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Research Article

On-farm Validation of *Coffea arabica* and *Aloe vera* Extracts for Management of Seed-Borne Bacterial Leaf Spot Disease of Tomato

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Abstract: In Tanzania, seed infection by bacterial leaf spot (BLS) pathogen (*Xanthomonas perforans*) causes yield losses up to 45% in tomato (*Lycopersicon esculentum* L.; Solanaceae family). Several studies have been conducted and wedged ecological organic agriculture (EOA) technologies (i.e., on botanicals/ biopesticides) which are significant to organic farmers in Tanzania. Nevertheless, these studies have been conducted in laboratory and greenhouse conditions, hence the technology cannot be disseminated to organic farmers for application before being validated. The current study was laid out as a 2x3 factorial experiment with five replications. Factor A was two common tomato cultivars "Rio grande" and "Malkia F1", while factor B was seed treatment with three levels of crude plant extracts namely *A. vera*, *C. arabica*, and *A. vera* + *C. arabica* and untreated/control. To make the individual crude extracts, the roasted *C. arabica* beans powder (5g) and *A. vera* juice (5 ml) were mixed into 50 ml of clean water to get 10% weight/volume (w/v), respectively, while *A. vera* + *C. arabica* combination was obtain at a volume (ml) ratio (1:1). Tomato seeds were then soaked in 1 ml of the 10% w/v plant extracts for 12 hours, then air-dried for 1 hour before sowing. The highest efficacy against *Xanthomonas perforans* was obtained from a combination of extracts from *A. vera* + *C. arabica* at volume (ml) ratio (1:1) hence, recommended for seed treatment. Organic tomato farmers need to adopt seed treatment practices that ensure seedlings' start-up and enhance crop growth and productivity. Although the results of validation comply with the recommendations from previous research findings, further study is needed to evaluate the effectiveness of plant extracts subject to seasonal variability among the production areas.

Keywords: *Aloe vera*; Bacterial leaf spot; *Coffea arabica*; Plant extracts; Seed-borne pathogens; Seed treatment; Tomato; Validation; *Xanthomonas perforans*

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1. Introduction

Vegetables are among the major horticultural crops grown in Tanzania, whereby the main production areas include the southern highlands, northern corridor, and the coastal zone [1]. There has been an increase in horticultural production including fruit vegetables for both export and domestic use due to huge demand for Tanzanian agricultural products. This has triggered intensified cultivation, reduced usage of traditional pest management methods, and increased reliance on synthetic agricultural inputs particularly inorganic fertilizers and pesticides [1]. These have resulted in negative impacts on soils, biodiversity, and the environment in general, thereby affecting crop, animal genetic diversity and welfare, human nutrition and increased costs for public health, and communities' vulnerability to external shocks [2]. In addition, negative impacts of climate change (i.e., low productivity, food security, and profitability both at household and national level) tend to dominate crop production, especially horticulture. The change in rainfall, temperature, atmospheric CO_2, solar radiation, and wind patterns as the climate variables have both direct and indirect significant impacts on plants, soils, insects, weeds, and diseases [3]. Reduction in plant available water as a result of increased evaporative demand of the atmosphere; decline in soil fertility from an increase in soil acidification, mineralization, erosion, degradation, and microbe imbalance; increase in incidence, the geographic distribution of pest populations, survival and their vigour are among the numerous and diverse mechanisms, effects, or responses of agriculture to the change in climatic conditions [4].

In efforts to resolve the negative impacts associated with conventional agriculture and climate change which also affect crop production through the reduction of natural resources' capacity to support productive agriculture; ecological organic agriculture (EOA) becomes inevitable. EOA initiative in collaboration with several actors including the Sustainable Agriculture Tanzania (SAT), set validation of different EOA technologies recommended from several studies, purposely to make them useful at farmers' level for improvement of the organic agriculture industry. EOA combines tradition, innovation, and science to benefit the shared environment and promote fair relationships and good quality of life for all involved. It involves the use of fertilizers of organic origin like manures and emphasizes techniques like crop rotation, mixed cropping, biological pests, and diseases control [5]. This type of agriculture relies on ecological processes, biodiversity and cycles adapted to local conditions rather than the use of inputs with adverse effects. However, under EOA, inputs with fewer chemicals like copper, elemental sulfur, and rock phosphate fertilizers can be used [6].

Considering the potential of vegetables in improving the nutrition and livelihood of communities, and the large yield losses caused by insect pests and disease-causing pathogens, more effective and affordable pest management is required. Among the feasible and economical strategies for pest control in vegetables is the use of botanicals/bio-pesticides [7]. Adoption of biopesticides may reduce overreliance on synthetic pesticides, prevent their detrimental effects on human health and the environment, as well the development of resistant pest and pathogen strains [7]. Moreover, botanicals are inexpensive, easily biodegraded, their sources are easily available, and most of them have low toxicity to non-target organisms. Hence, they can be incorporated into integrated pest management systems and contribute to sustainable agricultural production [7]. However, for some naturally occurring active substances like caffeine (1,3,7-trimethylxanthine) found in plant constituents such as coffee require appropriate risk assessment to non-target organisms so that to ensure that the vulnerable groups including toddlers, children and pregnant women are not exposed to levels of caffeine above the maximum level of no concern (3 mg/kg bw per day) as a consequence of its use as plant protection product since its adverse effects may result to main health concerns [8].

In Tanzania, seed infection by bacterial leaf spot (BLS) pathogens (*Xanthomonas perforans*) cause yield losses up to 45%. Seeds, plant debris, and volunteer plants enhance the survival of *Xanthomonas perforans* [9]. However, the management of BLS is limited to foliar applications of pesticides composed of copper-based compounds. Moreover, the abundance of Xanthomonas species, and races causing BLS symptoms in tomatoes [10] and the existence of numerous BLS pathogens strains with higher degrees of tolerance to copper-based compounds [11–17] have made the control of the disease difficult. In the attempt to manage the seed-borne disease, the study by Mbega et al. [9] was conducted on the tomato (*Lycopersicon esculentum*) crop of the Solanaceae family has wedged an EOA technology (i.e., botanicals/biopesticides) which is significant to organic farmers in Tanzania. Plant extracts from the true Aloe barbadensis Miller (*Aloe vera* L.), and Coffee (*Coffea arabica* L.) were potential for seed treatment against seed-borne *Xanthomonas perforans* [9]. In other countries such as Nigeria, an in vitro and in greenhouse application of crude extract of *Aloe vera* as seed dressing (at higher concentration of 50% cold water extract) also reduced percentage incidence of bacterial blight of long-staple cotton (*Gossypium hirsutum* L.) caused by *Xanthomonas axonopodis* pv. malvacearum (Smith) Dye (Xam) [18].

A previous study [19] reported that, Maillard, carbohydrate caramelization and thermal composition products are substances produced by the roasting process which have been associated with the antibacterial activity of coffee. However, with advancement of research and technology, several studies have reported that coffee beans, and its by-product extract have strong antibacterial activity due to the fact that, coffee beans contain numerous compounds such as phenolics, caffeine, caffeic acid (CA), diterpenes, and trigonelline, flavonoids, chlorogenic acid (CGA), and protocatechuic acid that exhibit powerful bioactivities [20–25] and as significant natural antimicrobial agents they play a vital role against enteric bacteria [21,26,27]. Polysaccha-

rides from *Aloe vera* (*Aloe barbadensis* Miller) parenchyma attributed the control of bacterial spot caused by *Xanthomonas gardneri* on tomato [28]. It's effect was related to the induction of resistance through increase in the activity of enzymes i.e., peroxidase, polyphenoloxidase, and glucanase that are responsible for the biosynthesis of plant-defense-compounds especially during the onset of bacterial spot, as well as the reduction of disease severity [28]. A significant increase in the level of these enzyme activities in the treated plants contributes to reduction of disease severity since the proteins create adverse conditions for the development of the pathogen in the plant particularly through deposition of lignin in plant cell walls, promoting disorganization of pathogen cell wall structure and cell death, consequently slowing disease progression in tomatoes [28]. Nevertheless, the previous research were conducted in a laboratory and greenhouse setting, hence the technology cannot be disseminated to organic farmers for application before being validated. Therefore, generally, the present study aimed to establish detailed efficacy of the recommended biopesticides through on-farm experimentation for dissemination through organized farm visits, where men, women, and youths were involved in the on-farm demonstration of the results.

2. Materials and Methods

2.1. Description of the Study Areas

The study was conducted in five randomly selected organic farms from two regions, one in Tanzania mainland and the other in the islands of Zanzibar. Among the five farms, three of them were selected from the Morogoro region in mainland Tanzania, where each farm was located at Menge and Mayanga villages in the Mvomero district (7° 2' 49.3" S, 39° 19' 57.1" E, 380m asl), and Mikese village in Morogoro district (6° 44' 1" S, 37° 55' 14" E, 402m asl). While the other two selected farms were located at Bungi village in Unguja island (6° 14' 20" S, 39° 19' 45" E, 22m asl) of Zanzibar. The villages in the Mvomero district receive a bimodal type of rainfall with peaks in April and December for long and short rains, respectively, while June to September remains relatively dry, where the average rainfall amounts to 1146mm per annum. The mean maximum temperature is 31°C from October to March; whereas the mean minimum temperature is 19°C between June to September [29]. The climate of Mikese village is hot and humid all year round. Mikese receives rainfall from October to May, with an average rainfall of 850mm per annum, whereas the driest months are from June to September [30]. On the Unguja Island of Zanzibar, the annual air temperature ranges between 27 and 35°C with an average rainfall of 1,600mm per annum. Southwest-driven monsoonal rains and high currents occur between March to May, while low precipitation and north-eastern currents are typical between November and March [31]

2.2. Plant Extracts and Seed Treatment

2.2.1. Plant Extracts Preparation

Crude extracts from two botanicals namely; *C. arabica* and *A. vera* were evaluated as biological control strategies (i.e., organic pesticides for seed treatment) to manage BLS disease. *C. arabica* beans and *A. vera* leaves for extractions were obtained from farmers in Mbeya and Morogoro regions, respectively. Coffee beans were roasted then ground to get coffee powder. Then the crude extract was made by mixing 5g of coffee powder into 50 ml of clean water to get 10% weight/volume (w/v). Aloe leaves were cut into small pieces, then ground using a mottle and pestle. Extraction was done by mixing 5 ml of *A. vera* juice with 50 ml of clean water to get 10% w/v. Each of these mixtures was left for 12 hours then filtered using a double-layered cotton cloth.

2.2.2. Seed Treatment

BLS-infected seeds of the common tomato cultivars "Rio grande" in the Morogoro region (3 locations) and "Malkia F1" in Zanzibar island (2 locations) were used for the tests. In each location, a total of 400 tomato seeds per cultivar (100 seeds for each of the four treatments) were randomly picked and soaked in a total of five plastic vials (20 seeds per vial) containing 1 ml of the 10% w/v plant extracts and sterile distilled water (as a negative control), respectively, then were placed on a table overnight (12 hours) at room temperature (25±3°C). After an overnight incubation, the treated seeds were placed on tissue papers and allowed to air-dry for 1 hour before sowing.

2.3. Experimental Design and Treatments Allocation

The study was laid out as a 2x3 factorial experiment with two factors and five replications. The five selected organic farms were used as replicates whereby three farms were selected from the Morogoro region in mainland Tanzania, where each farmer was located at Menge and Mayanga villages in Mvomero district and Mikese village in Morogoro district. While two farms were selected from farmers located at Bungi village in Unguja island of Zanzibar. Factor A was two common tomato cultivars "Rio grande" and "Malkia F1", while factor B was seed treatment with three levels of crude plant extracts namely *A. vera*, *C. arabica*, combination of *A. vera* + *C. arabica*, and untreated control.

2.4. Evaluation of Plant Extracts Efficacy in Farmers' Fields

2.4.1. Seed Germination and Seedling Vigour Test

The effect of selected plant extracts on seed germination and seedling vigour was evaluated on *Xanthomonas perforans* infected tomato seeds collected from tomato growers and treated with plant extracts as described in (Section 2.2.2). Seed germination tests were conducted using the

400 tomato seeds per cultivar (100 seeds for each of the four treatments). The standard International Seed Testing Association top of the paper method by ISTA [32] was followed. The seeds were sown uniformly (25 seeds for each of the four replicates) in polythene plastic trays (L x W x H = 56.5 x 26.5 x 6 cm) containing a mixture of forest soil, rice husks, and farmyard manure at weight (kg) ratio (3:1:1) and kept under nursery condition in farmers' fields for 14 days.

2.4.2. Seedlings Health and Crop Performance Experiment

The effect of selected plant extract on seedling health and crop growth was evaluated on *Xanthomonas perforans* infected tomato seeds collected from tomato growers and treated with plant extracts as described in (Section 2.2.2). The experiment on seedlings health and crop performance was conducted using the 400 tomato seeds per cultivar (100 seeds for each of the four treatments). Seeds were sown in polyethene plastic trays (L x W x H = 56.5 x 26.5 x 6 cm) containing a mixture of forest soil, rice husks, and farmyard manure at weight (kg) ratio (3:1:1) and kept under nursery condition in farmers' fields. Seven days after sowing, 48 seedlings of each cultivar per treatment were randomly pricked and transplanted to the farmers' fields (plot size 51.84m^2) at a spacing of 30x90cm.

2.5. Data Collection

Normal and abnormal seedlings and dead seeds were counted for the germination tests. The vigour test involved measurements of root and shoot lengths of seedlings and the percentage seed (normal seedlings) germination. The seedling vigor index (Vi) was calculated as Vi = (mean root length + mean shoot length) x (percentage germination). BLS incidence and severity in the tomato seedlings were assessed 21 days after sowing in the nursery (since symptoms can be seen from early stages of growth). Disease severity was determined based on visual observation using a BLS severity index of the 1-6 scale, where 1 = no disease, 2 = 0–3% of leaves with BLS symptoms, 3 = 3–12% of leaves with BLS symptoms, 4 = 12–25% of leaves with BLS symptoms, 5 = 25–50% of leaves with BLS symptoms and 6 = 50% of leaves with BLS symptoms [9]. . The efficacy of plant extract treatments in the control of BLS in tomato seedlings was calculated as the BLS reduction index (BLSRI%) = (C - T)/C x 100, where C is the incidence of BLS in tomato seedlings raised from seeds treated without treatment (negative control) and T is the incidence of BLS in tomato seedlings from infected seeds treated with a given plant extract [9]. The height (cm) of the plants was determined by measuring the aerial part of the plants from the soil surface to the node of the terminal developing leaf by using a tape measure.

2.6. Data Analysis

The collected data were subjected to analysis of variance (ANOVA) using Genstat 16th edition software (VSN International, UK) and means were separated using Tukey's Honest Test at ($p=0.05$).

3. Results

3.1. Seed Germination and Seedlings Health Enhancement

The results obtained from the evaluation of the seed treatments are shown in (Table 1). Tomato cultivars (factor A) had insignificant differences in seed germination ($p=0.136$ for normal seedlings and $p=0.051$ for abnormal seedlings), seedling vigor index ($p=0.865$), bacterial leaf spot severity ($p=0.789$), and plant height ($p=0.876$). The only significant differences were observed in the BLS incidence ($p=0.027$), and in BLS incidence and severity reduction ($p=0.019$, $p=0.003$).

As of seed treatment (factor B), the percentage seed germination (normal and abnormal seedlings), seedling vigour index, BLS incidence and severity differed significantly ($p<0.001$) among treatments. Seeds treated with *A. vera* + *C. arabica* had the highest normal seedlings (96.6%), seedling vigour index (1383.4), and lowest abnormal seedlings (3.2%) than control (untreated seeds). The lowest BLS incidence and severity was recorded when seeds were treated with a combination of *A. vera* + *C. arabica* (7.4%, 6%) than untreated seeds (59.2%, 71%). The seed treatments significantly reduced ($p<0.001$) the incidence and severity of BLS in tomato seedlings without significantly ($p=0.360$) affecting the growth of tomato seedlings. A combination of *A. vera* + *C. arabica* had the highest BLS incidence and severity reduction (51.5%, 63.3%) followed by *C. arabica* (49.5%, 58.3%) and *A. vera* (56.7%, 56.7%).

Interaction between seed cultivars (factor A) and seed treatments (factor B) caused insignificant effects on seed germination for normal seedlings ($p=0.081$) and for abnormal seedlings ($p=0.111$), seedling vigor index ($p=0.940$), bacterial leaf spot (incidence $p=0.638$ and severity $p=0.225$), and plant height ($p=0.983$). The reduction in BLS disease on tomato plant from treated seeds compared to tomato plant from untreated seeds (control) is illustrated in (Figure 1).

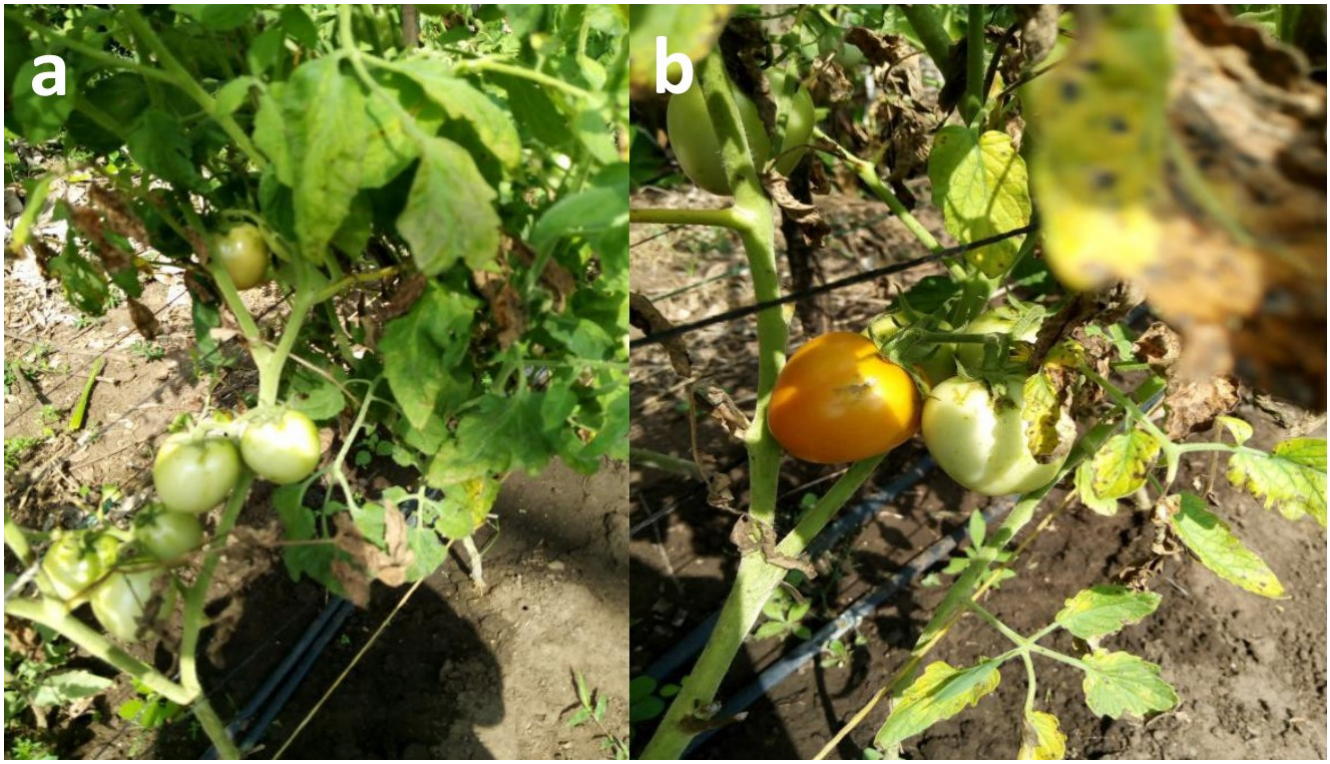

Figure 1. Reduction in BLS disease on (a) tomato plant from treated seeds (b) tomato plants from untreated seeds.

Table 1. Effect of plant extracts on seed and seedlings' health.

	Seed germination		Seedling Vigor	BLS Incidence	BLS incidence Reduction	BLS Severity	BLS Severity Reduction	Plant height
	NS (%)	AS %	index	%	%	%	%	(cm)
Factor A: Tomato variety								
Rio grande	93.17	6.83	961.2	22.3	36.5	25.4	47.9	54.5
Malkia F1	93.75	6.12	964.6	20.8	38.0	25.0	41.3	54.6
Mean	93.40	6.55	962.6	21.7	37.25	25.3	44.6	54.5
p-value	0.136	0.051	0.865	0.027	0.019	0.789	0.003	0.876
Factor B: Seed treatments								
A. vera	92.8b	7.2c	862.8b	10.8b	48.0b	13.0b	56.7b	54.7
C. arabica	94.2b	5.8b	934.5b	9.4ab	49.5bc	11.00ab	58.3b	55.1
A. vera + *C. arabica*	96.6c	3.2a	1383.4c	7.4a	51.5c	6.0a	63.3b	54.5
Untreated	90.0a	10.0d	669.6a	59.2c	0.0a	71.0c	0.0a	53.8
Mean	93.4	6.55	962.6	21.7	37.25	25.3	44.6	54.5
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.360
Interaction: AxB								
Rio grande x *A. vera*	92.0	8.0	864.7	11.0	49.3	13.3	60.0	54.8
Malkia F1 x *A. vera*	94.0	6.0	860.1	10.5	48.7	12.5	53.3	54.7
Rio grande x *C. arabica*	94.0	6.0	933.7	10.0	50.3	10.0	63.3	55.1
Malkia F1 x *C. arabica*	94.5	5.5	935.6	8.5	46.7	12.5	53.3	55.2
Rio grande x *A. vera* + *C. arabica*	97.0	3.0	1374.1	8.0	52.3	5.0	68.3	54.5
Malkia F1 x *A. vera* + *C. arabica*	96.0	3.5	1397.5	6.5	50.7	7.5	58.3	54.4
Rio grande x Untreated	89.7	10.3	672.5	60.3	0.0	73.3	0.0	53.6
Malkia F1 x Untreated	90.5	9.5	665.2	57.5	0.0	67.5	0.0	54.1
Mean	93.4	6.55	962.6	21.7	37.3	25.3	44.6	54.5
p-value	0.081	0.111	0.940	0.638	0.439	0.225	0.237	0.983

NS = Normal seedlings, AS = Abnormal seedlings, Means followed with the same letter(s) within a column are not significantly different based on Turkey's Honest Significance Test a *p=0.05*

Since, farmers (i.e., men, women, and youths) in the selected villages were engaged in the on-farm demonstration of the results, the majority of farmers learned and appreciated the safe tomato production through the seed treatment approach using biopesticides (a combination of *A. vera* and *C. arabica* being the best treatment followed by *C. arabica* then *A. vera*). This would encourage farmers to reduce and/or eliminate extensive usage and reliance on toxic synthetic chemicals in tomato production, and linked with promoting the development, dissemination, and application of sustainable EOA technologies in Tanzania, for enhanced health of plants, animals, humans, and the environment as a whole.

4. Discussion

Numerous biochemical compounds with antibiotic and antimicrobial properties are synthesized by plants including coffee and aloe, and the biochemicals can, therefore, be exploited as an alternative approach to manage BLS. Therefore, for farmers to utilize the eco-friendly pest management resources such as botanicals with relevance to the importance of each pest in tomato crop, more reliable biochemical data and positive controls among the studied botanicals will enable the formulation and commercialization of botanicals to ensure full adoption for pests' management by farmers [7].

In the current study, aloe and coffee plant extracts significantly exhibited their ability to control BLS pathogen (*Xanthomonas perforans*) on seeds of the selected cultivars of tomato grown in the selected areas of Tanzania without negatively affecting plant growth. This indicated their possible combination as seed treatment could enhance the modes of action of their phytochemicals against BLS pathogen. The direct effect on the bacterial cells attributes the antibacterial activity of extracts from A. vera can be associated with the presence of anthraquinones and saponin [33–35]. While, the presence of polysaccharides in aloe, can elicit plant defence responses that destroy bacteria [36–38] and contribute to hydrogen peroxide accumulation in epidermal and mesophilic cells [38,39]. Hence, leads to a significant reduction in bacterial spot incidence, severity, and an increase in the activity of enzymes such as the peroxidases, glucanases and polyphenol oxidases which are associated with plant defence and can be recognized by the plants as the damage-associated molecular patterns (DAMPs) [38,40]. Whereby, these molecules (DAMPs) are released by host cells when exposed to attack by pests and/ or microorganisms [39]. Thus, the molecules can elicit and cause the accumulation of phytoalexins, increasing glucanase and chitinase activity, promoting callose deposition and accumulating reactive oxygen species [41]. The polysaccharides are essential in the elicitation of defence mechanisms of various plants species including tomato, and it seems that they are plentiful, readily available, and can be derived from numerous natural-renewable sources like the aloe plants. Therefore, their use in organic tomato production for the management of the seed-borne BLS disease can be considered as an alternative to conventional/ synthetic chemical control of pathogens. Moreover, coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid have also been previously identified as compounds with the maximum antibacterial activity of the *A. vera* gel extract [42]. Hence, the broad spectral antimicrobial activity of the *A. vera* gel extract is also likely to be dependent on the synergistic effect of different compounds.

On the other hand, the plant extracts from processed coffee have also been reported to inhibit the growth of food-borne pathogens like Listeria spp., Yersinia spp., and *Escherichia coli* [43]. The coffee roasting process leads to carbohydrate caramelization, and also produces substances such as Maillard products, and thermal composition products which attribute the antibacterial activity [19] also compounds such as phenolics, caffeine, caffeic acid (CA), CGA, diterpenes, and trigonelline, flavonoids, chlorogenic acid, and protocatechuic acid that exhibit powerful bioactivities [20–25] and as significant natural antimicrobial agents they play a vital role against enteric bacteria [21,26,27]. Polysaccharides found in the inner leaf parenchymatous tissue attribute to enormous medicinal and/ or antibacterial effects of *A. vera* leaf extracts [28,38,40,44]. These biological activities are more of an additive and/or synergistic action of the compounds rather than a single chemical substance contained therein. As evidenced in previous studies, where benzoic acid, p-toluic acid, p-coumaric acid, psalicylic acid, protocatechuic acid, hydroxyphenylacetic acid, ferulic acid, aloe emodin, and vanillic acid were among the individual phenolic acids/polyphenols occurring in the highest concentrations in *A. vera* extract, but it is well-known that the protective benefits of these compounds are mainly through a combination of additive and/or synergistic effects between the individual compounds [44]. According to Daglia et al [45], caffeine synergistically enhances the antibacterial activity of R-dicarbonyl compounds and that glyoxal, methylglyoxal, and diacetyl in the presence of caffeine account for the whole antibacterial activity of roasted coffee. Among the major compounds of the coffee extracts i.e., chlorogenic acid (CGA) was found to effectively damage the bacterial cell membrane integrity that was exhibited following the release of high amounts of proteins and nucleic acids from bacteria, and the disruption of the bacterial cell membrane potential and permeability [46]. Sledz et al. [47] reported that, when bacterial cells were treated with caffeine, the RNA biosynthesis was highly affected in those cells unlike DNA and protein biosynthesis. Moreover, resistance to caffein was not induced by long duration (336 hours) treating bacteria (*Pectobacterium atrosepticum*) with caffeine, also disease symptoms on chicory leaves, potato slices, and whole potato tubers due to *Dickeya solani* were significantly reduced especially under storage conditions, thus indicating the potential of caffeine in the control of diseases caused by plant-pathogenic bacteria [47].

Moreover, a recent study by Sledz et al. [48] illustrated the antibacterial activity of caffeine (1,3,7–trimethylxanthine) an alkaloid from the *C. arabica* plant on several

bacteria strains such as *Clavibacter michiganensis* subsp. sepedonicus, *Dickeya solani*, *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. carotovorum, *Pseudomonas syringae* pv. tomato, *Ralstonia solanacearum*, and *Xanthomonas campestris* pv. campestris in several crops i.e., lettuce (*Lactuca sativa* L. var. capitata), tomato (*Solanum lycopersicum* L.), cabbage (*Brassica oleracea* L. convar. capitata), potato (*Solanum tuberosum* L., cv. Irga) and broad bean (*Vicia faba* L., cv. Hangdown white). Exogenously supplemented caffeine in concentrations lower than 5 mM significantly enhanced the seed germination rate of tomato plants. However, spraying with 0, 1, 5, or 8 mM of caffeine on the tomato plants grown in soil caused no phytotoxicity symptoms, and did not significantly affect their growth or development as indicated by the plant heights measured after 6 weeks post planting [48]. Seeds that were previously exposed to caffeine led to tomato plants exhibiting a higher accumulation of this compound in its leaf tissues than in the stem or root tissues. Therefore, the level of caffeine accumulation strongly relies on the caffeine application method and differs between the analyzed plant and/or its organs, whereby the latter is relative to the unequal supply of caffeine within plant species capable of synthesizing caffeine [48]. Despite the pest control benefits of these plant extracts, its application in the field has limitations particularly because the bacteria (*Xanthomonas perforans*) is a seed-borne pathogen hence seed dressing is of relevance, moreover and the plant extracts have short shelf-life given the high rate of biodegradability, consequently the residual efficacy under field conditions is also limited [49].

5. Conclusions

A combination of A. vera + C. arabica or *A. vera* and *C. arabica* applied singly had significant efficacy on the pathogen (*Xanthomonas perforans*) causing bacterial leaf spot (BLS). However, the most effective seed treatment was obtained when a combination of extracts from *A. vera* + *C. arabica* at volume (ml) ratio (1:1). Hence, these biological activities are more of an additive and/or synergistic action of the compounds rather than a single chemical substance contained therein. The validation study agrees that the selected EOA technology (seed treatment) from the recommendation of the previous study by Mbega et al. [8] is of significant impact to the organic tomato production in Tanzania. Organic tomato farmers need to adopt seed treatment practices that ensure seedlings' start-up and enhance crop growth and productivity. Hence, the application of *A. vera* and *C. arabica* singly or in combination at volume (ml) ratio (1:1) is recommended for seed treatment. Although the results of validation comply with the recommendations from previous research findings, further studies are needed to establish suitable techniques of getting the chemically stable extracts, also, the bioactive fractions from the plant extracts and their mechanisms of action need to be comprehensively studied to enable the formulation and commercialization of botanicals to ensure full adoption for pests' management by farmers.

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