Aerobic bacteria in bovine livers infested with *Fachiola gigantica*

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Summary

133 samples from bovine livers infested with *Fachiola gigantica* were aseptically collected from an abattoir and investigated for the presence of aerobic bacteria. Different genera and species of bacteria were isolated with variable incidences. These were *Proteus species, Escherichia coli, Pseudomonas species, Citrobacter aerogenes* , *Morganella morgani, Paracolon species, Klebsiella species, Bacillus species, Streptococcus species, Corynebacterium species, Staphyllococcus aureus* , other *Staphyllococcus species, Micrococcus species and Aerococcus species*. Some of these isolates were of potential human health hazard. It was concluded that such infested livers are unfit for human consumption and should not be passed during the postmortem examination.

Introduction

The incidence of bovine livers infested with *Fachiola gigantica* is one of the highest affections of livers encountered during routine postmortem inspection in Sudan. Most of these livers were condemned; some livers however are passed when moderately infested. Outside the townships there is no guarantee that fluke infested livers are not consumed by the public.

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This study was conducted to assess the bacteriological quality and safety of the bovine livers infested with *F.gigantica.* The assessment was based on the type of aerobic bacteria which are present in such infested livers.

Materials and Methods

133 samples from bovine livers infested with *F.gigantica* were aseptically collected and put in sterile glass containers, then placed inside a thermoflask and immediately taken for laboratory investigation. Part of the sample was inoculated into Selenite. F.broth and incubated at 37°c for 24 hours. The rest of the sample was used for streaking on blood agar and MacConkey agar. A loopfull from Selenite F.broth culture was streaked on Salmonella – Shigella agar.

The three inoculated plates for each sample were incubated at 37°c for 24 hours. Isolation and identification was made on the basis of biological and biochemical reactions as described by Cowan (1985).

Results

The aerobic bacteria isolated from the bovine livers infested with *F.gigantica* include genera and species of Gram –ve and Gram +ve bacteria. These include the following:

Proteus species, Escherichia coli, Pseudomonas species, Citrobacter aerogenes , Morganella morgani, Paracolon species, Klebsiella, Bacillus species, Streptococcus species, Corynebacterium species, Staphyllococcus aureus , other Staphyllococcus species, Micrococcus species and Aerococcus species.

The frequency of isolation of the different genera and species of bacteria were shown in tables 1, 2 then figures 1 and 2.

Table (1): The frequency of isolation of the Gram negative bacilli from bovine livers infested with *F.gigantica:*

Genera and species of bacteria	Frequency of isolation
Proteus species	**96 (72.18%)**
Escherichia coli	**81 (6..9.%)**
Pseudomonas species	**56 (42.11%)**
Citrobacter aerogenes	**64 (48.12%)**
Morganella species	**21 (15.79%)**
Paracolon species	**2. (15..4%)**
Klebsiella species	**7 (5.26%)**

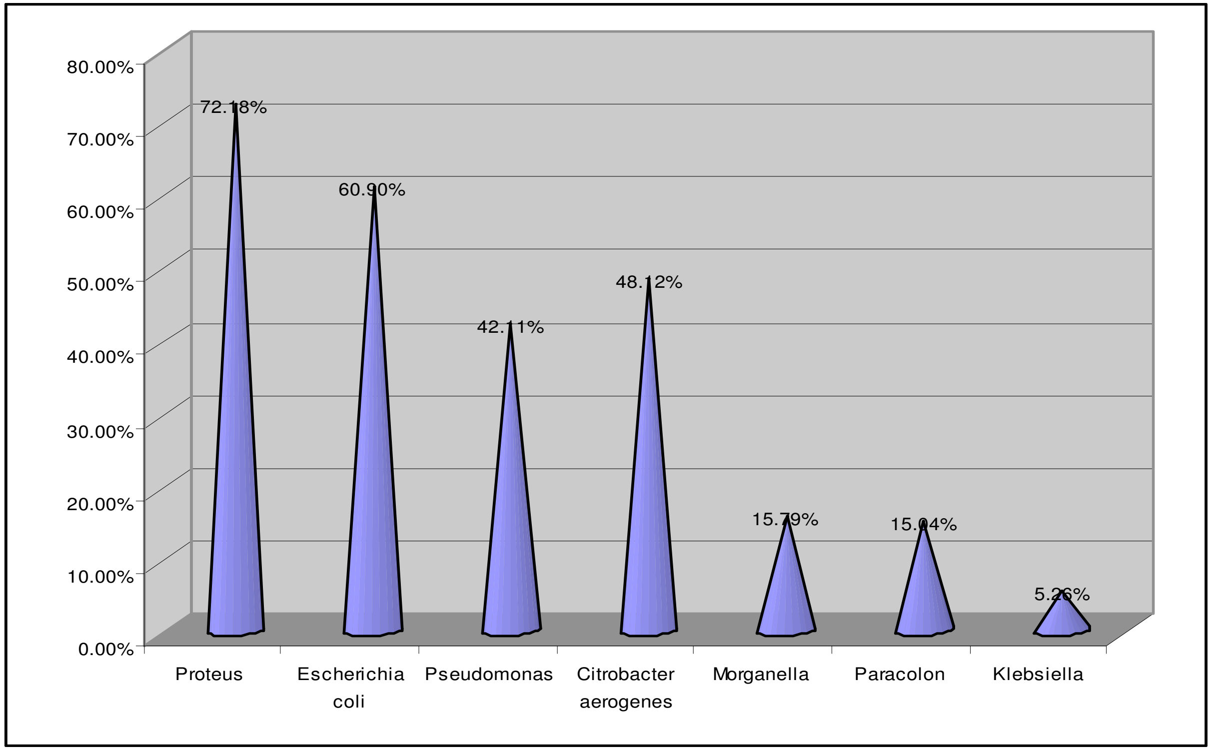

Figure (1): The frequency of isolation of the Gram negative bacilli from bovine livers infested with *F.gigantica:*

Table (2): The frequency of isolation of the gram positive bacteria from bovine livers infested with *F.gigantica:*

Genera and species of bacteria	**Frequency of isolation**
Bacillus species	**57 (42.86%)**
Corynebacterium species	**29 (21.8%)**
Streptococcus species	**56 (39..9%)**
Staphylococcus aureus	**22 (16.54%)**
Other staphylococcus species	**15 (11.28%)**
Micrococcus species	**15 (11.28%)**
Aerococcus species	**1. (7.52%)**

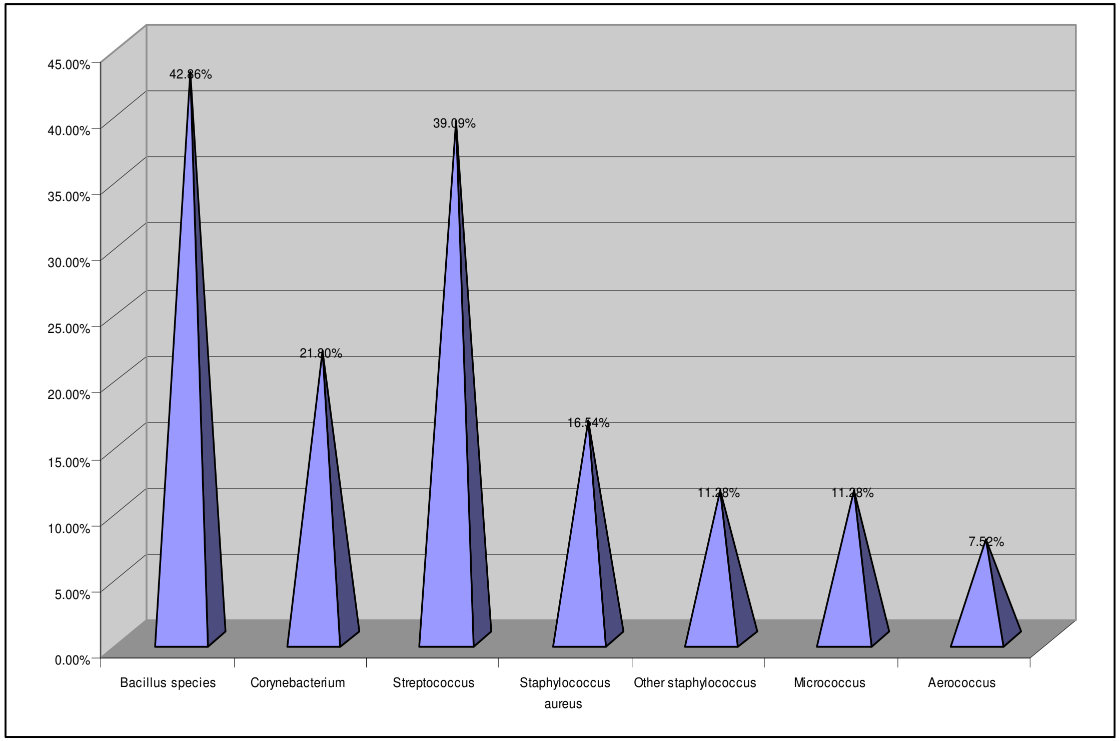

Figure (2): The frequency of isolation of the Gram positive bacteria from bovine livers infested with *F.gigantica:*

Discussion

The bacteriological findings show variation in incidence of the isolates. This may be due to the different pathological changes associated with the infestation. Michael (197.) stated that the PH variation of bile had an influence on the bacterial infection and that liver flukes had a bacteriostatic effect on the growth of some microrganisms. Isolation of *Escherichia coli* and *Corynebacterium pyogenes* were in conformity with Kruedener (1952) and that of *Citrobacter, Proteus, Paracolon, Pseudomonas, Staphylococcus and Streptococcus species* were in conformity with Michael (197.). The high incidence of isolation of genera and species of Enterobacteriaece may be attributed to the intestinal bacteria that passed through the intestinal epithelium, Mackey and Derrick (1979) or may be due to that the intestinal bacteria had been carried on the young flukes during excystation and migration, Sorokina (1987).

The high incidence of the different genera and species of bacteria constitute a potential hazard for human health. Food poisoning and intoxication is likely to occur when the bovine livers infected with *F. gigantica* are consumed raw or not well cooked. Beside that, Ingestion of such livers lead to pharyngeal infection (Halazon) and pitfalls in stool examination. Mitchel (1968) stated that in some parts of Africa goat livers which may be infested with flukes were eaten raw and in this way pharyngeal infection can occur with subsequent appearance of eggs in feaces. Samaha (1989) detected *Fachiola* ova in feacal samples of 29 (6.27%) out of 43. patient at abbis hospital, Alexandria

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Effects of Fungicide Treatment and *Rhizobium* Inoculation on Proximate Composition of Faba Bean Seeds

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Abstract:

The effects of fungicidal seed dressing and *Rhizobium* inoculation on the chemical properties of faba bean seeds were studied. Two fungicides, Captan and Fernasan-D at concentrations between zero and 10 g/kg seeds were used. Inoculation with *Rhizobium leguminosorum* bv. *viceae* strain TAL 1397 immediately before and after seed dressing or inoculation at the seedling stage was adopted.

Compaired to uninoculated plants *Rhizobium* inoculation by either method significantly (P≤0.05) increased 100-seed weight, seed moisture content, fat content, fibre and protein content.

Carbohydrate content was significantly (P≤0.05) decreased where as no effect was detected on ash content.

Unlike carbohydrate content, seedling inoculation resulted in a higher contents of all parameters tested. No clear differences were observed between inoculation immediately before or after seed dressing.

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Fungicide seed dressing significantly ($P \leq 0.05$) increased ash, moisture and carbohydrate contents when applied at the field recommended dose. Fat, fibre and protein contents were significantly ($P \leq 0.05$) decreased with different degrees depending on fungicide toxicity and concentration. Increasing the concentration of either fungicide resulted in lower contents of these parameters.

No differences between the two fungicides were observed in the contents of moisture, ash and crude fibre. Captan seed dressing resulted in a lower fat and crude protein content and higher carbohydrates content compared to Fernasan-D.

Introduction:

Legumes are unique in the high protein content of their seeds and their ability to fix atmospheric nitrogen. Faba bean (*Vicia faba* L.) is one of the major leguminous crops in the Sudan. It is mainly grown for human consumption. The popularity of faba bean may be due to high protein content and availability at relatively reasonable price. Chemical and physical properties of the seeds are indicators of quality and nutritional value.

Many efforts were directed to improve yield, protein content, and cookability and to decrease tannins and hard seed percentage through breeding, fertilization and/or genetic engineering. Biofertilization receives great attention for its minimal effect on the environment and its longer lasting effect.

Inoculation of faba bean has been found to increase yield and improve seed quality (Elsheikh and Osman,1995) Many factors act upon legume – *Rhizobium* symbiosis and may positively or negatively affect the yield and seed quality. These factors include cultivar, cultural practices and locality or environment (Elsheikh and Elzidany, 1997) Chemical and biological fertilizers were reported to increase protein content of faba bean (Babiker *et al.,* 1999). Fungicidal seed dressing is one of the factors that interfere with legume- *Rhizobium* association and affect chemical and physical properties of the seeds.

The objectives of this study was to assess the effects of fungicide treatment and the method of *Rhizobium* inoculation on protein content and proximate composition of faba bean seeds, variety "Agabat".

Materials and Methods:

A field experiment was conducted during the 1997/1998 season in the Demonstration Farm of the Faculty of Agriculture at Shambat (Latitude 15 ° 40/N, Longitude 32° 32/E)> The land was prepared by deep ploughing, harrowing and leveling; the area was then ridged and divided into 4 X 4 m plots, 80 cm between ridges and 5 north – south ridges per plot.

Seeds of faba bean cultivar Agabat were purchased from commercial sources from Shendi. *Rhizobium leguminesarum* by *viceae* strain TAL 1397 was supplied by ENNRI. The Crop Protection Department,
Ministry of Agriculture, Khartoum North, supplied the fungicides Captan and Fernasan -D.

Treatments Used include:

Captan or Fernasan – D Treatment at concentration of:

Zero g/kg seeds as control
2 g/kg seeds
4 g/kg seeds
6 g/kg seeds

8 g/kg seeds
10 g/kg seeds

Each of these Treatment was either inoculated in conjunction with fungicidal seed dressing or at the seedling stage in the furrow or otherwise uninoculated.

Five disinfected seeds were sown per hole thinned to three at the seedling stag. The crop was irrigated every10 – 15 days. The experiment was arranged in a complete randomized block design with three replicates.At havest, the seeds were carefully cleaned, then ground to passs a 0.4mm screen for proximate analysis.

AOAC (1984) methods were followed in the determination of moisture, crude fibre, crude protein and fat content. Carbohydrates content was determined by differences.

Statistical Analysis:

Each sample was analyzed in triplicates and figures were then averaged. Data were assessed by analysis of variance (ANOVA) (Snedecor and Cochran,1987) with the probability of $p \leq 0.05$.

Results and Discussion:

Moisture content:

As shown in Table (1), results of the first season indicated that moisture content is not affected by any of the treatments. In the second season *Rhizobium* inoculation significantly ($p \leq 0.01$) increased seed moisture content over the uninoculated control plants. The increment in moisture content of faba bean seeds due to *Rhizobium* inoculation was reported by Elsheikh and Elzidany, (1997). Fungicidal seed dressing by Captan or Fernasan – D significantly ($p \leq 0.01$) increased moisture content over the control plants up to the recommended dose but decreased again with increasing dose up to 10 g/kg seed. The differences between the results of the two seasons in the seeds moisture content could be attributed to the storage period as the first season harvest was analyzed after a long time.

Generally, the moisture content of legume seed was found to be affected by the relative humidity of the surrounding atmosphere at the time of harvest and during storage (Elsayed, 1994).

Ash conten:

Rhizobium inoculation by either method did not affect ash content of faba bean seeds (Table 2). Fungicide seed dressing significantly ($p \leq 0.01$) increased ash content of the seeds compared to untreated control plants. Fernasan-D resulted in higher ash content compared to Captan. Increasing the concentration of either fungicide from zero to the field recommended dose increased ash content by 2%,where increasing the concentration to 10 gm fungicide/Kg seeds decreased the percentage of ash content by 2.4% but still lower than the control plants. Similar findings were reported in fenugreek (Abdelgani, 1997) and (ElSheik and ElZidany,1997).

The ash content of foodstuff reporesents the residue remaining after the organic matter has burnt. The ash obtained is not necessarily of exactly the same composition as the mineral matter present in the original food as there may losses due to volatilization or as a result of some interactions between constituents.

Fat content:

As shown in Table (3), *Rhizobium* inoculation significantly ($p \leq 0.01$) increased fat content of faba bean seeds. Seedling inoculation resulted in higher fat content compaired to seed inoculation. Fungicide seed dressing significantly ($p \leq 0.01$) decreased it with different degrees depending on the fungicide toxicity. Captan which is more toxic resulted in lower fat content than Fernasan-D. Increasing the fungicide concentration above the recommended dose significantly ($p \leq 0.01$) decreased faba bean seeds fat content.

The increase in fat content of faba bean seeds due to biological fertilization was reported by Elsheikh and Elzidany, (1997), geroundnut (Elsheikh and Mohmedzein,1998) and fenugreek, (Abdelgani,1997). The range of fat content of faba bean was found to be in the range 0.9-1.8% which comparable to previous values of 0.7-0.92% (ElSheikh and ElZidany, 1997).

Crude Fibre Content:

Rhizobium inoculation by either method significantly ($p \leq 0.01$) increased crude fibre content of faba seeds over the control uninoculated plants. Fungicidal seed dressing at doses above the field recommended rates significantly ($p \leq 0.01$) decreased fibre content whereas no significant differences were observed between different fungicides (Table 4).

Regardless the treatment applied the crud fiber content of faba bean seeds was found to be in the range of 5.6-7.2% Reported values were 5.7-6.78% (Elsheikh and Elzidany, 1997).

In general, the crude fibre content is influenced by the environmental condition and varietal characteristics. In faba bean, the cultivear, location and time of harvest are the factors that leed to fibre content variation (EL Tinay *et al*,1989). The fibre content is an important constituent of human food and animal feed and is needed in reasonable proportion as it give the bulk to the diet and helps in movement of the food thrugh the animal digestive tract.

Crude Protein Content:

Legume seeds are rich in protein up to 35% (Wery and Grignac,1989) with a well-balanced amino a cid pattern. Faba bean contains a high protein content compared to other legumes amounting to 33.4% (Elsheikh *et al*.,2000).

Rhizobium inoculation significantly ($p \leq 0.01$) increased crude protein content of faba bean seeds over the uninoculated control plants by 7%. Seedling inoculation resulted in higher values than seed inoculation (Table 5). El Tilib *et al*.,(1994) reported that protein conten increases with improved plant nutrition and that *Rhizobium* inoculation and nitrogen fertilization were found to increase protein content of faba bean (Babiker *et al*., 1995), and indeed other legumes such as soybean (Mukhtar and Abu Naib, 1988) and fenugreek(Abdelgani,1997).

Fungicidal seed dressing significantly ($p \leq 0.01$) decreased crude protein content of the seeds. The amount of reduction was related to the fungicide toxicity and concentration as toxicity and high concentrations reduced the efficiency of nitrogen fixation. Captan was found to reduce crude protein of faba bean seeds by 20% compared to 19% reduction by Fernasan-D.

Faba bean can be used as a protein supplement to other staple food in Sudan such as sorghum and millet. This is because faba bean protein is rich in lysine with low methionine content (El Tinay et al., 1993), whereas sorghum and millet are deficient in lysine and contain moderate quantities of methionine (Salih and El Hardallou, 1986).

Carbohydrates content:

Rhizobium inoculation significantly ($p \leq 0.01$) decreased carbohydrates content compared to uninoculated control plants (Table 6). This result reflects the effect of inoculation on moisture, fat, fibre and protein contents where the increase in these constituents due to inoculation was countered by decrease in carbohydrates content. The reduction in carbohydrates content of faba bean seeds due to *Rhizobium* inoculation was reported by

ElSheikh and ElZidany, (1997). Fungicidal seed dressing by either fungicide significantly ($p \leq 0.01$) increased the carbohydrates content. Increasing the fungicide concentration over the recommended field rate also resulted in a significant ($p \leq 0.01$) increase in this parameter.

Generally, the carbohydrates content is inversely related to the protein content of faba bean seeds (ElSheikh and ElZidany, 1997).

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Table1: Effects of fungicides treatment and *Rhizobium* inoculation on moisture content of faba bean seeds.

Concentration g/kg seed	*Rhizobium* inoculation	First Season		Second Season	
		Fernasan-D	Captan	Fernasan-D	Captan
0	R0	4.15(± 0.07)	4.31(± 0.15)	4.37(± 0.09)	4.80(± 0.10)
	R1	4.13(± 0.11)	4.16(± 0.06)	4.77(± 0.12)	4.86(± 0.01)
	R2	3.68(± 0.60)	4.10(± 0.07)	4.15(± 0.09)	4.35(± 0.05)
2	R0	3.99(± 0.01)	3.64(± 0.79)	4.47(± 0.15)	4.44(± 0.10)
	R1	4.10(± 0.14)	4.25(± 0.01)	4.92(± 0.06)	4.78(± 0.08)
	R2	4.15(± 0.07)	4.13(± 0.04)	4.17(± 0.03)	4.63(± 0.15)
4	R0	3.95(± 0.07)	3.90(± 0.14)	4.49(± 0.31)	4.59(± 0.04)
	R1	4.03(± 0.11)	4.08(± 0.04)	4.80(± 0.10)	5.57(± 0.04)
	R2	4.04(± 0.09)	4.16(± 0.06)	5.63(± 0.09)	4.67(± 0.12)
6	R0	4.00(± 0.02)	4.17(± 0.06)	4.44(± 0.05)	4.45(± 1.00)
	R1	4.06(± 0.07)	4.19(± 0.05)	5.03(± 0.08)	4.55(± 0.13)
	R2	4.15(± 0.07)	3.99(± 0.16)	4.68(± 0.10)	5.11(± 0.04)
8	R0			4.82(± 0.05)	4.48(± 0.04)
	R1			5.66(± 0.06)	4.75(± 0.44)
	R2			4.22(± 0.04)	4.52(± 0.08)

10	R0			4.55(± 0.14)	4.75(± 0.39)
	R1			4.30(± 0.26)	4.90(± 0.37)
	R2			4.68(± 0.08)	4.69(± 0.27)

LSD for Fungicide (F) = NS ± 0.06
LSD for Dose (D) = NS ± 0.11
LSD for Inoculation (I) = NS ± 0.08
LSD for F × D = NS ± 0.18
LSD for F × I = NS ± 0.11
LSD for D × I = NS ± 0.19
LSD for F × D × I = NS ± 0.26

Ro = Control
R1 = Seed inoculation
R2= Seedling inoculation

Table 2: Effects of fungicides treatment and *Rhizobium* inoculation on ash content of faba bean seeds.

Concentration g/kg seed	*Rhizobium* Inoculation	First Season		Second Season	
		Fernasan-D	Captan	Fernasan-D	Captan
0	R0	2.9(± 0.4)	2.5(± 0.4)	3.19(± 0.02)	3.40(± 0.01)
	R1	3.0(± 0.1)	2.8(± 0.6)	3.30(± 0.05)	3.38(± 0.10)
	R2	3.2(± 0.3)	2.5(± 0.5)	3.36(± 0.04)	3.20(± 0.06)
2	R0	3.5(± 0.1)	3.0(± 0.1)	3.41(± 0.04)	3.41(± 0.03)
	R1	3.5(± 0.4)	2.9(± 0.3)	3.41(± 0.05)	3.35(± 0.04)
	R2	3.1(± 0.1)	2.5(± 0.5)	3.26(± 0.05)	3.38(± 0.02)
4	R0	2.9(± 0.6)	2.5(± 0.5)	3.42(± 0.04)	3.29(± 0.07)
	R1	3.4(± 0.5)	3.0(± 0.4)	3.45(± 0.12)	3.23(± 0.04)
	R2	3.5(± 0.6)	3.1(± 0.1)	3.40(± 0.09)	3.37(± 0.21)
6	R0	3.1(± 0.4)	2.8(± 0.2)	3.44(± 0.04)	3.22(± 0.02)
	R1	2.8(± 0.6)	3.0(± 0.1)	3.45(± 0.02)	3.32(± 0.10)
	R2	3.0(± 0.1)	3.4(± 0.4)	3.57(± 0.21)	3.36(± 0.11)
8	R0			3.25(± 0.02)	3.30(± 0.07)
	R1			3.45(± 0.02)	3.24(± 0.01)
	R2			3.38(± 0.02)	3.38(± 0.14)
10	R0			3.37(± 0.08)	3.25(± 0.10)
	R1			3.33(± 0.04)	3.25(± 0.05)
	R2			3.33(± 0.04)	3.25(± 0.09)

LSD for Fungicide (F)	=	± 0.155	± 0.03
LSD for Dose (D)	=	± 0.219	± 0.05
LSD for Inoculation (I)	=	NS	NS
LSD for F × D	=	± 0.310	± 0.07
LSD for F × I	=	NS	± 0.05
LSD for D × I	=	NS	± 0.09
LSD for F × D × I	=	NS	± 0.13

Ro = Control
R1 = Seed inoculation
R2= Seedling inoculation

Table 3: Effects of fungicides treatment and *Rhizobium* inoculation on fat content of faba bean seeds.

Concentration g/kg seed	*Rhizobium* Inoculation	First Season		Second Season	
		Fernasan-D	Captan	Fernasan-D	Captan
0	R0	1.47(± 0.31)	1.13(± 0.15)	1.28(± 0.02)	1.23(± 0.24)
	R1	1.83(± 0.33)	1.27(± 0.31)	1.24(± 0.06)	0.97(± 0.05)
	R2	1.77(± 0.15)	1.23(± 0.25)	1.06(± 0.07)	1.02(± 0.10)
2	R0	1.07(± 0.12)	1.20(± 0.01)	1.43(± 0.40)	1.02(± 0.04)
	R1	1.47(± 0.25)	0.90(± 0.50)	1.09(± 0.05)	1.07(± 0.04)
	R2	1.87(± 0.15)	1.20(± 0.17)	0.89(± 0.10)	0.96(± 0.16)
4	R0	1.10(± 0.17)	1.07(± 0.15)	1.20(± 0.07)	1.09(± 0.10)
	R1	1.37(± 0.15)	1.00(± 0.01)	0.89(± 0.04)	1.02(± 0.03)
	R2	1.43(± 0.12)	1.33(± 0.15)	0.91(± 0.19)	1.11(± 0.01)
6	R0	1.00(± 0.01)	0.93(± 0.12)	1.04(± 0.05)	0.76(± 0.09)
	R1	1.33(± 0.25)	1.07(± 0.21)	1.03(± 0.10)	0.89(± 0.05)
	R2	1.37(± 0.05)	1.30(± 0.01)	1.03(± 0.01)	.02(± 0.17)1
8	R0			1.05(± 0.04)	0.97(± 0.22)
	R1			0.97(± 0.14)	0.98(± 0.09)
	R2			0.97(± 0.15)	0.94(± 0.01)
10	R0			1.00(± 0.01)	0.84(± 0.12)
	R1			0.82(± 0.04)	0.79(± 0.05)
	R2			1.20(± 0.65)	0.82(± 0.11)

LSD for Fungicide (F)	=	± 0.09	± 0.06
LSD for Dose (D)	=	± 0.12	± 0.11
LSD for Inoculation (I)	=	± 0.12	± 0.08
LSD for F × D	=	NS	NS
LSD for F × I	=	± 0.17	NS
LSD for D × I	=	NS	NS
LSD for F × D × I	=	NS	NS

Ro = Control
R1 = Seed inoculation

R2= Seedling inoculation

Table 4: Effects of fungicides treatment and *Rhizobium* inoculation on crude fibre content of faba bean seeds.

Concentration g/kg seed	*Rhizobium* inoculation	First Season		Second Season	
		Fernasan-D	Captan	Fernasan-D	Captan
0	R0	5.8(± 0.7)	6.7(± 0.2)	7.63(± 0.44)	7.17(± 0.32)
	R1	6.5(± 0.4)	6.9(± 0.3)	5.75(± 0.01)	6.77(± 0.82)
	R2	6.7(± 0.3)	6.8(± 0.2)	6.47(± 0.01)	6.95(± 0.86)
2	R0	7.1(± 0.4)	6.5(± 0.3)	7.13(± 0.02)	7.37(± 0.44)
	R1	7.0(± 0.1)	7.0(± 0.1)	6.64(± 1.54)	6.70(± 0.58)
	R2	5.9(± 0.5)	7.0(± 0.1)	7.06(± 0.01)	6.49(± 0.34)
4	R0	6.5(± 0.4)	6.6(± 0.1)	8.01(± 0.36)	7.15(± 0.63)
	R1	6.8(± 0.3)	5.8(± 0.4)	6.70(± 0.59)	7.28(± 0.37)
	R2	6.8(± 0.4)	5.6(± 0.3)	5.15(± 1.78)	6.00(± 1.26)
6	R0	6.2(± 0.2)	6.7(± 0.3)	6.87(± 0.39)	6.47(± 1.03)
	R1	6.6(± 0.2)	6.8(± 0.1)	6.91(± 0.49)	6.46(± 1.12)
	R2	6.5(± 0.2)	6.9(± 0.3)	6.63(± 0.01)	6.85(± 0.42)
8	R0			6.48(± 0.48)	6.36(± 0.39)
	R1			6.87(± 0.33)	5.70(± 1.11)
	R2			6.51(± 0.34)	6.23(± 0.3)
10	R0			6.97(± 0.35)	6.17(± 0.20)
	R1			6.88(± 0.33)	6.25(± 0.39)
	R2			6.21(± 0.97)	6.11(± 0.01)

LSD for Fungicide (F)	=	NS	NS
LSD for Dose (D)	=	± 0.18	± 0.46
LSD for Inoculation (I)	=	± 0.18	± 0.33
LSD for F × D	=	± 0.25	NS
LSD for F × I	=	NS	NS
LSD for D × I	=	± 0.36	NS
LSD for F × D × I	=	± 0.51	NS

Ro = Control
R1 = Seed inoculation
R2= Seedling inoculation

Table 5: Effects of fungicides treatment and *Rhizobium* inoculation on crude protein content of faba bean seeds.

Concentration g/kg seed	*Rhizobium* inoculation	First Season		Second Season	
		Fernasan-D	Captan	Fernasan-D	Captan
0	R0	33.97(± 0.21)	31.90(± 1.70)	33.93(± 0.42)	31.50(± 1.72)
	R1	34.57(± 0.59)	33.00(± 0.01)	34.80(± 0.20)	32.21(± 1.85)
	R2	36.33(± 1.52)	35.40(± 0.50)	35.60(± 0.53)	33.55(± 1.37)
2	R0	32.70(± 0.20)	31.00(± 0.90)	32.80(± 0.36)	32.60(± 0.40)
	R1	32.70(± 0.60)	32.80(± 0.70)	33.33(± 0.32)	34.60(± 1.40)
	R2	34.87(± 0.31)	34.20(± 0.80)	34.93(± 0.21)	35.10(± 2.30)
4	R0	31.47(± 0.42)	30.80(± 0.80)	31.70(± 1.30)	33.40(± 1.70)
	R1	31.20(± 0.30)	31.70(± 0.60)	32.90(± 1.00)	34.60(± 1.80)
	R2	33.60(± 0.30)	34.00(± 0.01)	33.40(± 0.40)	35.00(± 0.01)
6	R0	29.90(± 0.40)	28.27(± 0.80)	30.40(± 1.20)	31.20(± 0.50)
	R1	30.80(± 0.40)	29.80(± 0.78)	29.97(± 1.15)	31.60(± 0.90)
	R2	33.83(± 0.35)	33.07(± 0.21)	32.17(± 0.50)	32.90(± 0.50)
8	R0			31.30(± 1.36)	29.00(± 0.01)
	R1			31.57(± 0.86)	31.00(± 0.01)
	R2			32.67(± 0.26)	32.40(± 1.10)
10	R0			29.40(± 1.24)	27.90(± 0.90)
	R1			30.70(± 1.21)	29.53(± 0.61)
	R2			32.53(± 0.50)	31.17(± 0.37)

LSD for Fungicide (F)	=	± 0.28	± 0.36
LSD for Dose (D)	=	± 0.40	± 0.62
LSD for Inoculation (I)	=	± 0.40	± 0.44
LSD for F × D	=	± 0.57	± 0.88
LSD for F × I	=	± 0.57	NS
LSD for D × I	=	NS	NS

LSD for F × D × I = NS NS

Ro = Control
R1 = Seed inoculation
R2= Seedling inoculation

Table 6: Effects of fungicides treatment and *Rhizobium* inoculation on carbohydrates content of faba bean seeds.

Concentration g/kg seed	*Rhizobium* inoculation	First Season		Second Season	
		Fernasan-D	Captan	Fernasan-D	Captan
0	R0	51.68(± 0.8)	53.49(± 0.4)	49.60(±0.71)	52.41(± 1.04)
	R1	49.97(± 0.3)	51.87(± 1.0)	50.14(± 1.27)	51.81(± 0.37)
	R2	48.32(±1.7)	54.69(± 0.8)	49.36(± 1.27)	50.92(± 0.37)
2	R0	51.64(± 1.0)	47.85(± 0.7)	50.70(± 0.21)	51.16(± 0.45)
	R1	51.23(± 0.4)	50.97(± 0.7)	50.91(± 0.09)	49.50(± 0.88)
	R2	50.11(± 0.1)	55.13(± 1.5)	49.66(±1.18)	49.44(± 0.88)
4	R0	53.18(± 0.8)	54.42(± 0.7)	51.18(± 0.37)	50.48(± 0.36)
	R1	53.20(± 0.3)	51.98(± 0.8)	51.08(± 0.27)	48.30(± 0.73)
	R2	50.60(± 0.1)	57.30(± 0.6)	51.51(± 0.36)	49.85(± 1.00)
6	R0	55.80(± 0.8)	51.68(± 0.8)	53.81(± 0.36)	52.91(± 0.28)
	R1	54.41(± 0.7)	55.16(± 0.2)	53.61(± 0.45)	53.16(± 0.37)
	R2	51.18(± 1.1)	51.24(± 0.8)	51.92(± 1.00)	50.76(± 0.20)
8	R0			53.10(± 1.10)	55.89(± 1.08)
	R1			52.48(± 0.64)	54.33(± 0.82)
	R2			52.37(± 0.64)	52.53(± 0.82)
10	R0			54.70(± 0.79)	57.09(± 0.18)
	R1			53.93(± 0.90)	55.38(± 0.62)
	R2			53.05(± 0.86)	54.94(± 0.73)

LSD for Fungicide (F)	=	± 0.33	± 0.31
LSD for Dose (D)	=	± 0.47	NS
LSD for Inoculation (I)	=	± 0.47	± 0.40
LSD for F × D	=	± 0.66	NS
LSD for F × I	=	± 0.66	± 0.61
LSD for D × I	=	± 0.94	± 0.87
LSD for F × D × I	=	± 1.33	NS

Ro = Control
R1 = Seed inoculation
R2= Seedling inoculation