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2 **DIVERSITY OF *Ralstonia solanacerum***

3 **STRAINS IN SOLANACEOUS CROPS**

4 **PRODUCTION REGIONS OF CENTRAL KENYA**

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15 **ABSTRACT**

Aims: Bacterial wilt disease is a very serious yield limiting problem in crops in the solanaceae family grown in regions of Kenya. The very much encouraged field sanitation and use of clean planting materials have been inadequate in combating this challenge. This has seen increased need for understanding the genetic and biochemical diversity of *Ralstonia solanacerum* strains common to these areas as a basis for better strategies in their control.

Methodology: A field survey accompanied by samples collection was conducted covering: Nyeri, Nyahururu, Kirinyaga, Kiambu, Nakuru Murang'a and Embu Counties exclusively for solanaceae crop farms. Purposeful sampling was employed.

Results: A total of 160 samples of bacterial wilt symptomatic plants were collected, 120 isolates were confirmed as *R. solanacearum* using biochemical tests. They were further subjected to biovar tests using sugars and alcohols, (Cellobiose, Lactose, Maltose, D-Ribose, Salacin, Dulcitol, Mannitol, Trehalose and Sorbitol). Dextrose and Salacin were used as positive controls while sterile distilled water was used as negative control respectively. The results enabled mapping of *R. solanacearum* biovars in these regions. Biovar 2 was the most prevalent in the study area with biovar 2T showing high prevalence in both highlands and lowlands; biovar 2A was common in lowlands. Other biovars identified were 1 and 3.

Conclusion: This survey findings indicated that *R. solanacerum*, biovars present in Kenyan Highlands and Lowlands included biovars 1, 2 and 3. These biovars are highly pathogenic on solanaceae crops throughout the world. The findings of the present study will be useful for designing the study of the population structures of *R. solanacearum* using the molecular approaches with special emphasis on its integrated management.

17 **Keywords:** *Bacterial wilt, Kenya, Biovars, Control*

18 **1. INTRODUCTION**

19 Tomatoes, capsicum and potatoes are among the most important vegetables in Kenya. Their
20 increased production and supply within the country is very important for food and nutritional
21 security. These crops are majorly affected by diseases and pests. With scenarios of
22 increased resistance of the pests to common control measures arising, there is need for
23 more research for betterment of their management. Amidst plant diseases, *Ralstonia* spp is
24 a phytopathogenic bacterium causing great losses in *Solanaceae* crops production in Kenya.
25 *R. solanacearum* is an aerobic, gram negative, non-spore forming non-capsulated rod
26 shaped bacterium and nitrate-reducing, ammonia-forming, aerobic, rod-shaped bacterium
27 [1]. The bacterium, is among world most destructive pathogens, [2] it was first reported in
28 South America and Asia in 1880's, [3] and is now spread worldwide, [4]. The pathogen is
29 highly heterogeneous with many distinct strains differing in natural host range, and
30 geographic distribution, genetic and biochemical characteristics. The host range of the
31 bacterium is expansive comprising of hundreds of plant species, [5]. Important cultivated
32 crops affected include: *Capsicum annum* (sweet pepper), *Solanum lycopersicum* (tomato),
33 *Solanum tuberosum* (potato), *Capsicum* spp (Chilies), *Solanum melongena* (Eggplant),
34 *Musa* spp (banana) *Arachis hypogaea* (groundnut) and *Nicotiana tabacum* (tobacco), [6].
35 Ginger, Sunflower, cassava, olive, and eucalyptus are as well susceptible. Many
36 asymptomatic weeds harbor the bacteria such as some ecotypes of *Arabidopsis thaliana*.
37 The common symptoms range from inhibited growth, poor leaf quality in tobacco,
38 deterioration of leaves, vascular damage, browning and necrosis of tubers (potato) [7-8].

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40 *R. solanacearum* is a species complex that has been classified into five races based on host
41 range [9] and into five biovars based on biochemical properties [10]. There are variations in
42 *R. solanacearum* strains in term of host relationships, phenotypic properties and phylogeny,
43 this accompanied by rapid adaptation and mutations are key in their pathogenicity,
44 influencing disease development and virulence factors of the strains [11]. *R. solanacearum*
45 races and biovar are now associated with specific hosts; Race 1 biovar 1, race1 biovar 3 and
46 race1 biovar 2; common to Asia, Africa, Australia, North America and south America attacks:
47 ginger, capsicum, olive, peanut, tobacco among other *Solanum* spp plants. Race 2 biovar 1,
48 common to Asia, Caribbean Central America, South America and Hawaii prefer *Musa* spp
49 (plantain and banana), peanut, tomato and some *Heliconia* spp. Race3 biovar 2, is found
50 worldwide (except Canada and United States), affecting *Solanum* spp and *Pelargonium* spp.
51 Race 4 biovar 3 and race 4 biovar 4 are common in Australia, India, Asia, and Hawaii,
52 attacking ginger. Race 5 biovar 5 common in China attacks, *Musa* spp. The pathogen has
53 been further categorized into phylotypes; phylotype i (biovars, 3, 4 and 5), phylotype ii
54 (biovars 1 and 2-both 2A and 2T), phylotype iii (biovars 1 and 2) and Phylotype iv (biovars 1,
55 2A and 2T), [12-14].

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57 *Ralstonia* spp, can survive in soil and infected plant debris over long periods [15]. This
58 bacterium may also survive by colonizing the rhizospheres of non-host plants [16]. Elevated
59 temperatures (30-35 °C) are favorable for bacterial wilt disease, whereas soil temperatures
60 below 20 °C are not suitable [8]. The disease prevails in diverse soil types [6]. During planting
61 season the bacteria may be introduced into the field through contaminated planting materials,
62 contaminated implements and old plant debris (diseased debris). Spreading of the bacteria
63 from one plant to another is increased by contaminated irrigation water [17-18]. The
64 objective of the study was to establish the diversity of *Ralstonia solanacearum* strains in
65 solanaceous crops production regions of central Kenya

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2. MATERIALS AND METHODS

2.1. Collection of diseased samples

A total of 160 diseased solanaceae crops (potato, tomato capsicum and black nightshade) were collected from highlands and lowlands of Nakuru, Nyahururu, Nyeri, Kirinyaga, Murang'a, Kiambu and Embu Counties. The diseased solanaceae crops were subjected to bacterial wilt simple bacterial streaming test and later cultured in the laboratory where they were confirmed as pathogenic *Ralstonia* spp by development of virulent colonies (fluidal, irregularly-shaped, white colonies with pink centers) 36 hours incubation on TZC media. This procedure was further supplemented with biochemical analysis for biovar determination.

2.2. Biovar testing

2.2.1. Inoculum preparation

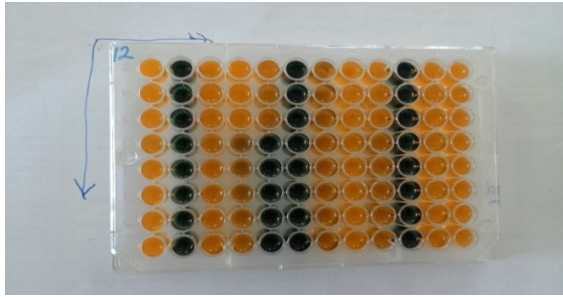
The *Ralstonia solanacearum* bacterial isolates were confirmed by growing the bacteria in SMSA Media and later multiplying it in CPG medium for 48 hrs at 28°C. A loop-full of bacteria was taken and mixed with 1 ml sterile water in a 2.0 ml eppendorf tube and shaken thoroughly to form a uniform suspension, [19].

2.2.2. Basal medium preparation

Media preparation for biovar testing was done according to standards by the international plant diagnostic network (IPDN). Ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$) 0.35g, Potassium hydroxide (KOH) 0.07g, Bromothymol blue 0.0105g, Magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) 0.07g, Peptone 0.35g, water 350 ml and Agar 1.05g were dissolved and autoclaved at 121°C for 18 minutes. The medium was left to cool to 60°C and amended with sugars and alcohols. Ten ml of each prepared sugars/alcohols was added to 90 ml of the prepared basal medium. Cellobiose (Cel), Lactose (Lac), Maltose (Malt), D-Ribose (D-Rib), Salacin (Sal), Dulcitol (Dul), Mannito (Man), Sorbitol (Sorb), L-Tryptophan (L-Tryp), Dextrose (Dex), Trehalose (Tre) were used. Sterile distilled water was used as negative control while salacin and dextrose were used as a positive control [20].

2.2.3. Biovar testing procedure

The isolates of *R. solanacearum* were differentiated into biovars based on their ability to utilize disaccharides (sucrose, lactose, maltose) and sugar alcohols (manitol, sorbitol and dulcitol) as described previously by [9-10]. The biovars were determined in the mineral medium ($\text{NH}_4\text{H}_2\text{PO}_4$ 1.0 g, KCl 0.2 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.2 g, Difco bacto peptone 1.0 g, Agar 3.0 g and Bromothymol blue 80.0 mg per litre) containing 1% sugar. About 150 µl of the melted medium was dispensed into the wells of microtitre plate. Inoculums for each group of isolate was prepared by several loop full of bacteria from 24-48 hours old cultures to distilled water to make suspension containing about 10⁸ CFU/ml. Then 20 µl of bacterial suspension was added to the wells of microtitre plate incubated at 28°C. The plates were then examined after 3-5 days of inoculation for changing pH which was indicated by the change of colour. The colour of the medium changed to yellow for positive results while those that remained blue-green colour exhibited negative results (Plate 1). Descriptive statistics were carried out to generate percentages of biovars on crops and study areas and graphs used to present the results.



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116 Plate 1: A 96 well plate displaying positive (yellow) and negative (blue-green) results. Source
117 Kago, E.18.7.2016.

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119 2.2.4 Biovar diversity determination

120 *R. solanacearum* biovars diversity in the study areas was determined based on sugar
121 utilization using a number of diversity indices: Dominance_DShannon_H, Berger-Parker,
122 Simpson_1-D, Brillouin, Margalef, Fisher_alpha, Evenness_e^H/S, Menhinick, Equitability_Jand
123 Chao-1. This enabled development of diversity profile. A dendrogram was drawn grouping
124 the biovars isolated into clusters. Biochemical diversity indices were calculated using PAST
125 software version 3. The diversity profiles were drawn based on Renyi index which is a
126 generalization of Shannon's informational measure of diversity extrapolated to particular
127 moments of the same function with a scale parameter ($\alpha = 0, 0.25, 0.5, 1, 2, 4, 8, 16, 32,$
128 infinity using PAST software version 3. Dendrograms and PCA for biochemical diversity were
129 drawn using PAST software version 3.

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131 3. RESULTS AND DISCUSSION

132 3.1 Biovars isolated on solanacea crops and variability in study region

133 Diseased crop samples collected from Nakuru, Nyahururu, Nyeri, Kirinyaga, Kiambu and
134 Nyandarua counties comprised of potato, tomato, capsicum and black nightshade. These
135 diseased crop samples showing symptoms of bacterial wilt (stunted and vascular damage)
136 were subjected to a series of tests to confirm the causal organisms of the disease. These
137 proved true for *Ralstonia solanacearum*. Biochemical analyses of the bacterium indicated
138 three biovars: biovar 2 (88.3%), biovar 1 (6.7%), and biovar3 (5.0%), (table 1).

Biovar Type	Biochemical Test	Test crop	% BIOVAR ISOLATE	% BIOVAR TYPES (1, 2,3)
1	Cel-, Lact-, Malt- & Dulci-, Mant-, Sorb.-	Potato	5.00	6.7
		Tomato	0	
		Capsicum	1.70	
2	Cel+, Lact+, Malt+ & Dulci-, Mant-, Sorb.-	Potato	55.80	88.3
		Tomato	29.20	
		Capsicum	1.70	
		Black	1.70	

		nightshade		
3	Cel+, Lact+, Malt+ &Dulci+, Mant+, Sorb.+	Potato	4.20	5.0
		Tomato	0.80	
		Capsicum	0	

Table 1: Bacterial wilt biovars obtained from diseased samples from Kenyan highlands and lowlands.

Among these biovars, the predominant biovar 2 was highly exhibited on potato (55.80 %) and tomato (29.20%) and to a lower percentage on black nightshade (1.70%) and capsicum (1.70%). This biovar had been noted to cause huge losses in potato and tomato farming. There has been strict control at the market, to prevent continued spread of this bacteria to other parts, with EPPO, imposing strict measures preventing marketing of Solanacea crop products from regions where the pathogen is suspect to be present [10]. Currently there is a big challenge in managing *R. solanacearum* race 3 biovar ii [20], since the pathogen is known to spread over long distances through potato tubers and planting materials, and some of the suggested control measures are inclusive of utilization of high quality-pathogen free irrigation water, use of certified planting materials and strict checks of the presence of the pathogen, especially at seed production units/ nurseries [18].

Biovar 1 was common on potato (5.00%) and Capsicum (1.70%), while biovar 3 was only present in isolates from potato (4.20%) and tomato (0.80%). Biovar 1 was more from samples from lowlands and less from isolates obtained from highlands. Biovar 2 was prevalent in isolates from both highlands and lowlands; biovar 3 was common to isolates from highlands and less common in isolates from lowlands, (figure1).

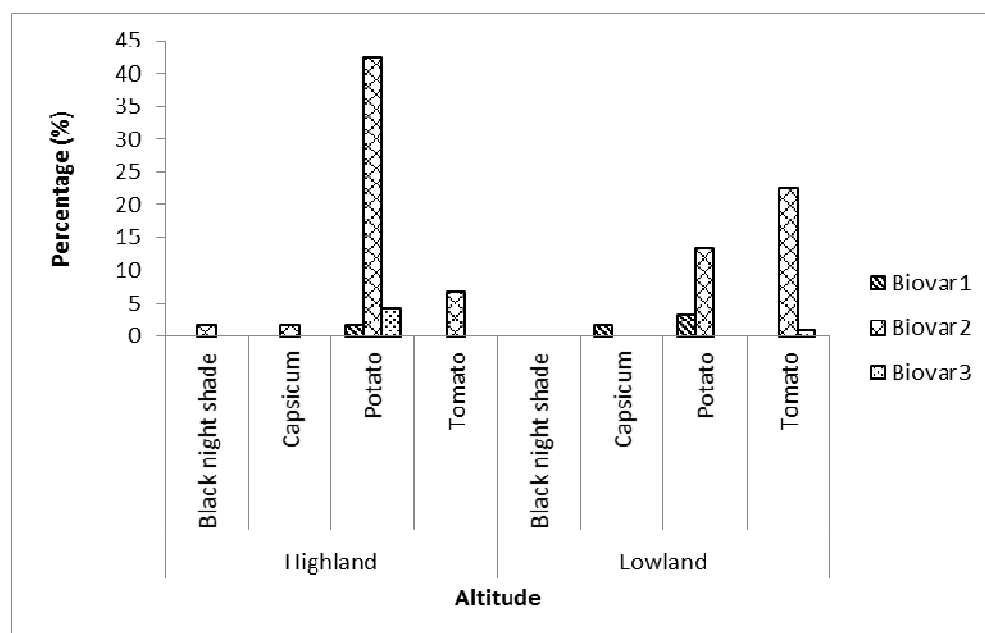
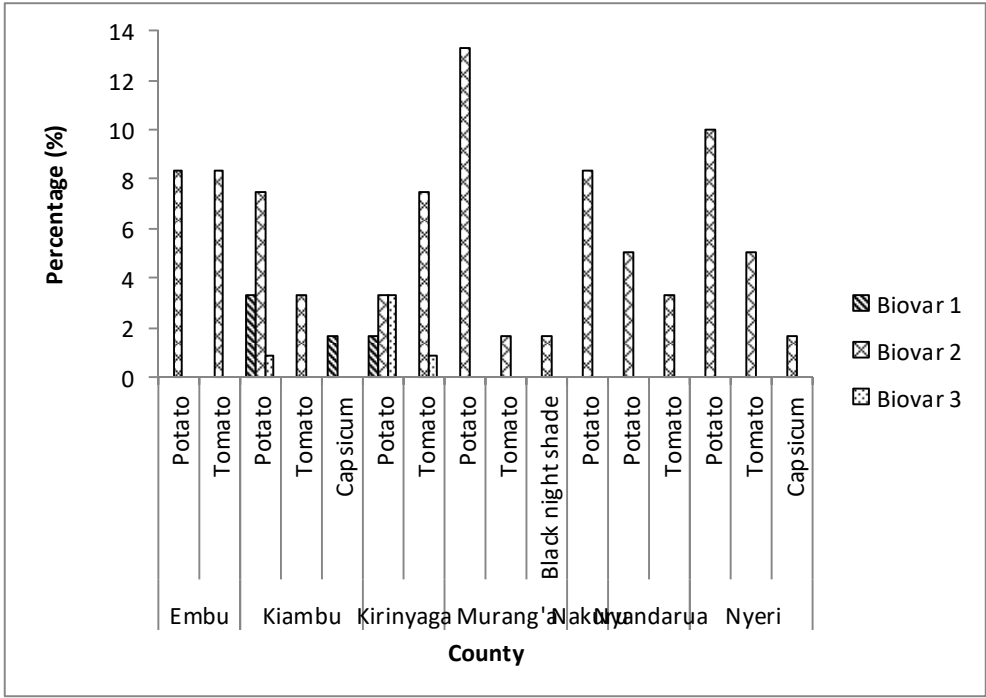


Figure 1. *R. solanacearum* biovars isolated from black nightshade, capsicum, potato and tomato, for samples from both highlands and lowlands.

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Among the three *R. solanacearum* biovars (biovar 1, 2 and 3) isolated from the Kenyan highlands and lowlands, biovar 2 was the most common, and was predominant on potatoes and capsicum. Many cases of bacterial wilt disease in Central Kenya region of potato production is likely to be due to race 3 biovar 2. This pathogen had severally been credited to huge losses in potatoes and tomatoes in Kenya. Biovar 1 was found on both potatoes and capsicum in lowlands, but on potatoes only in highlands. Biovar 2 was found attacking black nightshade, capsicum, potato and tomato in highlands, and only affecting potatoes and tomatoes in lowlands. Biovar 3 was found attacking tomatoes in lowlands and potatoes in highlands. Biovar 1 had been identified as causal organism of moko disease in bananas, brown rot in potato, it was also reported to affect tomato. Biovar 3 is pathogenic to eggplant, pepper and tomato [21-23].

Isolates from diseased samples obtained from Embu County (potato and tomato) tested positive from biovar 2 only, this was similar to isolates from Murang'a (potato, tomato and black nightshade), Nakuru (potato), Nyandarua (potato and tomato) and Nyeri (potato, tomato and capsicum). Kiambu (potato, tomato and capsicum) and Kirinyaga (Potato and tomato) counties' isolates had the most diverse composition of *R. solanacearum* biovars; biovars 1, 2 and 3, (figure 2).



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Figure 2. Counties, solanaceae crops grown and biovars isolated

Tryptophan, D-ribose and Trehalose were used to further differentiate biovar 2 into 2A and 2T. Among the biovar 2 isolated, a larger percentage was biovar 2T (85.90%) with biovar 2A (14.10%) being relatively low. Biovar 2T was prevalent in highlands (46.20%) and lowlands (39.60%), with biovar 2A appearing prevalent in highlands (13.20%), and less prevalent in lowlands (0.90%), (table 2).

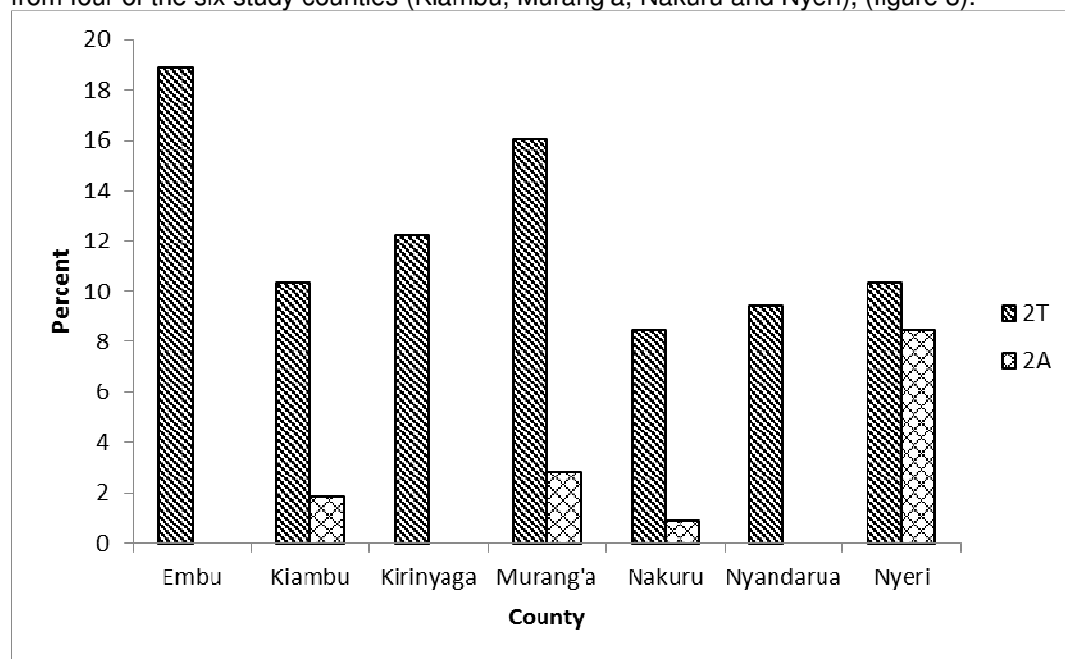
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Table 2: *R. solanacearum* biovars 2A and 2T zonations in Kenyan highlands and lowlands

Biovar Type	Biochemical Test	Zonation	% BIOVAR ISOLATE	%BIOVAR (2A/2T)
2A	Cel+, Lact+, Malt+, L(-) Trypt-, D-Rib-, Tre- &Dulci-, Mant-, Sorb.-,	Lowland	0.90	{14.10}
		Highland	13.20	
2T	Cel+, Lact+, Malt+ L(-) Trypt+, D- Rib+, Tre+ &Dulci-, Mant-, Sorb.-	Lowland	39.60	{85.90}
		Highland	46.20	

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Isolates from all the seven study counties (Embu, Kiambu, Kirinyaga, Murang'a, Nakuru, Nyandarua and Nyeri), tested positive for biovar 2T. Biovar 2A was only present in isolates from four of the six study counties (Kiambu, Murang'a, Nakuru and Nyeri), (figure 3).



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Figure 3. *R. solanacearum* biovars 2A and 2T as isolated from samples collected from the seven study counties.

Among the solanaceae crops used in the study, biovar 1 was predominant on capsicum (1.70%) and potato (5.00%). Biovar 2 was found on capsicum (1.70%), black nightshade (1.70%), potato (55.80%) and tomato (29.20%). Biovar 3 was found on potato (4.20%) and tomato (0.80%) only (figure 4).

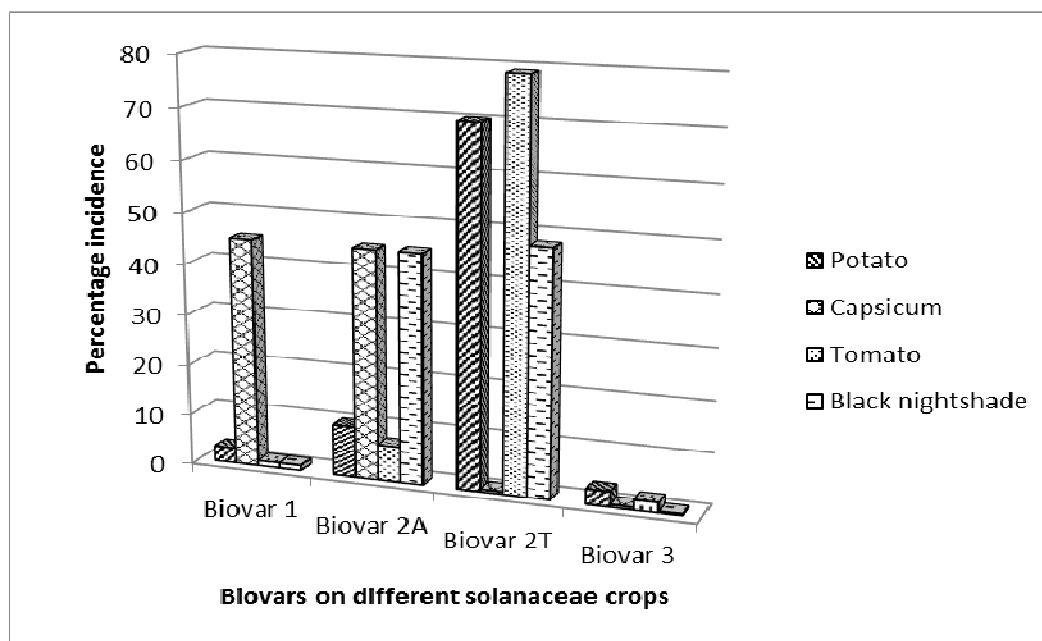


Figure 4. *R. solanacearum* biovars 1, 2 and 3 Isolated from solanaceae crops

In the highlands and lowlands biovar 2 was the most prevalent found on black nightshade, capsicum, tomato and potato in highlands, and found on potato and tomatoes in the lowlands.

The high diversity of *R. solanacearum*, is likely to confer survival in varied ecological conditions as seen in the isolates.

The results showing biovar 1 attacking both capsicum and potatoes in lowlands indicates higher virulence of the pathogen to solanaceae crops in the warm conditions, this is in line with the work by Meng et al., [24], who described biovar 1 as the most common in warmer areas. In highlands that are presumed relatively cooler, biovar 1 could only be obtained from potato derived isolates. The study found *R. solanacearum* biovar 2 to be significantly common in Kenyan highlands and lowlands. These findings are in line with work by other researchers who had singled out biovar 2 (race 3 biovar 2) as the one adapted to diverse ecological conditions. This biovar had been isolated in Cameroon's potato and tomato growing regions comprising of both highlands and lowlands, [23, 24]. The biovar is exceptionally adapted to cold conditions. Milling noted high survival of biovar 2 in tubers spanning 4 months at low temperatures of about 5 °C [25]. It was further discovered that this *R. solanacearum* biovar could retain viability in infected geranium debris for periods exceeding 6 months in very harsh temperature conditions of -20°C, [24]. The adaptations of are not unique to cold conditions, but also to lowlands, as Milling reported high virulence of biovar 2 at temperature of 20°C [23]. The same biovar had been majorly implicated in bacterial wilt disease at an average temperatures of 29.5°C [22].

Biovar 3 of *R. solanacearum* isolated was significantly present in highlands and lower in lowlands, this contradicts earlier reports by Prior et al, who had described this biovar as one for the warmer conditions. The likely explanation for this may be linked to the possibility of occurrence in latent infections without causing disease. But it had initially been reported that biovar 3 could prevail in diverse soil pH, temperatures and be able to infect tomato, sweet pepper and eggplants in such conditions, [23].

Pathogenicity of *R. solanacearum* biovars to solanaceae family was observed to vary among the study crops (Potato, tomato, black nightshade and capsicum). Biovars 1 occurrence on potato and tomato is in line with similar observations by Milling et al [26] and Zulperi et al [21], who found this biovar highly virulent to tomato with low pathogenicity to potato

According to the current study, Biovar 2 (2A and 2T), was the most common biovar affecting all the study crops (tomato, capsicum, potato and black nightshade). This is in line with the earlier studies that had implicated this biovars on potato, tomato and many other solanaceae crops in Cameroon, Kenya and east Africa at large [27]

R. solanacearum bv2 has thus been detected in the highlands of the tropics as well as in several (sub) tropical and some temperate areas throughout the world [28]. This study identified Biovar 3 incidence on tomato and potato. This corresponds to knowledge of its pathogenicity to affect tomato, eggplant and capsicum, [20, 25].

3.2 Biovar Diversity in the study region

Using varied diversity indices, the study areas varied in diversity of *Ralstonia solanacearum* isolated. Kirinyaga highland (KRH), Kiambu highland (KBH) and Kiambu lowlands exhibited the highest diversity in comparison to all the other areas. Murang'a highland (MRH), Murang'a lowland (MRL), Nyandarua highland (NYRH), Nakuru highland (NKRH), Nyeri highland (NYEH), Nyeri lowland (NYEL), Kirinyaga lowland (KRL), Embu highland (EBH) and Embu lowland (EBL) exhibited the low diversity as shown by figure 5.

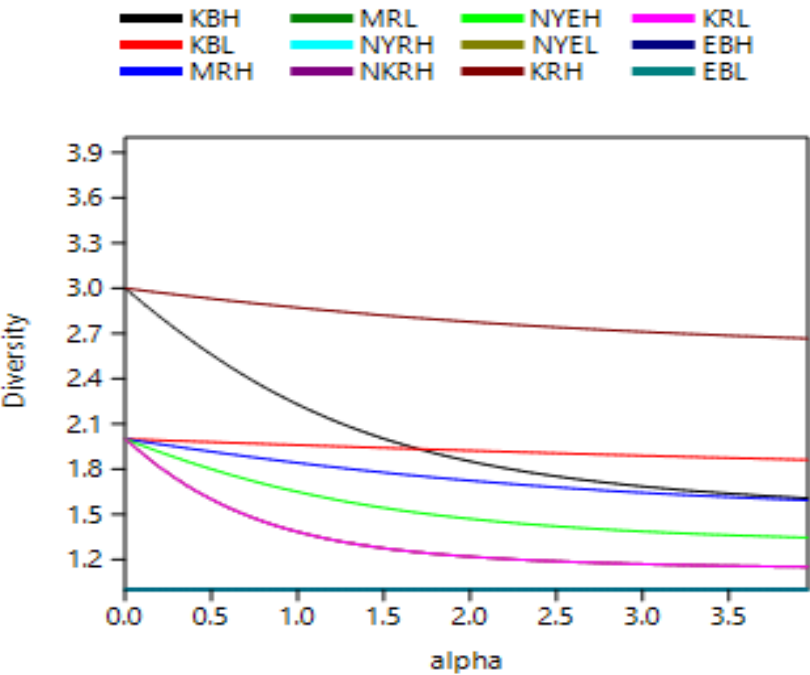
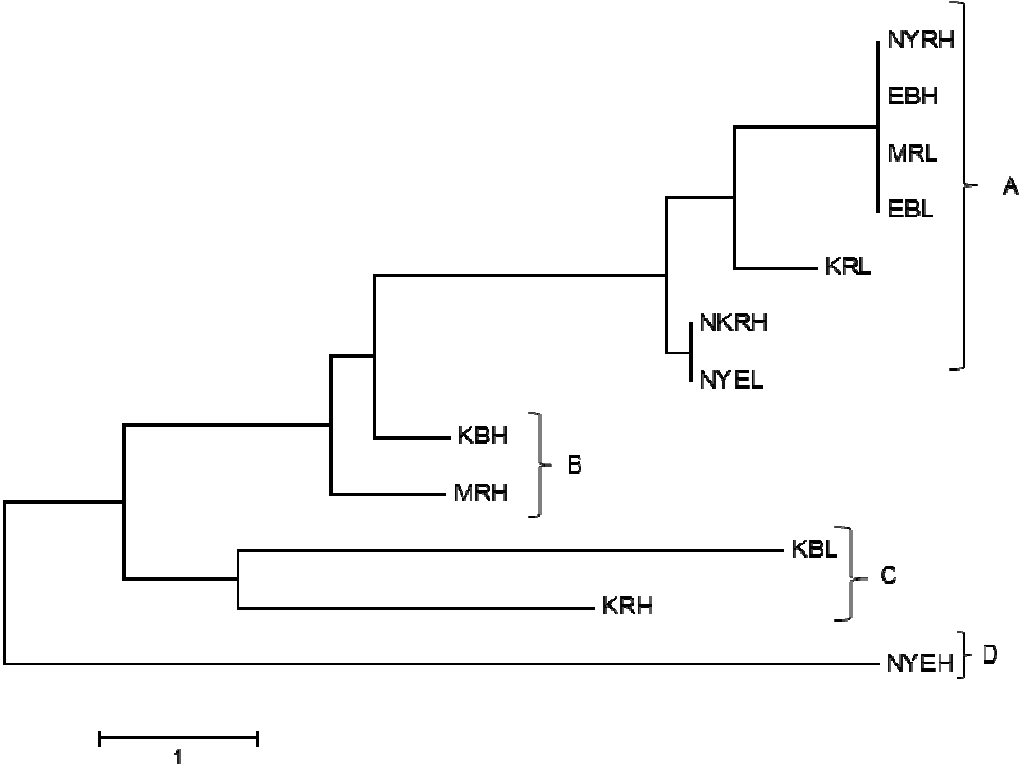


Figure 5: Diversity profile of the ralstonia isolates based on sugar utilization

According to the Neighbor joining distance dendrogram based on sugar utilization of the isolates, the various regions were grouped into four clusters (A, B, C, D). Cluster A included regions NYRH, EBH, MRL and EBL which were closely associated with isolates from regions

270 KRL, NKRH and NYEL. Cluster B included regions KBH and MRH. Region C included KBL
 271 and KRH while Region NYEH was an independent out group and was placed in cluster D,
 272 (Figure 6). Cluster C representing **Kiambu lowland and Kirinyaga highland** had significantly
 273 higher isolates of biovars 1 and 2 in comparison to all the other regions and had the highest
 274 diversity of the biovars isolated as shown in **figure 6**.
 275
 276 Kiambu and Kirinyaga regions are the most important potato and tomato producing regions
 277 in Kenya. Poor seed production systems common to these regions may contribute to the
 278 high diversity of ***R. solanacerum*** biovars observed. Reports of high transmission of the
 279 bacterium through infected seeds coming from the highlands are common **[26, 28, 29]**.
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 283 **Figure 6: Distance dendrogram drawn based on Neighbor joining method and Jaccard**
 284 **similarity index showing the diversity of the isolates based on sugar utilization.**
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286 *R. solanacerum*, has been found to express varied gene encoding cell wall and cell
 287 membrane synthesis, and similar to those that function in carbohydrate transport and
 288 metabolism. With this Milling's suggestion of presence of a great variation in chemical
 289 structure of the bacterium's extracellular polysaccharides, the two explain the high
 290 biochemical and pathogenic variability of this bacterium. The varied genes contribute to the
 291 complex difference in composition of extracellular polysaccharides, which contributed too to
 292 disease development in the susceptible plants. In general, the identification of biovars
 293 affecting solanaceae crops in highlands and lowlands of Kenya is a step towards better
 294 techniques in management of the bacterial wilt disease in major solanaceae crops growing
 295 regions **[24-26, 28- 29]**.
 296

297 **4. CONCLUSION**

298 This survey findings indicated that *R. solanacearum*, biovars present in Kenyan Highlands
299 and Lowlands included biovars 1, 2 and 3. These biovars are highly pathogenic on
300 solanaceae crops throughout the world. The differential prevalence of this biovars, and their
301 identity permits easy understanding of the pathogenicity of *R. solanacearum* for improved
302 control measures of bacterial wilt disease.

303 **ACKNOWLEDGEMENTS**

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305 Nyeri, Nakuru, Nyandarua and Kirinyaga Counties for enabling the researchers access the
306 potato, capsicum, black nightshade and tomato growing parts in these areas.

307 **COMPETING INTERESTS**

308 The authors have no competing interests to declare

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310 **AUTHORS' CONTRIBUTIONS**

311 Author A did the sample collection, isolated and performed the biochemical analysis of
312 biovars, analyzed data and prepared the first draft, author B, provided the working protocols
313 for biovar analysis and guided (Supervised) the activity, authors C and D provided general
314 guidance on the experiment and edited the manuscript. All the authors read and approved
315 the manuscript.

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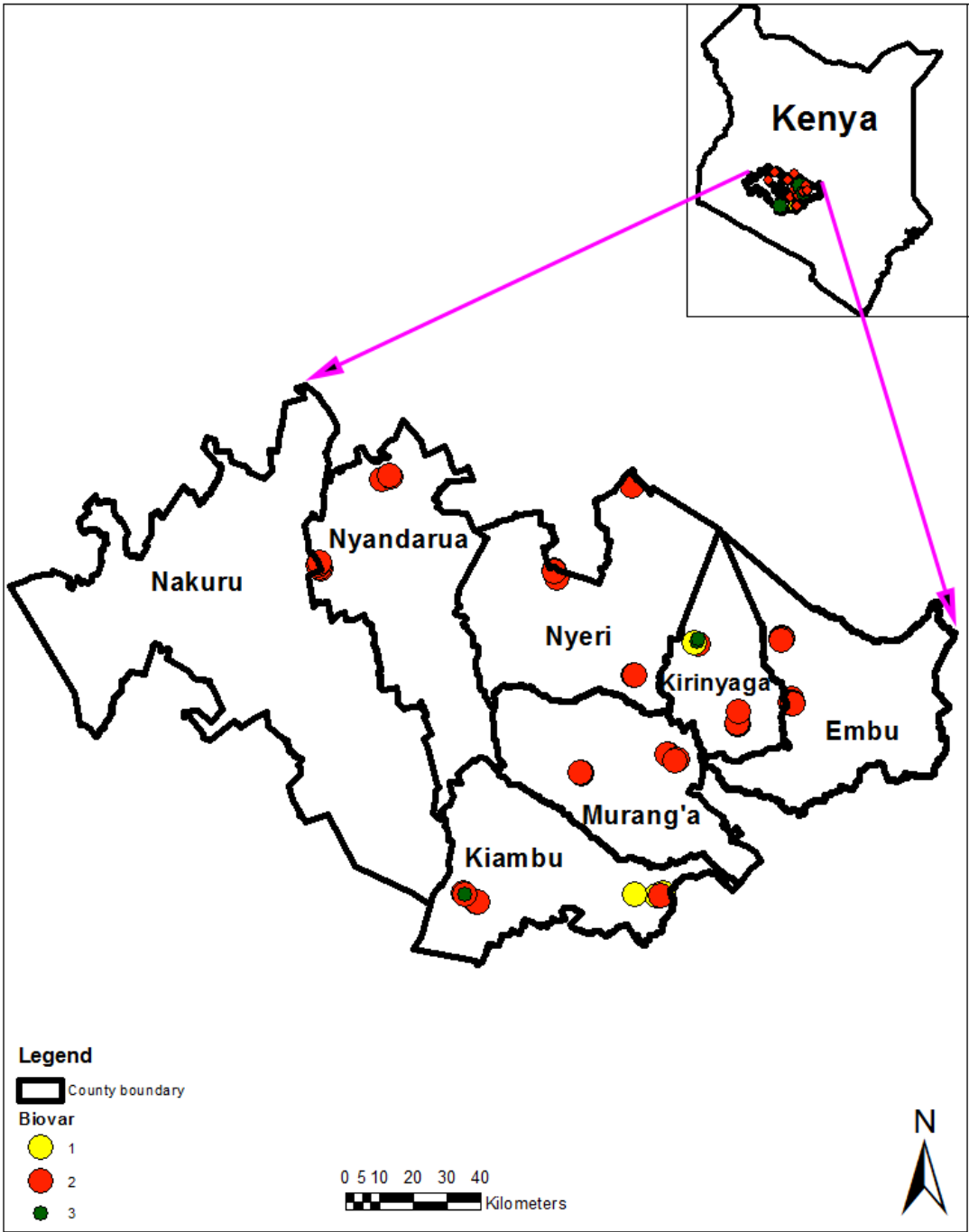
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416 **DEFINITIONS, ACRONYMS, ABBREVIATIONS**

417 0°C:	Degrees celsius
418 CPG:	Casamino acid-Peptide-Glucose (CPG) medium
419 Cel:	Cellobiose
420 D-Rib:	D-ribose
421 Dulci:	Dulcitol
422 EEPPPO:	European and Mediterranean Plant Protection Organization
423 Malt:	Maltose
424 Mant:	Manitol
425 Lact:	Lactose
426 L-Trypt:	L (-)Tryptophan
427 Sorb:	Sorbitol
428 Tre: D(+):	trehalose
429 TZC:	Triphenyltetrazolium chloride (TTC or TZC) medium

430

431 APPENDIX 1: MAP SHOWING R. SOLANACERUM BIOVARS DISTRIBUTION IN
432 KENYAN HIGHLANDS AND LOWLANDS



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435

APPENDIX 2: ISOLATE DIVERSITY BASED ON SUGAR UTILIZATION

DIVERSITY INDICES	KBH	KBL	MRH	MRL	NYR H	NKR H	NYE H	NYEL	KRH	KRL	EBH	EBL
TAXA_S	3	2	2	1	1	2	2	2	3	2	1	1
INDIVIDUALS	10	10	10	10	10	10	10	10	10	10	10	10
DOMINANCE_D	0.54	0.52	0.58	1	1	0.82	0.68	0.82	0.36	0.82	1	1
SIMPSON_1-D	0.46	0.48	0.42	0	0	0.18	0.32	0.18	0.64	0.18	0	0
SHANNON_H	0.801 8	0.673	0.610 9	0	0	0.325 1	0.500 4	0.325 1	1.055	0.325 1	0	0
EVENNESS_E^H/ S	0.743 2	0.980 1	0.921	1	1	0.692 1	0.824 7	0.692 1	0.957 2	0.692 1	1	1
BRILLOUIN	0.588 6	0.534 7	0.478 7	0	0	0.230 3	0.380 7	0.230 3	0.805 5	0.230 3	0	0
MENHINICK	0.948 7	0.632 5	0.632 5	0.316 2	0.316 2	0.632 5	0.632 5	0.632 5	0.948 7	0.632 5	0.316 2	0.316 2
MARGALEF	0.868 6	0.434 3	0.434 3	0	0	0.434 3	0.434 3	0.434 3	0.868 6	0.434 3	0	0
EQUITABILITY_J	0.729 8	0.971	0.881 3			0.469	0.721 9	0.469	0.960 2	0.469		
FISHER_ALPHA	1.453	0.751 7	0.751 7	0.276 6	0.276 6	0.751 7	0.751 7	0.751 7	1.453	0.751 7	0.276 6	0.276 6
BERGER-PARKER	0.7	0.6	0.7	1	1	0.9	0.8	0.9	0.4	0.9	1	1
CHAO-1	3	2	2	1	1	2	2	2	3	2	1	1

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