, insect repellent, insecticidal and antibacterial properties[12,13]. The plant extracts with their recognized systemic modes of action have already proven to be effective in strengthening the defense system of the plants enabling them to be more vigorous and resistant to diseases[14]. *Annona muricata* seeds have already been the subject of numerous phytochemical studies, which have demonstrated their insecticidal, fungicidal and bactericidal potential[15-17]. Numerous studies showed that the extract of seeds of *A. muricata* are rich in secondary metabolites (phenolic compounds, alkaloids, saponins, terpenoids, sterols etc.) which have a pesticide property[18,19]. Nevertheless, very few studies have highlighted the effect of the aqueous extract of *A. muricata* seeds against late blight in order to optimize tomato yield in the field. Thus, the objective of this study was to evaluate the efficacy of the aqueous extract of *Annona muricata* seeds on late blight and yield parameters of tomato in the field while preserving tomato yield.

2 MATERIALS AND METHODS

2.1 Study Site

The experiment was carried out in a mid-fallow (2 years old) in the locality of Éssazok (03° 46′ 00″ North and 12° 15′ 00″ East), located in the Centre Region of Cameroon. This locality belongs to the agro-ecological zone 5 of Cameroon (humid forest zone with bimodal rainfall). The zone is characterized by a Congo-Guinean sub-equatorial climate. The average rainfall is 1633mm/year, distributed into a small rainy season (March-June) and a long rainy season (September-November). The average annual temperature is relatively constant (around 23 to 27°C) and the average relative humidity is over 80%. Tomatoes are the main vegetable crop, and the soil is red laterite, sandy, black or clay in some places[20].

2.2 Plant and Chemical Material

Plant material consisted of seeds of two tomato varieties (Rio Grande and Lindo F1) was chosen based on of their agronomic performance and different susceptibility to diseases. Both varieties were obtained at Semagri, which is an approved company for the development and sale of agricultural seeds. On the other hand, seeds of *Annona muricata* obtained from the already ripe fruits at local market reported to the National Herbarium for identification and confirmation. The chemical material consisted mainly of a synthetic fungicide commercially called PENNCOZEB 80 WP which is a contact fungicide, with a very broad spectrum of action, powdery appearance and its active ingredient being Mancozeb 800g/kg.

2.3 Nursery and Experimental Design

Two ridges (5m x 1m) were formed using hoes and incorporated poultry manure (two 15kg bags). The duration of the tomato plants in the nursery was 23 days, after which the tomato plants were transplanted to the field in cool weather in afternoon. The experiment was conducted during February to July 2019. A split-plot design with four replications was used. Varieties constituting the main plot randomly in replication with two levels (Rio Grande and F1 Lindo). The treatments represented the sub-plots randomized within the main plot (T0: control, T1: synthetic fungicide and T2: aqueous extract of *Annona muricata* seeds (AqEAM)). Each experimental unit had 15 tomato plants for a total of 90 plants per block. The experimental units measured 3m x 1.5m and were separated by 1m paths and contained three rows of five seedlings. The four blocks were spaced 1.5m apart.

2.4 Preparation and Application of Treatments

For the preparation of the aqueous extract of *A. muricata*, the seeds isolated from the ripe fruits were dried in the shade for three weeks and kept in an air-permeable container. The dried seeds were crushed with a grinder and 210g of *A. muricata* seed powder was taken and macerated in 2L of water for at least 24h. The resulting solution was filtered through a cotton cloth and the collected contents were diluted with 1L of soapy water and then introduced into a knapsack sprayer for exclusive use in the eight experimental units involved in the treatment. The soap powder (10g) associated with the extract was used as a wetting agent to reinforce the adhesion of the product on the parts of the plant to be treated. The dose used was 70g/L or 58kg of *A. muricata* powder for 833 liters of water per hectare[21].

For the synthetic fungicide treatment, the rate was in accordance with the recommendations prescribed in the registration, i.e. 5g/L. The mixture obtained was homogenized and then introduced into a sprayer for specific use in the eight experimental units examined.

The aqueous extract of *A. muricata* seeds and the synthetic fungicide were applied five times throughout the experiment at regular intervals of 10 days.

2.5 Parameters Measurements

2.5.1 Identification of Late Blight

Late blight of tomato, using various diseased plant samples collected in the field, was identified in the laboratory by microscopic observations of sporocysts[22]. The method consisted in placing a drop of distilled water on a slide, then bringing and dissociating in the drop a sample to be observed; the slide was covered by a slide and the observation was made through a binocular optical microscope of the brand OLYMPUS CH-2 at 40X magnification.

2.5.2 Assessment of the Incidence and Severity of Late Blight

The incidence of disease was recorded 40 days after transplantation at 12 days intervals in different experimental units using formula proposed by Tchoumakov and Zaharova[23].

$$\%\,I = \frac{n}{N} \times 100$$

With I = Incidence; n = Number of diseased plants; N = Total number of plants

The severity of late blight was calculated according to the formula proposed by Tchoumakov and Zaharova (1990)[23].

$$\%\,S = \frac{\sum (ab)}{N} \times 100$$

With S = Severity; $\sum$ (ab) = sum of multiplications of the number of diseased plants (a) by the corresponding degree of infection (b); N= total number of diseased plants.

Degree of infection was attributed using the scale varying from 0 to 5 with modification (Table 1)[24]. The score was assigned by the plant pathologist based on their eye estimation.

2.5.3 Assessment of Growth and Development Parameters

Data collection was carried out weekly from 12 days after transplanting (DAT) until the end of the experiment on seven randomly selected tomato plants per experimental unit for growth parameters. The height of the tomato plants was measured with a ruler. The leaves and the flowers per tomato plant were assessed by counting. The fruit set rate was calculated using the formula used by Tikarrouchine[25]:

$$\text{Fruit set rate} = \frac{\text{Number of knotted flowers}}{\text{Total Number of flowers}} \times 100$$

2.6 Assessment of Yield Parameters

The means of number of fruits, fruit mass and fruit diameter of tomato plants were evaluated considering fruits damaged by late blight. Tomato yield in tons per hectare was expressed from the following formula[26, 27]:

$$\text{Yield in t/ha} = \frac{NF \times MF}{S} \times \frac{10000\text{m}^2}{1\,\text{ha}} \times \frac{1\text{t}}{10^6\text{ g}}$$

With NF = Mean number of fruits per plot; MF = Mean mass of fruits per plot (g); S = Plot area (m^2)

2.7 Statistical Analysis

The data obtained were subjected to a one-and two-ways analysis of variance (ANOVA) using IBM SPSS Statistic 20.0 software. Normality (Shapiro-Wilk test; P>0.05) and homogeneity of variance (Levene test; P>0.05) were verified. Then a multiple comparison of means using the Student-Newman-Keuls (SNK) test at the 5% threshold was determined.

3 RESULTS

3.1 Identification of Late Blight of Tomato

Light microscopic observations of the late blight (Figure 1) structures from the various samples obtained in the field confirmed the effective presence of late blight on tomato plants during the experiment (Figure 1A). Morphological study under the microscope showed that the sporocysts of *Phytophthora infestans* were ovoid-ellipsoid to limoniform in shape with one pedicel and one papilla each. The mean lengths of the sporocysts ranged from 43.35μm to 51.85μm and the mean widths of the sporocysts from 23.96μm to 35.30μm (Figure 1B).

3.2 Epidemiological Parameters of Late Blight in the Field

3.2.1 Incidence of Late Blight

The results reveal significant differences (P<0.05) between the treatments for the incidence of late blight in the field at 40, 52, 64 and 76 DAT (Table 2). The highest incidence of 61.73%, 59.62%, 61.48% and 61.53% was

Table 1. Field Key to Estimate the Degree of Severity of Late Blight[24]

Grade	% Blighted Foliage	Nature of Infection
0	0%	No disease
1	10%	Small lesion area less than 10% of the whole leaflet
2	11%-20%	Lesion area between 11% and 20% of the whole leaflet
3	21%-30%	Lesion area between 21% and 30% of the whole leaflet
4	31%-60%	Lesion area between 31% and 60% of the whole leaflet
5	>60%	Lesion area over 60% of the whole leaflet

Figure 1. Late blight of tomato. (A): Symptoms observed on a tomato plant; (B): Sporocysts of *Phytophthora infestans* observed under a binocular optical microscope (40 X magnification).

showed in the control compared to the AqEAM treatment which recorded the lowest incidence of 29.65%, 26.13%, 24.32% and 27.80% at 40, 52, 64 and 76 DAT respectively. Regarding the variety effect, no significant difference (P>0.05) in incidence between the two tomato varieties was observed.

3.2.2 Severity of Late Blight

The data on late blight severity are presented in Table 3. A highly significant difference (P<0.01) is recorded between varieties and treatments at 40, 52, 64 and 76 DAT. The AqEAM treatment showed the lowest severity of 8.1%, 5.93%, 5% and 7.23% followed by the fungicide treatment (10.7%, 6.82%, 6.52% and 8.63%) at 40, 52, 64 and 76 DAT respectively. The control treatment recorded the highest severity in all periods. Regarding the variety effect, the variety V2 (Lindo F1) showed the lowest severity compared to the variety V1 (Rio Grande) in all periods.

3.3 Effect of Treatments and Varieties on Tomato Growth Parameters

The plant height and number of leaves of the two varieties in all treatments are presented in Table 4. No significant difference (P>0.05) between treatments for plant height was observed. However, a very highly significant difference (P<0.01) was showed between the varieties. The Lindo F1 variety (58.31±3.28cm) presented the highest plant height compared to the Rio Grande variety (42.05±7.59cm) at 50 DAT. For the number of leaves, there significant difference (P<0.05) at 50 DAR between treatments was observed. Plot treated with AqEAM recorded the highest number of leaves (37.50±4.57) compared to the synthetic fungicide (32.60±3.44) and the control (31.50±34). No significant difference (P>0.05) was recorded between the varieties for the number of leaves.

3.4 Effect of Treatments and Varieties on Tomato Development Parameters

The results of the mean number of flowers show a significant difference (P<0.05) between the AqEAM (65.19±5.89) and the control (57.46±8.99) at 59 DAT. On the other hand, at 80 DAT, there was no significant difference (P>0.05) between the treatments. For the varieties, there was no significant difference (P>0.05) during the experiment. Regarding the fruit set rate, there were significant differences between the treatments at 86 (P<0.05) and 105 (P<0.001) DAT. Overall, the AqEAM treatment recorded better fruit set rates of 59.38% and 65.19% compared to the control 33.83% and 57.46% respectively at 86 and 105 DAT. The variety Lindo F1 recorded significantly higher fruit set rates than the variety Rio Grande during the experiment (Table 5).

3.5 Effect of Treatments on Yield-related Parameters

3.5.1 Number of Fruits

The number of fruits was significantly higher in the

Table 2. Effect of Antifungal Treatments and Varieties on the Incidence (%) of Late Blight

Treatments	40 DAT	52 DAT	64 DAT	76 DAT
T0	61.73±16.51b	59.62±19.42b	61.48±20.18b	61.53±21.38b
T1	48.62±17.24ab	43.80±19.11ab	40.63±22.51ab	45.10±22.22ab
T2	29.65±19.77a	26.13±19.64a	24.32±19.42a	27.80±20.28a
Varieties				
V1	51.46±26.50a	49.61±23.69a	48.60±30.43a	50.94±29.49a
V2	40.50±18.27a	37.30±17.27a	36.88±18.96a	38.33±19.10a
Varieties	ns	ns	ns	ns
Treatments	*	*	*	*
Interaction	ns	ns	ns	ns

Notes: *P*: 0 '***' 0.001 '**' 0.01 '*' 0.05; ns: not significant. In each column, the values (means ± standard deviations) followed by the same letters do not differ significantly using the Student-Newman-Keuls (SNK) test at the 5% threshold. T0: Control; T1: Synthetic fungicide; T2: AqEAM; V1: Rio Grande; V2: F1 Lindo; DAT: Days after transplanting.

Table 3. Effect of Antifungal Treatments and Varieties on the Severity (%) of Late Blight

Treatments	40 DAT	52 DAT	64 DAT	76 DAT
T0	34.80±12.35b	32.72±12.51b	32.42±12.80b	33.65±12.08b
T1	10.70±3.24a	6.82±2.12a	6.52±2.17a	8.63±2.75a
T2	8.10±3.85a	5.92±3.37a	5.00±2.71a	7.23±3.84a
Varieties				
V1	23.42±25b	20.28±25.36b	20.10±25.60b	21.50±24.81b
V2	7.00±5.13a	5.68±4.10a	5.25±3.73a	6.80±5.20a
Varieties	**	**	**	**
Treatments	**	**	**	**
Interaction	*	*	**	*

Notes: *P*: 0 '***' 0.001 '**' 0.01 '*' 0.05; ns: not significant. In each column, the values (means ± standard deviations) followed by the same letters do not differ significantly using the Student-Newman-Keuls (SNK) test at the 5% threshold. T0: Control, without any treatment; T1: Synthetic fungicide; T2: AqEAM; V1: Rio Grande; V2: F1 Lindo; DAT: Days after transplanting.

Table 4. Evolution of Tomato Plant Growth in Relation to Antifungal Treatments and Varieties

Treatments	Plant Height (cm)		Number of Leaves	
	20 DAT	50 DAT	20 DAT	50 DAT
T0	22.50±6.43a	46.32±9.61a	6.10±1.29a	31.50±3.34a
T1	23.01±7.76a	49.13±8.59a	8.50±3.50a	32.60±3.44a
T2	23.68±11.06a	50.76±13.36a	8.50±2.46a	37.50±4.58b
Varieties				
V1	30.08±9.39b	42.05±7.59a	9.15±4.75a	34.00±4.01a
V2	18.54±2.81a	58.31±3.28b	6.95±1.43a	35.45±5.71a
Varieties	***	***	ns	ns
Treatments	ns	ns	ns	**
Interaction	ns	ns	ns	**

Notes: *P*: 0 '***' 0.001 '**' 0.01 '*' 0.05; ns: not significant. In each column, the values (means ± standard deviations) followed by the same letters do not differ significantly using the Student-Newman-Keuls (SNK) test at the 5% threshold. T0: Control, without any treatment; T1: Synthetic fungicide; T2: AqEAM; V1: Rio Grande; V2: F1 Lindo; DAT: Days after transplanting.

AqEAM treatment (6.1±1.37) compared to the synthetic fungicide treatment (4.8±0.92) and the control (4.7±1.56) at 93 DAT. For the variety effect, a significant difference (P<0.01) was recorded at 93 and 106 DAT. The Lindo F1 variety had the highest number of fruits (5.1±1.37 and 9.75±2.33) during these two periods (Table 6).

3.5.2 Effect of Treatments and Varieties on Yield

The effect of treatments and varieties on mean fruit diameter per plant, mean fruit mass per plant and yield were presented in Table 7. Indeed, the AqEAM treatment with 6±0.59cm recorded better diameters compared to the synthetic fungicide treatment (5.35±0.38cm) and the control (4.96±0.61cm). The results on the mean fruit diameter per plant showed no significant difference (P>0.05) between the two varieties. In terms of mean fruit mass per plant and yield in tons per hectare, the AqEAM treatment recorded the best values (52.34±11.25g and 11.40t ha^{-1}) compared to the synthetic fungicide (46.80±3.72g and 8.47t ha^{-1}) and the control (44.19±5.94g and 8.42t ha^{-1}). The variety Lindo F1 had significantly better fruit weight (54.7±7.44g) and yield (10.8t ha^{-1}) than the variety Rio Grande (41.80±2.61g and 6.60t ha^{-1}).

4 DISCUSSION

The assessment of the efficacy of the aqueous extract of *A. muricata* seeds on the epidemiological parameters revealed a significant decrease in the incidence and severity of late blight of tomato compared to the control. This result would be due to annonain which is an acetogenin contained in the seeds of *A. muricata* and which would have antifungal properties. Thus, this secondary metabolite would have inhibited the development of *Phytophtora infestans* spores and consequently, significantly limited the expansion of late blight of tomato in the field. Le Ven[16] demonstrated by analysing the properties of the phytochemical components of *A. muricata* that the annonain, terpenoids and phenolic acid contained in the seeds would have antifungal potentialities. These results are similar to those obtained on the antifungal activities of acetogenins from *Annona squamosal* against various plant pathogens[28]. Similarly, Rizwana et al.[29] show that extract of *A. muricata* contain important bioactive compounds that possess antifungal activity when they evaluated the antifungal activities of *A. muricata* on tomato Alternaria. Also, Naik and Sellappan[30] and Boli et al.[31] demonstrate that phytochemical screening of the aqueous extract of *A. muricata* seeds shows that it is rich in phenols, terpenoids, glycosides, saponins, flavonoids, alkaloids and sterols. The use of aqueous extract of *A. muricata* seeds (AqEAM) in the protection of tomato plants resulted in significant improvements in leaf number. These effects of AqEAM are believed to be due to its composition of acetogenins, amino acids, vitamins, cytokinin and auxin. Indeed, these substances affect the cellular metabolism of plants and contribute considerably to increasing their productivity[5,32]. Furthermore, statistical analyses did not reveal significant differences in plant height between all treatments. This could be explained by the fact that the height growth of tomato plants is mainly influenced by the physio-chemical composition of the soil, as the poultry manure were added to the soil to enrich it with nitrogen plays an important role in plant growth. Indeed, Hanitriniony[33] when assessing the efficacy of *Fourcroya gigantea* in the control of Alternaria and late blight in tomato, observed a homogeneity of the growth in height of tomato plants according to the treatments applied. Similarly, Aghofack-Nguemezi et al.[5] had no significant effects for height when applying *Spirulina plantensis* and *Jatropha curcas* extracts to tomato plants. On the order hand, Fangue et al.[34] demonstrate that extracts of *Tithonia diversifolia* and *Thevetia peruviana* affected significantly plant height of tomatoes in field. Other studies also showed that crude extract of *Gleicheni linearis* at the rate of 100mg/L was the most effective in increasing plant height[35]. Regarding the fruit set rate, a good development of tomato plants treated with AqEAM was observed compared to the control. These results can be explained by the fact that the annonain contained in the seeds of *A. muricata* would act against the fungal diseases that cause dysfunctions capable of causing flower bud abortion and poor fruit set. Indeed, Hanitriniony[33], in assessing the effectiveness of *Fourcroya gigantea* against Alternaria and late blight of tomato, noted that the formation of floral clusters was favoured by the decrease of fungal diseases. Regarding the number of flowers, no significant difference was observed between treatments. These results could be due to the fact that phytohormones such as gibberellin, ethylene and cytokinin involved in flowering induction acted independently of the treatments applied[36]. Comparing the mean fruit weights and diameters as well as the yield per hectare obtained at the different treatments, it was found that overall the AqEAM-treated plots showed significantly better values than the other treatments. This result could support the fact that AqEAM is effective from a phytotechnical point of view. Indeed, the decrease of pests contributes to the improvement of the quantity and quality of the crop yields. In addition, the mean number of fruits per plant in each treatment did not reveal significant differences between AqEAM and the other treatments. Tounou et al.[37] showed that plant extracts applied to cowpea plants did not result in significant differences between treatments on the average number of cowpea pods in southern Togo. The seeds of *A. muricata* due to their insecticidal, fungicidal, and bactericidal properties[15,16], their richness in acetogenins, essential oils, amino acids, vitamins, auxin and cytokinin as demonstrated by Le Ven[16] in his analysis of the phytochemical components of *A. muricata*, would favour by the combination of these factors, the obtaining of a good durable phytosanitary follow-up and consequently contribute to the obtaining of a bigger growth, a better development and a higher yield of the crops. During the trial, a better varietal response of

Table 5. Effect of Antifungal Treatments and Varieties on Tomato Development

Treatments	Number of Flowers		Fruit Set Rate (%)	
	59 DAT	80 DAT	86 DAT	105 DAT
T0	2.80±1.87a	12.00±2.94a	33.83±44.12a	57.46±8.99a
T1	5.00±2.16a	16.00±2.45a	46.66±13.14ab	70.84±6.67b
T2	6.20±4.78b	15.30±5.01a	59.38±28.76b	65.19±5.89b
Varieties				
V1	4.70±4.20a	14.60±4.39a	24.75±26.77a	54.53±14.67a
V2	4.45±1.76a	14.45±3.37a	60.19±28.73b	67.21±11.87b
Varieties	ns	ns	***	***
Treatments	ns	ns	*	***
Interaction	*	ns	ns	***

Notes: *P*: 0 '***' 0.001 '**' 0.01 '*' 0.05; ns: not significant. In each column, the values (means ± standard deviations) followed by the same letters do not differ significantly using the Student-Newman-Keuls (SNK) test at the 5% threshold. T0: Control, without any treatment; T1: Synthetic fungicide; T2: AqEAM; V1: Rio Grande; V2: F1 Lindo; DAT: Days after transplanting.

Table 6. Effect of Antifungal Treatments and Varieties on the Number of Tomato Fruits

Treatments	87 DAT	93 DAT	99 DAT	106 DAT
T0	2.30±1.05a	4.70±1.56a	7.00±2.16a	8.50±2.22a
T1	3.20±1.75a	4.80±0.92a	7.60±1.50a	8.80±2.44a
T2	3.60±1.50a	6.10±1.37b	7.70±2.50a	10.20±3.22a
Varieties				
V1	2.35±1.60a	3.85±2.37a	6.30±2.90a	7.50±2.70a
V2	3.00±1.65a	5.10±1.37b	7.10±1.62a	9.75±2.33b
Varieties	ns	**	ns	**
Treatments	ns	**	ns	ns
Interaction	ns	ns	ns	ns

Notes: *P*: 0 '***' 0.001 '**' 0.01 '*' 0.05; ns: not significant. In each column, the values (means ± standard deviations) followed by the same letters do not differ significantly using the Student-Newman-Keuls (SNK) test at the 5% threshold. T0: Control, without any treatment; T1: Synthetic fungicide; T2: AqEAM; V1: Rio Grande; V2: F1 Lindo; DAT: Days after transplanting.

Table 7. Variation in Mean Fruit Diameter, Mean Fruit Mass and Tomato Yield in Tons Per Hectare According to Antifungal Treatments and Varieties

Treatments	MFD (cm)	MFM (g)	Yield (t ha-1)
T0	4.96±0.61a	44.19±5.94a	8.42±0.20a
T1	5.35±0.38a	46.80±3.72a	8.47±0.33a
T2	6.00±0.59b	52.34±11.25b	11.4±0.76b
Varieties			
V1	5.30±0.56a	41.80±2.61a	6.60±0.20a
V2	5.58±0.78a	54.70±7.44b	10.80±0.41b
Varieties	ns	***	***
Treatments	**	***	***
Interaction	ns	***	***

Notes: *P*: 0 '***' 0.001 '**' 0.01 '*' 0.05; ns: not significant. In each column, the values (means ± standard deviations) followed by the same letters do not differ significantly using the Student-Newman-Keuls (SNK) test at the 5% threshold. T0: Control, without any treatment; T1: Synthetic fungicide; T2: AqEAM; V1: Rio Grande; V2: F1 Lindo; MFD: Means fruit diameter; MFM: Means fruit mass.

the Lindo F1 variety was observed overall compared to Rio Grande both in epidemiological, growth, development and yield parameters. On the one hand, this could be due to genetic modifications that allow the introduction of genes of interest to obtain tomato cultivars that perform as well as Lindo F1, or on the other hand, the cultivar Lindo F1 was simply more adapted to the growing conditions presented during the experiment.

5 CONCLUSION

In view of the results obtained and the analyses that follow from this trial whose objective was to evaluate in the field, the effect of the aqueous extract of *Annona muricata* seeds against late blight of tomato and yield parameters, it can be maintained that, the aqueous extract of *A. muricata* seeds had remarkable effects on the reduction of the incidence and severity of late blight in comparison with the control. The application of aqueous extract of *A. muricata* seeds contributed to improve the growth, development and yield of tomato especially in terms of number of leaves, number of flowers, average fruit diameter per plant and average fruit mass per plant. Regarding the varietal effect, the tomato variety F1 Lindo is a recommendable variety for tomato cultivation based on the results obtained. The seeds of *A. muricata* could be a natural resource for sustainable agriculture. Thus, the aqueous extract of *Annona muricata* seeds could be used as an alternative method to synthetic fungicides; thus, falling within the framework of the agroecological transition, which nowadays remains a major concern. The further development of this trial in an *in vitro* environment would constitute an axis for future research.

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Conflicts of Interest

The authors declared no conflict of interest.

Author Contribution

This work realized with the collaboration of all authors. Ndongo B. designed the study; Tsala R collected data and wrote the first draft of manuscript; Ngatsi PZ coordinated the field and lab study and reviewed the manuscript; Temegne NC performed the statistical analyses; Lontsi SLD, Tueguem WNK and Nsangou ANK reviewed the manuscript. All authors approved the final version.

Abbreviation List

AqEAM, Aqueous extract of Annona muricata seeds
DAT, Day(s) after transplanting
MFD, Means fruit diameter
MFM, Means fruit mass
MF, Mass of fruits
NF, Number of fruits

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