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Efficacy of Mancozeb and *Ocimum gratissimum* Extract in the Management of Late Blight Disease in Tomato Varieties

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Authors' contributions

This work was carried out in collaboration among all authors. Author MEB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AN and NTN managed the analyses of the study. Author BNT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Tomato late blight is an economic disease that causes 100 % yield loss of tomato in Cameroon. The objective of the study was to determine the efficacy of mancozeb and *Ocimum gratissimum* extract in managing late blight disease in Nadira and Rio-grande tomato varieties. Seedlings of each tomato variety were planted in replicates of three in a complete randomized block design comprising of three treatments (T1 –*Ocimum gratissimum* extract (1666.7 g/15 L); T2 –Mancozeb (50 g/15 L); T3 –control. Fifty grams of mancozeb (50g) and 1666.7 g of *O. gratissimum* extract dissolved and mixed with water in a 15 L knapsack sprayer was applied to control tomato late blight from the onset of blight symptoms, at two days intervals, to control Data for the disease incidence, severity, and yield parameters were collected at weekly intervals for four weeks from plants in the middle of the ridges. Mancozeb and *O. gratissimum* extract significantly reduced the disease

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severity of Nadira and Rio-grande tomato varieties by 0.2., resulting in a mean fruit number of 18.31 and 16.31 fruits and fruit weight of 1.44 g and 1.13 g. There was no significant difference (p =0.05) between plants sprayed with Mancozeb and *O. gratissimum* extract. The Nadira variety was resistant to the tomato late blight caused *by Phytophthora infestans*. Thus biopesticides (*O. gratissimum*) can be effectively used as alternatives to synthetic fungicides (mancozeb) –which pose risks to human and environmental health.

Keywords: Mancozeb; O. gratissimum; phytophthora infestans; late blight, tomato.

1. INTRODUCTION

Tomato (*Solanum esculenta*) is a fruit that belongs to the Solanaceae family and, the fruit is consumed as fresh fruits or as a paste [1]. The fruit is rich in vitamins B, C, and minerals such as iron and phosphate [2]. Despite the importance of this crop in Cameroon, pests and diseases reduce its yield. The tomato late blight disease (TLBD) caused by *Phytophthora infestans* is amongst the most devastating diseases and accounts for 100 % yield loss of tomatoes in Cameroon [3]. Annually, the disease reduces the production and fruit quality of tomato globally [4]. The disease severity of late tomato blight is high in the humid environments characterized by high rainfall and is low in dry areas [5]. It also causes yield loss of 90 % in cool and wet weather conditions and reduced fruit quality and, consequently, the marketability values are reduced [4,6]. It also destroyed tomato stems, fruits, and reduced leaves within 10 to 15 days of infection [7,8].

Synthetic fungicides effectively control tomato late blight disease in the field. However, these fungicides are toxic, expensive, have a long degradation period, and cause a lot of damage to the organism in the environment and human health [9]. Therefore, it is necessary to seek for non-toxic alternative pesticides that can replace synthetic pesticides. Bio-pesticides are environmentally friendly, non-toxic, and less expensive pesticides [10]. Low concentrations of antifungal-active ingredients in plant extracts can kill fungus pathogen [11]. For instance, sterilized and un-sterilized crude extract of *O. gratissimum* significantly reduces the radial growth of *Cercospora purpurea* in *Persea americana* [12]. *O. gratissimum* extract also significantly inhibits the growth of *S. rolfsii* and as well reduces its disease severity [13]. The antifungal-active ingredient in plant extracts retards the reproduction, growth of plant pathogenic fungi and prevents tomato from damaged by late blight disease [14]. Therefore, the current study aimed todetermine the efficacy of fungicide and plant extract in the control of tomato late blight disease in the field.

2. MATERIALS AND METHODS

2.1 Experimental Sites

This study was conducted from 13th March to 5th July 2020, at the Institute of Agricultural Research for Development (IRAD), situated between latitude 32^{o}, 0627' N, and longitude 0659' E, at an altitude of 1263 m above sea level. The plant samples were analysed in the Phytopathology Laboratory in the School of Tropical Agriculture and Natural Resource Laboratory, Catholic University of Cameroon, Bamenda.

2.2 Preparation of Plant Extract

O. gratissimum extract was prepared by crushing sun-dried leaves after washing thoroughly with sterilized distilled water. The leaves were kept at room temperature of 25 ± 2 0C for 72 hours before extraction. The leaf extract was prepared by crushing 100 g of sun-dried leaves with 300 ml of sterilized distilled water and filtered through a 3-layer muslin cloth. The supernatant was passed through a millipore filter of 0.22 µm pore size using a Swinger filter adaptor, and the final product was stored in a refrigerator at 10 ^{o}C. The different concentrations of *O. gratissimum* (100 %, 75 %, and 50 %) in a serial dilution of the stock extract in the ratio of 1:2:3 ml of distilled water were prepared.

The fungicidal activity of *O. gratissimum* extract in vitro was tested by using different concentrations of the plant extract (100 %, 75 % and 50 %) in a serial dilution of the stock extract in the ratio of 1:2:3 ml of distilled water. The different concentrations of plant extract were added to the Phytophthora infestans culture to evaluate their antifungal effects. The plant extract concentration that resulted in the highest fungal growth inhibition in vitro was used in the field experiment [15].

2.3 Nursery Preparation

Seedbeds of 5m in length by 1m in width were prepared in the nursery and fertilized with poultry manure mixed with soil at a ratio of 3 kg poultry: 1kg soil. The seeds of Rio-grande and Nadira varieties were broadcasted on separate seedbeds and covered with a plant mulch, and later removed after germination. Watering of the plants was done twice per day.

2.4 Field Planting and Experimental Layout

After four weeks in the nursery, the healthy seedlings of each tomato variety (15 - 25 cm tall), were transplanted in the field. Each seedling was planted in a 10 m ridge at a depth of 5cm, in an intra-row and inter-row spacing of 60 cm x 1 m. Mulch was applied to prevent erosion, conserve water, and control weeds. Four weeks after transplanting, 5 g of fertilizer NPK (20, 10, 10) and Yaramila (13,13, 21) were applied to the plants to enhance plant growth. Weeds were removed from the field by hand weeding and, the ridges molded after four weeks of transplanting. The experimental layout was a complete randomized block design with three replicates and three treatments (T1 –*O. gratissimum* extract (1666.7 g/15 L); T2 –Mancozeb (50 g/15 L); T3 – control.

2.5 Field Application of Fungicide (Maneb) and Biopesticide (*O. gratissimum*) Extract

At the onset of the first tomato late blight disease symptoms, 50 g of fungicides (Mancozeb) and 1666.7 g plant extract (*O. gratissimum*) was applied separately using a 15 L knapsack sprayer. The 1666.7 g *O. gratissimum* extract per 15L knapsack sprayer corresponded to the best concentration (100%) which was active against *P. infestans* invitro. The plants were sprayed continuously at two-day intervals to control tomato late blight. The disease incidence and severity data were collected from the middle ridges weekly for four weeks [16].

2.6 Determination of Disease Incidence of Tomato Late Blight

Percentage incidence was calculated using the standards adopted from Fokunang et al. [17]

$$\text{Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \text{ X } 100$$

2.7 Evaluation of Disease Severity of Tomato Late Blight

Disease severity was scored using a scale of 1-5 as used by Fukonang et al. [18] where;

1 = No symptom
2 = Low percentage (0 – 30 %) leaf infection
3 = Moderate percentage (30 – 50 %) of plant leaves infected with late blight
4 = High percentage (50 – 70 %) of plant leaves affected with late blight
5 = High percentage (70 – 100 %) leading to complete damage of leaves

2.8 Yield Assessment of Tomato Fruits

Three months after planting (at maturity), yield parameters like fruit number and weight were evaluated. The number of tomato fruits in each plant from the middle ridge was counted and recorded. The fruit weights were measured with an electronic balance.

2.9 Pathogenicity Assessment of Tomato Varieties for *Phytophthora infestans* in a Screen House

Infected tomato leaves with fungus lesions were collected randomly from each treatment in the field, preserved in plastic bags, and transported to the laboratory for isolation and observation of *P. infestans* spores. The leaves were cut into pieces of 2 mm from the advancing edge of the disease, surface-sterilized in 5 % diluted solution of sodium hypochlorite for 30 seconds, and rinsed trice with sterile distilled water for 3 minutes.The pure cultures were observed under the light microscope to identify fungi spores. The pure cultures were used in preparing spore suspensions by flooding the surface of the growing colonies in each Petri dish with 5 ml of sterile distilled water and the mycelia dislodged with a sieve brush. The spore suspension was centrifuged for 5 minutes, and the supernatant filtered through a two-layered sterilized muslin cheesed cloth. A drop of Tween 80 per 100 ml inoculum was added to the suspension as a wetting agent and, the spore suspension was used in inoculating the forty-day-old tomatoes plants in the screen house (six spots per tomato leaf). In the screen house, tomatoes were planted in sterile soils in pots and arranged in a complete randomized design with three replicates of ten plants per replicate. The tomato plants in the screen house were inoculated with the pathogen (six spots per tomato leaf) using a

syringe. A metre rule used was to measure the length and width of fungus lesions for six days, and the lesion area was computed [19].

2.10 Statistical Analysis

The data collected for disease incidence, disease severity, yield parameters, and lesion area for the two varieties of tomatoes were subjected to analysis of variance (ANOVA) using statistical software (JMP11). The treatment means were separated using the Turkey HSD least significant difference at the statistical significance of 95 % confidence interval.

3. RESULTS

3.1 The Effects of Mancozeb and *O. gratissimum* Extract on the Disease Incidence Tomato Late Blight

The tomato late blight disease appeared in the field at 36 days after transplanting. Water-soaked spots appeared on the leaf surfaces of both tomato varieties (Nadira and Rio-grande). For the control, the Rio-grande tomato variety had a higher disease incidence than the Nadira variety (Fig.1). Plant leaf surfaces in treatments T1 (mancozeb) and T2 (*O. gratissimum*) developed water-soaked spots that later dried off, leaving newly sprouted shoots. The water-soaked spots on leaf surfaces of plants in T3 (control) continuously increased and finally dried out. Both Nadira and Rio-grande tomato varieties in T1, T2, and T3, recorded the highest mean disease incidence of 100 % at 3 and 4 weeks of disease infection (Fig.1). Within the first week of the disease, infection Rio-grande variety in T1 and T2 scored the lowest mean disease incident of 5 %. There was a significant difference (p=0.05) in disease incidence between the tomato varieties at one and two weeks of disease infection, with the mean disease incident ranging from 6 – 100 % in the Nadira variety and from 4 – 100 % in the Rio-grande variety (Fig.1). There was no significant difference in diseases incidence at the third and fouth week amongst the treatments.

3.2 The effect of mancozeb and *O. gratissimum* Extract on the Disease Severity Tomato Late Blight

The fungus lesions on the leaf surfaces had water-soaked spots that later developed in brown-blighted areas with an indefinite margin. In

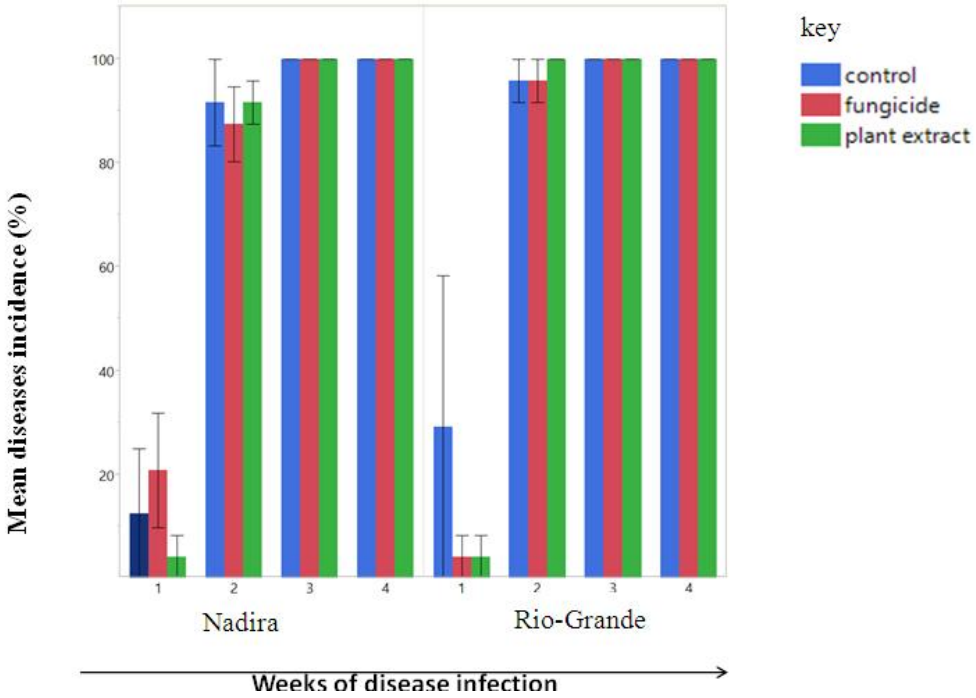

Fig. 1. Effect of mancozeband *O. gratissimum* extract on disease incidence tomatoes late blight at 1 to 4 weeks interval

Bars represent means of disease incidence with standard errors; 1 = First week from disease infection; 2 = Second week from disease infection;3 = Third week of disease infection; 4 = Fourth week from disease infection

T1 and T2, the gray mold and fungus lesions on leaf surfaces did not extend to the stems. However, in T3 (control), the fungi lesions spread to the tomato stems and caused severe to the leaves. There was a significant variation (P=0.05) in the disease severity amongst the treatments (T1, T2, and T3). The Rio-grande variety in T3 (control) scored the highest mean disease severity (3.7) after four weeks of infection, while plants in T1 and T2 scored the least mean disease severity (0.2) at one week of fungi infection (Fig. 2).

3.3 Yield Assessment of Tomato Fruits at 3 Months of Harvest

3.3.1 The Effect of Mancozeb and *O. gratissimum* Extract on the Mean Number and Weight of Tomatoes

After fruit harvesting, the Nadira variety had a higher number of fruits relative to the Rio-grande tomato variety. At harvest, the Nadira variety treated with T1 (mancozeb) and T2 (*O. gratissimum)* scored the highest mean number of fruits (18.31 and 16.31fruits). The Rio-grande tomato variety in T3 (control) scored the least mean number of tomato fruits (10.75 fruits) (Table 1).

The Nadira and Rio-grand tomato varieties showed variation in fruit weight. In all treatments, the Nadira variety produced larger fruits and more weight compared to Rio-grande. In all treatments (mancozeb, *O. gratissimum* and control, there was a significant variation (P=0.05) in fruit weight between the two tomato varieties (Table 1). After harvest, the Nadira variety in treatments with mancozeb and *O. gratissimum* scored the highest mean fruit weight (1.44 g and 1.13 g). Based on the overall weight score, the Rio- grand tomato variety in the control scored the least mean fruit weight (0.76g) (Table 1).

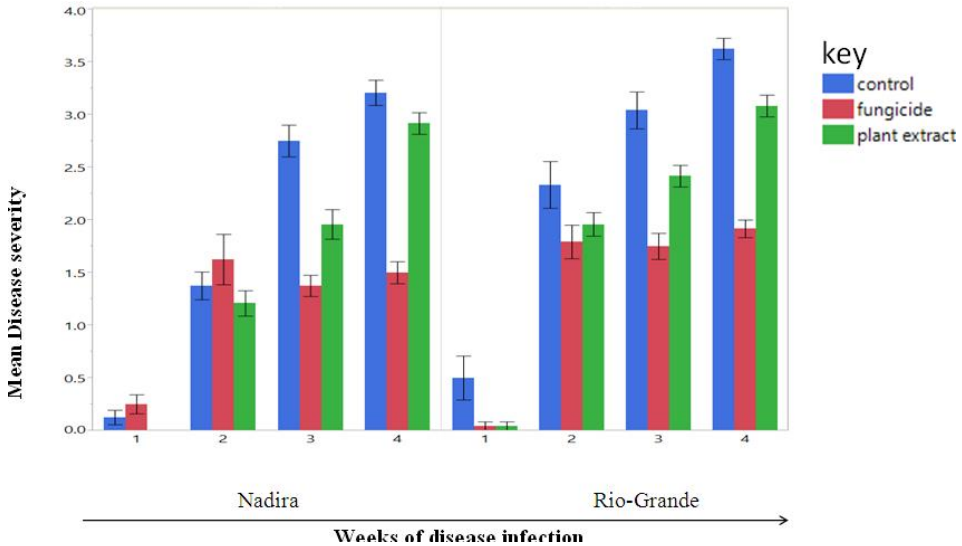

Fig. 2. Effect mancozeb and *O. gratissimum* extract on disease severity tomatoes late blight at 1 to 4 weeks interval

Bars represent means of disease severity with standard errors.1 = First week from disease infection; 2 = Second week from disease infection; 3 = Third week from disease infection; 4 = Fourth week from disease infection

Table 1. Number and weights of fruits at three months of harvest of tomato

Variety	Treatment	Mean number of fruits	Mean weight of fruits (g)
Nadira	Control	13.56±1.53^{d}	0.65±0.11^{de}
Nadira	Mancozeb	19.44±1.05^{a}	1.4±0.07^{a}
Nadira	*O. gratissimum*	18.31±1.83^{b}	1.13±0.12^{b}
Rio-Grande	Control	10.75±1.16^{f}	0.54±0.07^{e}
Rio-Grande	Mancozeb	16.31±1.14^{c}	0.89±0.08^{c}
Rio-Grande	*O. gratissimum*	11.63±1.25^{e}	0.76±0.09^{d}

Means followed by the same latter in the same column are not significantly different at p=0.05 (T HSD) values are mean number and weights of fruits followed by standard errors

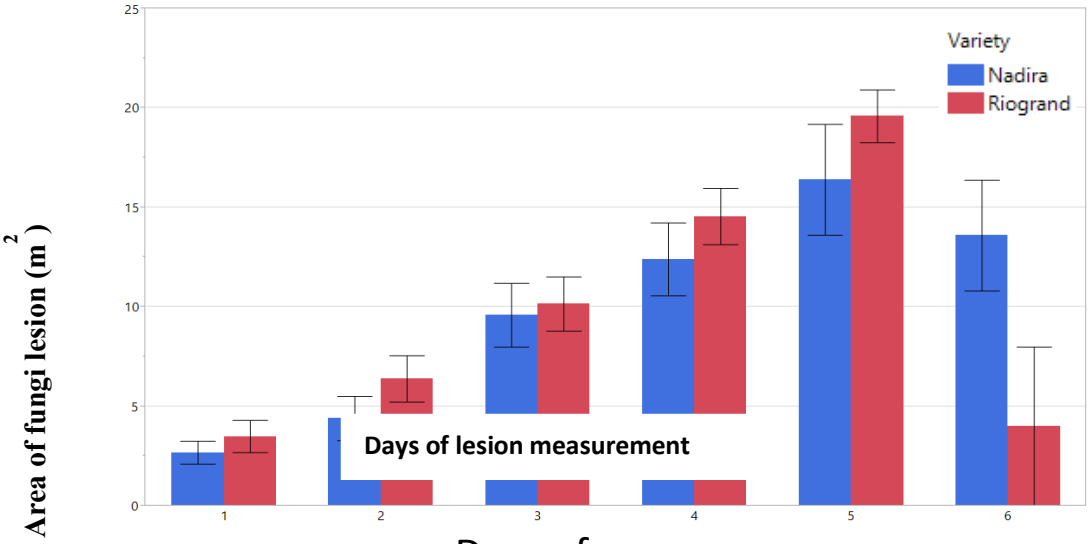

Fig. 3. Size of fungus lesions in the screen house
Bars represent means area of lesion measurement with standard errors

3.4 Screen House Assessment of Tomato Varieties for *Phytophthora infestans*

Two days after inoculation, fungus lesions appeared on the leaf surfaces. These fungus lesions increased progressively from 2nd to 5th day, followed by leaf destruction on the 6th day, with the Rio-grand tomato variety scoring the highest number of destroyed leaves. However, the Rio-grand tomato variety had the highest number of destroyed leaves (Fig. 3). The plants in the control did not show disease symptoms. The lesion area on the leaf surfaces varied with the variety of tomatoes following inoculation. The Rio-grand variety recorded the maximum lesion area (21 cm^2) on the 5th day of lesion measurement and a minimum lesion area of 4 cm^2 was recored on the 6th day of lesion measurement (Fig. 3).

4. DISCUSSION

For both Rio-grand and Nadira tomato varieties, the disease incidence and severity increased from 36 – 57 days after transplanting in all the treatments (mancozeb, *O. gratissimum* and control). This result may be due to the high temperatures and humidity, and rainfall, which favored rapid disease development and proliferation. This finding is in concordance with those of Majid et al. [20], who also attributed the late blight disease severity to high humidity and heavy rainfall Srivastava and Handa [5] also found that tomato late blight disease has a devastating effect in areas with high humidity and cool temperature levels. Generally, fungicides significantly reduced disease severity and increased the yield of crops in the field [21,22]. Likewise, plant extracts controlled or slow down the rate of proliferation of pathogenic fungi (20). In the current study, the water-soaked spots on leaves of plants sprayed with mancozeb and *O. gratissimum* dried off, leaving newly sprouted leaves and shoots. Plants in T1 (Mancozeb) and T2 (*O. gratissimum*) show no significant variation in disease incidence and severity (p =0.05) from plants in T3 (control). This result indicates that both the chemical (mancozeb) and bio-pesticides *(O. gratissimum)* may have similar anti-fungi properties, effective against tomato late blight diseases caused by Phytophthora infestans, though their effects may vary especially with cultivar. For instance, the yield of tomatoes treated with 5% *O. gratissimum* extract was comparable to the yield obtained when is treated with fungiforce fungi [13].

The current result supports the study by Gondal et al. [23], who found mancozeb significantly reduced severity and incidence of tomato late blight disease in the field. The anti-fungi active ingredient present in *O. gratissimum* is phenylpropanoid. This substance is a product obtained from the synthesis of phenylalanine and tyrosine [24]. This active ingredient has been shown to kill many pathogenic micro-organisms

and repelled insect pests [24]. For instance, *O. gratissimum* extract effectively controls diseases caused by Alternaria*, Colletotrichum capsici* Ark*,* and *Sclerotium rolfsii* [25] However, in the current study, the anti-fungi effects of mancozeb were higher than that of *O. gratissium* leaf extract, applied on both tomato varieties.

Fungicides also have varying effects on the yield of tomatoes [26]. Tomatoes in T1 and T2 had a higher number of fruits and fruit weight than tomatoes in T3. However, the Nadira tomato variety had a higher fruit number and fruit weight than the Rio-grande tomato variety. This difference shows the variation in the inherent disease and yield characters of the two tomato varieties [27,28]. The yield of both tomato varieties in T3 (control) was low because of the devastating effects (defoliation and premature flower and fruit drop) of the tomato late blight disease caused by *P. infestans*. This finding is in concordance with the assertion made by Amin et al. [29], who attributed tomato yield losses of 60 – 70 % during harvest to fruit drop of infected fruits and difficulties to produce crops without chemicals protection during the rainy season. An earlier study by Deahl et al. [30] showed that tomato late blight disease causes failure of fruit set and fruit and leaf defoliation.

5. CONCLUSION

This study demonstrated that mancozeb and *O. gratissium* extract reduced the incidence and severity of tomato late blight disease in the field. The Nadira tomato variety in T1 (mancozeb) and T2 (*O. gratissium*) had the highest mean number of fruits (18.31 and 16.31 fruits) and, highest mean fruit weight (1.44 g and 1.13 g). The yield of the two tomato varieties in T1 and T2 were significantly different (p =0.05) from the yield of plants in T3 (control). The late bight disease in the Nidira variety responded to the mancozeb and *O. gratissium* extract treatment better than the Rio-grand tomato variety, which was severely damaged. In the screen house, the two tomato varieties were highly susceptible to *P. infestans* and expressed symptoms identical to field symptoms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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