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RESEARCH ARTICLE

Management of bean root rots by intercropping and legume diversification

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Abstract

Root rots are a major biotic factor impacting negatively on the production of common beans (*Phaseolus vulgaris* L.) in smallholder farming systems. The search for revamping approaches toward the management of root rots is welcome. Therefore, this study evaluated the effect of intercropping and legume diversification on the intensity of the root rot disease complex of common beans in Western Kenya**.** Farm saved bean seeds of GLP2 (*Rose coco*) bean variety and seeds of varieties KATX56 and KK8 sourced from the local market and Kenya Agricultural and Livestock Research Organization (KALRO), respectively, and subsequently planted on ten farms. The treatments of this study consisted of intercrops of each of the three bean varieties with maize compared to sole bean crops. Two legumes, cowpea and groundnuts, variety K80 and Red Valencia, respectively were incorporated to achieve diversification. Data was collected on the population of soil-borne fungal pathogens causing root rots before planting, at two and four weeks after emergence; plant stand and root rot intensity. *Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina, Pythium* spp., and *Rhizoctonia solani* were isolated from both sample soils and symptomatic bean plants. Intercrops and diversification cropping systems resulted in a significant ($p \leq 0.05$) decrease in the incidence of root rot pathogens and intensity of root rot. The results showed that bean sole cropping is more prone to root rots than when intercropped with maize and other legumes. These two cropping systems, intercropping and diversification are effective approaches for the suppression of bean root rot pathogens.

Keywords: cereal-legume intercrop; disease management; soil borne pathogens; *Phaseolus vulgaris.*

1. Introduction

Among the grain legumes common bean (*Phaseolus vulgaris* L.) stands out as the most produced and consumed within and beyond East Africa. It is a key source of cheap protein, income earner to farmers and traders, and its interaction with *Rhizobium* spp. improves soil health and its foliage is utilized as fodder (Cong *et al.*, 2015; Karuma *et al.*, 2016). The legume is an integral component in diverse cropping systems and particular crop mixtures. Common bean has been used as an intercrop with Maize, rotational crop of choice, and double cropping, all found to contribute immensely but not limited to ecosystem stability (Blair, 2013; Liu *et al.*, 2017; Nurk *et al.*, 2017). However, production of the common bean is constrained by biotic factors which make realization of potential yields impossible especially in open field production in western Kenya. Arthropod pests and plant pathogenic microorganisms have been found to threaten the production of common beans in open fields (Ojiem *et al., 2006*; Muthomi *et al.*, 2007; Kimiti *et al.*, 2009). The continued infestation and infection of beans by pests and plant pathogens, respectively is aggravated by susceptible bean varieties, poor soil health and ineffective crop and pest management practices

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(Buruchara, 1990; Scott *et al.,* 2003; Okalebo *et al.,* 2006).

Soil-borne diseases of common bean result from singular or multiple infections by pathogens which to a large extent are also seed-borne (Makelo, 1997; Muthomi *et al.*, 2007; Kimiti *et al.,* 2009). According to Makelo. (1997) common bean smallholders either use their saved seeds or seeds borrowed from neighbors. In the event your saved seeds or seeds borrowed are not available then sourcing from local markets becomes the option. The use of certified and recommended seeds is a foundational approach for integrated bean-root rot management worth to be adopted by smallholder farmers (Makelo, 1997; Karavina *et al.,* 2011; Naseri and Hemmati, 2017). However, smallholder farmers are handcapped adopting integrated management programs in line with bean production. Such delimiting factors are poverty-stricken status, limited access to extension services and information, and continued embrace of poor agronomic practices coupled with poor-quality bean seeds (Katungi *et al.*, 2009). Further, Mahasi *et al.* (2010) and Naseri and Hemmati (2017) argue that epidemiological understanding as far as root rot pathogens are concerned cannot be overlooked in strategizing how to manage root rots.

Root rot complex is the major soil-borne disease of beans following infection by diverse species of *Fusarium*, *Macrophomina Pythium*, *Rhizoctonia* and,*Sclerotinia* (Mwang'ombe *et al.,* 2008; Nzungize *et al.,* 2012). The disease is prevalent in bean-growing areas globally (Naseri and Hemmati, 2017), however, in Kenya it is pronounced in Central, Eastern, and Western regions (Mwang`ombe *et al.,* 2007). Root roots have increasingly affected smallholder farmers in western Kenya and a remarkable yield loss (>70%) has been reported when susceptible bean varieties are planted under favorable conditions (Otysula *et al.,* 2003). An overwhelming number of common bean smallholder farmers seldom use fertilizers in their continuous bean cropping. These unsustainable agronomic practices in western Kenya explain why soil fertility and shrinking bean productivity are current realities (Njeru *et al.,* 2009; Odundo *et al.,* 2009; Kenya Agriculture and Livestock Research Organization (KALRO), 2011). The study findings by KALRO, (2011) affirmed that failure to incorporate fertilizer in the soil increases stiff competition for limited nutrients among bean plants resulting in decreased vigour hence predisposing beans to soil-borne pathogens.

Intercropping, two or more crops in proximity is flagged as an inexpensive and potent alternative approach to managing plant diseases in the tropics (Sacred Africa, 2002). Accoladed aspects of intercropping range from improving soil health, weed species suppression, and maintenance of ecological stability to decimated plant disease severity (Odhiambo and Ariga, 2001; Njeru *et al.,* 2009; Odundo *et al.,* 2009; Belel *et al.*, 2014). Practically, studies have shown that growing varietal mixtures of beans and intercropping maize with beans reduce bean foliar-associated diseases such as anthracnose and further leads to increased bean yield (Trutmann *et al.,* 1993; Fininsa and Yuen, 2002). To the best of the authors' knowledge, there is scanty information on the implication of growing bean varietal mixtures and beans as an intercrop with maize on root rot inoculums and resultant disease.

The functional mechanism of intercropping in suppressing pest and disease infestation and development, respectively, and later spread is by maintaining predation and spatial increment among intraspecies (Carlson, 2008; Fininsa and Yuen, 2001). Legume diversification is associated with but not limited to reducing soil-borne pathogens, but it is also attributed to increased crop production and enhancing beneficial microbial community within the soil (Barbieri *et al.,* 2019; Yang *et al.,* 2020). Cong *et al.* (2015) inferred that increased productivity in mixed cropping systems is associated with legumes capacity to produce secondary plant compounds which trigger the nodulation process.

The suppression of soil-borne by free-living soil microorganisms in legume diversification as a cropping system is because the latter has a competitive edge in terms of niches and nutrients. The interaction of pathogens and free-living soil bacteria is said to be that of antagonism (Yang *et al.,* 2020).

To decipher the extent and importance of root rot pathogens, soil samples from farms of bean smallholder farmers in Busia County, Western Kenya were collected. It is worth noting that smallholder farmers in the study site grow maize, beans, cowpea, and groundnut without necessarily intercropping or observing a particular cropping system in managing pests. This study aimed to evaluate the effect of intercropping and legume diversification on the intensity of root rot of common beans in Busia County in Western Kenya.

2. Materials and Methods

2.1. Experimental treatments and design

The field experiments were conducted from April to July (long rain cropping season) 2015, in farmers' farms in Alupe, Bujumba, and Madola, sites that fall under Lower midland One (LM1) in Busia County, Western Kenya. Bean seeds of GLP2, KK8, and KATX56 varieties were obtained from farmers, KALRO, and the local market, respectively, and were planted in 10 x 10 m plots. The intercrop treatments were: i) GLP2 bean variety with maize, ii) intercrop of mixture bean varieties GLP2, KATX56, and KK8 with maize, iii) intercrop three bean varieties (GLP2, KATX56, and KK8) together with two other legumes, cowpea (variety K80) and groundnuts (variety Red Valencia), plus maize. The spatial arrangement within the intercrop plots consisted of alternate double rows of each legume variety planted between two rows of maize. The control plots consisted of the sole crop of each of the three varieties of bean (GLP2, KATX56, and KK8) planted in 5 x 5 m plots. The spacing of 75 × 30 cm and 30 × 15 cm were for maize and beans, respectively. The maize was top dressed with CAN fertilizer (30,30,30 NPK kg/ha) at V6 developmental stage (Berglund et al., 1999).

2.2. Isolation and identification of root rot pathogens from soils

Soil samples were collected before planting from each experimental plot. In each plot, soil was picked from four corners and at the center resulting in five soil sub-samples per plot. Soil from each plot was composited by mixing the sub samples in a paper bag until evenly mixed. Half a kilogram of each composite sample was considered for analysis. In the plant pathology laboratory, Upper Kabete Campus, University of Nairobi, 10 of the finely sieved soil extracted from each 0.5 kg composite sample was suspended in 100 ml sterile distilled water (SDW) and mixed on a mechanical shaker (Unimax 1010 DT, Heidolph, Germany) for 30 minutes. The fully mixed soil suspension was subjected to serial dilution.to prompting plating of aliquots 1 ml of 10^{-2} and 10^{-3} dilutions on potato dextrose agar (PDA) media amended with streptomycin and tetracycline both at a concentration of 50 mg/l (Mueller *et al.,* 2004).

The isolation plates were incubated at standard conditions, room temperature (23 ± 2 °C) for 7 days. Quantification of soil-borne fungus was by counting colonies of each fungus and presented as colony forming units/gram (CFU/g) of soil. To identify respective soil fungal isolates, pure cultures were obtained by sub-culturing on a PDA medium for all fungi. Synthetic nutrient agar (SNA) medium (Nirenberg, 1981) was used to completely characterize *Fusarium* spp. whose sub-culturing was on for *Fusarium* spp. PDA medium was used to stimulate color development while SNA was used to stimulate the development of microconidia and macroconidia. Fungal cultural and morphological attributes namely colony color, growth type, mycelial orientation, septation, and spore or conidia shape (Nelson *et al.,* 1983; Leslie and Summerell, 2006) were tenets of fungal identification.

2.3. Assessment of bean seedling plant stand

Plant stand parameter was considered at two-time points during the seedling stage of beans in the experimental plots. The first count was at two weeks after emergence, where the number of bean seedlings was determined from two inner double rows in each plot. This was repeated four weeks after emergence. Bean seedlings stand count was expressed as a percentage of the total number of plants of two inner double rows over predetermined planted seeds for the same rows per plot.

2.4. Assessment of intensity of root rots

To determine the intensity of root rots in the three study sites, three aspects were assessed: distribution, incidence, and severity at the second and fourth week after the emergence of beans. This study adopted Arabi and Jawhar (2013) disease distribution scale of 0 - 2, where 0 = no disease, 1 = spots, and 2 = whole field. Symptomatic bean plants in two inner double rows were identified counted and expressed as a percentage of the total number of bean plants for the same double rows per plot which represented disease incidence. Root rot severity was assessed by observing symptoms on stem bases and scoring on a scale of 0 – 3 (where 0 = no disease; 1 = mild infection; 2 = moderate infection; 3 = severe infection. Root rot intensity was the sum of scores of distribution, incidence, and severity.

2.5. Determination of root rot pathogens from symptomatic bean stem bases

From each plot 10 symptomatic bean plants were sampled. The stem bases were washed in running tap water to remove foreign matter before chopping each stem base into five 1cm-long pieces. The cut plant tissue pieces were subjected to aseptic conditions (1.3% sodium hypochlorite solution; rinsed thrice with SDW). The tissues were plated on PDA media and section 2.2 protocol for culturing and identification of fungal isolates was followed.

2.6. Statistical analysis

Data on population and frequency of soil-borne pathogens, bean seedling stand count, root rot incidence, and root rot intensity were subjected to analysis of variance (ANOVA), using GENSTAT software version 12, and differences among treatments compared using Fisher's protected Least Significance Difference (LSD) test at 5% probability level.

3. Results

3.1. Root rot pathogens isolated from the soil

Fungi that cause root rots found in Busia County farmer fields in respect to the three study sites were found to be from genera *Fusarium*, *Macrophomina*, *Pythium,* and *Rhizoctonia.* Two species of genus *Fusarium*, *Fusarium solani*, *F. oxysporum* were the most prevalent followed by *Pythium* spp. Generally, there was significant difference ($P \leq 0.05$) in the incidence of each root rot pathogen in soils in each study site. *Fusarium solani* and *F. oxysporum* were the most prevalent in Bujumba and least in Alupe and Madola. The number of *F. solani* in Alupe was statistically not different from Madola while *F. oxysporum* was (Figure 1). Alupe soils harboured more species of *Pythium* and *R. solani* statistically significant from Madola. The incidence of *Macrophomina* spp. in soil samples from the three sites was the lowest but Alupe led in the incidence (Figure 1). The isolation frequency of root rot pathogens in soils had a similar trend as that of their population across the three sites. The species of *Fusarium* were the most isolated in Bujumba soil samples while the other three genera were the most isolated in Alupe soil samples (Table 1).

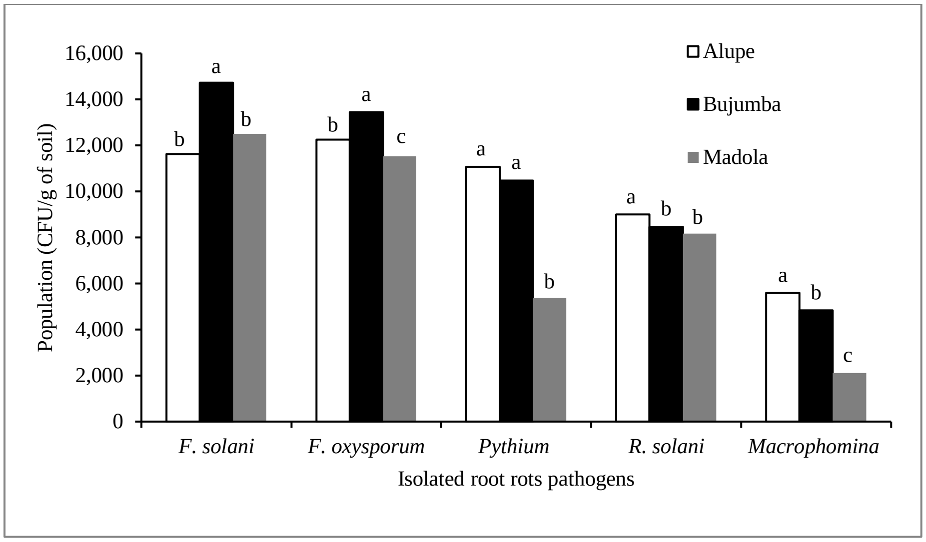

Figure 1. Population (CFU/g soil) of various root rot pathogens isolated from soils. Bars labels as letters per study site indicate significant differences following Fisher's protected LSD test at 0.05 probability level.

Table 1. Isolation frequency (%) of root rot pathogens recovered from soils

Site	*F. solani*	*F. oxysporum*	*Pythium* spp.	*R. solani*	*Macrophomina* spp.
Alupe	29.6^{b}	23.0^{a}	9.5^{a}	11.5^{a}	5.4^{a}
Bujumba	48.9^{a}	23.4^{a}	3.9^{b}	6.9^{ab}	0.2^{b}
Madola	32.7^{b}	26.6^{a}	7.4^{ab}	2.0^{b}	0.1^{b}
Mean	37.1	24.3	7.0	6.8	1.9
LSD ($P \leq 0.05$)	9.2	9.3	4.7	5.4	2.1
CV (%)	64.9	100.2	179.6	207.1	268.1

Same letter(s) as superscript after means in each column indicate no significant differences (P ≥ 0.05) in isolation rates within sampled soils; LSD: Least significant difference, CV: Coefficient of variation.

3.2. Root rot pathogens isolated from bean stem bases

The species of root rot pathogens isolated from symptomatic common bean stem bases showed that the disease in Busia County is as a result of multiple infections. The fungal pathogens were *F. solani, F. oxysporum, R. solani, Macrophomina* spp., and *Pythium* spp. in the order of decreasing incidence (Figure 2). There was significance difference ($P \leq 0.05$) in the frequency of isolation of root rot pathogens between symptomatic bean stem bases samples collected from intercrops and those from sole crops. Sole crops recorded a higher incidence (Mean = 31.6%) of root rot pathogens in compared to intercrops (Mean = 14.1%) in all the sites. The treatment with the highest diversity of legumes resulted in the least frequency (Mean = 9.6%) of the root rot pathogens on diseased bean stem bases in all three sites (Figure 2).

3.3. Bean seedling stand count

Generally, intercropping and legume diversification resulted in almost 56% and 35% higher bean seedling stand count at two weeks and four weeks after the emergence of beans, respectively across the three study sites (Figure 3). On the other hand, bean sole crops had seedling stands of up to 35% and 27% at two and four weeks after emergence, respectively across the three sites, However, there was no significant difference ($P \geq 0.05$) in seedling stand count following intercropping and legume diversification among the sites. There was a remarkable decrease in bean seedling stand count in the fourth week after emergence.

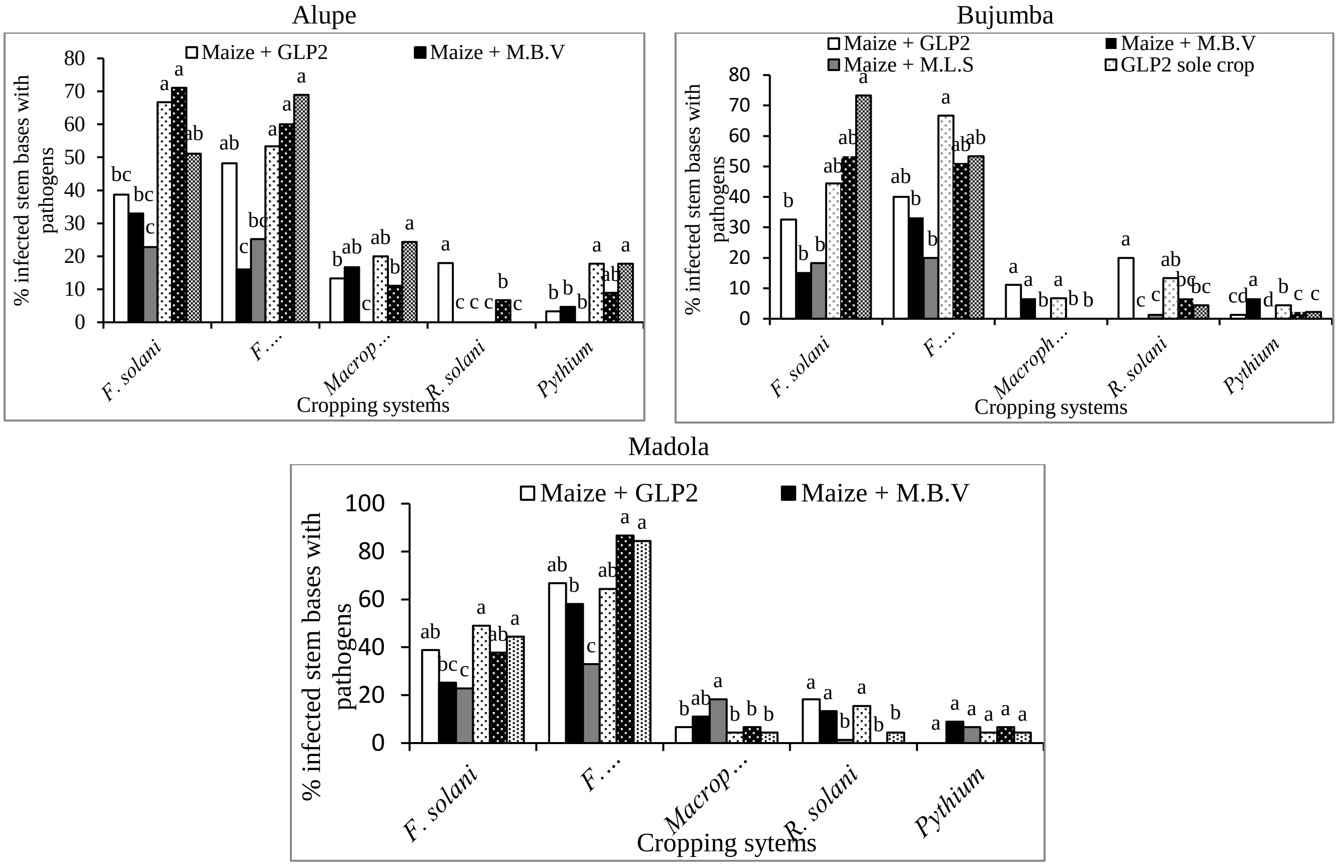

Figure 2. Percentage of stem bases infected with root rot pathogens from symptomatic bean plants in different treatments at four weeks after emergence in three sites in Busia County in western Kenya

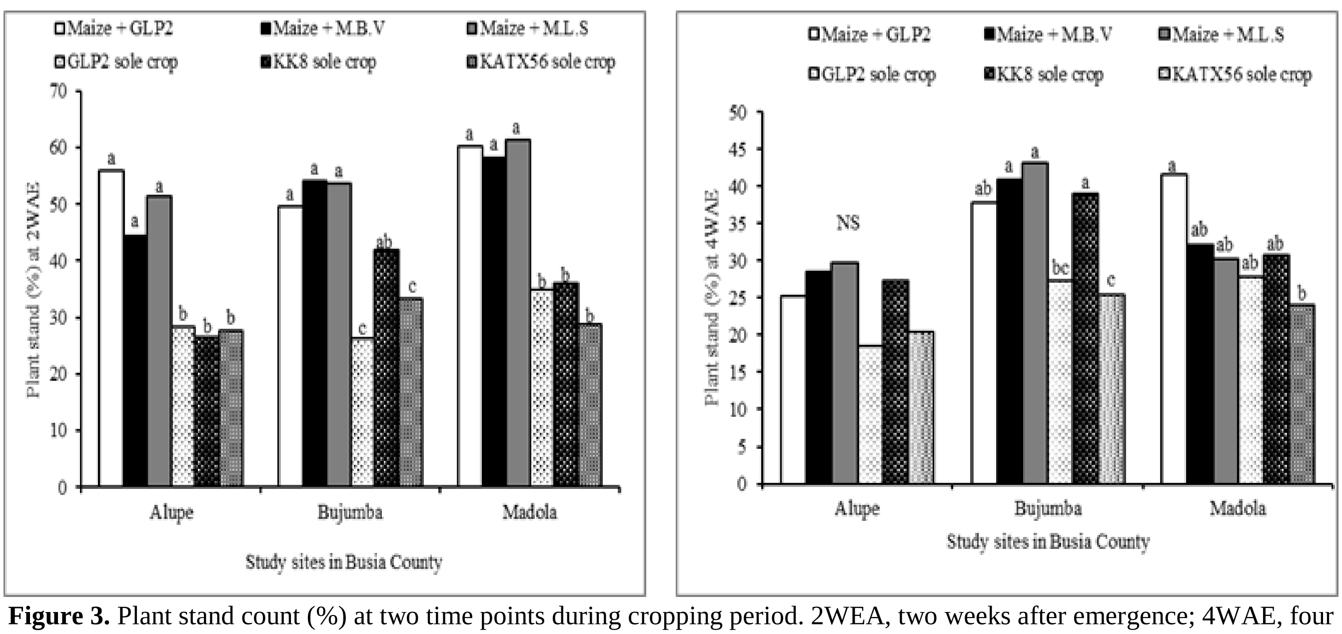

Figure 3. Plant stand count (%) at two time points during cropping period. 2WEA, two weeks after emergence; 4WAE, four weeks after emergence; M.B.V, mixed bean varieties (GLP2, KATX56 and KK8), M.L.S, mixed legume species (cow pea, GLP2, groundnuts, KATX56 and KK8); Bars labels as letters per study site indicate significant differences following Fisher's protected LSD test at 0.05 probability level; NS, not significant.

3.4. Intensity of root rot at the seedling stage of beans

The intensity of root rot varied across the three sites, Madola and Alupe highly and least affected, respectively at two weeks after the emergence of beans (Table 2). There was no significant difference ($P \geq 0.05$) in root rot intensities at each site among intercrop and legume diversification systems at two weeks after emergence. Notably, root rot intensity in monocrops, intercropping, and legume diversification systems were differently significant ($P \leq 0.05$) in Alupe at four weeks after emergence (Table 2). GLP2 and KATX56 sole crops in Alupe had 5.6- and 4.0-fold increases from the second to four weeks after emergence, respectively. On average, at four weeks after emergence, sole crops had a higher percentage of root rot intensity relative to the intercrops. Root rot disease intensity increased from two weeks (42.9%) to four weeks (57%) after emergence for the two cropping systems. There was no statistical difference in root rot disease intensity between intercrops. Root rot intensity increased drastically in the sole crop (44%) system between the second and fourth week after emergence in comparison to the intercrop system (18%) where disease progress was reduced.

Table 2. Intensity of root rot (%) for different cropping systems at two and four weeks after emergence of beans

Treatment	Alupe	Bujumba	Madola	Alupe	Bujumba	Madola
2 WAE				4 WAE		
Maize+ GLP2	40.2^{a}	38.5^{ab}	70.9^{a}	22.8^{b}	51.0^{a}	61.7^{a}
Maize + M.B.V	32.8^{ab}	49.3^{ab}	65.6^{ab}	64.1^{a}	49.1^{a}	68.7^{a}
Maize + M.L.S	30.9^{ab}	44.0^{ab}	63.2^{ab}	64.7^{a}	49.7^{a}	67.2^{a}
GLP2 sole crop	11.6^{b}	29.8^{b}	51.4^{b}	65.0^{a}	49.0^{a}	69.3^{a}
KK8 sole crop	28.6^{ab}	23.3^{b}	65.7^{ab}	55.0^{a}	53.3^{a}	69.7^{a}
KATX56 sole crop	11.9^{b}	59.1^{a}	54.4^{ab}	47.8^{ab}	45.1^{a}	72.6^{a}
Mean	26.0	40.8	61.9	53.2	49.6	68.2
LSD ($P \leq 0.05$)	21.9	26.1	19.4	30.9	37.1	36

Note: Tabulated values represent mean percentage intensity of root rot and they are followed by uppercase letters depicting significantly different at 0.05 probability levels within each column.

Abbreviations: M.B.V, mixed bean varieties (GLP2, KATX56 and KK8), M.L.S, mixed legume species (cow pea, GLP2, groundnuts, KATX56 and KK8).

4. Discussion

Root rot pathogens stand out as be an important common bean production constraint that smallholder farmers face mostly as the unseen biotic enemy. In this current study, five root rot pathogens were identified from soils and symptomatic plants in high potential bean growing regions. The fungal isolates from the soil were *F. solani, F. oxysporum, Macrophomina* spp., *Pythium* spp., and *Rhizoctonia* spp. affirming that root tot is caused by a complex of pathogens in the study area. The complexity of root rots in legumes especially in common beans has also been registered in previous studies within and beyond East African borders (Mwang'ombe *et al.*, 2008; Okoth and Siameto, 2010; Nzungize *et al.*, 2012; Naseri and Hemmati, 2017). In these cited previous and current studies it is clear that the genera of *Fusarium*, *Macrophomina*, *Pythium*, and *Rhizoctonia* are ubiquitous in common bean cropping systems. It was also found that *Fusarium solani* was the predominant pathogen threatening common bean production, an aspect reported by Abawi and Pastor-Corrales (1990), Naseri (2008), Naseri and Mousavi, (2015). Ostensibly, the close association of these soil-borne pathogens with common beans could be a result of exudates from roots that trigger pathogen multiplication and aid in the infection process (Steinkellner *et al.,* 2007). The prevalence of root pathogens like the ones identified in this study in soil is attributed to soil health. Cropping systems contribute immensely to the status of soil health and possibly Busia County soils have been compromised over the years. Gichangi *et al.* (2012) found that root rot pathogens build up rests squarely on poor farming practices. It has been documented that smallholder farmers use their own saved seeds or locally sourced, season-to-season bean production, and legume-legume rotations (Opole *et al.*, 2003; Gichangi *et al.*, 2012). This study shows a direct relationship of soil-borne inocula abundance and with intensity of root rot disease such that *F. solani, F. oxysporum, R. solani, Macrophomina* spp., and *Pythium* spp. in the order of decreasing population and incidence. Such findings are critical in the search for epidemiological information on managing root roots. It can be deduced that inasmuch as Busia County soils continue to harbor inocula of species of *Fusarium, Rhizoctonia, Macrophomina* and *Pythium* bean production under current systems will register declining yields over the years. The infection could also be attributed to favourable environmental conditions during the cropping season (rainy and warm conditions) during which a study was carried out which is i congruent with studies by Jaeztold *et al.* (2005) and Naseri and Marefat (2011).

Since prior studies depict cropping systems cum farmer practices as the mainstay of bean root rot in bean-growing areas such as Busia County in Kenya, this current study sought to investigate the impact of intercropping and legume diversification primarily on root rot complexity at the seedling stage of beans. It was found that symptomatic bean seedlings under intercropping and legume diversification systems recorded a low frequency of root rot pathogens relative to the bean monocropping system. Maize intercropped with mixed varieties (GLP2, KK8, and KATX56) of beans, cowpea, and groundnuts had fewer root rot pathogens. This observation is concurrent with a recent study finding by de-Medeiros et al. (2019) that when two legumes, pigeon pea, and beans were intercropped with cassava, root rot was suppressed by 50% relative to cassava planted as a sole crop. Growing crops in an intercrop system jeopardizes plant disease progression and more often makes the target plant evade the delirious effects of diseases such as those of root rot (Skelsey *et al.,* 2005; Dane and Laugale, 2014). Crop diversification is not limited to soil-borne pathogen reduction but among other accrued benefits it also enhances the proliferation of beneficial microorganisms (Yang *et al.*, 2020). Similar mechanisms have been lauded when intercropping is practiced (Altieri, 1994; Dwivedi *et al.*, 2015) which culminates into improved soil health and quality.

It can be further argued that maize-legumes diversification increases compatibility with beneficial soil microbes that keep the soil pathogens at bay (Yang *et al.*, 2020). Intercrops had resulted in a higher stand count than the sole crops. These results suggest that intercropping mixed bean varieties and legume diversification increase common bean vigor to fend off soil pathogens at an early stage of growth. Bean seedling stand count is reduced between the two-point times irrespective of the cropping system. This study suggested that the observed decimated stand count at the seedling stage was under the deleterious effects of the root rot disease complex coupled with the soil health status of Busia County. These observations concur with the findings that bean mortality is pronounced at the seedling stage following infection of root rot pathogens and acidic soils (Medvecky *et al.*, 2007). Farooq *et al.* (2011) and Naseri and Marefat (2011) also reported seedling loss due to soil-borne pathogens.

Intercrop cropping system resulted in reduced stem bases infected with root rot pathogens relative to sole crops. Further, there was a remarkable root rot disease intensity disparity between the sole crop system and intercrop system over time. The two cases showed that common beans under the intercrop system were more resilient against root rot infection than sole crop beans during the growth period ranging from two weeks to the fourth week after emergence.

5. Conclusion

The two cropping systems, intercropping and crop diversification reduced the population of all the identified soil-borne pathogens and root rot disease progress. This resulted in reduced damage to bean seedlings and improved crop stand which would translate to higher yields. The use of intercropping and legume diversification is promising since both approaches are effective, simple, affordable, and sustainable in the management of bean root rot disease complex. The approach is compliant with the existing cropping systems in the study region and it will also contribute to the maintenance of the agro-ecosystem.

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Author's contribution

J.W.M. was the team in charge of leader overall design of the study, development of study methodology, and refining of the final manuscript; M.J.W. oversaw the developing of the field and laboratory sampling and data processing; B.W.M. performed experiments, wrote the paper, and analyzed. A.M.F edited and refined the manuscript. All authors approved the final version of the manuscript.

Competing interest

All the authors have no competing interest.

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