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Effect of Soil Fertility and Intercropping on the Incidence and Severity of Root Rot Diseases of Common Bean (*Phaseolus vulgaris* L.)

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Abstract High occurrence of root rots is attributed to continuous and inappropriate cropping systems, low soil fertility levels, low moisture in soil, use of farm saved seeds and use of root rot susceptible bean (*Phaseolus vulgaris* L.) varieties. This study evaluated the effect of soil fertility and intercropping on the incidence and severity of root rot diseases of common bean. Soil samples were collected at the start of the 2016 short rain cropping season to determine the soil nutrients status, and the incidence and population of soil borne fungal pathogens. The soil samples were analyzed for total nutrient status and pH levels. Soil borne fungal pathogens were isolated from the soil and stem bases by pour plate technique. Farm saved seeds of bean varieties KK8 and GLP2 were planted in field experiments at three sites in pure stand, intercropped with maize, applied with and without fertilizer. Data collected included seedling emergence, stand count, bean fly incidence, root rot distribution, incidence and severity, and yield. The pathogens isolated from soil and stem bases included *F. oxysporum*, *F. solani*, *Pythium* spp, *Macrophomina* and *Rhizoctonia* spp, with *Fusarium* spp. being the most predominant at 40% incidence and mean population of 3000 CFU/g of soil. Bean intercropped with maize had 22% lower intensity of root rot compared to the sole crop. The findings of this study demonstrate that low soil fertility, use of farm saved seeds and high inoculum levels of soil borne pathogens in the soil contributed to the high incidence of root rots in the study sites. In addition bean varieties intercropped with maize had a 17% lower incidence of root rot pathogens compared to bean varieties from sole crop. It was observed that intercropping system reduces pests and diseases. However, root rot pathogens isolated from bean intercropped with maize had a significantly lower incidence than the sole crops.

Keywords: *soil fertility, intercropping, root rot, soilborne pathogens, common bean*

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1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important grain legume in Eastern Africa, which is grown primarily as a food crop and to generate income by smallholder farmers [1]. In Kenya, common bean is grown in both the long rain season (April to June) and the short rain season (July to October). The crop has a role in sustaining agricultural systems by improving soil fertility and crop yield thereby reducing reliance on inorganic fertilization [2]. However, common bean production has been declining worldwide due to various biotic and abiotic constraints such as insect pests, diseases and poor soil fertility [3].

The crop is mainly grown in Kenya by smallholder farmers either as pure stand or intercropped with other crops such as maize, bananas and tuber crops [4]. In Western Kenya, the crop is produced continuously in the same fields due to decreased land size with limited allocation of resources for soil improvement [5]. This has led to a decline in soil fertility and build-up of pathogens in the soil thereby contributing to high disease pressure [3,6]. One of the major constraints to common bean production is low soil fertility. A global perspective of fertility decline is as a result of continuous and inappropriate cropping systems with little or no external nutrient input to replenish nutrients in the soil [7]. Phosphorus is a major nutrient required for bean production, and unlike nitrogen, it requires application of external nutrients [8]. Low productivity of common bean is as a result of poor soil fertility, nutrient depletion, low quality seed as well as high incidences of soil borne pathogens and insect pests [9]. According to [10], high intensity of root rot occurs in areas with low soil fertility especially low nitrogen and phosphorus. Declining soil fertility decreases the ability of common bean to fix atmospheric nitrogen [7]. Common bean losses due to low soil fertility in Eastern, Central and Southern Africa are estimated at 1,128 million tons per year [7].

Root rot in common bean is caused by individual or a complex of fungal pathogens mainly *Fusarium* spp.

Pythium spp, *Rhizoctonia* spp and *Macrophomina* spp [11]. Root rots reduce seedling emergence, limit crop establishment, cause crop failure thereby lowering the crop's yield [12]. Root rot infections occur at all plant growth stages with high inoculum of the pathogens in the soil resulting in higher incidence and severity of the disease [11]. It is estimated that the annual yield loss worldwide caused by root rot is 20 to 40% [13].

Intercropping is advantageous in maximizing land use efficiency and also reduced pest and diseases [14]. Maize and common bean are important components of intercropping systems in improving soil fertility, controlling weeds, diseases and insects [3]. Common bean is a potential source of plant nutrients that complement inorganic fertilizers for cereal crops, mainly through biological nitrogen fixation [3,5]. Intercropping common beans with maize, sorghum and ground nut has effects on soil borne fungal and bacterial pathogens, thereby lowering diseases and improving the growth and yield of the crop [3]. Intercropping common bean with different crops produces a greater yield and is a better management approach in enhancing seed quality and soil fertility levels [2]. Intercropping system therefore provides higher cash returns to smallholder farmers than growing monocrops [15].

Common bean cropping systems have soil quality benefits which include increasing soil organic matter, improving soil porosity, recycling nutrients, improving soil structure and water holding capacity as well as decreasing soil pH in the soil [16]. Common bean has greater roles in cropping systems, especially in regions where accessibility and affordability of fertilizer is a challenge [17]. In common bean cropping systems, there is an increase in soil fertility as a result of nutrient rich residues provided by the crop [2]. Intercropping as a cropping system is advanced as one of the integrated soil fertility management practices [2,17]. The objective of this study was therefore to determine the effect of soil fertility and intercropping on the incidence and severity of root rot diseases of common bean.

2. Materials and Methods

2.1. Description of the Study Area

Field experiments were conducted in farmers' fields at three sites during the long rain cropping system of 2016 in Alupe, Busire and Butula of Lower Midland zone one (LM1) in Busia County, western Kenya. Busia County is located at the extreme Western part of Kenya between longitude 33º 55' and 34º 25' East and latitudes 0º 30' and 0º 45' North [18]. The County is in the Lower Midland (LM) zone and it is divided into four agro-ecological zones (AEZ): lower Midland 1, 2, 3 and 4 [18]. Busia County has varying climatic conditions with annual rainfall ranging between 800 mm to 2000 mm with 50% of the rain falling in the long rain season which starts in April and continues into June, while 25% of the rain falls in the short rain season which starts in late August and continues into October. The County has an average temperature of 22°C and altitude range between 1216 m and 1520 m above sea level [18].

2.2. Collection of Soil Samples and Determination of Levels of Soil Nutrients

Soil samples were collected before planting from each experimental plot to determine soil nutrient status and population of soil borne pathogens. Soils were sampled from the top 0-10 cm at four equidistant positions in each plot using a sterile spatula and were mixed thoroughly to obtain a composite sample of approximately 1kg per plot for analysis [19]. The soil samples were put in Kraft bags and stored in a refrigerator at 4ºC before microbial analysis. Analysis of the soil samples for soil nutrients and other characteristics such as the pH level was done at the Kenya Agricultural and Livestock Research Organization (KALRO) laboratories. The analysis was carried out for total N by Kjekdahl digestion method [20], organic carbon by calorimetric method [21], and the available phosphorous by Olsen method [22]. Soil pH was determined in a 1:1 soil water suspension with a pH meter. Available elements such as iron, zinc, copper, potassium, calcium manganese and magnesium were extracted in a 1: 10 ratio using Mehlich double acid method [20].

2.3. Determination of the Population and Diversity of Soil Borne Fungal Pathogens

Soil sub-samples were obtained from the composite samples described in Section 2.2. One gram was placed in 10 ml of sterilize distilled water and mixed on a mechanical shaker for 40 minutes. The suspension was serially diluted up to 10^3 and one milliliter aliquots of 10^2 and 10^3 dilutions placed in each Petri dish in which approximately 20 millilitres of molten Potato Dextrose Agar amended with 50ppm streptomycin and 40ppm tetracycline was added [23]. The content was gently swirled, allowed to solidify and incubated at room temperature (23 ± 2°C) for 5 to 7 days [24]. Fungal colonies showing different cultural characteristics were observed and recorded and the total number of colony forming units (CFU) per gram of soil calculated using the formula by [25].

$$\text{CFU}/\text{ gram soil} = \text{Total number of colonies} \times \text{dilution factor.} \quad \text{(I)}$$

Each fungal colony type was sub-cultured separately on fresh PDA media. *Fusarium* species were also sub cultured on Synthetic Nutrient agar (SNA) media containing 1g KH_2PO_4, 1g KNO_3, 0.5g $MgSO_4$.7H2O, 0.5g KCl, 0.2g glucose, 0.2g sucrose and 20g agar in 1000ml distilled water [23]. The SNA plates were incubated for 10 to 14 days in a dark room to allow sporulation. The fungi were identified by cultural and morphological features such as colony color and type of growth supplemented with microscopic identification using identification keys [23].

To allow for undisturbed fungal structures, riddle slides were prepared by placing a sterilized cover slip over a block of sterile agar on microscopic slides and placed over V- shaped glass rod on moist filter paper [26]. Seven-day-old cultures were used in preparing fungal colonies for examination and identification. The agar was inoculated with fungal mycelia and incubated at room temperature

for seven days. The growth of each pathogen extended over onto the coverslips and the microscopic slide. The cover slip was removed and mounted directly on to a microscope slide with appropriate stain [27]. The observed structures such as microconidia, macroconidia and chlamydiospores, conidia, and sporangiophores were used to identify the fungal pathogens. *Fusarium* spp. were identified based on the *Fusarium* laboratory manual by [28], while the identity of other soil borne pathogens was confirmed using fungal identification keys described by [23].

2.4. Field Assessment of Root Rot Infection on Bean Plants

Field assessment of root rot infection was carried out at the second and fourth weeks after crop emergence by observing and counting infected plants showing root rot symptoms such as stunted growth, yellowing of leaves, wilting, brown dark- colored roots and root rotting. Assessment of disease distribution was scored using a scale of 0-2, where 0 = no disease, 1 = disease occurs in localized spots and 2 = disease distributed in whole field [29]. The incidence of root rots was determined by counting the number of infected plants within each plot, and the percentage incidence calculated as follows:

$$\text{Percentage incidence} = \frac{\text{Total number of infected plants}}{\text{Total number of plant per plot}} \times 100. \quad \text{(II)}$$

Severity of the disease was assessed by observing symptoms on the leaves and the pods using a rating scale of 0 – 3, where: 0 = No disease; 1 = Mild infection; 2 = Moderate infection ; 3 = Severe infection [30,31]. The total percentage disease index of 0 – 100 was calculated using the following formulae [32]:

$$\text{Percent disease index} = \frac{\text{Disease distribution} + \text{incidence} + \text{severity}}{\text{Sum of the disease score}} \times 100. \quad \text{(III)}$$

2.5. Isolation of Root Rot Pathogens from Stem Bases

At emergence, ten symptomatic and ten non-symptomatic bean plants were randomly sampled from each plot. The stem bases were cut off after washing in running tap water. Each stem base was surface sterilized in 1.3% sodium hypochlorite solution for 30 seconds and then rinsed in three changes of sterilize distilled water and blot dried. The root and stem tissues (≈ 2mm long) were placed on PDA amended with 50ppm streptomycin and incubated for 7 - 14 days at room temperature (23 ± 2°C). Fungal colonies of different cultural characteristics were recorded. Root rot pathogens were identified based on their morphological and cultural characteristics as described in Section 2.3.

2.6. Assessment of Bean Fly Incidence

Data on the incidence of bean fly was taken on the 4th and 6th weeks after emergence. Bean plants in each plot were examined for bean fly infestation symptoms such as swollen cracked tunneling through stem tissues and discolored rotten stem. Bean fly distribution was scored using a scale of 0-2, where 0 = no damage, 1 = slight damage, 2 = moderate damage and 3 = severe damage [29]. The incidence was expressed as the number of infested plants per plot [31].

2.7. Data Analysis

Data on soil nutrients status, incidence and population of soil borne pathogens, percentage plant emergence and stand count, bean fly incidence and root rot disease index were subjected to analysis of variance (ANOVA) using GENSTAT software version 14. The means were separated using Fisher's protected least significant different (LSD) at 5% level of significance.

3. Results

3.1. Soil Nutrient Levels

There was significant variation ($P \leq 0.05$) in soil pH, level of copper and available nutrients which included nitrogen, carbon, potassium, magnesium, calcium, sodium, and manganese in the three study sites in Busia County (Table 1). Soils from Butula had the lowest levels of soil nutrients compared to Alupe and Busire. Soils in Butula and Busire were acidic (Mean pH of 4.8) while those in Alupe had a near neutral pH of 5.5. Soils from Alupe and Busire had a 0.2% higher level of nitrogen, while soils from Butula had the lowest level of nitrogen. The level of manganese was 0.6% higher in soil from Busire compared to levels in soils from Alupe and Butula while there was no significant variation ($P \geq 0.05$) in levels of phosphorus, iron and zinc across the three study sites. Levels of potassium, calcium, magnesium, carbon and sodium were significantly higher in Alupe compared to the other sites.

Table 1. Level of nutrients (ppm and percentage) in soil sampled from three study sites in Busia County

Site	pH	N	C	P	K	Ca	Mg	Mn	Cu	Fe	Zn	Na
Alupe	5.5 a	0.2 a	1.8 a	6.7 a	0.5 a	4.2 a	2.9 a	0.4 b	6.5 b	57.1 a	12.1 a	0.4 a
Busire	4.8 b	0.2 a	1.7 a	6.7 a	0.1 b	2.3 ab	2.4 a	0.6 a	13.0 a	46.0 a	9.7 a	0.1 b
Butula	4.6 b	0.1 b	0.7 b	5.0 a	0.1 b	1.8 b	0.8 b	0.5 ab	2.0 c	65.9 a	9.5 a	0.1 b
Mean	5.0	0.1	1.4	6.1	0.3	2.8	2.0	0.5	7.2	56.3	10.4	0.2
LSD ($P \leq 0.05$)	0.2	0.1	0.9	6.0	0.1	2.1	0.9	0.21	1.1	22.7	9.7	0.1
CV (%)	1.9	20.3	29.4	43.1	10.2	34.0	19.1	18.6	7.0	17.8	40.9	13.6

Means accompanied by the same letter(s) in each column are not significantly different at $p \leq 0.05$; LSD - Least significant difference at $P \leq 0.05$; CV - coefficient of variation.

3.2. Incidence and Population of Soil Borne Fungal Pathogens

The fungal pathogens isolated from soils sampled from the three study sites in Busia County were *Fusarium*, *Pythium*, *Macrophomina*, *Rhizoctonia*, *Penicillium* and *Sclerotinia*. There were significant differences ($P \leq 0.05$) in the incidence of soil borne pathogens in soils from the three study sites (Table 2). The root rot pathogens isolated from soil were *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Pythium ultimum* and *Macrophomina phaseolina*. There was no significant difference ($P \geq 0.05$) in the incidence of *F. solani*, *Rhizoctonia* and *Macrophomina* across the study sites; while the incidence of *Pythium* and *F. oxysporum* varied significantly ($P \leq 0.05$) across the three study sites. Soil samples from Butula had significantly ($P \leq 0.05$) higher incidence of *F. solani*, *F. oxysporum* and *R. solani*. There were significant differences in the population of soil borne pathogens across sites (Table 3). Soil from Alupe had a 45% higher population of soil borne pathogens compared to soil samples from Butula which had the lowest population.

3.3. Root Rot Infection and Effect on Emergence and Plant Stand

There was no significant variation ($P \geq 0.05$) in the plant stand of bean seedlings across sites and among the treatments (Table 4). However, the plant stand of bean varieties intercropped with maize was higher than the sole crops. The highest stand count at sixth week after planting was in Alupe compared to other sites. Percent root rot disease index at second and fourth week after planting varied significantly ($P \leq 0.05$) between sites and among the treatments (Table 5, Table 6, Table 7). Root rot disease index in Butula was 17% higher than in the other study sites. Alupe had the lowest root rot disease index of about 48%, while the bean varieties intercropped with maize had significantly lower ($P \leq 0.05$) root rot disease index compared to the sole crops in the three study sites. The bean variety KK8 had significantly lower disease index of root rot than the bean variety GLP2 across the three study sites.

Table 2. Incidence (%) of soil borne pathogens in soils sampled from three study sites in Busia County

Site	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*	Others
Alupe	22.6 a	19.2 b	11.8 a	15.3 a	11.5 a	19.6 a
Busire	20.7 a	23.0 ab	9.9 a	15.4 a	10.4 a	20.6 a
Butula	23.7 a	25.0 a	11.7 a	10.9 b	11.8 a	17.04 a
Mean	22.3	22.4	11.1	13.9	11.2	19.1
LSD ($P \leq 0.05$)	10.7	11.1	10.3	11.4	10.8	14.0
CV (%)	29.2	30.1	56.4	50.1	58.5	44.8

Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV- coefficient of variation.

Table 3. Population (CFU/g) of fungal pathogens in soils sampled from three study sites in Busia County of western Kenya

Site	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*	Others
Alupe	1917 c	2083 b	917 b	917 c	1000 b	1458 b
Busire	2375 b	2625 a	1125 b	1750 b	1208 b	2417 a
Butula	3458 a	2917 a	1792 a	2333 a	1750 a	2958 a
Mean	2583	2542	1278	1667	1319	2278
LSD ($P \leq 0.05$)	1269	1383	1081	1320	1223	1675
CV (%)	30	33	52	48	56	45

Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV- coefficient of variation.

Table 4. Percent plant stand of two common bean varieties in eight treatments at four weeks after emergence in three study sites in Busia County

Treatment	Alupe	Busire	Butula	Mean
KK8 + maize	69.6 a	70.4 a	67.5 ab	69.2 a
GLP2 pure	33.7 d	47.2 bc	42.0 c	40.9 b
GLP2 + maize	70.0 a	74.2 a	64.2 b	69.4 a
KK8 pure	43.8 cd	47.3 bc	43.2 c	44.8 b
GLP2 pure + fertilizer	43.8 bcd	39.5 c	48.0 c	43.8 b
KK8 +maize + fertilizer	67.1 ab	58.3 ab	70.0 ab	65.1 a
GLP2+maize + fertilizer	60.8 abc	60.0 ab	77.9 a	66.3 a
KK8 pure + fertilizer	37.7 cd	45.5 bc	38.2 c	40.4 b
Mean	53.3 a	55.3 a	56.4 a	55.0
LSD ($P \leq 0.05$)	21.8	15.7	10.6	
LSD ($P \leq 0.05$)	Site; 5.39	Treatment; 8.8	Site * Treatment; 15.25	
CV (%)	23.3	16.2	10.7	16.9

GLP2 – Rose coco, KK8- Kakamega 8: Bean varieties. Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD –Least significant difference at $p \leq 0.05$; CV- coefficient of variation.

Table 5. Percent plant stand of two common bean varieties in eight treatments at six weeks after emergence in three study sites in Busia County

Treatment	Alupe	Busire	Butula	Mean
KK8 + maize	94.6 a	96.3 a	87.9 ab	92.9 a
GLP2 pure	80.5 ab	73.0 b	71.7 c	75.1 c
GLP2 + maize	95.4 a	94.6 a	80.4 bc	90.1 a
KK8 pure	72.8 ab	69.8 b	69.7 c	70.8 c
GLP2 pure + fertilizer	85.0 ab	81.7 b	70.0 c	78.9 bc
KK8 +maize +fertilizer	71.2 b	78.7 b	87.5 ab	79.2 bc
GLP2+maize + fertilizer	82.1 ab	78.8 b	94.2 a	85.0 ab
KK8 pure + fertilizer	74.5 ab	74.8 b	76.3 c	75.2 c
Mean	82.0 a	81.0 a	79.7 a	80.9
LSD (P ≤ 0.05)	20.5	11.3	10.2	
LSD (P ≤ 0.05)	Site; 4.95	Treatment; 8.09	Site * Treatment; 14.01	
CV (%)	14.3	8.0	7.3	10.5

GLP2 – Rose coco, KK8- Kakamega 8: Bean varieties. Means accompanied by the same letter(s) in each column are not significantly different at P ≤ 0.05; LSD –Least significant difference at p ≤ 0.05; CV- coefficient of variation.

Table 6. Percent root rot disease index of two common bean varieties at two weeks after planting in the three study sites in Busia County

Treatment	Alupe	Busire	Butula	Mean
KK8 + maize	40.7 a	39.8 a	57.7 bc	46.1 b
GLP2 pure	35.3 a	51.7 a	77.0 a	54.7 ab
GLP2 + maize	52.7 a	41.3 a	66.0 abc	53.3 ab
KK8 pure	46.1 a	56.9 a	79.7 a	60.9 a
GLP2 pure + fertilizer	46.7 a	58.2 a	59.1 bc	54.7 ab
KK8 + maize + fertilizer	58.3 a	52.2 a	52.6 c	54.4 ab
GLP2 + maize + fertilizer	53.4 a	47.4 a	53.3 c	51.4 ab
KK8 pure + fertilizer	45.7 a	57.0 a	69.3 ab	57.3 ab
Mean	47.3 b	50.6 b	64.3 a	54.1
LSD (P ≤ 0.05)	23.6	16.9	13.7	
LSD (P ≤ 0.05)	Site; 6.88	Treatment; 11.2	Site * Treatment; 19.5	
CV (%)	28.5	19.0	12.1	21.9

GLP2 – Rose coco, KK8- Kakamega 8: Bean varieties. Means accompanied by the same letter(s) in each column are not significantly different at P ≤ 0.05; LSD- Least significant difference at P ≤ 0.05; CV- coefficient of variation.

Table 7. Percent root rots disease index of two common bean varieties at fourth week after planting in three study sites in Busia County

Treatment	Alupe	Busire	Butula	Mean
GLP2+ maize	41.2 bc	52.2 ab	64.5 ab	52.6 cd
KK8 pure	68.8 a	69.6 ab	70.5 ab	69.6 a
KK8 + maize	35.6 c	46.9 b	59.4 b	47.3 d
GLP2 pure	62.9 ab	62.7 ab	85.7 a	70.4 a
GLP2 pure + fertilizer	58.0 abc	74.5 a	70.1 ab	67.5 ab
KK8 + maize + fertilizer	35.8 c	c57.3 ab	64.1 ab	52.4 cd
GLP2 + maize + fertilizer	36.3 c	c55.7 ab	75.6 ab	55.9 bcd
KK8 pure +fertilizer	46.3 bc	74.3 a	74.8 ab	65.1 abc
Mean	48.1	61.7 b	70.6 a	60.1
LSD (P ≤ 0.05)	23.4	23.4	22.1	
LSD (P ≤ 0.05)	Site; 7.29	Treatment; 11.9	Site * Treatment; 20.6	
CV (%)	24.2	21.7	17.9	20.9

GLP2 – Rose coco, KK8- Kakamega 8: Bean varieties. Means accompanied by the same letter(s) in each column are not significantly different at P ≤ 0.05; LSD- Least significant difference at P ≤ 0.05; CV- coefficient of variation.

3.4. Incidence of Bean Fly

Incidence of bean fly at the fourth and sixth week after planting varied significantly (P ≤ 0.05) across sites and various treatments (Table 8, Table 9). During the fourth week, the incidence of bean fly in Butula was 7% higher than the overall mean, while the incidence of bean fly in Busire was 5% lower than the overall mean. The bean varieties KK8 and GLP2 intercropped with maize had lower bean fly incidence compared to the sole crops, while the variety KK8 had significantly lower (P ≤ 0.05) bean fly incidence across the sites than variety GLP2. During the sixth week, Butula had the highest bean fly incidence of about 70% compared to the other sites.

Table 8. Percent incidence of bean fly at fourth week after planting

Treatment	Alupe	Busire	Butula	Mean
GLP2 + maize	34.0 b	28.1 b	58.2 b	40.1 bc
GLP2 + maize + fertilizer	46.4 b	34.2 b	51.4 c	44.0 b
KK8 pure	64.3 a	68.3 a	69.9 a	67.5 a
KK8 pure + fertilizer	63.8 a	63.4 a	69.6 a	65.6 a
KK8 + maize	33.9 b	28.3 b	51.5 c	37.9 c
KK8 + Maize + fertilizer	45.8 b	34.2 b	51.4 c	43.8 b
GLP2 pure	69.0 a	68.7 a	69.6 a	69.1 a
GLP2 pure + fertilizer	69.7 a	68.9 a	70.2 a	69.6 a
Mean	53.4 b	49.3 c	61.5 a	54.7
LSD ($P \leq 0.05$)	12.8	10.3	5.9	
LSD ($P \leq 0.05$)	Site; 3.34	Treatment; 5.46	Site * Treatment; 9.45	
CV (%)	13.7	12.0	5.4	10.5

GLP2 -Rose coco, KK8- Kakamega 8: Bean varieties. Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD- Least significant difference at $P \leq 0.05$; CV- coefficient of variation.

Table 9. Percent incidence of bean fly at sixth week after planting

Treatment	Alupe	Busire	Butula	Mean
GLP2 + maize	50.9 b	51.0 d	69.5 abc	57.2 bc
GLP2 + maize + fertilizer	57.7 b	62.9 bc	62.7 bc	61.1 b
GLP2 pure	74.1 a	68.6 ab	80.4 a	74.4 a
GLP2 pure + fertilizer	68.4 a	68.5 ab	75.1 ab	70.7 a
KK8 + maize	51.3 b	51.1 d	57.5 c	53.3 c
KK8 + maize + fertilizer	51.4 b	57.5 cd	57.8 c	55.6 bc
KK8 pure	68.9 a	68.6 ab	80.8 a	72.7 a
KK8 pure + fertilizer	68.8 a	74.0 a	80.2 a	74.4 a
Mean	61.4 b	62.8 b	70.5 a	64.9
LSD ($P \leq 0.05$)	9.3	9.7	14.6	
LSD ($P \leq 0.05$)	Site; 4.05	Treatment; 6.61	Site * Treatment; 11.45	
CV (%)	8.6	8.8	11.8	10.7

GLP2 - Rose coco, KK8- Kakamega 8: Bean varieties. Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD- Least significant difference at $P \leq 0.05$; CV- coefficient of variation.

Table 10. Incidence (%) of root rot pathogens isolated from symptomatic stem bases of common beans in Alupe

Treatment	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*
GLP2 + maize	31.1 a	17.8 a	25.6 a	18.9 a	6.7 a
GLP2 + maize + fertilizer	27.8 a	27.8 a	16.7 a	22.2 a	5.6 a
GLP2 pure	28.9 a	32.8 a	5.6 a	17.8 a	15.0 a
GLP2 pure + fertilizer	40.0 a	20.0 a	13.3 a	6.7 a	20.0 a
KK8 + maize	33.3 a	26.7 a	13.3 a	26.7 a	0.0 a
KK8 + maize + fertilizer	26.1 a	24.4 a	15.0 a	15.0 a	19.4 a
KK8 pure	28.3 a	21.7 a	21.7 a	13.3 a	15.0 a
KK8 pure + fertilizer	35.0 a	20.0 a	8.3 a	21.7 a	15.0 a
Mean	31.3	23.9	14.9	17.8	12.1
LSD ($P \leq 0.05$)	18.9	32.1	21.5	19.4	21.4
CV (%)	34.5	76.8	82.1	62.2	101.2

Root rot pathogens, other.Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV- coefficient of variation.

3.5. Root Rot Incidence and Infection on Stem Bases

A complex of five root rot pathogens were isolated from symptomatic and asymptomatic stem bases of the various treatments in the study sites. The five pathogens were generally isolated in higher incidence in symptomatic stem bases (Table 10; Table 11; Table 12). There was no significant variation ($P \geq 0.05$) in the incidence of root rot pathogens from symptomatic stem bases across sites. However, there were general variations in the incidence of *F. solani*, *F. oxysporum* and *Pythium* being high on bean stem bases from Butula. The incidence of *Macrophomina phaseolina* was high in stem bases from Alupe, while the incidence of *Rhizoctonia* was high in stem bases from Busire.

There was a significant variation ($p \leq 0.05$) in the incidence of root rot pathogens isolated from asymptomatic stem bases across sites (Table 13, Table 14, Table 15). Asymptomatic stem bases from Busire had a 23% higher incidence of *F. solani* and 19% of *F. oxysporum* compared to stem bases from Alupe which had the lowest incidence of *F. solani* and *F. oxysporum*. Asymptomatic stem bases from Butula had higher incidence of *M. phaseolina* and *R. solani*; while the stem bases from Alupe had a 38% higher incidence of *Pythium*.

Table 11. Incidence (%) of root rot pathogens isolated from symptomatic stem bases of common beans in Busire

Treatments	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*
GLP2 + maize	27.5 a	27.6 a	11.4 a	19.5 a	14.0 a
GLP2 + maize + fertilizer	33.4 a	26.4 a	12.5 a	15.3 a	12.3 a
GLP2 pure	25.1 a	22.7 a	12.0 a	15.3 a	24.8 a
GLP2 pure + fertilizer	28.3 a	36.0 a	8.6 a	11.9 a	15.2 a
KK8 + maize	28.3 a	25.6 a	11.1 a	12.7 a	22.3 a
KK8 + maize + fertilizer	28.5 a	26.0 a	10.4 a	18.1 a	17.0 a
KK8 pure	38.4 a	23.2 a	9.1 a	14.6 a	14.6 a
KK8 pure + fertilizer	30.3 a	25.7 a	7.0 a	22.4 a	14.5 a
Mean	30.0	26.7	10.3	16.2	16.8
LSD ($P \leq 0.05$)	18.6	18.0	13.5	10.8	11.7
CV (%)	35.4	38.6	75.0	37.8	39.5

Root rot pathogens.Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV- coefficient of variation.

Table 12. Incidence (%) of root rot pathogens isolated from symptomatic stem bases of common beans in Butula

Treatment	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*
GLP2 + maize	24.2 bc	16.7 a	15.0 a	27.3 a	12.2 a
GLP2+maize+fertilizer	30.6 abc	13.9 a	10.4 a	27.8 a	8.3 a
GLP2 pure	36.1 abc	27.8 a	15.1 a	26.4 a	17.0 a
GLP2 pure +fertilizer	40.3 ab	25.7 a	22.4 a	18.8 a	18.6 a
KK8 + maize	30.0 abc	24.4 a	7.9 a	18.9 a	6.7 a
KK8 + maize+fertilizer	17.0 c	21.7 a	10.4 a	26.1 a	12.2 a
KK8 pure	40.2 ab	19.6 a	14.3 a	25.7 a	5.6 a
KK8 pure +fertilizer	46.3 a	30.0 a	13.1 a	15.7 a	10.0 a
Mean	33.1	22.5	13.6	23.3	11.3
LSD ($P \leq 0.05$)	19.6	15.5	18.6	19.1	18.8
CV (%)	33.8	39.4	78.3	46.7	95.0

Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV-coefficient of variation.

Table 13. Incidence (%) of root rot pathogens isolated from asymptomatic stem bases of common beans in Alupe

Treatment	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*
GLP2 + maize	22.2 a	17.8 a	12.2 a	41.1 a	6.7 a
GLP2 + maize + fertilizer	8.3 a	20.6 a	12.2 a	46.7 a	12.2 a
GLP2 pure	22.2 a	0.0 a	25.0 a	47.2 a	5.6 a
GLP2 pure + fertilizer	18.1 a	18.1 a	6.7 a	52.4 a	4.8 a
KK8 + maize	15.1 a	10.3 a	18.7 a	45.6 a	10.3 a
KK8 + maize + fertilizer	13.3 a	20.0 a	6.7 a	46.7 a	13.3 a
KK8 pure	20.0 a	13.3 a	8.3 a	50.0 a	8.3 a
KK8 pure + fertilizer	15.0 a	6.7 a	15.0 a	52.2 a	11.1 a
Mean	16.8	13.3	13.1	47.7	9.0
LSD ($P \leq 0.05$)	28.6	22.5	9.6	32.1	21.7
CV (%)	97.4	96.1	20.7	38.4	137.2

Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV-coefficient of variation.

Table 14. Incidence (%) of root rot pathogens isolated from asymptomatic stem bases of common beans in Busire

Treatment	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*
GLP2 + maize	47.2 a	44.4 a	0.0 a	0.0 b	8.3 a
GLP2 + maize + fertilizer	28.4 a	45.1 a	4.8 a	11.4 ab	10.3 a
GLP2 pure	36.5 a	35.4 a	9.3 a	14.0 ab	4.8 a
GLP2 pure + fertilizer	33.3 a	27.8 a	11.1 a	11.1 ab	16.7 a
KK8 + maize	30.6 a	41.7 a	11.1 a	5.6 ab	11.1 a
KK8 + maize + fertilizer	38.9 a	25.0 a	11.1 a	19.4 a	5.6 a
KK8 pure	37.3 a	42.9 a	4.8 a	10.3 ab	4.8 a
KK8 pure + fertilizer	38.9 a	40.3 a	4.2 a	4.2 ab	12.5 a
Mean	36.4	37.8	7.0	9.5	9.3
LSD ($P \leq 0.05$)	25.0	26.1	15.3	14.1	18.0
CV (%)	39.3	39.3	123.8	84.7	111.3

Means accompanied by the same letter(s) in each column are not significantly different at $P \leq 0.05$; LSD - Least significant difference at $p \leq 0.05$; CV-coefficient of variation.

Table 15. Incidence (%) of root rot pathogens isolated from asymptomatic stem bases of common beans in Butula

Treatment	*F. solani*	*F. oxysporum*	*Macrophomina*	*Pythium*	*Rhizoctonia*
GLP2 + maize	38.9 ab	16.7 a	22.2 a	11.1 a	11.1 a
GLP2 + maize + fertilizer	30.6 abc	22.2 a	19.4 a	8.3 a	19.4 a
GLP2 pure	35.6 abc	22.2 a	6.7 a	6.7 a	28.9 a
GLP2 pure + fertilizer	13.3 c	40.0 a	20.0 a	20.0 a	6.7 a
KK8 + maize	44.4 a	19.4 a	13.9 a	16.7 a	5.6 a
KK8 + maize + fertilizer	19.4 bc	44.4 a	11.1 a	0.0 a	25.0 a
KK8 pure	23.3 abc	23.3 a	23.3 a	15.0 a	15.0 a
KK8 pure + fertilizer	38.3 ab	25.0 a	15.0 a	15.0 a	6.7 a
Mean	30.5	26.7	16.5	11.6	14.8
LSD (P≤ 0.05)	21.2	27.4	25.4	21.2	25.0
CV (%)	39.7	58.7	88.3	104.6	96.5

Means accompanied by the same letter(s) in each column are not significantly different at P ≤ 0.05; LSD - Least significant difference at p ≤ 0.05; CV- coefficient of variation.

Table 16. Correlation among soil nutrients, population of soil borne pathogens, plant stand count, root rot intensity and infection on stem bases

	Soil Nitrogen	Soil Carbon	Soil Potassium	Soil Phosphorus	Soil PH	Soil borne inoculum	Root rot intensity	Root rot infection	Stand count
Soil Nitrogen	-								
Soil Carbon	0.5*	-							
Soil Potassium	0.5*	1.0**	-						
Soil Phosphorus	1.0**	0.5*	0.5*	-					
Soil PH	0.7*	1.0**	1.0**	0.7*	-				
Soil borne inoculum	0.8**	0.9**	0.9**	0.8**	1.0**	-			
Root rot intensity	-0.8**	-0.9**	-0.9**	-0.8**	-1.0**	-1.0**	-		
Root rot infection	-1.0**	-0.5*	-0.5*	-1.0**	-0.7*	-0.8**	0.8**	-	
Stand count	0.9**	0.8**	0.8**	0.9**	0.9**	1.0**	-1.0**	-0.9**	-

*- significant; ** - highly significant at 5% level of probability.

3.6. Correlation among Soil Nutrients, Population of Soil Borne Pathogens, Plant Stand Count, Root Rot Intensity and Infection on Stem Bases

There was a negative correlation among the soil nutrients, soil borne inoculum, root rot intensity and root rot infection on stem bases (Table 16). The soil nutrients positively correlated with the plant stand count; however soil borne inoculum positively correlated with the root rot intensity and the disease infection on the stem bases. In contrast, the soil borne inoculum negatively correlated with the plant stand count.

4. Discussion

There were significant levels of soil pH and available nutrients such as nitrogen, carbon, potassium magnesium, calcium, sodium, manganese and copper in this study. Soils from Butula had the lowest levels of nutrients compared to Alupe and Busire. Soil samples were below the recommended levels for total nitrogen (0.2 to 0.5) and carbon (2.7 to 5.3) in soils from the study sites. The low soil nutrients status could be attributed to continuous cultivation of the land, removal or burning of crop residues, loss of nutrients through soil erosion, and continuous application of acidic fertilizers. The results of the current study concur with the findings by [4] who reported that low soil nutrient status was due to inadequate use of inorganic fertilizers and soil erosion that have led to inadequate food production per capita in smallholder farms in Western Kenya.

pH of the soil samples from the three study sites was slightly acidic and in the range of 4.6 to 5.5. A study by [33] reported low soil pH in the range of 4.0 to 5.5 for soils in Western Kenya. The low soil pH values (pH < 5.5) are strongly acidic and have potential to cause toxicity problems and deficiency of some essential plant nutrients as well as affect soil microbial activities [33]. Stunting and yellowing of leaves are symptoms of acidic soils which result in limited plant growth and declining yields worldwide [34]. Micronutrients were high in Butula and generally low in Busire and Alupe which could be due to different soil properties in different sites. A study by [35] indicated that availability of micronutrients positively correlated with silt, clay, organic carbon, pH and CEC of soils.

Higher incidences of root rot pathogens inoculum were observed in soils from the study sites. This could be explained by poor fertility status, amount of inoculum in the soil and low soil moisture [36]. Higher incidence of root rot pathogens was observed in Butula where the soil pH and fertility levels were the lowest among the study sites. Higher incidence of root rot pathogens were obtained in low soil fertility areas [12]. Conventional and intensive farming practices lead to a decline in soil structure and fertility as well as higher microbial diversity and high population of soil and root pathogens [37]. The same authors also reported that root diseases are more

devastating in low fertility soils. Another study by [38] indicated that soil moisture and soil pH influence the densities of root rot pathogens in soil. In the current study there was significantly lower population of root rot pathogens in high fertility soil. Microbial population in soil is determined by various factors such as soil depth, organic matter, and soil pH [39]. These microorganisms are known as plant growth promoters and are beneficial in increasing crop yield [39].

Infection on stem bases varied significantly among sites and treatments, and the major root rot pathogens isolated from the stem bases were *F. oxysporum*, *F. solani*, *Pythium* spp, *Macrophomina* and *Rhizoctonia* spp. Butula had the highest infection compared to Alupe and Busire. This can be attributed to low soil nutrient status where Butula had the lowest soil pH and fertility levels among the study sites. According to [12], higher incidence of root rot pathogens is prevalent in areas of low fertility soils. The incidence of root rot pathogens isolated from symptomatic and non-symptomatic stem bases of the beans was similar and high across sites. This could be attributed to the farmers' reliance on planting farm saved seeds. Recycling of farm saved seeds leads to build-up of inoculum and loss of resistance of the seed against fungal and bacterial pathogens [40]. Farm saved seeds serve as a source of inoculum that is detrimental to crop production [40]. Survival structures of the pathogens are stored within the seed and the pathogens may cause failure of the seed to germinate, infect the germinated seedlings and the mature plants. There were differences in the intensity of root rot between the different bean varieties with KK8 intercropped with maize having a lower disease index compared to GLP2 intercropped with maize. This could be attributed to differences in resistance and susceptibility of a bean variety. The results in the current study concur with the findings by [41] who reported low incidence of root rot in variety KK8 which is tolerant to the disease compared to the susceptible variety GLP2.

There was variation in the rate of emergence and plant stand among the treatments of the experiment in the study sites. Difference in the rate of emergence between intercrops and sole crop could be due to competition for resources such as nutrients and light [42]. The current study findings concur with results by [43] who reported that intercropping maize and beans increased percent emergence, reduced diseases and increased yields of both bean and maize compared to the monocrop system. The plant stand varied among the various treatments in the study sites, with higher percentage recorded in the intercrops compared to the sole crops. The variation could be explained by the ability of intercropping system in reducing pests and diseases.

Bean fly (*Ophiomyia* spp.) is one of the insect pests that seriously affect production of common bean and losses of up to 40% have been reported [36]. Due to scarcity of land, majority of the farmers do not practice rotation with non-host crops which are known to reduce bean fly infestation [44]. Low soil fertility aggravated by low application of inorganic fertilizers by the farmers leads to weakly growing bean plants which are vulnerable to bean fly infestation [45].

Severe bean fly infestation may result into total yield losses, especially under low soil fertility and drought conditions [36]. There was significant variation in the incidence of bean fly in both bean varieties and treatments across the three study sites. Bean varieties KK8 and GLP2 intercropped with maize had lower incidences of bean fly (*Ophiomyia* spp) compared to the sole crops. This could be attributed to the advantages of intercropping that include reduction in damages caused by insect pests, diseases and weeds. The study findings are in agreement with [44] who reported that the incidence of *Ophiomyia* spp. decreased with increasing plant population. The study further indicated that low counts of insect pest were recorded in intercrops and stem damage was higher in pure bean plots, compared to the intercrops. Findings by [45] indicated that intercropping system has the ability to reduce damage caused by pests and diseases.

Intercropping beans with maize reduced infestation of beans by *Ophiomyia phaseoli* and the bean fly count was significantly lower in mixed stands than in pure stands [46]. Bean fly is often considered the most important field pest of beans in Africa, and cultural practices that include site selection, intercropping, crop rotation, and cultivar and seed selection, may to a certain degree reduce crop infestation by the pest [47]. A contrary observation was made by [48] who reported that bean fly incidence in the maize bean intercrops was not significantly different from the sole crops.

Bean fly incidence was significantly higher in Butula compared to Alupe and Busire, hence the high root rot infection in the respective site. This could be attributed to bean fly being a predisposing factor of root rot disease and the damage caused by bean stem maggot which creates avenues for entry of root rot pathogens. This agrees with findings by [36] who reported that bean root rot diseases and bean fly occur in a complex and there is a positive correlation especially where soil fertility is low. Bean fly is a serious constraint to bean production creating entry for root rot pathogens, thus causing high root rot intensity, lowering plant stand count, and reducing yield. Therefore, cultural practices that include intercropping, crop rotation and resistant varieties may to a certain degree reduce infestation of bean fly in the field [47]. There was a negative correlation between soil nutrients, soil borne inoculum and root rot. This can be explained by the soil fertility levels influencing soil microbes [12]. A study by [12] reported that soil pH negatively correlated with *Fusarium* root rot severity and index across different soil environments. A study by [49] indicated that soils with low fertility increase the population of soil borne pathogens.

5 Conclusions

Low soil fertility status in the study sites influenced the population of root rot pathogens and grain yield. Due to reduced land size, smallholder farmers do not practice crop rotation and therefore continuous cultivation of common beans has led to low soil nutrients status and high buildup of fungal and bacterial pathogens. From the study findings, the site with poor soil fertility had high incidence of root rots infection and reduced yield. This implies that low fertility levels in the study sites played an important role in increasing the incidence and severity of

soil borne pathogens. Smallholder farmers should incorporate field sanitation, intercropping system, and crop rotation as common bean disease management and soil fertility improvement measures.

Competing Interests

The Authors have no competing interest

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