Effect of Canola and Compound Fertilizer on Potato (*Solanum Tuberosum* L.) Bacterial Wilt Management

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ABSTRACT

Bacterial wilt is a problematic disease affecting potato production in Kenya and the available management methods are not efficient. Field, semi-field and laboratory experiments were conducted to evaluate the effect of canola green manure and compound fertilizer on bacterial wilt management. Laboratory experiment was conducted at Egerton university biological laboratory to evaluate the effect of canola extract on *R. solanacearum* population density in-vitro. Four levels of canola extract quantities were used; 0, 100, 200, 300 microlitres, and enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) was used as a positive control. Inoculum was prepared from infested soil and Selective Medium South Africa (SMSA) (Casamino acids, Bacto-Peptone, Glycerol, and Bacto-Agar) was used as growth medium in a completely randomized design. Field experiment was carried out in two sites (Elburgon site is in Upper high land zone two (UH2) and Mau-Narok is in Upper high land zone one UH1). Two levels of canola green manure (with and without green manure application), four levels of fertilizer (NPK+Ca+Mg+ micronutrients) applied at 0(F1), 250(F2), 575(F3), 900 (F4) kg ha⁻¹ and diammonium phosphate (DAP500 kg ha⁻¹) +Calcium ammonium nitrate (CAN300 kg ha⁻¹) (F5) as a positive control. The experiment was carried out in randomized complete block design (RCBD) split plot replicated three times, canola green manure as main plot and fertilizer and varieties combination as sub plots. Semi-field experiment (in pots) was conducted at Egerton university farm, five levels of canola green manure (100, 75, 50 and 0 g kg⁻¹ soil) and four levels of the compound fertilizer as used in the field experiment were used in a completely randomized design (CRD) with three replicates. The data shows that canola extract did not have a significant (Pr <0.05) effect on bacterial population density (CFU), where chemical treatment (DDD) restricted the growth of *R. solanacearum* under laboratory experiment. Under field and semi-field experiments, canola green manure and fertilizer treatments had no significant (Pr <0.05) effect on bacterial population density in the soil nor bacterial wilt incidence. *Kenya karibu* variety it did not show any wilting symptoms of bacterial wilt across the sites and semi-field experiment. Canola did not suppress the growth of *R. solanacearum*; further investigation should be done on other brassica family plants.

Keywords: Green manure, Canola extract, Biofumigation, compound fertilizers, Bacterial wilt, enrich immunomodulator.

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I. INTRODUCTION

Bacterial wilt also known as brown rot is potato disease and is one of the most destructive diseases of potato, caused by the pathogen called *R. solanacearum*. [1] reported that is the second most damaging potato disease after late blight in tropical and sub-tropical areas. Globally, the disease is estimated to damage over 950 million USD annually and about 1.7 million hectares of potato are affected by bacterial wilt disease in approximately 80 countries [2]. It is a constraint for potato production mostly in Sub-Saharan Africa. Kenya is one of the most affected country in Africa and affecting over 70% of potato farms and causing yield losses of 50 to 100%. The speed of spread is associated with limited farmer's skills on disease management and

prevention, and unavailability of enough healthy potato seeds [3].

The bacteria appear in three races and five biovar, race 3, biovar 2 being important for solanacea family including potato [4], [5]. The bacteria are soil borne, that can spread through infested potato seeds, infested running water, farm equipment, crops residues and infested shoes [32]. The bacteria infest plant from soil through wound that may be caused by nematode, mechanical damage and/ or insect damage on root cortex and colonize the whole plant through xylem vessels, this leads to the obstruction of the vessels and the subsequent development of the typical wilting symptoms due to impaired water conductance [7]. The bacteria can persist in soil for a long period up to five years [8] and the population increases easily with the presence of host plants [9]. Wilting, yellowing, and stunted growth are

common symptoms. At early infestation, wilting mostly appear on young parts of plants but later the whole plant wilts and eventually dries [10].

Several measures have been used in management of this disease, as chemical [11] cultural and soil solarization methods [12], however it seems difficult to manage. A number of soil chemical fumigants like chloropicrin have been tested for bacterial wilt control in many crops; however, fumigation is not economically feasible over large areas and has environmental hazards [4]. Some chemicals are prohibited in some countries due to the risks associated to the pesticide operators, aquatic organisms, birds, and bees [13]. [14] reported that, much emphasis should be put on use of certified disease-free seed, quarantine measures on infected fields and farms, sufficient crop rotation, reliable and early detection of the pathogen, removal of volunteer plants and management of nematodes where present, avoidance of surface water for irrigation and control of weed hosts. However, crop rotation, which is commonly recommended, requires long periods and strict elimination of volunteer plants and alternate host for its efficiency. This may not be possible with intensive cultivation to meet food demand due to small land holding and limited farmer's knowledge on disease cycle [15]. It is difficult to reclaim fields that are highly infested with *R. solanacearum* due to its wide range host, biological variation, and conducive climate particularly in tropical highlands [13].

Biofumigation using brassica plants (e.g., Canola, Mustard, and cabbages) is another method that has been used in management of soil borne pathogens [16], [17] yet little is known on their efficacy on potato bacterial wilt management. Brassica plants produce compounds called glucosinolates that are released from plants through rupturing of plants tissues. Glucosinolates undergo further process (hydrolysis) to make isothiocyanates in the presence of enzymes myrosinase and moisture. Glucosinolates are not toxic at this stage, the isothiocyanates are the ones known to have broad biocidal activity including insecticidal, nematicidal, fungicidal, antibiotic, and phytotoxic effects [18]. Although disease reduction associated with green manures can be variable and provides only partial control, it can be used in an integrated disease management program [19]. Different authors revealed some levels of inhibition on fungal pathogen and reduction of fungal disease severity and incidences due to brassica green manure application [16], [20]-[22]. There is less information on the use of brassica plants on bacterial diseases management including potato bacterial wilt, therefore experiments were conducted to evaluate the effect of canola and compound fertilizer on potato bacterial wilt disease management.

II. MATERIALS AND METHODS

A. Site Description

Field experiment was conducted at Mau Narok and Elburgon sub counties while semi-field experiment (pot) was conducted at Egerton University farm, all sites are located in Nakuru County, Kenya. Egerton university site lies between longitude 35° 35' E, latitude 0° 23' S, and at an altitude of 2238 meter above sea level (m.a.s.l). Mau-Narok

is located in Njoro sub-county at an altitude of 2,900 m.a.s. land lies between longitude 36°0'E and latitudes 0°36'S. The area receives an average annual rainfall of 1,200-1,900 mm. The minimum temperatures of 6-14 °C and maximum of 22-26 °C [23]. The soil is well drained, deep to very deep, very dark greyish brown, friable and smeary, clay loam, with thick humic topsoil (mollic andosols) [24]. Elburgon is located in Molo sub-county at an altitude of 2,200 m.a.s.l and lies between longitude 35° 41'E and latitudes 0°12'S. This area experiences mean annual rainfall of 1000-1400 mm and mean temperatures of 13.7-20 °C [24]. The soils of Elburgon are acidic, well drained, deep, dark reddish brown with a mollic A horizon, and classified as mollic Andosols [25]. According to [26], Elburgon site is in Upper high land zone two (UH2) and Mau-Narok is in Upper high land zone one (UH1). The sites were selected because they are suitable for potato production.

B. Description of Varieties Used in the Study

Shangi and Kenya karibu potato varieties were used in these experiments. Shangi is popular and the most grown variety in Nakuru, it grows well in the altitude above 1500 meter above sea level (m.a.s.l). It matures early in about 3.5 months with the yield ranging between (30,000-40,000 kg ha⁻¹). It is moderately susceptible to late blight [6]. Kenya karibu variety is one of the popular potato varieties after Shangi, it grows well at altitudes between 1800-2600 m.a.s.l. The variety is a high yielder (35,000-45,000 kg ha⁻¹) and tolerant to late blight [6]. The varieties were selected because they are suitable for experimental sites and are grown by most of the farmers in Nakuru.

C. Laboratory Experiment

Laboratory experiment was conducted at Egerton University biological science laboratory. The experiment was laid down in a completely randomized design and replicated three times. Canola (*Brassica napus* L.) was grown at Egerton University field (7) for two months. Early flowering, fresh leaves, and shoots (20 g) were collected, washed in water and surface-sterilized by dipping them in 1% sodium hypochlorite for 1 minute followed by three rinses in sterile distilled water and dried on absorbent filter paper. The leaves were chopped and thoroughly macerated with a mortar and pestle [21]. The extracts were obtained by squeezing (by hands) macerated products and then poured in a sealed bottle for use in subsequent experiments.

A bacteria inoculum was prepared from infested soil with the initial bacterial colony density range of 2-5×10³ Cfu/ml. Five grams of soil was weighed into a flask, then 50ml of distilled water added. The mixture was shaken vigorously for 15min and allowed to settle for 5 min. A bacterial suspension was taken to prepare two serial dilutions. From dilution two, (1ml) of bacterial suspension was inoculated into each petridish. Zero, one hundred, two hundred and three hundred microliters of canola extract were poured into petridishes using micropipette. Enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) was used as a positive control (0.5 g was diluted in 1ml of water and poured into petridishes). A specific medium for *R. solanacearum* "Selective Medium South Africa (SMSA)" composed of; Casamino acids, Bacto-Peptone, Glycerol,

Bacto-Agar Crystal violet, Polymyxin β sulfate, Bacitracin, Chloromycetin and Penicillin was prepared following [27] and [28] method. The medium at 40-45 °C was poured in each petridish containing (inoculum and canola extracts), mixed vigorously, and allowed to solidify. Bacterial cultures were transferred to incubator for 72 h at 28 °C.

D. Field and Semi-field (pots) Experiments

1. *Ralstonia solanacearum* test in soil

Before experiments were set, soil test for *R. solanacearum* population density determination and identification were done by taking composite samples in all sites. The samples were analyzed at biological science laboratory, Egerton University by using serial dilution procedure as described by [27]. The population of *R. solanacearum* was calculated using the following formula [20]:

$$\text{colony forming unit } \left(\frac{\text{Cfu}}{\text{ml}} \right) = \frac{\text{Number of colonies counted} \times \text{dilution factor}}{\text{Quantity plated}} (1)$$

2. Experimental procedure

Field experiments were laid in randomized complete block design split-plot arrangement, canola green manure as main plot and combination of variety and fertilizer treatments as sub-plots replicated three times. After land preparation, canola seeds were sown on (3rd August 2019) at Mau-Narok site and (12th September 2019) at Elburgon at a rate of 6 kg ha⁻¹ as recommended by [29] and allowed to grow for a period of two months. Weed management was done in all plots (the one with green manure and the one without green manure). At early flowering stage (60 days after sowing), canola was uprooted, chopped (Approximately 1cm) and incorporated into the soil in equal quantities (Mau-Narok 100g m⁻² and Elburgon 300 g m⁻² (based on each site's productivity) at depth of 15 cm [30]. Canola production at Mau-Narok was low because of heavy rainfall that washed away seeds before germination. The whole plot (with and without green manure) was covered with a polyethylene sheet for two weeks to avoid glucosinolate compounds from volatilization [21]. Two weeks after incorporation, certified potato seeds Shangi and Kenya karibu varieties sourced from Agricultural Development Cooperation (ADC) Molo were planted at a spacing of 75×30 cm (75 cm between the rows and 30 cm between the plants) with planting depth of 10 cm. The plot size was 3×1.5m with 4 rows and 5 plants per row. NPK + Ca + Mg + micronutrients fertilizer treatments (Table 1) were applied in splits, two third at planting and one third at flowering stage [31]. Recommended DAP and CAN were used as positive control at rate of 500 kg ha⁻¹ during planting and 300 kg ha⁻¹ at flowering stage, respectively. The rates for fertilizer treatments were calculated according to farmers practice and recommended nitrogen rate (90 kg ha⁻¹) as indicated by [32], [33].

TABLE 1: FERTILIZER RATES TREATMENTS

Fertilizer	Treatments	Nitrogen <-----kg ha ⁻¹ ----->	Phosphorus	Potassium
Compound	F1	0	0	0
fertilizer (NPK	F2	25.0	65.0	25.0
10:26:10+ Ca, S,	F3	57.5	149.5	57.5
Mg, Cu, Zn, B,	F4	90.0	234.0	90.0
Mn, Mo).				
DAP 18% N and		90.0	230.0	-
46% P as Basal				
application and	F5	81.0	-	-
CAN 27% N as				
top dressing				

CAN: Calcium ammonium nitrate, DAP: Diammonium phosphate.

Semi-field (pot) experiment was laid out in completely randomized design in factorial arrangement with three replicates. Treatments combinations are shown in Table 2. Canola seeds were grown at Egerton University farm for two months following the same management as for field experiments. The treatments were four levels of canola and five fertilizer treatments. The pot size of 20 cm diameter and 30 cm length were each filled with 5 kg of topsoil infested with *R. Solanacearum*. A hundred and two pots were used in this experiment. At early flowering stage (60 days after sowing) canola was chopped and mixed into the soil in the pots at the rate of 0, 50, 75, 100 g kg⁻¹ of soil and then covered with polyethylene sheet for two weeks before planting of potato seeds [21]. Certified seed potato (*Shangi* and *Kenya Karibu* variety) was planted one in each pot and fertilizer treatments applied at five rates as shown in the (Table 1). NPK + Ca + Mg + micronutrients fertilizer was applied in two splits, two third at planting stage and the remaining at flowering stage while DAP was applied at planting followed by CAN at flowering stage as for field experiment. The fertilizer treatments applied in the pots, were calculated based on the amount of soil per hectare (2,000,000 kg ha⁻¹) f.s (furrow slice) and converted to the amount of soil used in the pot (5 kg).

TABLE 2: TREATMENT COMBINATIONS FOR SEMI-FIELD EXPERIMENT

Treatment	Combination	Treatment	Combination
T1	V1F1G1	T21	V2F1G1
T2	V1F1G2	T22	V2F1G2
T3	V1F1G3	T23	V2F1G3
T4	V1F1G4	T24	V2F1G4
T5	V1F2G1	T25	V2F2G1
T6	V1F2G2	T26	V2F2G2
T7	V1F2G3	T27	V2F2G3
T8	V1F2G4	T28	V2F2G4
T9	V1F3G1	T29	V2F3G1
T10	V1F3G2	T30	V2F3G2
T11	V1F3G3	T31	V2F3G3
T12	V1F3G4	T32	V2F3G4
T13	V1F4G1	T33	V2F4G1
T14	V1F4G2	T34	V2F4G2
T15	V1F4G3	T35	V2F4G3
T16	V1F4G4	T36	V2F4G4
T17	V1F5G1	T37	V2F5G1
T18	V1F5G2	T38	V2F5G2
T19	V1F5G3	T39	V2F5G3
T20	V1F5G4	T40	V2F5G4

Key: V1: Shangi, V2: Kenya Karibu, F5: DAP500 + CAN300 kg ha⁻¹, F4: NPK900 kg ha⁻¹, F3: NPK575 kg ha⁻¹, F2: NPK250 kg ha⁻¹, G1: no green manure, G2: green manure 50 g kg⁻¹ of soil, G5: green manure 75 g kg⁻¹ of soil, G5: green mnaure100 g kg⁻¹ of soil.

3. Crop management practices

Experiments were kept weed free and earthing up in the

field experiments were done twice, first at two weeks and the second at sixth week after emergence. Late blight was controlled by alternating *Equation pro* (Famoxadone 225 gkg⁻¹+Cymoxanil300gkg⁻¹) sprayed at the rate of 10 g/20 L of water and *Ridomil gold* MZ 68 WG (Metalaxyl-M 40 gkg⁻¹+Mancozeb 640 gkg⁻¹) sprayed at a rate of 50 g/20 L of water. Spraying was done weekly during intensive rainy period and at two-week intervals in sunny period. Pests of canola such as aphids and flea beetles among others were monitored and controlled using: Cypertox 250Ec (cyhalothrin 25 gL⁻¹). Canola pest attack were severe at early stage (2-4 weeks after planting), regular monitoring was done and sprayed twice depending on the attack.

4. Data collection for laboratory experiment

Three days after incubation, number of colonies were counted in each treatment. For cell shape, crystal violet dye was used to stain the bacterial cells and visualized using light compound microscope (10× eyepiece and 40× objective lens). For bacterial colony margin visualization, Leica zoom stereo-microscope (eyepiece 10× built-in, zoom range 7× to 30× and 100mm working distance) was used.

5. Data collection for semi-field and field experiments

Data on *R. solanacearum* population density was collected at potato flowering stage and seven days after harvesting. Because of high cost of analysis, composite soil samples were collected from selected treatments, (DAP500 kg ha⁻¹, NPK 900kg ha⁻¹, NPK 0 kg ha⁻¹ with green manure and without green manure application). The soil samples were analyzed using Selective Medium South Africa (SMSA) (Casamino acids, Bacto-Peptone, Glycerol, and Bacto-Agar) [27]. Five grams soil sample was added in 50mls of distilled water in conical flask, then shaken vigorously. The sample was allowed to settle for 2 minutes, then 1ml suspension drawn from top using pipette. One mill suspension was diluted twice to make 10⁻² dilution factor. One milliliter suspension was taken from 10⁻² bottle after shaking then added in petridish. SMSA medium at temperature 45-50°C was added, then mixed with inoculum. The petridish was taken to incubator at 28 °C for 2 days. After two days colony forming unit (Cfu/ml) counted and then the actual (Cfu) in original sample calculated using formula (1).

6. Assessment of bacterial wilt incidence and severity under field experiment

Disease incidence, a sample of 10 plants from middle rows were monitored weekly by counting number of symptomatic plants in each plot. Disease severity was assessed 10 plants from middle rows using a scale of 0 - 4 disease, where 0 = no symptoms of wilting, 1 = 0 to 25% of stems showing wilting, 2 = 26 to 50% of stems showing wilting, 3 = 51 to 75% of stems showing wilting and 4 = 76 to 100% showing wilting [34]. Disease incidence was calculated as % diseased plants over the total number of plants. Disease severity grades were converted to percentage severity index (PSI) using the formula:

$$PSI = \frac{\sum \text{individual numerical rating}}{\text{Total number of plant assessed} \times \text{maximum score in the scale}} \times 100$$

E. Data analysis

The data were subjected to normality test and the appropriate transformation (log or square root) was done to achieve normal distribution and meet the assumptions of ANOVA. General Linear Model (GLM) procedures of SAS (9.3) was used for ANOVA at $P \leq 0.05$ (Bacterial colony density in plate, bacterial density in the soil, disease incidence and severity). Dunnette test at 5% level of significance was performed to compare negative control with the other treatments. The significantly different treatment means were separated using Tukey's honest significant difference (HSD) test at 5% level of significance [35].

III. RESULTS

A. Laboratory Experiment

1. Effect of canola extract on *Ralstonia solanacearum* colony density and growth

The result showed that, there was a significant difference among the treatments though, the three levels (100, 200 and 300 µl) of canola extract had no effect on *R. solanacearum* colony density. Negative control (no canola extract) exhibited slightly lower level of *R. solanacearum* colony density compared to canola extract treatments. The positive control treated with chemical enrich immunomodulator (Di-bromo Di-nitro propane 1-3-diol) (DDD) had zero colony forming units (Cfu) (Table 3). Dunnette test showed that, negative control was significantly different from the 300 microliters canola extract treatment (Table 4).

After 48 hours of incubation, all bacterial colonies appeared in all petridishes (excluding positive control), but after 72hours incubation canola extract treatments seemed to enhance the growth of bacterial colonies (Plate 1).

TABLE 2: EFFECT OF CANOLA EXTRACT ON *R. SOLANACEARUM* DENSITY
IN-VITRO EXPERIMENT

Canola extract	Means (Cfu/ml) × 10 ²
300 (µl)	59.33 ^a
200 (µl)	28.00 ^{abc}
100 (µl)	33.67 ^{ab}
0 (µl)	20.00 ^{bc}
Chem (DDD)	0.00 ^c
MSD	32.26

The means followed by the same letters are not significantly different using Tukeys' honest significant difference (HSD) test at 5% level of significance. Cfu: Colony forming unit, MSD: minimum significant difference. Chem (DDD): Di-bromo Di-nitro propane 1-3-diol.

TABLE 3: COMPARISON OF CANOLA EXTRACT TREATMENT WITH
CONTROL

Canola extracts comparisons	Differences between means (CFU) × 10 ²
300-0(µl)	39.33 ^{***}
200-0(µl)	8.00
100-0(µl)	13.67
Chem (DDD)-0(µl)	-20
MSD	28.33

Cfu: Colony forming unit, MSD: minimum significant difference, Chem (DDD): Di-bromo Di-nitro propane 1-3-diol. ***: $P < 0.0001$.

Plate 1. Effect of canola extract on *R. solanacearum* colony density at 72 hours of incubation. A: 0.5 g l⁻¹ (Di-bromo Di-nitro propane 1-3-diol), B, C, D and E are: 0, 100, 200 and 300 (µl) of canola extract respectively.

2. Effect of canola extract on colony margin and cell shape

A microscope was used to visualize the margin of colonies as affected by canola extracts. Plate 2 shows that there was no effect of canola extract on colony margin. The shape of cell was visualized also using a microscope, though no differences observed among the treatments.

Plate 2. Effect of canola extract on *R. solanacearum* colony margin. A, B, C and D are 0, 100, 200, and 300 microliters of canola extract respectively.

B. Semi-field and Field Experiment

1. Effect of green manure and fertilizer treatments on *Ralstonia solanacearum* population density in the soil

Preliminary soil sample analyses for bacterial population density were done in all sites and the results showed that they were 103×10^3 , 11.5×10^3 , and 7.5×10^3 colony-forming unit per milliliter (Cfu/ml) at Mau-Narok, Egerton and Elburgon respectively. The soil at Mau-Narok site had high level of bacterial population density compared to the other sites.

The results show that canola green manure and fertilizer

did not have a significant effect on soil bacterial population density at 5% level of significance under field and semi-field experiments. The higher soil bacterial population density was observed in two fertilizer treatments (NPK 900 kg ha⁻¹ and DAP500 kg ha⁻¹) compared to control and the trend was the same across the sites and semi-field experiment (Table 5 and 6). The interaction between fertilizer levels and canola green manure did not have significant effect on bacteria population density at 5% level of significance and interaction of NPK 900kg ha⁻¹ and DAP500 kg ha⁻¹ with green manure had the highest level of bacteria density while control had the lowest. The same trend was observed across all sites and semi-field experiment (Fig. 1 and 2). The population density (Cfu) increased as the fertilizer levels were varied 0-900 kg ha⁻¹, the highest increase was observed at GM100 NPK900 followed by GM0 NPK900 and GM 50 DAP. However, the lowest population was observed at GM100 NPK0, followed by GM50 NPK0 and GM75 NPK0 (Fig. 3). Unexpectedly, the high reduction of bacterial population density was observed between the samples taken before experimental setup and at flowering stage, with slight reduction on samples collected after potato harvesting 89.00, 55.73 and 90.70 % at Mau-Narok, Elburgon and Egerton (semi-field experiment), respectively (Fig. 4).

TABLE 4: EFFECT OF CANOLA GREEN MANURE AND FERTILIZER LEVELS ON *R. SOLANACEARUM* POPULATION DENSITY IN THE SOIL UNDER FIELD EXPERIMENT

Fertilizer treatments	Mau-Narok (Cfu/ml×10 ³)		Elburgon (Cfu/ml×10 ³)	
	flowering stage	After harvesting	flowering stage	After harvesting
NPK 900kg ha ⁻¹	12.17 ^a	10.50 ^a	4.03 ^a	3.40 ^a
DAP500				
+CAN300kg ha ⁻¹	13.00 ^a	11.50 ^a	3.70 ^a	3.07 ^a
NPK 0 kg ha ⁻¹	8.83 ^a	8.00 ^a	2.22 ^a	2.18 ^a
MSD	Ns	Ns	Ns	Ns
Canola green manure treatments				
Green manure	11.78 ^a	10.22 ^a	3.53 ^a	3.06 ^a
No green manure	10.89 ^a	10.89 ^a	3.10 ^a	2.71 ^a
MSD	Ns	Ns	Ns	Ns

The means followed by the same letter are not significantly different at $P < 0.05$. DAP: Diammonium phosphate, CAN: Calcium ammonium nitrate, MSD: minimum significance difference, Cfu: colony-forming unit. Ns: not significantly different.

TABLE 5: EFFECT OF CANOLA GREEN MANURE AND NPK FERTILIZER ON *R. SOLANACEARUM* POPULATION DENSITY UNDER SEMI-FIELD EXPERIMENT (Cfu/ML×10³)

Fertilizer treatments	At flowering stage	After harvesting
NPK 900kg ha ⁻¹	1.22 ^a	0.98 ^a
DAP500 +CAN300kg ha ⁻¹	1.20 ^a	0.97 ^a
NPK 0 kg ha ⁻¹	0.79 ^a	0.65 ^a
MSD	Ns	Ns
Canola green manure treatments		
GM100g kg ⁻¹ of soil	1.09 ^a	0.85 ^a
GM75 g kg ⁻¹ of soil	1.06 ^a	0.86 ^a
GM50 g kg ⁻¹ of soil	1.08 ^a	0.86 ^a
GM0 g kg ⁻¹ of soil	1.04 ^a	0.89 ^a
MSD	Ns	Ns

The means followed by the same letter are not significantly different at $P < 0.05$. GM: canola green manure, DAP: Diammonium phosphate, MSD: minimum significance difference, Cfu: colony-forming unit. Ns: not significantly different.

Fig.1. Effect of selected interactions between fertilizer and canola green manure treatments on *R. Solanacearum* population density under field experiment (means and standard error bars). A: Mau-Narok, B: Elburgon. Cfu: Colony forming Unit, F1: NPK 0 kg ha⁻¹, F5: DAP (Diammonium phosphate) 500 kg ha⁻¹+ CAN (Calcium ammonium Nitrate) 300 kg ha⁻¹, F4: NPK 900 kg ha⁻¹.

Fig.2. Effect of selected interaction between fertilizer and canola green manure on *R. Solanacearum* population density under semi-field experiment (means and standard error bars). (A): soil sample collected at potato flowering stage, (B): soil sample collected after potato harvesting. Cfu: Colony forming Unit, Gm: canola green manure in grams per kilogram of soil. F1: NPK 0 kg ha⁻¹, F5: DAP (Diammonium phosphate) 500 kg ha⁻¹+ CAN (Calcium ammonium Nitrate) 300 kg ha⁻¹, F4: NPK 900 kg ha⁻¹.

Fig. 3. Means and standard error bars of *R. Solanacearum* population density as affected by fertilizer and canola green manure treatments at different sites at different sampling times during potato growth (combined data for fertilizer and canola green manure).

2. Effect of canola green manure and fertilizer treatments on disease incidence and severity

The result shows that fertilizer and canola green manure had no significant (at 5% level of significance) effect on disease severity and disease incidence in all sites. Even though no significant difference was observed statistically, NPK 900 kg ha⁻¹ and DAP500 kg ha⁻¹ treatments had higher level of potato bacterial wilt incidence and severity than control. High level of disease severity and incidence were observed on the plant from NPK 900 kg ha⁻¹ treatment followed by DAP500 kg ha⁻¹ at Mau-Narok site with slight difference at Elburgon, because NPK 900 kg ha⁻¹ was followed by control (Table 7 and 8). Interaction between fertilizer treatments and green manure levels seems to be influenced by fertilizer levels, because the two treatments NPK 900 kg ha⁻¹ and DAP 500 kg ha⁻¹ exhibited the high level of potato plant incidence interacted either with or without canola green than no fertilizer application (Fig. 4). Mau-Narok site exhibited high level of potato plant incidence and severity than Elburgon site but this was due to differences observed initially before experimental set up. The disease incidence seems to be positively correlated to disease severity in both sites (Fig. 4 and 5). Potato varieties showed differences, Shangi was most susceptible variety while Kenya Karibu did not show any symptom of wilting and the same trend was observed across the sites.

TABLE 6: EFFECT OF CANOLA GREEN MANURE AND FERTILIZER TREATMENTS ON POTATO BACTERIAL WILT DISEASE INCIDENCE (DATA COMBINED FOR ALL VARIETIES)

	Mau-Narok (%)	Elburgon (%)
Fertilizer treatments		
NPK 900 kg ha ⁻¹	28.61 ^a	4.26 ^a
DAP500 +CAN300 kg ha ⁻¹	27.41 ^a	0.83 ^a
NPK 575 kg ha ⁻¹	19.44 ^a	0.83 ^a
NPK 250 kg ha ⁻¹	18.58 ^a	1.76 ^a
NPK 0 kg ha ⁻¹	15.65 ^a	2.50 ^a
MSD	Ns	Ns
Canola green manure treatments		
Green manure	23.05 ^a	2.74 ^a
No green manure	20.83 ^a	1.33 ^a
MSD	Ns	Ns

The means followed by the same letter are not significantly different at Pr<0.05. DAP: Diammonium phosphate, CAN: Calcium ammonium nitrate, MSD: minimum significance difference. Ns: not significantly different.

Fig.3. Effect of interaction between fertilizer and canola green manure on potato bacterial wilt incidence (means and standard error bars). A and B are Mau-Narok and Elburgon site respectively. T1: NPK 0kg ha⁻¹, T2: NPK 250 kg ha⁻¹, T3: NPK 575 kg ha⁻¹, T4: NPK 900 kg ha⁻¹, T5: DAP (Diammonium phosphate) 500 kg ha⁻¹+ CAN (Calcium ammonium Nitrate) 300 kg ha⁻¹

Fig.5. Effect of interaction between fertilizer and canola green manure on potato bacterial wilt severity (means and standard error bars). A and B are Mau-Narok and Elburgon site respectively. T1: NPK 0 kg ha⁻¹, T2: NPK 250 kg ha⁻¹, T3: NPK 575 kg ha⁻¹, T4: NPK 900 kg ha⁻¹, T5: DAP (Diammonium phosphate) 500 kg ha⁻¹+ CAN (Calcium ammonium Nitrate) 300 kg ha⁻¹

TABLE 7: EFFECT OF CANOLA GREEN MANURE AND FERTILIZER TREATMENTS ON POTATO BACTERIAL WILT DISEASE SEVERITY (DATA COMBINED FOR ALL VARIETIES).

	Mau-Narok (PSI)	Elburgon (PSI)
Fertilizer treatments		
NPK 900 kg ha ⁻¹	19.01 ^a	3.61 ^a
DAP500 +CAN300 kg ha ⁻¹	17.69 ^a	0.21 ^a
NPK 575 kg ha ⁻¹	12.20 ^a	0.83 ^a
NPK 250 kg ha ⁻¹	8.91 ^a	0.21 ^a
NPK 0 kg ha ⁻¹	7.71 ^a	2.80 ^a
MSD	Ns	Ns
Canola green manure treatments		
Green manure	14.24 ^a	2.15 ^a
No green manure	11.97 ^a	0.92 ^a
MSD	Ns	Ns

The means followed by the same letter are not significantly different at $P < 0.05$. PSI: Percentage severity index, DAP: Diammonium phosphate, CAN: Calcium ammonium nitrate, MSD: minimum significance difference. Ns: not significantly different.

IV. Discussion

A. Laboratory Experiment

1. Bacterial colonies population density and growth as affected by canola extract

The growth of *Ralstonia solanacearum* bacteria on SMSA growth medium occurs in 2-5 days at 28 °C of incubation [28]. Bacterial growth on plate is controlled by medium components and agar concentration, and if agar remain constant and nutrients increases may enhance growth of bacteria [36]. Bacto peptone, Casamino acid and glycerol are ones of the reagents used to culture the bacteria and are the main source of nutrients to the bacteria. In this study, the bacteria seemed to grow better in treatments where canola extract was added. This may be due to additional nutrients from canola extract. Moreover, canola extract did not show a biofumigation potential in suppressing *Ralstonia solanacearum* growth; this may be associated with low glucosinolate contents. [37] reported that canola has low, rapeseed, yellow mustard and oilseed radish have moderate while mustard duchess, mustard blend and oriental mustard have high glucosinolate content. [38] reported that canola contains less than 3 mg/g of glucosinolates and this has achieved through breeding program. However, different authors found some positive effect of brassica against soil borne pathogens. [16] found that, the macerated leaf tissue from Indian mustard exhibited total inhibition of fungal pathogens (*R. solani*, *Phytophthora erythroseptica*, and *Pythium ultimum*) while canola resulted in lower inhibition under in-vitro. [21] Sintayehu, reported that Ethiopian mustard and rapeseed extracts showed higher inhibition of *Fusarium oxysporum* growth compared to control in vitro-experiment. Several types of plant extracts have been tested for their efficacy in management of *R. solanacearum*. [39] evaluated plant derived resveratrol and coumarin concentration on *R. Solanacearum* in tobacco, found that, increasing concentration of phytochemicals significantly decreased growth rate of the bacteria. [40] on the study that was evaluating antibacterial and mechanism of action of plant-derived chemicals against *R. solanacearum* observed that Dimethylsulfoxide (DMSO) had no effect on the growth of *R. solanacearum* but Protocatechualdehyde was able to inhibit *R. solanacearum* completely at concentrations of 40 µg/mL on agar media.

2. Colony margin and cell shape as affected by canola extract

The result showed that canola extract had no effect on *R. solanacearum* colony margin. The colonies were red/pinkish red in centres and whitish periphery with fluidal and irregular in shape as supported by [27]. Normally, the bacterial colonies are circular in shape with smooth margin. [41] reported that *R. solanacearum* colonies on SMSA nutrient agar medium were smooth circular, raised and dirty white. [28] reported that on solid medium SMSA, bacterial colonies appear fluidal, irregular in shape, and white with pink centers and may appear 2-5 day after incubation at 28 °C. The results indicated that the colonies had pinkish red in centers and whitish periphery with irregular margins. [35] reported that, bacterial colony can exhibit a great diversity of forms depending on culture conditions (agar concentration and nutrient availability). There are no early researches on the effect of canola extract on bacterial colony margin available, but some other plant derived extracts have been tested on their effect on *R. solanacearum*. In vitro inhibition assay that was done by [42] to evaluate the effect of three concentrations of *Allium fistulosum* extract on *Ralstonia solanacearum*, and inhibition of bacterial growth were observed, where strongest inhibition was observed at concentrations of 50 and 100%. [43] evaluated the efficacy of botanical plants extracts by the zone of inhibition assay technique against *Ralstonia solanacearum* causing bacterial wilt of tomato using water and ethanol extracts, undiluted *Ocimum gratissimum* extract showed highest inhibition zone of 28.66 mm.

Bacterial cell shape is genetically determined and primarily dictated by a polymeric macromolecular structure that surrounds the cytoplasmic membrane called peptidoglycan (PG) sacculus. Bacterial cell shape remains unchanged though, throughout the cell division variations may occur, and mostly influenced by environmental conditions (nutritional condition and stress response). In this experiment, the results showed that canola extract did not affect bacterial cell shape. The cells were rod shaped without any deformation. [41], [27], reported that *R. solanacearum* cells are gram negative, small straight rod shaped. Even though, there are limited information of brassica's effect on bacterial cell shape, other plant-derived products have been studied.[39] in the study that was evaluating plant derived resveratrol and coumarin concentration on *R. Solanacearum* in tobacco, observed effect of phytochemical on bacterial cell. With control, the cells were intact and uniform in size, with a clear boundary and a smooth surface but when treated with high concentration of phytochemical the cell appeared incomplete and turned flat in a dissolved shape, with an unclear boundary that was partially ruptured. [40] on the study that was evaluating the effects of the antibacterial and mechanism of action of Protocatechualdehyde against *Ralstonia solanacearum*, found that Protocatechualdehyde (PCA) treated bacteria, clearly destroyed the surface structure of the bacterial cells and shapes.

B. Semi-field and Field Experiments

1. Effect of canola green manure and fertilizer treatments on *Ralstonia solanacearum* population density in the soil, bacterial wilt incidence and severity

The results show that, canola green manure did not have a significant effect on *Ralstonia solanacearum* population density in the soil, bacterial wilt incidence and severity. The observed increase in bacterial density in the soil were mostly associated with plant growth and vigour due to nutrients. [45] found that nitrogen content was positively correlated ($r^2=0.2659$) with *R. solanacearum* abundance, this could be the reason why NPK 900kg ha⁻¹ and DAP500 kg ha⁻¹ treatments had higher bacterial population, incidence and severity compared to control.

The occurrence and prevalence of soil-borne diseases including bacterial wilt are closely related to soil quality [46]. [47] on the study that was evaluating the effect of soil amendments and management on potato brown rot (Bacterial wilt) incidence and severity, reported that NPK fertilizer application may reduce or increase disease depend on some soil condition. They found that NPK treatment amended in sandy soils significantly increased the percentage-wilted plants from 8.3% to 28.4% and wilt severity from 4.3% to 27.1% in clay soils. They also found higher *R. solanacearum* population, disease severity and incidence in organically amended soil, and then concluded that it may be due to the high availability of substrate. Although, there is no clear literature focusing on relationship between soil fertility, potato growth and bacterial wilt incidence and severity.

The findings on the biofumigation potential in management of disease has been inconsistent. [48] found that canola residue had higher population of *Pseudomonas spp* compared to other treatments and no canola application. However, there are some other studies revealed that canola suppressed growth of soil pathogen mostly for fungus. [22] evaluated the effect of different parts of *Brassica juncea* green manure, above-ground parts (AP), below-ground parts (BP) and combination of both (AP+BP). He found that AP and AP+BP suppressed *Rhizoctonia solani* by 54 and 63%, respectively, and *Gaeumannomyces graminis* var. *tritici* by 40 and 40%, respectively, compared with control. Fulya et al. [20] found that green manure of yellow mustard (*Sinapis alba*), turnip (*Brassica rapa*), arugula (*Eruca vesicaria*), Mighty mustard (*B. juncea*), rape (*B. napus*), mustard green (*B. carinata*) and brown mustard (*B. juncea*) cover crops were effective in suppressing *Rhizoctonia solani* and *Phytophthora nicotianae*. [16] evaluated the effect of brassica crops, including canola, rapeseed, radish, turnip, yellow mustard, and Indian mustard on potato soil borne pathogens. Found that the growth of (*Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, and *Fusarium sambucinum*) inhibited by brassica crops, with the highest inhibition of (80 to 100%) resulted from Indian mustard application under in-vitro experiment. In the same study, under field experiment, Indian mustard was most effective for reducing powdery scab and common scab diseases, whereas rapeseed and canola were most effective in reducing *Rhizoctonia* diseases. In this study, observed inefficacy of canola green manure may be associated with low glucosinolate contents

compared to other brassica family. [18] reported that biofumigation potential varies from species to species, he found that oriental or Indian mustard (*Brassica juncea*) has the highest biofumigation potential than rapeseed and canola (*Brassica napus*). This was supported by [37] who reported that canola has low, rapeseed moderate and mustard high level of glucosinolates. Furthermore, canola has been bred from rapeseed first around 1960s [38] for reducing erucic acid content and glucosinolate contents in the oil and feedstuffs. These compounds are believed to cause diseases to animals when in higher quantity. Canola has less than 30 micromoles of glucosinolates and less than 2% of erucic acid reported [49] and this was confirmed by [38], who reported that contains less than 2% erucic acid and the meal less than 3 mg/g of glucosinolates.

Overall observation of bacterial population density reduction was observed across sites. The high reduction was observed on the samples taken at potato flowering stage compared to initial samples before experiment set up. This reduction may be as a result of solarization produced by a black polyethylene sheet used to cover experiment after incorporation of canola green manure for a period of two weeks as recommended by [20], [21], to void volatilization of glucosinolate compound. Soil solarization is based on the use of solar energy to raise soil temperature by 10 to 15 °C to the normal and this may affect the survival of the soil pathogen even at depths of approximately 20 cm [50]. *R. Solanacearum* is very sensitive to soil temperature fluctuation, the temperature below 20 °C and above 35 °C affect its growth [51]. [52] on the study that was evaluating effect of heat treatment on *Ralstonia spp.*, found that the bacteria populations were reduced from $2.4-7 \times 10^8$ colony forming units (Cfu) g^{-1} to 0-115, and 11-173 Cfu g^{-1} under 60 °C for 120 min and 45 °C for 48 hours respectively and reduced bacterial wilt incidence by 50-75%. [53] on the study that was evaluating the effect of soil solarization for the control of *R. Solanacearum*, found that no wilted tomato plants in the plots solarized for 60 days. The same result was observed by [12], who found that soil solarization for 60 days using transparent plastic mulches reduced the incidence of bacterial wilt of tomato.

Two potato varieties Kenya karibu and Shanghi were used in this study and Kenya karibu showed to be tolerant to bacterial wilt disease than Shanghi. Kenya karibu did not show any symptom of bacterial wilt in all sites and semi-field experiments. It is reported that there is no potato variety that has been found to be resistant to bacterial wilt 100%, but some level of tolerance may be observed [6], [54]. The absence of potato varieties resistance to bacterial wilt could be attributed to the high genetic variability of strains within the *R. solanacearum* species complex [55]. Through breeding, moderate to highly resistant potato varieties have been released, though those varieties are not effective against all the strains of the pathogen and high frequency of latent infection in tubers is still a major problem in breeding program [56]. [54] on the study that was assessing tolerance of potato cultivars (Tigoni, Asante, Kenya Karibu, Kenya Sifa and Dutch Robjyn) on bacterial wilt in Kenya, found that cultivars like Kenya Sifa and Kenya Karibu were the most tolerant to bacterial wilt, while Dutch Robjyn and Tigoni were the most susceptible to bacterial wilt disease. This was confirmed by [3], who found

that Kenya Karibu, Kenya Sifa and Ingabire were three most bacterial wilt resistant potato genotype among thirty-six evaluated potato genotypes. Even if no physical symptoms, tolerant varieties carry latent infection to the next progeny once planted as seeds [57]. [58] reported the same that potato varieties were found to have some degree of tolerance to potato bacterial wilt but it not safe because it may carry latent infections to the next progeny.

V. CONCLUSION

Potato is a short duration, high yielder and most disease susceptible crop that require intensive care for better production. In Kenya, potato bacterial wilt is the second most problematic disease after late blight causing high potato yield loss. Biofumigation is one of the environmentally friendly methods of management of soil borne pathogen. In the present study, canola green manure and compound NPK fertilizer application in Nakuru agro-ecological zone did not show a promising result in management of potato bacterial wilt. The higher rate of fertilizer application (NPK 900 kg ha^{-1}) seemed to enhance bacterial wilt incidence and severity compared to other treatments. Kenya karibu variety showed a potential to be tolerant to bacterial wilt disease than Shanghi and this may be exploited by breeders to produce a resistant cultivar. Further investigation should be done on other brassica family crops.

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