Original Research Article

Field efficacy of Gliding Arc Plasma Activated Water and natural products on tomato (*Solanum lycopersicum* L.) late blight [*Phytophthora infestans* (Mont.) de Bary] severity

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Abstract

This study aimed to evaluate the field efficacy of plasma-activated water (PAW) as a novel technology in agriculture together with vinegar, sodium bicarbonate, and plant aqueous extracts against tomato late blight in a complete randomised block design. Ten treatments were evaluated: Plasma activated water (PAW), a mixture of sodium bicarbonate and vinegar at different ratios (25/75, 50/50 and 75/25) and different mixtures of aqueous plant extracts made up of ten plants: *Ageratum conyzoides*, *Eucalyptus saligna*, *Azadirachta indica*, *Panax quinquefolius*, *Callistemon viminalis*, *Euphorbia hirta, E. cordifolia Laggera pterodonta*, *Ocimum gratissimum* and avocado pit powder. Plantizeb and distilled water were used as positive and negative controls, respectively. There was a significant difference ($p < 0.05$) in the growth variables between treatments at 10 weeks after transplanting (WAT). All the treatments reduced late blight severity compared to the negative control (100%) at 10 WAT. Late blight severity on plants treated with Plantizeb, PAW, the mixture of bicarbonate and vinegar (B+V) at 50/50 (33.24%) and the mixture of *E. hirta* + *E. cordifolia* extract (33.33%) was the lowest and significantly comparable ($p < 0.05$) to Plantizeb (31.67%). Tomato yield was 2.24 t/ha (negative control), 28.56 t/ha (Plantizeb), 27.23 t/ha (PAW), 30.32 t/ha (B+V at 50/50), and 27.69 t/ha (mixture of the aqueous extract of *E. hirta* + *E. cordifolia*). The PAW, the mixtures between B+V at 50/50, and the aqueous extract of *E. hirta* and *E. cordifolia* could be exploited for their bioactivity in late blight management in field conditions.

Keywords: bioactivity; Plasma Activated Water; *Euphorbia hirta; Euphorbia cordifolia;* tomato; natural products; late blight

INTRODUCTION

In Cameroon, tomato is cultivated in all the country's five agro-ecological zones, providing substantial income to farmers. Tomato consumption is a source of diverse vitamins and minerals (Willcox et al. 2003) and it is ranked as the second most consumed fresh or processed vegetable after potato (INRA, 2010). The national tomato production is 1,215,466 tons compared to the total vegetable production which is 3,107,164 tons (FAOSTAT, 2019), representing 39.1% of total vegetable

production. However, in the western region of Cameroon where tomato production represents 62% of the total national production, its cultivation is exposed to pests and diseases (Gaucher et al. 1998). Late blight (*Phytophthora infestans*) is the most destructive among disease constraints. Indeed, when the crop is not treated and the environmental conditions are favourable to the disease, crop loss can reach 100% (Fontem et al., 1998; Konje et al., 2019). In the main tomato production zones of Cameroon, late blight is controlled through the use of synthetic chemical fungicides. The most effective fungicides used are Metalaxyl, Mancozeb, Maneb, carbendazim, and chlorothalonil (Konje et al., 2019). However, previous studies have shown that the continuous usage of fungicides poses a potential threat to the environment, ranging from mere irritation to being very toxic to human beings and organisms and resistance development (Isin and Yildirim, 2007; Nawab et al., 2015; Damalas and Koutroubas, 2016). Based on these facts, we investigated Plasma Activated Water (PAW) as an alternative method to chemical control of tomato late blight in this study. The Gliding Arc Plasma Activated Water is distilled water prepared by exposure to non-thermal plasma produced by a continuous positive corona discharge in a transient spark regime. The activation of the water is carried out in the atmosphere of various surrounding gases (air, nitrogen, carbon dioxide, and argon). The presence of ions in water greatly affects the conductivity and production of hydrogen peroxide (which plays a significant role in the antimicrobial properties of PAW) (Hu et al., 2022; Hadinoto et al. 2023). Several studies have shown the efficiency of PAW on plant physiology and disease control. The mechanism of plasma action on harmful microorganisms is multistage and it involves mainly permanent damage to the cell wall, cytoplasmic membrane, and then intracellular structures, genetic material, and the enzyme apparatus (Moisan et al., 2002). Plasma also positively affects plant germination, and seedlings and enhances plant defence mechanisms against pathogens (Zhai et al., 2019; Zambon et al., 2020 Jiang et al., 2022). This was the case for the bacterial leaf spot of tomato caused by *Xanthomonas vesicatoria* (Perez et al., 2019) and *Fusarium* head blight of wheat and barley due to *Fusarium graminearum* (Jiang et al., 2022). Vinegar is one of the natural products that has a long medical history, and its consumption has long been used to cure diabetes. Its use in medicine is thus very old (Chen et al., 2016). The bioactive compounds in natural vinegar constitute a large heterogeneous group of secondary metabolites (polyphenols), organic acids, melanoidins, and tetramethylpyrazine (Xia et al., 2020). In plant protection, sodium bicarbonate has been used to control several diseases like the post-harvest decay fungus of orange (*Penicillium digitatum*), anthracnose of papaya (*Colletotrichum gloeosporioides*) (Gamagae et al., 2003; Pimenta et al., 2010). Even if biopesticides represent nowadays only 5% of the overall pesticide market (Kumar et al., 2021; Rakshit et al., 2021), it is established that plant extracts constitute a real alternative to chemicals for the future both for human and plant diseases. Their efficacy is due to the plant's ability to synthesise aromatic secondary metabolites, like phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins (Cowan, 1999; Lahlali et al., 2022). The components with phenolic structures, like carvacrol, eugenol, and thymol, were highly active against the pathogen. These groups of compounds show antimicrobial effects and serve as plant defence mechanisms against pathogenic microorganisms (Das et al., 2010). The objective of this study was to contribute to the development of control measures against tomato late blight respecting the environment and the health of tomato consumers through the use of natural products. The research hypothesis of this study is as follows: the Gliding Arc Plasma-Activated Water, the vinegar + sodium bicarbonate mixture, and plant aqueous extracts control tomato late blight.

MATERIAL AND METHODS

Production of the Gliding Arc plasma-activated water

The plasma-activated water was prepared in the Inorganic Chemistry Lab of the Faculty of Science, University of Yaoundé 1 (Cameroon). The production of water activated by plasma was carried out with a sliding discharge at atmospheric pressure belonging to the family of non-thermal plasmas. Saturated air, used as feed gas in the experiment, is supplied by a compressor and passes through a bubbler containing distilled water before entering a reactor. The flow rate of gas used was 800 L/h. The electrodes were connected to a high-voltage transformer which delivers an average current intensity of 160 mA (Hu et al., 2022). An electric arc is produced between the electrodes and at the inter-electrode space, containing excited species and free radicals. Discharge in humid air induces acidifying and oxidizing effects in a humid environment. A volume of 500 mL of distilled water is used as a target, the electric arc formed comes to touch the surface of the water kept under agitation and at the plasma-distilled water interface, a transfer of species takes place

generated from the bow to the water (Mogo et al., 2022). The total exposure time is 30 minutes after which the water enriched with oxidising chemicals, also called Plasma Activated Water, is ready for use.

Preparation of natural products

The aqueous plant extracts were obtained by maceration in water. Leaves and stems of *Ageratum conyzoides*, *Laggera pterodonta* (Asteraceae), *Euphorbia hirta* and *Euphorbia cordifolia* (Euphorbiaceae), and leaves of *Eucalyptus saligna*, *Callistemon viminalis* (Myrtaceae), *Ocimum gratissimum* (Lamiaceae) were collected in Dschang locality (latitude: 5°26′38.29″ N, longitude: 10°03′11.95″ E), as well as the avocado pit *Persea americana,* Lauraceae). The roots of *Panax quinquefolius* (Araliaceae) were collected in the locality of Bandrefam (latitude: 5°13′0″ North, longitude: 10°30′0″ East) during the same month. The leaves of *Azadirachta indica* (Meliaceae) were collected in the locality of Garoua (latitude: 9°18′5.15″ N, longitude: 13°23′51.76″ E) where they grow naturally. In Cameroon, these plants (Figure 1) are commonly used in the traditional pharmacopoeia to cure many human diseases (Adjanohoun et al., 1996; Kuete and Efferth, 2010; Ngene et al., 2015).

The plant's organs were harvested in the afternoon, washed with tap water, and fragmented into small pieces. They were then dried in the shade for 14 days and ground separately using an electric grinder to obtain fine powder. One hundred grams (100 g) of each plant powder was macerated in 15 L of solvent (water) for 24 hours. The filtrate obtained (with a concentration of 6.67 g/L) was directly used for field application (Djeugap et al., 2023). The natural vinegar solution was obtained from ripe and unripe banana peels through fermentation. Ripe and unripe banana peels were cut into small pieces, dried and crushed into fine powder. Then, 150 g of the powder was fermented in 15 L (10 g/L) of water for 5 days. The fermented solution was directly applied without any dilution. The natural sodium bicarbonate (baking soda) was obtained from the local shops.

Phytochemical screening and determination of total phenols, flavonoids and tannins content in *Euphorbia* spp. extract

The identification of phytochemical compounds was carried out only with *Euphorbia* spp. (*E. hirta* and *E. cordifolia*) extract that showed a high antifungal potential. This screening was performed using qualitative staining methods according to Harbone (1973).

The total phenol content was determined by the method described by Ramde-Tiendrebeogo et al. (2012). The reagent is a mixture of phosphotungstic acid and phosphomolybdic acid. It is reduced, during the oxidation of the phenols, to a mixture of blue oxides of tungsten and molybdenum. These blue pigments have a maximum absorption that varies according to the qualitative and/or quantitative composition of the phenolic mixtures in addition to the pH of the solutions, usually obtained by adding sodium carbonate. The results were expressed as milligrams equivalent of gallic acid per gram of powder.

The total flavonoid content was determined using the aluminium chloride colorimetric method (Chang et al., 2002). A volume of 100 µL of extracts (2 mg/mlL) was mixed with 50 µL of aluminium chloride (1.2 %), then 50 µL of potassium acetate (120 mM) was added. The total flavonoid content was calculated using the quercetin calibration curve (quercetin concentration ranged from 0.015 to 2 mg/mlL) and the results were expressed as milligram equivalent quercetin per gram of powder.

Figure 1. Plant material used for the preparation of aqueous extracts. *Eucalyptus saligna* (A), *Azadirachta indica* (B), *Callistemon viminalis* (C), Avocado pit (D), *Ageratum conyzoïdes* (E), *Panax quinquefolius* (F), *Euphorbia hirta* (G), *E. cordifolia* (H), *Ocimum gratissimum* (I) and *Laggera pterodonta* (J).

The Folin-Ciocalteu method as described by Govindappa et al. (2011) determined the total tannin content. Here, the reaction mixture consisted of 100 µL of extract, 500 µL of Folin-Ciocalteu reagent (diluted 10-fold in water), 1000 µL of 35% sodium carbonate solution, and 8.4 ml of distilled water. The mixture was stirred and incubated at room temperature for 30 min, and then the absorbance was measured with a spectrophotometer at 700 nm. The results were expressed as milligrams equivalent of tannic acid per gram of powder.

Field experimentation

The study was carried out in Dschang sub-division (Latitude: 5°26′45.3804″ N, Longitude: 10°2′49.758″ E and Elevation: 1327 m) from April to August 2022. The experimental design was a completely randomised block design made of 10 treatments. The different treatments were as follows: Plasma activated water (PAW), Sodium bicarbonate + Vinegar (25:75), Sodium bicarbonate + Vinegar (50:50), Sodium bicarbonate + Vinegar (75:25), Mixture of aqueous extract of *Ageratum conyzoides* + *Eucalyptus saligna* + *Azadirachta indica*, Mixture of aqueous extract of *Panax quinquefolius* + *Callistemon viminalis*, Mixture of aqueous extract of *Euphorbia hirta* + *E. cordifolia*, Mixture of aqueous extract of *Laggera pterodonta* + Powder of avocado pit + *Ocimum gratissimum*. The chemical fungicide Plantizeb (active ingredient: Mancozeb) and water were considered positive and negative control, respectively. The number of repetitions (block) was five and each experimental unit (EU) occupied a surface area of 6 m². The EU and the blocks were separated from each other by a distance of 1 m and 1.5 m, respectively. The total exploited area was 604.5 m² (19.5 m × 31 m). One-month-old tomato seedlings grown in the nursery near the experimental site were transplanted at a crop density of 28,571 plants/ha (0.7m × 0.5m). The tomato variety used was NADIRA, a hybrid variety with a cycle of 65 to 70 days after transplanting and yielding 28 t/ha. Poultry manure (10 t/ha) was applied one week before transplanting. The fertilisers NPK (20-10-10) and NPK (13-13-21) were applied at two and four weeks after transplanting (100 g/m²), respectively; NPK (13-13-21) was repeated when the first flowers appeared (Djeugap et al., 2016). Weeding was done manually once a month with a hand hoe. Natural infection was allowed to develop in the field like in the tomato farmer plantations. However, after the establishment of natural infection, samples showing late blight symptoms were collected and carried to the laboratory for pathogen isolation and identification. This was a requirement for field application of treatments and necessary to confirm that the natural infection observed was due to *Phytophthora infestans*. The PAW and the different combinations of sodium bicarbonate with vinegar were used directly without dilution. The mixtures of plant extracts were prepared at a ratio of 50:50 and tested at 6.67 g/L while Mancozeb (positive control) was applied at the recommended dose (3.33 g/L). The water was used as a negative control. The treatments were applied on all the aerial parts of the tomato crops with a 15 L Knapsack sprayer. The extracts and Mancozeb were applied at the frequency of 10 days as recommended

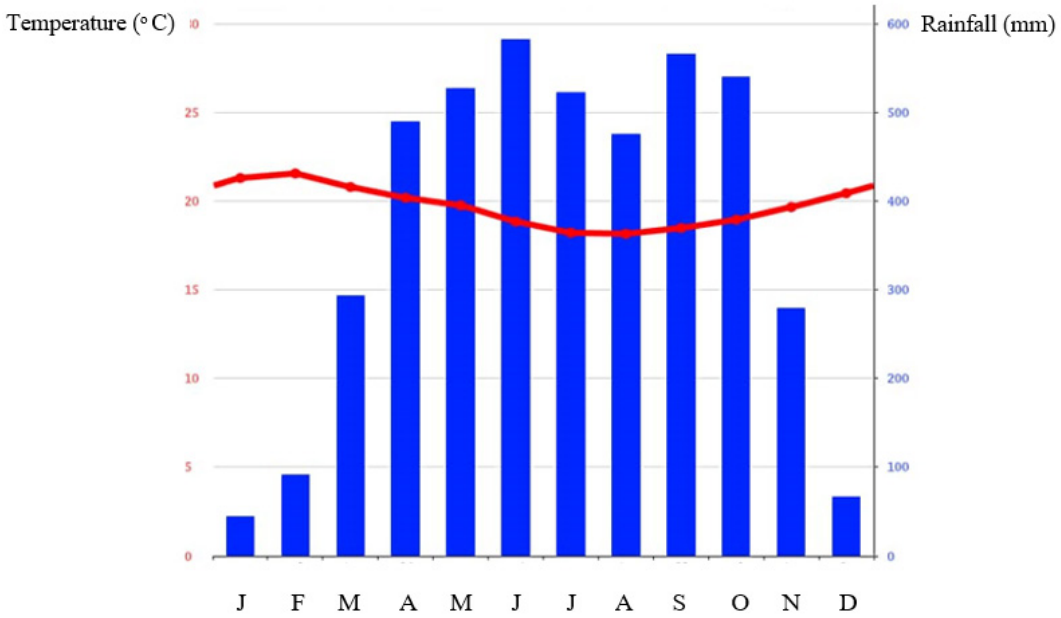

Figure 2. Ombrothermic diagram of the experimental site, 2022

for the chemical during homologation (8 to 14 days). Imidacloprid (systemic insecticide) was used to control insects in the field. All the treatments were repeated five times. The rainfall (mm) and temperatures (°C) were recorded (Figure 2) in the meteorological station of the National Institute of Research in Agriculture located about 500 m from the experimental site.

Data collection

Data were collected on six plants located at the center of each experimental unit and related to growth, late blight, and yield. The measurement of growth variables started 4 weeks after transplanting (WAT) and they included the plant height (cm), and the number of leaves and branches. Late blight incidence and severity were assessed weekly as follows: Incidence = 100 × (number of diseased plants/total number of plants). The severity is the percentage of the infected area of the plant over the total area of the plant considered (Campbell and Madden, 1990). The marketable yields were obtained by recording the mass of mature fruits as follows: Yield (t/ha) = (mi × 28,571) / 6 where mi is the cumulated mass of harvested fruits of each treatment in t/ha and 28,571 is the crop density (in plants/ha) and 6, the number of plants on which data were collected.

Data analysis

The data were introduced to Microsoft Office Excel 2013 and histograms were plotted by the same software. Then, they were subjected to a two-way analysis of variance test using the R software package, version 4.0.1 (R Core Team, 2021). Data in percentage were submitted to angular transformation (or the arcsine square root transformation) before analysis of variance. The data were tested using the Shapiro-Wilk test and were also homogeneous (Levene test) at 5%. For each variable having a significant effect, mean separation was carried out by LSD test (least significant difference) at a 5% significance level.

RESULTS AND DISCUSSION

Effect of treatment application on tomato growth variables

There was a significant difference ($p < 0.05$) in plant height, number of leaves, and the number of branches between treatments. The plant height of the tomato varied from 34.21 cm (control) to 45.75 cm (Plantizeb). The height of plants treated with PAW (43.83 cm) and with the mixture of the extract of *E. hirta* and *E. cordifolia* was similar to the effect of Plantizeb, 10 weeks after transplanting (Table 1).

The highest number of leaves was obtained with Plantizeb (11.17) similar to PAW (9.85). The lowest leaf number was obtained in plants treated with water (control) and with the mixture of Sodium bicarbonate and Vinegar (50:50). The effect of treatments on the number of branches was not significant at 5%. The application of PAW and the plant extracts caused a significant improvement in the growth variables of the tomato plants which were all superior to the control. This could be explained by the fact that these extracts were rich in growth compounds like vitamins, cytokinin and auxin. Cytokinin and auxin are known to affect positively the cellular metabolism of plants increasing their growth, and consequently, their yield. These results corroborate the work of Aghofack et al. (2015) who showed that the extracts from the powder of *Spirulina platensis* and *Jatropha curcas* significantly improved the size, collar diameter, and biomass of the aerial parts. The effect of plasma-activated water on growth variables was highly significant and comparable

Table 1. Effect of PAW, plant extracts, bicarbonate, and vinegar on the height (cm), number of leaves and ramifications of tomato, 10 weeks after transplanting

Treatments	Plant height (cm)	Number of leaves	Number of branches
Positive control Plantizeb (fungicide)	45.75 ± 3.03^{a}	11.17 ± 3.40^{a}	6.67 ± 2.23^{a}
Negative control (water)	34.21 ± 2.01^{c}	3.12 ± 4.16^{c}	5.33 ± 1.23^{a}
Plasma activated water (PAW)	43.83 ± 3.11^{a}	9.85 ± 2.78^{a}	6.58 ± 1.38^{a}
Sodium bicarbonate + Vinegar (25:75)	37.13 ± 3.56^{ab}	3.75 ± 1.22^{c}	5.15 ± 1.65^{a}
Sodium bicarbonate + Vinegar (50:50)	38.25 ± 3.21^{bc}	6.67 ± 3.70^{bc}	5.83 ± 1.11^{a}
Sodium bicarbonate + Vinegar (75:25)	39.42 ± 2.21^{bc}	5.13 ± 2.97^{bc}	5.51 ± 1.45^{a}
Extract of *A. conyzoides* + *E. saligna* + *A. indica*	38.53 ± 3.17^{ab}	5.67 ± 3.2^{bc}	5.17 ± 1.34^{a}
Extract of *P. quinquefolius* + *C. viminalis*	40.83 ± 3.34^{bc}	5.17 ± 3.46^{bc}	5.75 ± 1.60^{a}
Extract of *E. hirta* + *E. cordifolia*	42.33 ± 3.25^{a}	7.58 ± 3.55^{bc}	6.25 ± 1.66^{a}
Extract of *L. pterodonta* + Powder of avocado pit + *O. gratissimum*	37.75 ± 2.09^{bc}	5.51 ± 1.31^{bc}	5.12 ± 1.41^{a}

*Means in the column followed by the same letter are not significantly different at the 5% level according to the Kruskal-Wallis test.

Table 2. Some secondary metabolites and phenolic compounds present in aqueous extract of *Euphorbia hirta* and *E. cordifolia*

Secondary metabolites and phenolic compounds	*Euphorbia hirta*	*Euphorbia cordifolia*
Alkaloids	+	+
Phenols	+	+
Flavonoids	+	+
Sterols	-	-
Triterpenoids	+	+
Tannins	+	+
Saponins	-	-
Anthocyanins	-	-
Anthraquinones	-	-
TPC (mg GAE/g of extract)	66.79 ± 0.15	82.55 ± 0.22
TFC (mg EQ/g of extract)	3.91 ± 0.27	5.16 ± 0.11
TTC (mg TAE/g of extract)	44.5 ± 0.29	52.3 ± 0.44

+ Presence – absent; TPC: Total phenol content, TFC: Total flavonoid content, TTC: Total tannin content; GAE: Gallic acid equivalent; EQ: Equivalent of quercitrin; TAE: Tannic acid equivalent.

to Plantizeb. This result was also reported in previous studies showing a significant positive effect of Plasma Activated Water on promoting seed germination and plant growth (Thirumdas et al., 2018; Mogo et al., 2022). Moreover, it was recently established that PAW and aqueous extracts of some medicinal plants increase potato growth variables (Njopkou, 2019). This efficiency could be explained by the higher value of nitrates and nitrite ions in the PAW (Thirumdas et al., 2018). Indeed, nitrate plays an important role in the photosynthesis process with accelerated plant growth.

Chemical composition of aqueous extracts of *Euphorbia hirta* and *E. cordifolia*

The most active plant extract with high antifungal potential was the *E. hirta* and *E cordifolia* mixture. The phytochemical screening of aqueous extract of this plant showed the presence of secondary metabolites such as alkaloids, phenols, flavonoids, triterpenoids, and tannins (Table 2). The total phenol content was higher in the two plant extracts (66.79 and 82.55 mg GAE/g of extract) than the total flavonoid content and the tannins.

Due to their high content of various bioactive compounds, plants are the main raw material for the production of valuable, and useful bio-products with both growth regulators and antimicrobial potential (Galani et al., 2013; Luh et al., 2020; Mkindi et al., 2020). For example, soybean leaf extracts have been shown to contain higher levels of flavonoids such as salicylic, 4-hydroxybenzoic, valillic, 4-hydrooxycinnamic, ferulic, caffeic, gentisic, and quercetin with high protective properties (Mkindi et al., 2020). It was shown that terpenes and flavones may cause the breakdown of microbial cell membranes (Urzua et al., 2006) while tannins can inhibit electron transport through membranes and can alter ions like iron and copper thus inhibiting the activity of some enzymes which may be essential for microbial life.

Bioactivity of treatment application on tomato late blight and yield variables

The effect of PAW, aqueous plant extracts mixture, sodium bicarbonate and vinegar mixture on tomato late blight incidence and severity significantly varied between treatments 6 and 10 weeks after transplanting (WAT). The incidence of late blight was total (100%) in plants treated with water from the 6th WAT and with all the mixtures of plant extracts at 10 WAT. Plants that received Plantizeb, PAW, the mixture of sodium bicarbonate and vinegar, and the mixture of *E. hirta* and *E. cordifolia* extract gave the lowest late blight incidence and severity (Table 3). Also, the values of incidence and severity obtained from these treatments were comparable with each other at 10 WAT. Late blight severity was 31.67%, 35.83%, 33.24%, and 36.33% on plants treated with Plantizeb, PAW, sodium bicarbonate, and vinegar (50:50) and the extract of *E. hirta* + *E. cordifolia*, respectively. Among the mixture of plant extracts tested, the association of aqueous extracts of *A. conyzoides* + *E. saligna* + *A. indica* and *L. pterodonta* + Powder of avocado pit + *O. gratissimum* gave the highest disease severity.

The yields obtained from plants that received PAW (27.23 t/ha), sodium bicarbonate + vinegar mixture (30.32 t/ha) and the mixture of *E. hirta* + *E. cordifolia* (27.69 t/ha) were significantly comparable to yields of plants treated with Plantizeb (28.56 t/ha) (Table 3). The plants that did not receive PAW or extracts (control) gave the lowest yield values (2.24 t/ha). The plant

Table 3. Effect of the treatments on tomato late blight incidence and severity (6 and 10 weeks after transplanting) and on the yields (t/ha)

Treatments	Incidence (%)		Severity (%)		Yields (t/ha)
	6WAT	10WAT	6WAT	10WAT	
Positive control Plantizeb	62.64 ± 8.52^{c}	93.94 ± 3.16^{b}	25.12 ± 3.13^{cd}	31.67 ± 5.31^{e}	28.56 ± 1.31^{a}
Negative control (water)	100 ± 0.0^{a}	100 ± 0.0^{a}	50.51 ± 6.22^{a}	100 ± 0.0^{a}	2.24 ± 0.43^{d}
Plasma activated water	64.31 ± 7.84^{c}	96.12 ± 2.4^{b}	25.25 ± 4.32^{cd}	35.83 ± 3.94^{e}	27.23 ± 2.47^{a}
Sodium bicarbonate + Vinegar (25 :75)	90.64 ± 6.82^{b}	98.67 ± 2.1^{a}	27.52 ± 6.22^{bcd}	54.33 ± 6.31^{c}	20.15 ± 2.31^{b}
Sodium bicarbonate + Vinegar (50 :50)	60.48 ± 5.91^{c}	91.33 ± 2.25^{b}	23.13 ± 3.86^{d}	33.24 ± 4.46^{e}	30.32 ± 2.21^{a}
Sodium bicarbonate + Vinegar (75 :25)	64.37 ± 5.52^{c}	95.33 ± 2.67^{b}	28.33 ± 6.51^{bcd}	48.42 ± 6.8^{dc}	25.37 ± 1.31^{b}
Extract of *A. conyzoides* + *E. saligna* + *A. indica*	85.13 ± 9.54^{b}	100 ± 0.0^{a}	42.17 ± 8.32^{b}	64.58 ± 6.87^{b}	22.85 ± 1.14^{b}
Extract of *P. quinquefolius* + *C. viminalis*	68.27 ± 7.81^{c}	100 ± 0.0^{a}	20.15 ± 5.37^{d}	44.66 ± 4.22^{d}	25.45 ± 1.63^{b}
Extract of *E. hirta* + *E. cordifolia*	88.24 ± 6.56^{b}	100 ± 0.0^{a}	27.33 ± 7.42^{bcd}	33.33 ± 4.31^{e}	27.69 ± 1.9^{a}
Extract of *L. pterodonta* + Powder of avocado pit + *O. gratissimum*	87.86 ± 6.99^{b}	100 ± 0.0^{a}	46.67 ± 4.31^{ab}	68.75 ± 6.31^{b}	19.11 ± 1.3^{c}

*Means in the column followed by the same letter are not significantly different at the 5% level according to the Kruskal-Wallis test. WAT = week after transplanting.

extracts tested significantly reduced the development of late blight compared to the negative control (Figure 3).

This efficacy could be attributed to the fact that these plants possess well-known antimicrobial components such as emamectin benzoate and lambda-cyhalothrin for *Ageratum conyzoides* (Amoabeng et al., 2013), terpenes for *Eucalyptus saligna* and *Azadirachta indica,* flavonoids, alkaloids, saponosides and tannins for species of the Euphorbiaceae family (*Euphorbia hirta* and *E. cordifolia*) (Liu et al., 2007; Kiem et al., 2009). These results corroborate those of Kpatinvoh et al. (2017) who obtained significant fungal growth inhibition of an aflatoxin-producing strain of *Aspergillus flavus* with *A. indica* leaves and seeds extract (Abyaneh et al., 2005). The efficacy of *Callistemon viminalis* observed in this study was also reported by Djeugap et al. (2011) against *Phytophthora infestans* causative agent of late blight of huckleberry and *Trichoderma harzianum, Aspergillus* sp. and *Penicillium* sp. (Audy et al., 2000). The efficiency observed with Plasma Activated Water on late blight control corroborates findings reported by previous works on other plant pathogens. PAW was proven effective against the potato bacterium pathogen *Erwinia carotovora* subsp. *atroseptica* which was able to cause the release of the bacterial genomic DNA from the cell and induce the release and dimerization and/or aggregation of cell proteins (Moreau et al., 2007). On the contrary, PAW was not able to show direct antimicrobial activity against bacterial leaf spots of tomatoes caused by *Xanthomonas vesicatoria* during *in vitro* experiments but it was able to enhance the tomato plants' defences by significantly reducing the disease severity after field inoculation of the pathogen (Perez et al., 2019). Moreover, the conidial germination of *Pyricularia oryzae* (blast disease of rice) in laboratory conditions and the percentage of infected seedlings in the field were reduced after seed treatment with Plasma Activated Water for 120 s (Hasan et al., 2020). This efficacy could be due to the presence in PAW of the reactive oxygen species (ROS), such as O_2, OH, and H_2O_2, which are known to be one of the upstream responses of the plant during a pathogen attack (Alvarez et al., 1998) and therefore, corroborate the hypothesis that the plasma generated by glidarc discharge is acting on pathogens essentially through oxidative mechanisms and activator of defense mechanisms of the plant host (Konola et al., 2000).

Figure 3. Late blight severity on tomato plants treated with: Plantizeb (A), Plasma activated water (B), mixture of *E. hirta* + *E. cordifolia* extracts (C) and control, water (D), 10 WAT. WAT = week after transplanting

The efficacy of vinegar observed in the present study was reported earlier. Vinegar residues substrate made up of compost, peat, and vermiculite mixtures (at a ratio of 3:2:1) significantly promote tomato growth and suppress the bacterial wilt (*Ralstonia solanacearum*) in field conditions (He et al., 2020). Moreover, wood vinegar from six tree species (pine, pomegranate, pistachio, almond, walnut, and cypress) was shown to inhibit the growth of *Phytopythium* sp. causing tomato collar rot in Iran and allowing plant protection against oomycetes diseases (Bouket et al., 2020). The use of vinegar from plant products to protect plants against fungal diseases in this study shows that it could be used as an alternative to plant disease control. This efficacy could be due to the presence of lactic acid, alcohol, and esters as the main compounds in vinegar composition and were proven to inhibit the pathogen's development or their phenolic compound (Oramahi and Yoshimura, 2013). Natural vinegar was also shown to improve soil physico-chemical properties as well as the microbiome content, including plant-growth-promoting rhizobacteria (Sivaram et al., 2022).

As a well-known chemical fungicide, Plantizeb is homologated in Cameroon for the management of late blight and other fungal diseases. Indeed, several previous studies have shown the effectiveness of this active ingredient in controlling cryptogamic diseases such as downy mildew in tomatoes, black nightshade, and potatoes. This could be explained by its ability to generate isothiocyanate which deactivates thiol enzymes and metabolites in fungal cells (Ragsdale, 1992). In an aqueous solution (boiled), it decomposes to release ethylene bis sulfide thiocyanate which in turn under the action of UV light is converted into ethyl isothiocyanate; these two derivative compounds deactivate certain essential biochemical processes taking place in fungal cells such as respiratory function and spore germination (Gullino et al., 2010). This action of Plantizeb 80 WP in the control of tomato late blight in this study is close to that obtained by other authors on various diseases. This was the case, for example, in the control of rice helminthosporiosis due to *Bipolaris oryzae*, which made it possible to increase yield by 38 to 120 % (Percich and Huot, 1989), vine downy mildew due to *Plasmopara viticola*, whose application inhibits spore germination and provides complete control of disease (Wong and Wilcox, 2001) and *Ricinodendron heudelotii* seedling shoot blight caused by *Lasiodiplodia theobromae* (Djeugap et al., 2015).

CONCLUSION

In this study, natural products such as Plasma Activated Water, a mixture of sodium bicarbonate and vinegar from banana peels at different ratios and different mixtures of aqueous extracts from Cameroonian medicinal plants were tested on late blight development in the field. Results obtained from the field show that PAW and the mixture of aqueous extract of *Euphorbia hirta* and *E. cordifolia* (6.67 g/L) improved significantly tomato growth variables as Plantizeb compared to other treatments. These treatments and the mixture of sodium bicarbonate and banana peel vinegar (50:50) reduced late blight severity and increased tomato yields as well as Plantizeb. Based on these results, the foliar application of plasma-activated water (as manufactured without dilution), banana peel vinegar, and aqueous extract of the *Euphorbia* spp. could be exploited as an alternative to Plantizeb for the sustainable management of late blight.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicting interests.

ETHICAL COMPLIANCE

The authors have followed ethical standards in conducting the research and preparing the manuscript.

AUTHORS' CONTRIBUTIONS

Conceptualization of the work: Djeugap F.J. and Njopkou T.A.M; Methodology: Djeugap F.J. and Njopkou T.A.M; Data collection: Njopkou T.A.M., Pianta T.F., Kuenbou M.J. and Kamseu M.J.P; Software and data analysis: Djeugap F.J.; Writing-original draft preparation: Njopkou T.A.M. and Kuenbou M.J.; Writing-review and editing: Djeugap F.J. All authors have read and agreed to the published version of the manuscript.

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