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Effectsof Pre-Emergence Herbicides, *Rhizobium* Strains and their Integration on Black Root Rot and Faba Bean Productivity

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የባቄላ የስር አበስብስ በሽታ በባቄላ ምርታማነት ላይ ከፍተኛ ጉዳት የሚያስከትል በሽታ ነው። የባቄላ የስር አበስብስ በሽታን ለመቆጣጠር የሚያስችል ምርምር በአምቦ የዕፅዋት ጥበቃ ምርምር ማዕከል እና በሆለታ ግብርና ምርምር ማዕከል ስታስቲክስ ዲዛይንን በመጠቀም ተሰርቷል። የምርምሩ ውጤት እንደሚያሳየው በአምቦ እፅዋት ጥበቃ ምርምር ማዕከል እና በሆለታ ግብርና ምርምር ማዕከል ከፍተኛ ምርት (በሄክታር2.26 እና3.64 ተን) በራይዞብየም ባክቴርያዝርያ 1035 (በሄክታር 500 ግራም) ከታከመው ማሳ ላይ ማግኘት ተችሏል። ከዚህም በተጨማሪ በራይዞብየም ባክቴርያ ዝርያ 1035 ከታከመው ማሳ ላይ ዝቅተኛ የባቄላ የስር አበስብስ በሽታ ክስተት (34.94 እና 13.68) እና የበሽታው የማጥቃት ደረጃ (25.38 እና 14.39) ተመዝግባል። እንዲሁም ከፍተኛ የበሽታው ክስተት (61.77 እና 29.03) እና የበሽታው የማጥቃት ደረጃ (66.23 እና 32.69) ካልታከመው ማሳ ላይ በአምቦ እፅዋት ጥበቃ ምርምር ማዕከል እና በሆለታ ግብርና ምርምር ማዕከል ተመዝግባል። ምጣኔ ሀብታዊ ጥቅሙን ስናይ ከፍተኛ ጥቅም በራይዞብየም ባክቴርያ ዝርያ 1035 ከታከሙት ማሳዎች ላይ በሁለቱም ማዕከላት ተመዝግባል። ስለዚህ ራይዞብየም ባክቴርያን በመጠቀም ምርታማነትን ማሻሻል እንዲሁም ሰብሉን ከባቄላ የስር አበስባሽ በሽታ መጠበቅ እንደሚቻል በተደረገው ጥናት ተረጋግጧል።

Abstract

*Black root rot disease **(Fusarium solani)** is considered as a major problem in faba bean crops causing great losses in seed yield and low productivity. A randomized complete block design with nine treatments and three replications were employed at Ambo Plant Protection Research Center (APPRC) and Holetta Agricultural Research Center fields. The analysis of variance revealed that highly significant ($P \leq 0.001$) variation among the herbicides and Rhizobium strains treatments for grain yield, thousand seeds weight and Black root rot disease incidence and severity on faba bean. Thus, higher grain yields of 2.26 and 3.64t ha^{-1}were recorded from plots treated with Rhizobium strain FB1035. However, plots treated with herbicides plus Rhizobium strains gave lower yield than plots treated with Rhizobium strain alone. Plots treated with Rhizobium strain FB1035 showed less disease incidence (34.943 and 13.6767%) and severity (25.377 and 14.391%) respectively. However,the highest disease incidence (61.77 and 29.026 %) and severity (66.23 and 32.687 %) were recorded from the control plot. The disease severity was found negatively correlated with yield and yield components at both locations. Considering economic benefits, Partial budget analysis revealed that the highest total gross yield benefit (birr 35030 ha^{-1} and birr 56420ha^{-1}) were obtained from plots treated with Rhizobium strain FB1035, proving that the application of Rhizobium strains alone were profitable.Thus, it is recommended to use these Rhizobium strains for the best protection against faba bean black root rot and improve the productivity of faba bean.*

Introduction

Black root rot disease caused by *Fusarium solani* is one of the main constraints contributing to the low productivity of faba bean resulting in yield losses of up to 78 % in Ethiopia (Bogale *et al*., 2009). In the highlands of Ethiopia, faba bean black root rot is among the most important disease in black clay soils, where water- logging is a big problem (Eshetu *et al*., 2013).

Many species of *Rhizobium* are reported to inhibit significantly the growth of pathogenic fungi (Estevez de Jensen *et al*., 2002). *Rhizobia* are reported as effective bio-control agent for the inhibition of soil-borne plant pathogens (Mazen *et al*. 2008).

In addition, in recent years farmers have begun to apply several herbicides to soil, not only for weed control but also because of an unconfirmed belief in a secondary controlling effect on root rot (Johnston, *et al.,* 1980). However, the presence of herbicide residues in soil could have direct impacts on soil microorganisms is matter of great concern. The effects of herbicides on soil fungi varied amongst herbicides depending on their application rates and the type of herbicide used (Sebiomo *et al*., 2011). When herbicides applied frequently, they are accumulated in to the soil and at elevated levels impair the metabolic activities resulting in reduced growth of *rhizobia*, legumes or both (Khan *et al.*, 2004).

In view of the above, the present study was carried out with the objectives to determine the effect of pre-emergence herbicides and *Rhizobium* strains on black root rot and faba bean productivity and to assess the cost-benefit of herbicides and *Rhizobium* strains for faba bean production.

Materials and Methods

Planting and experimental materials

Experimental materials used for this field experiments were: Two *Rhizobium* strains, *Rhizobium* strain 1017 and *Rhizobium* strain 1035 at the rate of 500 gram per hectare, (carrier based powder inoculants) identified and released from HARC soil microbiology department were inoculated on the seed by using seed dressing method; Two pre- emergence herbicides, Dual Gold 960 EC at rate of 1.5 l/ha and Codal Gold 412.5 DC at the rate of 2.5 l/ha were sprayed immediately after planting. The faba bean Wolki cultivar released for Vertisols soil area from HARC was used as testing cultivar. The causal pathogen of black root rot on faba bean *Fusarium solani* was inoculated in the sick plot soil at Ambo. At Holetta, field experiment was conducted without inoculation of black root rot pathogen however; the presence of natural infestation of the pathogen was checked. These herbicides and *Rhizobium* strains alone and in combination (i.e. Dual Gold 960 EC alone, Codal Gold 412.5 DC alone, *Rhizobium* strain 1017 alone, *Rhizobium* strain 1035 alone, Dual Gold 960 EC + *Rhizobium* strain 1017, Dual Gold 960 EC + *Rhizobium* strain 1035, Codal gold 412.5 DC+ *Rhizobium* strain 1017,Codal Gold 412.5 DC + *Rhizobium* strain 1035 and *Fusarium solani* alone at APPRC and Un inoculated at HARC) were used as a treatments.

Experimental design and management

The treatment combinations were tested using faba bean cultivar Wolki in a randomized complete block design with three replications at both locations. The net plot size was 3.2 m x 4 m. Spacing of 0.4 m between rows and 0.1m between plants with 8 rows per plot. Diammonium phosphate (DAP) fertilizer was applied at 100kg ha $^{-1}$ with 18 kgha^{-1} Urea at planting. The trials were kept weed-free by hand weeding.

Disease assessment

Disease severity

Randomly selected 10 Plants from 2nd and 7th rows were removed from the soil and roots were washed with tap water and disease severity was estimated visually by assessing necrotic lesions on the roots and hypocotyls using a rating scale of 0-4 described by Ondrej *et al*., (2008) where 0:hypocotyls and roots white and firm, no root pruning, 1:slightly brown or discolored hypocotyls and roots, slight root pruning, 2:moderately discolored hypocotyls and roots, extensive root pruning, 3: darkly discolored hypocotyls and roots, hypocotyls completely collapse or, severe root pruning and 4:Dead or dying.

$$\text{Disease severity} = \frac{\sum(\text{ab})}{\text{AK}} * 100$$ ………… Bekriwala*et al*., (2016)

a= number of diseased plants having the same degree of infection, b= degree of infection,
A= total number of examined plants, K= highest degree of infection

Disease incidence

It was measured as counting the dead plants out of the total plants.

$$\text{Disease incidence}(\%) = \frac{\text{Number of infected plant}}{\text{Total number of plant}} \text{ X } 100$$

Phenological and growth parameters

Days to 50% emergence, Day to 50% flowering, Days to 90% maturity, Plant height (cm), Stand count at seedling emergency and Stand count at harvest were recorded from the middle four rows for each plot.

Yield and yield components data

Number of pods per plant, Number of seeds per pod, 1000 seed weight (g), Total grain yield (t/ha), Viable nodule count and Non-viable nodule count were measured from five randomly taken plants from the middle four rows for each experimental unit and the means were recorded.

Relative yield loss

Percent relative grain yield loss was calculated as follows:

$$RYL\ (\%) = \frac{(\text{Yp} - \text{Yt})}{\text{Yp}} \text{ x } 100$$

Where, RYL = relative yield loss in percent, Yp = yield from the maximum protected plots and Yt = yield from other plots.

Correlation between yield and disease parameters and regression analysis

Correlations among the disease parameters and with the all yield component were tested at 5% probability level. The reliable yield loss was estimated based on the severity level by employing regression equations.

Cost - benefit analysis

The price of Faba bean seeds (birr/kg) was assessed from the local market and the total price of the yield of faba bean obtained from each treatment was computed on hectare basis. Input cost of herbicides and labor was converted into plot based. Since there was significant difference between mean yields of treatments, the obtained data was analyzed using the partial budget analysis method (CIMMYT, 1998). Then the treatment with the highest rate of return was recommended for use by the farmers. Marginal rate return was calculated using the following formula:

$$\mathrm{MRR} = \frac{\mathrm{DNI}}{\mathrm{DIC}}$$

Where, MRR is marginal rate of returns, DNI is difference in net income compared with control, DIC is difference in input cost compared with control

Data analysis

All the data collected from field experiments were used and the analysis of variance (ANOVA) was performed using the PROC GLM in SAS (SAS Institute, 2012). The data on disease incidence was subject to square root transformation before analysis. The correlation coefficients of selected yield components were calculated using the SAS procedure PROC CORR (SAS Institute, 2012).

Results and Discussion

Disease assessment in the field

Days to disease onset

The analysis of variance (ANOVA) exhibited that there was highly significant ($P< 0.001$) difference among treatments in days to disease onset both at APPRC and at HARC. The longest (33.00 and 37.667 days) period to disease onset was recorded on the plot treated with the *Rhizobium* strain FB1035 and *Rhizobium* strain FB1017 at APPRC and plot treated with the *Rhizobium* strain FB1035 at HARC, while the shortest, 26.667 and 34.667 days period to disease onset were observed on the control plots at Ambo and Holetta, respectively. Similarly, the longest period to disease onset (30.667 and 37.333 days) was recorded on the plot treated with Codal gold + *Rhizobium* strain FB1035 and plot treated with *Rhizobium* strain FB1017 at Ambo and Holetta, respectively (Table 1).

Disease incidence

There was highly significant ($P \leq 0.001$) difference among treatments on the black root rot incidence at APPRC and HARC. The highest disease incidences (61.77 and 29.026 %) were recorded on the control plots at APPRC and HARC, respectively and the lower disease incidences (34.943 and 13.6767 %) were recorded from plots treated with *Rhizobium* strain

FB1035 at APPRC and HARC, respectively (Table 1). Many species of *Rhizobia* were found to promote plant growth and to inhibit the growth of various soil-borne pathogens including *Macrophomina phaseolina, Rhizoctonia solani,* and *Fusarium solani* in both leguminous and non-leguminous plants (El-Batanony *et al*. 2007). In this line Buonassisi *et al*. (1986) was observed a significant reduction in root rot of bean caused by *Fusarium solani f.sp. Phaseoli* by inoculating the seeds with strains of *Rhizobium leguminosarum pv. Phaseoli*.

Thus, treatment of faba bean with effective strains of *Rhizobium leguminosarum* alone or in combination with other beneficial microorganisms may be preferred over the fungicides, because of their multiple potentials to fix nitrogen, control disease, improve of soil fertility, and increase crop productivity besides reducing the negative environmental impact associated with chemical use (Huang and Erickson, 2007).

Disease severity

The analysis of variance (ANOVA) indicated that there was highly significant (P< 0.001) difference among treatments in disease severity at both locations, APPRC and HARC. The highest disease severities of 66.23and 32.69% were recorded on the control plots, while the lowest 25.377 and 14.391%were recorded from the plots treated by the *Rhizobium* strain FB1035 at APPRC and HARC, respectively (Table 1). Several previous studies reported that *Rhizobia* increase seed germination significantly and improve plant growth and yields through a reduction of soil-borne pathogens (Mazen *et al*. 2008). In this line, Rakib Al- ani *et al*, (2012) stated that, root rot severity caused by *Fusarium solani* was reduced by the addition of *Rhizobia* to the contaminated soil with the pathogen. Correspondingly, Samavat *et al*. (2011) also found that Common bean seeds treated with *Rhizobia* and *Pseudomonas fluorescens* isolates reduced root rot and damping-off severity.

Table 1. Black root rot disease onset, incidence, and severity

DDO			DI (%)		DS (%)	
Treatment	APPRC	HARC	APPRC	HARC	APPRC	HARC
FB1017	33.00a	37.33ab	37.55fg	15.31e	32.01g	16.31f
FB1035	33.00a	37.66a	34.94g	13.68e	25.38h	14.39f
CG+ FB1035	30.67b	35.00ef	39.34f	18.1d	46.05e	22.64cd
DG+ FB1035	30.00bc	35.67de	42.24e	18.56cd	38.96f	17.14ef
DG	30.00bc	35.67de	56.95b	20.4c	59.87b	31.42a
CG	29.67c	36.67bc	54.05c	19.97cd	55.39c	27.09b
DG+ FB1017	28.33d	36.00cd	51.99c	18.72cd	51.98d	19.77de
CG+ FB1017	28.33d	35.00ef	47.29d	22.49b	47.81e	24.01c
FS (control)	26.67e	34.67f	61.77a	29.03a	66.23a	32.69a
Mean	29.96	35.96	47.35	19.58	47.08	22.83
LSD	0.726**	0.726**	2.78**	2.04**	2.19**	2.96**
CV (%)	1.399	1.17	3.39	6.02	2.69	7.49

*Where: DDO: Days to disease onset, DI: Disease incidence, DS: Disease severity, FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold + Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobium strain FB1017, FB1017: Rhizobium strain FB1017, **- significant at p≤0.001.*

Phenological data

The analysis of variance (ANOVA) revealed that highly significant differences (P< 0.0001) among the treatments on days to 50% emergence and days to 90% maturity at APPRC. Similarly, the ANOVA indicated that highly significant differences (P<0.0001) on days to 90% maturity and significant difference (P<0.005) on days to 50% emergence at HARC (Table 2). The results showed that days to emergence were varying over locations. This difference can be explained by due to variation in sowing depth, land preparation and environment influences, which affected the germination and emergence of the seedlings (Pal, 2004). The plots treated with *Rhizobium* strain FB 1035 and *Rhizobium* strainFB1017 were found to decrease days to emergence while, the plot treated with the treatment Dual gold + *Rhizobium* strain FB1017 and Dual gold + *Rhizobium* strain FB1035 took longer days to seedling emergence at APPRC and HARC, respectively.

Statistically, significant (P<0.05) difference was observed for days to 90% maturity among the treatments at both locations APPRC and HARC (Table 2). The longest days to 90% maturity i.e. 136 and 145 days were recorded from plots treated with Codal Gold alone, Codal Gold with *Rhizobium* strain FB 1017 and control at APPRC, and Dual Gold alone and Codal Gold with *Rhizobium* strain FB1017at HARC, respectively. However, the plot treated with *Rhizobium* strain FB1017 and Dual gold + *Rhizobium* strain FB1017 reached to 90% maturity relatively in a shorter period of 134 and 143 days at APPRC and HARC, respectively. The reduction in days to maturity at APPRC might be due to the variation in the environmental conditions and disease pressure in the growing season. Thus, the variability in attaining maturity for the treatments might be attributed to environmental condition and the effect of the disease (Wulita, 2015).

Table 2. Number of days to 50% emergence, days to 50% flowering and days to 90% maturity

Treatment	Days to 50% emergence		Days to 50% flowering		Days to 90% maturity	
	APPRC	HARC	APPRC	HARC	APPRC	HARC
FB1017	6.00bc	8.33c	49.67ab	56ab	134.33d	143.67bc
FB1035	5.66c	9abc	49.00b	55.67b	134.67cd	143.67bc
CG+ FB1035	5.67c	8.67bc	49.00b	56.33ab	134.67cd	144.67ab
DG+ FB1035	6.33bc	10a	49.33ab	56ab	135.33abc	144abc
DG	6.67a	10a	49.00b	57a	135.00bcd	145a
CG	6.00bc	8.35c	49.33ab	57a	136.00a	144abc
DG+ FB1017	8.67a	9.33abc	50.00a	56.33ab	135.67ab	143.33c
CG+ FB1017	6.00bc	8.67bc	50.00a2	56.33ab	136.00a	145a
FS (control)	6.33bc	9.67ab	49.33ab	56.67ab	136.00a	144.33abc
Mean	6.37	9.11	49.407	56.37	135.3	144.19
LSD	0.86**	1.21*	0.91 ns	1.13 ns	0.97**	1.09**
CV (%)	7.78	7.65	1.06	1.16	0.41	0.44

*Where: FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold +Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobiumstrain FB1017, FB1017: Rhizobium strain FB1017, ns: non-significant, *- significant at 5%, **Significant at p≤0.001.*

Growth parameters

The analysis of variance (ANOVA) revealed significant plant height differences among treatments ($P \leq 0.001$) at APPRC and ($P \leq 0.05$) at HARC (Table 3). At Ambo the tallest plant height measurement was recorded from the plots treated with *Rhizobium* strain FB1035, CG + *Rhizobium* strain FB1035 and *Rhizobium* strain FB1017. This result was similar to the report of Sameh *et al.*, (2017) who found significantly higher plant height (127–134 cm) from plants inoculated with *Rhizobium* strains NGB-FR62, 126 and 128, than un-inoculated control plants. Sharma *et al*. (2000) also reported that the significant effect of seed inoculation on plant height and biomass dry mater compared to control. The shortest (60.93 cm) plant height was recorded from the control plot. This might be due to the presence of severe *Fusarium* root rot on the control plot. At Holetta, the tallest (118 cm) plant height was recorded from the treatment Codal gold + *Rhizobium* strains FB1035whereas, the shortest plant height 103.33 cm was recorded from the control plot (Table 3). The mean plant height recorded at HARC (111.11cm) was greater than the mean plant height recorded at APPRC (76.822 cm). This might be due to the presence of high inoculum of *Fusarium solani* at APPRC field.

The data of stand count at seedling growth and at harvest stage from both locations (Table 3) showed highly significant ($P<0.001$) difference among treatments. The highest number of stand count at seedlingi.e. 158.7 and at harvest 117.3 were recorded from the plots treated with the treatments *Rhizobium* strains FB1035 at APPRC and 154.7 and 119.7 were recorded from plots treated with *Rhizobium* strains FB1017 and *Rhizobium* strains FB1035 at HARC for stand count at seedling stage and at harvest stage respectively. The lowest number of seedlings i.e. 147.3 and 144.0 were recorded from the control plot, plots treated with the herbicide Dual gold at APPRC and HARC, respectively, and smallest stand number at harvest (86.00 and 102.00) was recorded from the control treatment at the two locations APPRC and HARC, respectively.

Table 3. Effects of treatments on plant height stand count at seedling and stand count at harvest

Treatment	PH (cm)		SCS		SCH	
	APPRC	HARC	APPRC	HARC	APPRC	HARC
FB1017	86.467a	104.33d	156ab	154.67a	107bc	116b
FB1035	86.533a	113.33ab	158.67a	150bc	117.33a	119.67a
CG+ FB 1035	86.53a	118a	153.33bc	148.33cd	113ab	114bc
DG+ FB 1035	74.4b	114.67ab	156.33ab	147.33cde	112.33ab	112.67c
DG	66.47c	111bc	151.33c	144e	106bc	112.67c
CG	76.73b	115.33ab	156.33ab	152.33ab	103.33cd	115bc
DG+ FB 1017	68.53c	107cd	155.67ab	146.33cde	96.33d	105.33e
CG+ FB 1017	73.73b	113ab	155.33bc	148.33cd	98.67cd	108.67d
FS (control)	60.93c	103.33d	147.33d	144.67de	86e	102f
Mean	75.64	111.11	154.48	148.44	104.44	111.78
LSD	4.74**	5.06*	3.6**	3.82**	8.45**	3.13**
CV (%)	3.62	2.63	1.35	1.49	4.67	1.62

PH: plant height, SCS: stand count at seedling, SCH: stand count at harvest, FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold + Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobium strain FB1017, FB1017: Rhizobium strain FB1017,- significant at 5%, **- Significant at p≤ 0.001.*

Yield and yield components

Rhizobium strains application significantly affect the number of pods per plant compared to non-inoculated treatments at the two locations APPRC and HARC. Significant ($P \leq 0.05$) variations were observed among the treatments on number of pods per plant and on number of seeds per pod at both locations (Table 4). In this study, the highest number of pods per plant was recorded from plot treated with *Rhizobium* strain FB1035 at APPRC and from plot treated with *Rhizobium* strain FB1035 and Dual gold with *Rhizobium* strain FB1035 at HARC. The highest number of seeds per pod was recorded from plot treated with *Rhizobium* strain FB1035 at APPRC and from plots treated with both *Rhizobium* strains alone, plot treated with Codal gold and Dual gold with *Rhizobium* strain FB1017 at HARC. This is in agreement with the study by Rodriguez-Navaro *et al*. (2000) observed significantly increase in pod numbers per plant among different bean cultivars inoculated by *Rhizobium* strain. Argaw and Tsigie (2015) also reported that the inoculation of *Rhizobium* increased the seed number of common bean. The lowest number of pods per plant and number of seeds per pod (4.7 and 1.83) at APPRC and (5.0 and 2) at HARC were obtained from the control plot, respectively (Table 4).

Analysis of variance also showed highly significant ($P<0.001$) statistical difference among the treatments at both study fields, on viable nodule count and non-viable nodule count. The highest number of viable nodules (449 and 467.7) was obtained from plots treated with *Rhizobium* strain FB1035 and the lowest number of viable nodules (242.67 and 270.67) was observed from the control plots both at APPRC and HARC, respectively (Table 4). These finding is in agreement with Habtemichial *et al*. (2007) who found that *Rhizobium* inoculation increased the nodule number and fresh weight of faba bean in north Ethiopia by 53% and 95% over un-inoculated control plots, respectively. The current result also in line with the study conducted on lentil by Beshur *et al*., (2015) observed that *Rhizobial* inoculation increased nodule number and dry weight. Similarly, Mehrpouyan (2011) reported significant increase in nodule number and dry weight in common bean cultivars when inoculated with *Rhizobium leguminosarum* strain Rb117.

The lowest (286 and 310) viable nodule count next to the control plot was recorded on plots treated with herbicide Codal gold at both APPRC and HARC, respectively. This is in line with the finding of Khan *et al*., (2006) who stated that the common use of herbicides may negatively affect nodulation either directly by affecting *Rhizobium*, or indirectly by reducing photosynthate allocation to the nodules for N fixation, or by restricting root growth and hence the number of root sites available for infection. Also other studies showed that pendimethalin when applied under field conditions significantly decreased nodulation on soybean (Vaziritabar *et al*.2014).

The largest number of non-viable nodule (40 and 39.7) was recorded from the plot treated with *Rhizobium* strain FB1017. Sheng (1993) reported that besides fixation of atmospheric nitrogen, the nodulation effect of rhizobial isolates is due to the production of plant growth regulators such as Auxins and cytokinins like substance.The smallest number of non viable nodule (20.7 and 25.7) was recorded from the plots treated with Codal gold + *Rhizobium* strain FB1017 at the two locations, APPRC and HARC, respectively.

Table 4.Yield and yield components of faba bean on different treatmentsduring the main cropping season, 2016

Treatment	NPP		NSP		VNC		NNC	
	APPRC	HARC	APPRC	HARC	APPRC	HARC	APPRC	HARC
FB1017	10.67b	7.67ab	2.67ab	3a	445.67a	457.33ab	40a	39.67a
FB1035	14.67a	8.33a	3a	3a	449a	467.67a	26d	31.33ab
CG + FB1035	9.33b	7abc	2.33abc	2.3b	443.33b	447.67b	31c	27.33b
DG+ FB 1035	7.67c	8.33a	2.33abc	2.67a	405c	417.33c	30.33c	30.67ab
DG	10.33b	6.33bcd	2.33abc	2.67a	304.33e	327.33e	22.67ef	32.67ab
CG	9.33b	7abc	1.92c	3a	286f	310f	22.67ef	27.33b
DG+ FB 1017	6.67cd	6cd	2bc	3a	360.67d	368d	33.67b	29b
CG+ FB 1017	5.67de	7.33abc	2bc	2.67a	310e	320ef	20.67f	25.67b
FS (control)	4.67e	5d	1.83c	2b	242.67g	270.67g	24.33de	26.67b
Mean	8.78	7	2.27	2.67	359.63	376.22	27.93	30.04
LSD	1.567*	1.61*	0.69*	0.58*	7.36**	16.36**	2.40**	9.99**
CV (%)	10.31	13.26	17.79	12.5	1.18	2.51	4.97	19.22

*Where: NPP: number of pods per plant, NSP: number of seeds per pod, VNC: viable nodule count, NNC: non-viable nodule count, FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold + Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobium strain FB1017, FB1017: Rhizobium strain FB1017, *- significant at 5%, **- Significant at p≤ 0.001.*

Thousand seed weight and total grain yield

Analysis of variance results revealed the presence of highly significant (P≤ 0.001) differences at APPRC and significant (P≤ 0.05) variation at HARC among the treatments for thousand seed weights and highly significant (P ≤ 0.001) variation among the treatments for grain yield at both locations (Table 5). At APPRC and HARC, the highest thousand seed weight of 541.23 and 545.1 g were obtained from the plots treated with *Rhizobium* strain FB1035 and the lowest thousand seed weight of 400.63 and 477.27 g were recorded from the control plots, respectively. Elsheikh and Elzidany (1997) reported that *Rhizobium* inoculation significantly increased hundred seed weight of faba bean. Similarly, Kazemi *et al*. (2005) reported that seed inoculation by *Rhizobial* bacteria significantly increased the number of pods and seed per plant, thousand grain weights and finally the yield of soybean.

The highest (2.26 and 3.64 t/ha) grain yield were also obtained from the plots treated with *Rhizobium* strain FB1035. In this line, Abbasi *et al*. (2010) reported that soybean seed yield quadratically increased by *Rhizobium* inoculation and phosphorous application. Elsheikh and Elzidany (1997) also found that *Rhizobium* inoculation and 40 kg N ha^{-1} application of inorganic N gave a comparable amount of grain yield increase. However, the lowest grain yields of 1.63and 2.99 t/ha was observed from the control plots at APPRC and HARC, respectively. The production of faba bean is constrained by several yield-limiting factors, of which diseases are the main factors (Eshetu *et al*., 2013). The production is threatened by black root rot caused by *Fusarium solani* (Bogale *et al*., 2009).

Table 5.Yield and thousand seed weights of faba bean tested at APPRC and HARC during the main cropping season, 2016

Treatment	TSW (g)		TGY (t ha^{-1})	
	APPRC	HARC	APPRC	HARC
FB1017	514.17ab	529.33ab	2.25b	3.59b
FB1035	541.23a	545.1a	2.26a	3.64a
CG+ FB1035	491.53bc	506.17bc	2.01e	3.3e
DG+ FB1035	500.3bc	514.57ab	1.95f	3.28f
DG	479.47c	497.63bc	2.11d	3.43d
CG	486.3bc	503.73bc	2.16c	3.46c
DG+ FB1017	437.23d	479.17c	1.85g	3.22g
CG+ FB1017	424.43de	498.37bc	1.78h	3.18h
FS (control)	400.63e	477.27c	1.63i	2.99i
Mean	475.03	505.70	1.62	2.72
LSD	2.83**	3.41*	0.23**	0.45**
CV (%)	3.45	3.33	0.52	0.95

*Where: TSW: thousand seed weight, TGY: total grain yield,FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold + Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobium strain FB1017, FB1017: Rhizobium strain FB1017, *- significant at 5%, **- Significant at P≤ 0.001.*

Relative yield loss

Grain yield losses and thousand seeds weight losses were computed relative to the average grain yield and thousand seeds weight of plots with the maximum protection against the disease (Plots with lowest disease severity in the treatments). The highest relative grain yield losses (39.04% and 21.6%) and thousand seeds weight losses(35.1% and 14.21%) were occurred on control plots.Complete crop losses could occur in severe infection conditions and when favorable conditions prevail for the pathogen. In farmers' fields, a loss of about 45% was estimated due to this disease (Nigussie *et al*., 2008). The lowest yield losses(0.75% and 1.3%) and thousand seed weight losses(5.26 % and 2.98 %) were recorded on plots treated with *Rhizobium* strain 1017 at APPRC and HARC, respectively (Table 6). This indicated thatmany species of *Rhizobium* are inhibiting significantly the growth of pathogenic fungi and reduce yield loss due to diseases (Estevez de Jensen *et al*., 2002).

Table 6.Yield loss of faba bean due to black root rot disease at APPRC and HARC on different treatments during the main cropping season, 2016.

A) APPRC

Treatment	Yield and relative yield loss					
	Yield (t/ha)	RYL (ton)	RYL (%)	TSW	loss (g)	Loss (%)
FB1035	2.26a	0	0	541.23a	0	0
FB1017	2.25b	0.017	0.75	514.17ab	27.06	5.26
CG	2.16c	0.095	4.4	486.3bc	54.93	11.3
DG	2.11d	0.015	7.18	479.47c	61.76	12.88
CG+ FB1035	2.01e	0.025	12.61	491.53bc	49.7	10.11
DG+ FB1035	1.95f	0.032	16.28	500.3bc	40.93	8.18
DG+ FB1017	1.85g	0.042	22.53	437.23d	104	23.79
CG+ FB1017	1.78h	0.048	27.19	424.43de	116.8	27.52
FS (control)	1.63i	0.064	39.04	400.63e	140.6	35.1

B) HARC

Treatment	Yield and relative yield loss					
	Yield (t/ha)	RYL (ton)	RYL (%)	TSW	loss (g)	Loss (%)
FB1035	3.64a	0	0	545.1a	0	0
FB1017	3.59b	0.047	1.3	529.33ab	15.77	2.98
CG	3.46c	0.177	5.11	503.73bc	41.37	8.21
DG	3.43d	0.212	6.2	497.63bc	47.47	9.54
CG+ FB1035	3.3e	0.339	10.28	506.17bc	38.93	7.69
DG+ FB1035	3.28f	0.361	11.01	514.57ab	30.53	5.93
DG+ FB1017	3.22g	0.415	12.87	479.17c	65.93	13.76
CG+ FB1017	3.18h	0.455	14.31	498.37bc	46.73	9.38
FS(control)	2.99i	0.646	21.6	477.27c	67.83	14.21

RYL: Relative yield loss, TSW: thousand seed weight, FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold + Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobium strain FB1017, FB1017: Rhizobium strain FB1017,

Correlation between yield and disease parameters

Correlation

Correlation analysis showed that black root rot severity had strongly negative correlation (r = -0.801and r = -0.811) with grain yields at APPRC and HARC, respectively (Table 7). The correlation coefficient between disease severity and number of pods per plant (r = -0.617 and r = -0.77) were not significant (P> 0.05) at APPRC and HARC, respectively. A strong and negative association with correlation coefficient of r = -0.805 and r = -0.614 were observed between the number of seeds per pod and disease severity values at APPRC and HARC, respectively.

Ahighly and positive correlation coefficient was recorded between yield and number of seeds per pod were r = 0.806 and r = 0.583 at APPRC and HARC, respectively (Table 7). Similarly, the thousand seed weight showed highly significant (P< 0.01) correlation with yield and disease severity at HARC and significant (p< 0.05) with disease severity at APPRC. A strong and negative association with correlation coefficient of r = -0.759and r = -0.801 values were observed between thousand seed weight and disease severity (Table 9).

Table 7. Correlation coefficients (r) among the traits of black root rot disease yield and yield components of faba bean evaluated at both locations in 2016 during the main cropping season

A) APPRC

Parameter	NPP	NSP	TSW (g)	TGY (kg/ha)	DS (%)
NPP	1				
NSP	0.879**	1			
TSW (g)	0.909**	0.861**	1		
TGY (t/ha)	0.955**	0.806**	0.899**	1	
DS (%)	-0.617ns	-0.805**	-0.759*	-0.801**	1

B) HARC

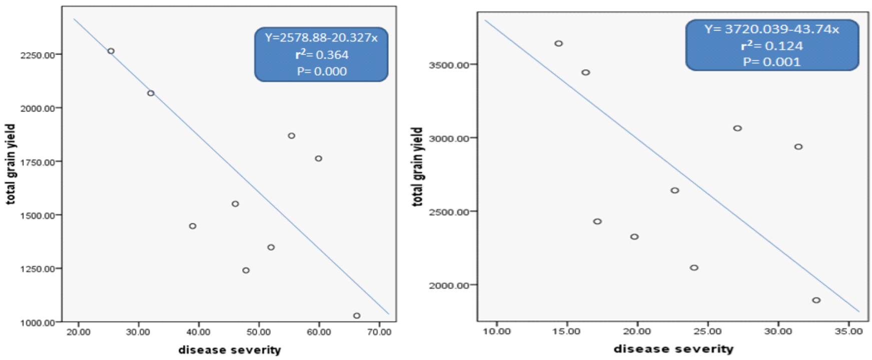
Parameter	NPP	NSP	TSW (g)	TGY (kg/ha)	DS (%)
NPP	1				
NSP	0.468ns	1			
TSW (g)	0.871**	0.422ns	1		
TGY (t/ha)	0.582ns	0.583ns	0.833**	1	
DS (%)	-0.77ns	-0.614ns	-0.801**	-0.811**	1

Where: NPP: number of Pod per plant, NSP: number of Seed per pod, TSW: thousand seed weight, TGY: total grain yield, DS: disease severity,: refers to mean square values significant at α=0.05, **: refers to mean square values significant at α=0.01, ns: refers to mean square values not significant at α=0.05*

Regression

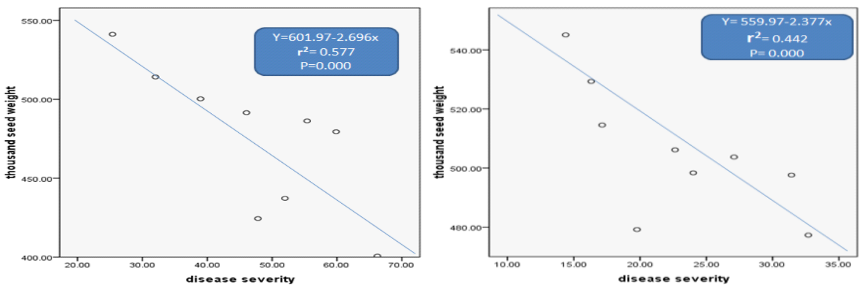
Linear regression model fitted to disease severity at APPRC and HARC as predictor and grain yield as dependent variable were regressed to estimate the yield loss due to the disease. Regression analysis of disease severity with yield revealed significant ($P \leq 0.001$) relationship. Thus, the linear regression indicated for every one unit increases in severity (%), there is a corresponding 0.02and 0.043 t/ha loss in yield of faba bean at APPRC and HARC, respectively (Figure 1). The regression equation: thousand seed weight (g) = -2.696X + 601.97, (R^2 = 57.7%, P = 0.000) and thousand seed weight (g) = -2.377X + 559.97, (R^2 = 44.8%, P = 0.000) demonstrated reduction of about 2.696 and 2.337 gram in thousand seed weight with the increase of 1% severity at APPRC and HARC, respectively (Figure 2).

As the severity increased there was a decreasing trend in yield and thousand seed weight, and declined towards zero asymptote, which indicated the reverse relation between yield and severity. In addition, the R^2 value of 36.4 and 12.4% indicated that these amounts of the variation in yields were explained by disease severity at APPRC and HARC, respectively.



a) b)

Figure 1: Linear regression of grain yield and disease severity at a) APPRC and b) HARC



a) b)

Figure 2: Linear regression of thousand seed weight and disease severity at a) APPRC and b) HARC

Cost - benefit analysis

Net benefit variations were obtained among treatments (herbicides and *Rhizobium* strains) at APPRC and HARC. Partial budget analysis was calculated based on cost of variable inputs during the main cropping season of the year 2016 and net benefit was estimated based on mean values of local market price when most of farmers supplied the produce to market.

Partial budget analysis revealed that the highest (birr 35030per hectare and birr56420per hectare) total gross yield benefit were obtained from plots treated with *Rhizobium* strain FB1035 at APPRC and HARC, respectively (Table 8). On the other hand, the lowest total gross yield (birr25265and birr46345per hectare) were obtained from the control plots at APPRC and HARC, respectively.

The highest (birr34870 and 56260per hectare)net profit was obtained from plots treated with *Rhizobium* strain FB1035, followed by birr34715 and 55485 per hectareobtained from plots treated with *Rhizobium* strain FB1017, whereas the lowest birr25265 and 46345 per hectare net benefit was obtained from the control plots at APPRC and HARC, respectively.

Generally, the highest values of marginal rate return of 60.03125 and 61.96875% were obtained from plots treated with *Rhizobium* strain FB1035, followed by 59.0625 and 57.125% obtained from plots treated with *Rhizobium* strain FB1017 at APPRC and HARC, respectively.

In this study, the application of *Rhizobium* strain FB1035 and *Rhizobium* strain FB1017 are found best to reduce black root rot disease of faba bean and to increase the yield. The economic analysis on these strains also showed more profit than the other treatments at current market prices.

Table 8. Partial budget analysis for herbicides and *Rhizobium* strains treated for the management of the faba bean black root rot disease at APPRC and HARC.

A) APPRC

Treatment	General cost benefit						
	(A) adj. yield(t/ha)	(B) price (birr/ t	(C) sale revenue (A*B)	(D) marginal cost (birr/ha)	(E) net profit (birr) (C-D)	(F) marginal benefit (birr)	MRR (F/D)(%)
FB1035	2.26	15500	35030	160	34870	9605	60.03125
FB1017	2.25	15500	34875	160	34715	9450	59.0625
CG	2.17	15500	33635	750	32885	7620	10.16
DG	2.11	15500	32705	600	32105	6840	11.4
CG+ FB1035	2.01	15500	31155	910	30245	4980	5.472527
DG+ FB1035	1.94	15500	30070	760	29310	4045	5.322368
DG+ FB1017	1.85	15500	28675	760	27915	2650	3.486842
CG+ FB1017	1.78	15500	27590	910	26680	1415	1.554945
FS (control)	1.63	15500	25265	0	25265	0	0

B) HARC

Treatment	General cost benefit						
	(A) adj. yield(t/ha)	(B) price (birr/ t	(C) sale revenue (A*B)	(D) marginal cost (birr/ha)	(E) net profit (birr) (C-D)	(F) marginal benefit (birr)	MRR (F/D)(%)
FB1035	3.64	15500	56420	160	56260	9915	61.96875
FB1017	3.59	15500	55645	160	55485	9140	57.125
CG	3.46	15500	53630	750	52880	6535	8.713333
DG	3.43	15500	53165	600	52565	6220	10.36667
CG+ FB1035	3.3	15500	51150	910	50240	3895	4.28022
DG+ FB1035	3.28	15500	50840	760	50080	3735	4.914474
DG+ FB1017	3.23	15500	50065	760	49305	2960	3.894737
CG+ FB1017	3.19	15500	49445	910	48535	2190	2.406593
FS (control)	2.99	15500	46345	0	46345	0	0

MRR: marginal rate return,FS: Fusariumsolani, DG + FB1035: Dual gold + Rhizobium strain FB1035, CG + FB1035: Codal gold + Rhizobium strain FB1035, DG: Dual gold, CG: Codal gold, DG + FB1017: Dual gold + Rhizobium strain FB1017, FB1035: Rhizobium strain FB1035, CG + FB1017: Codal gold + Rhizobium strain FB1017, FB1017: Rhizobium strain FB1017,

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