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Effect of Legume Extracts on Germination, Seedling Health of Beans (*Phaseolus vulgaris* L.) and Soil Microorganisms

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Authors' contributions

This work was carried out in collaboration among all authors. Authors OOO, JO and JWM designed the study. Authors OOO, JWM, RN and JHN wrote the protocol. Author OOO collected data and performed the statistical analysis and prepared the draft manuscript. Authors JWM, JO, RN and JHN reviewed and approved the final manuscript.

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ABSTRACT

Application of undecomposed green manure has been reported to cause poor emergence and establishment of common beans in the field. Therefore, to understand the mechanisms' contributing to the poor crop establishment, the effect of extracts from fresh and decomposed legume green manures on bean seed germination, fungal mycelial growth, spore germination and germ tube elongation were evaluated. The extracts were prepared in either ethanol or distilled water. Data was collected on percentage seed germination, seedling length, mycelial radial growth, spore germination and germ tube elongation. Ethanol extracts from fresh lablab inhibited bean germination by 56%, increased mean germination time to 8 days, and decreased germination index while ethanol extracts of groundnut and beans caused highest inhibition in bean shoot length and reduced biomass. Ethanol extracts from fresh green manures significantly inhibited fungal mycelial

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growth while the aqueous extracts from beans, groundnuts and soybean had significant level of antifungal activity while aqueous lablab extracts stimulated mycelial. Aqueous extract of lablab and soybean enhanced spore germination by over 70% with more pronounced effect on germ tube length and number of germ tubes by 8.0% and 13% respectively. The study comparatively reveals that the extract of lablab was inhibitory to common bean germination compared to other legume extracts and also stimulated the growth of root rot pathogens that may have resulted in poor establishment of beans.

Keywords: legume extracts; microbial decomposition; Phaseolus vulgaris; root rot pathogens.

1. INTRODUCTION

Common bean is an important food crop as well as soil improvers. Regardless of their economic importance, farmers have not been successful to realize potential yields because of several limiting factors chief among them, low soil fertility. Green manures have been introduced as way of improving soil nutrient fertility [1]. However, upon decomposition these crops introduce other problems as they release secondary metabolites that can be phytotoxic to succeeding crops [2]. However, there phytotoxicity depends on the amount of plant residues, the environment of decomposition, duration of decomposition, residue placement and weathering [2, 3]. These phytotoxins have specific communication in terms of growth inhibition and stimulation and they are either inhibitory or stimulatory to crop growth and microorganisms in the soil [4, 5].

The toxic chemicals released into the soil during breakdown of the residues may cause severe inhibition to germination, however, the concentrations phytotoxins decline as decomposition proceeds [3,6]. The chemicals released alter the plant environment which may result in either poor crop germination or reduced growth [7] and the seeds allowed to germinate in such environments require more time for germination [8]. Lertmongkol et al. [9] reported that continuous cropping of mung bean led to plant growth inhibition by between 10 to 25% of successive crop growth. Substances capable of inhibiting germination and growth of seedlings arise under some conditions of decomposition. Production of phytotoxic substances depends on residue maturity, water content, pH and length of decomposition [10]. Aqueous extracts of crop residues contain toxic substances that can greatly delay germination and reduce shoot and root length of crops [11-13]. However, the chemical contents of the residues differ based on the nature of the solvents used in the extraction process as higher quantities of phenolics have been consistently isolated in alcohol extracts [14]. Therefore, seeds treated with extracts results in lower germination percentage and the inhibition or stimulation is as a result of phytotoxins released by a crop during the growth or when decaying.

Compost extracts improve soil quality by changing chemical and physical properties of soil, increasing organic matter content, water holding capacity, general diversity of microbes, providing macro- and micro-nutrients crucial for plant growth and suppressing diseases thereby improving plant health. The effect of the green manure residues in the soil raises the question about the use of green manure in the field. While this practice has practical value in enhancing soil nutrients, the residues contain substances that affect germinations and growth of beans. The objective of this study was to investigate the effect of lablab extracts in comparison with other legume extracts on establishment of common beans and on the growth of soil microorganism.

2. MATERIALS AND METHODS

2.1 Preparation of Legume Extracts

Compost was prepared following the method described by Ingham [15] with minor modifications. Chopped portions of green manure were piled in compost bin then a thin layer of soil was added, covered and allowed to decompose, with occasional addition of water to maintain 60% moisture content. The compost was turned once a week to maintain porosity and facilitate homogenous decomposition. Maturity of the compost was determined by taking periodic temperature readings using a soil thermometer until a constant temperature of about 30.5°C was obtained. The decomposed plant materials were macerated using a blender in sterile distilled water or in 80% ethanol in the ratio of 1:10 to make the legume compost extracts [16]. Fresh legume extracts were prepared by macerating chopped legume foliage as described above. The

mixtures were left to stand for two hours and then strained through three layers of sterile cheese cloth followed by filtration using Whatman no.1 filter paper (Whatman plc, Maidstone, Kent, UK). The solvent was evaporated at 40°C under reduced pressure in rotary evaporator [17] and the extracts were stored at 4°C until further use.

2.2 Determination of the Effect of Legume Extracts on Bean Seed Germination

Four legume extracts from common bean, lablab, soybean and groundnut at 100% concentration were used for seed bioassay. Common bean seed variety GLP2 were washed in tap water and surface sterilized in 5% sodium hypochlorite for two minutes. The seeds were rinsed in four changes of sterile distilled water after which fifty uniform bean seeds were soaked overnight in 200 ml of each of the legume extracts then sown in moist chamber lined with sterile absorbent paper towel. In each moist chamber, 20 ml of each aqueous extract was used to wet the seeds while sterile distilled water was used for the control [12]. The treatments were replicated four times in a completely randomized design and repeated thrice. The seeds were allowed to germinate and data collected on number of seeds germinated, shoot length and seedling dry weight. The germination percentage determined using the formula:

> Percent germination (%) =. (number of seeds germinated / number of seeds used in bioassay) × 100

Mean germination time was calculated using the equation by Dezfuliet al. [17]. MGT = ΣDn/ Σnwhere N: Number of seeds which were germinated on day D, D: Number of days counted from the beginning of germination

Shoot length was determined on 10 randomly selected seedlings using a ruler and digital slide calipers. Dry weights of seedling were measured by electric digital balance after fourteen days.

2.3 Evaluation of the Effect of Legume Extracts on Fungal Mycelial Growth

Antifungal activity of the legume extracts was determined using poison food technique using *Fusarium oxysporum* as the test pathogen [18]. Two milliliters of each legume extract was premixed with 15 ml of molten potato dextrose agar (PDA) in petri dishes added. Controls consisted of mixing two millilitres of sterile distilled water with media instead of PDA. The plates were gently rotated to ensure even spread of the extracts and allowed to solidify. The plates were then inoculated with 5 mm of mycelial discs cut from actively growing 8 day-old cultures of *Pythium, Fusarium, Aspergillus* and *Trichoderma* at the centre of each plate. Each legume extract and fungal pathogen treatment was replicated four times in a completely randomized design and repeated twice. Radial growth of each of the fungal pathogens was measured each day from the second day of incubation at 25°C, until the 6th day. The percentage growth inhibition of each extract was calculated by the formula

> Percent inhibition = (growth in control – growth in sample / growth in control) [19]

2.4 Determination of the Effect of Legume Compost Extracts on Spore Germination

Spore germination assay was done according to Nollet and Rathore [20]. Spores were harvested from 10 day-old of well sporulated *Fusarium* cultures grown on PDA medium. The spores were collected by adding 5ml of sterile distilled water with tween 80 0.1% (v/v) to each petri dish and scrapping the surface using sterile glass slide. The suspension collected was centrifuged at 25°C at 2000 r/min for five minutes and the supernatant was discarded and pellet re-centrifuged until a highly concentrated spore solution remained. Spore concentration was adjusted to approximately 10^2 spores/ ml using haemocytometer slide. Using sterile pipette, 50 µl of spore suspension was mixed with 50 µl of the extract on sterile slides. The slides were incubated in moist chambers lined with moist paper towel. The experiment was done in duplicate and replicated four times. The number of germinated spores, number and the length of germ tubes on each spore were determined after 24 hours by observation under microscope. The length of germtube was measured using ocular micrometer and percentage spores that germinated were calculated according to Amadiet al., [21] thus:

$$\text{percent germination} = \frac{\text{no. of germinated spores}}{\text{total number of spores}} \times 100$$

While Spore germination inhibition was determined using the formula:

Spore germination inhibition= (spores germinated in control – spores germinated in treatments) / spore germinated in control × 100

2.5 Determination of the Effects of the Legume Extracts on Bacterial Growth

Activity of legume extract against *Bacillus* spp. was determined using the well diffusion method [22]. The test organism, *Bacillus* spp., was isolated into pure culture from the soil using serial dilution method. A bacterial suspension of *Bacillus* spp. was prepared by mixing loopfuls of bacterial growth in 5ml of sterile distilled water. One millilitre of the suspension was transferred into sterile petri dishes and mixed by gentle swirling with 20 ml of molten nutrient agar [23]. After the media solidified, two uniform wells were cut on the surface of inoculated agar medium using 8mm cork borer. About 100 µl legume extracts was added into each well and allowed to diffuse at room temperature for 20 minutes. Sterile distilled water was used in the control plates. The plates were incubated at 37°C for 24 h and diameters of the zones of inhibition (mm) were measured. The experiment was done in duplicate for each of the legume plant extracts extracts.

2.6 Data Analysis

The data collected was analyzed statistically using the Fisher's analysis of variance technique by Genstat statistical computer package version 15 [24] and least significant differences (LSD) tested at 5% probability to compare the treatments' means

3. RESULTS

3.1 Effect of Legume Green Manure Extracts on Bean Seed Germination and Seedling Growth

The results of analysis of variance revealed that all the tested extracts had different effect on seed germination (Tables 1 and 2). In both experiments there was significantly ($P \leq 0.05$) high germination percentage in aqueous legume extracts in comparison to ethanol extracts. However, seeds treated with aqueous lablab extracts constantly had the lowest germination percentage 42% and 46% respectively while the maximum seed germination was recorded in seeds treated with aqueous groundnut extracts (85%) and bean compost extracts (91%). Seeds treated with ethanol extracts had significantly ($P \leq 0.05$) low germination percentages this was followed by lablab compost extracts and by fresh lablab extracts. On the other hand, in both experiments, regular recording of germination percentage showed a delayed phase in germination of treated beans, however, inhibition of germination depended on the type of the extract used. In both experiments, seeds treated with aqueous extracts had significantly shortened mean germination time except for those seeds treated with fresh lablab extracts. The maximum mean germination time of 7.8 days was recorded in seeds treated with soybean compost ethanol extracts and fresh bean ethanol extracts. The minimum mean germination time was recorded in seeds treated with sterile distilled water followed by seeds treated with aqueous bean compost extracts. Similarly, ethanol extracts had inhibitory effects on the shoot and dry weight of beans. The sensitivity of seedling growth to the extracts was higher compared to the germination rate of beans. In addition, shoot growth was more sensitive and maximum shoot length (12.6 cm) was recorded in sterile distilled water treated seeds while the minimum value (0.2 cm) was recorded in seeds treated with lablab fresh ethanol extracts while the greatest weight was recorded in seeds treated with sterile distilled water.

The mean germination time (MGT) of all seed treatments was related to shoot length, seedling vigour index and hypocotyl length in the laboratory germination tests after 14 days. Seeds germinating earlier over a shorter period of time with lower MGT produced long shoots, larger seedlings, and higher seedling vigour index (Fig. 1) that were less variable. The relative mean germination time values were closely related (R2 = 0.54, 0.66, 0.71, p=0.05)

3.2 Effect of Legume Extracts on Fungal Mycelial Growth

Legume extracts had varied degree of inhibition compared to control (Tables 3 and 4). Even though some legume extracts exhibited certain levels of antagonism against mycelia of the tested fungi, fresh aqueous and ethanol extract of lablab and soybean significantly ($P \leq 0.05$) inhibited mycelial growth of *Pythium* and *Fusarium*. Fresh aqueous extracts from beans and groundnut extracts were found to have low antifungal activities on *Pythium* but had strong antifungal effect on *Fusarium*. In both experiments the highest percentage inhibition

(70%) in *Pythium* was observed in fresh soybean ethanol extracts while the least percentage inhibition was observed in fresh aqueous groundnut extracts. The highest percentage inhibition (53.3%) in *Fusarium* was observed in fresh soybean ethanol extracts while the least was observed in bean compost extracts. The fresh aqueous and compost extracts were found to stimulate the growth of *Trichoderma* except for fresh aqueous lablab and soybean while all the ethanol extracts inhibited mycelial growth of *Trichoderma*. However, the same extracts inhibited the growth *Aspergillus* by percentages ranging from 4% - 46%. Increase in

Table 1. Percentage germination, mean germination time, shoot length and dry weight of bean seeds treated with different legume extracts in the initial experiment

Legume extracts	Fresh				Compost			
	GP	MGT (Days)	S.L	DW	GP	MGT (Days)	S.L	DW
Water								
Lablab	57.5_{d}	4.4_{cd}	5.9_{bc}	16.9_{b}	60.0_{c}	3.9_{cd}	4.8_{c}	18.6_{a}
Bean	72.5_{c}	3.9_{cd}	4.6_{c}	13.4_{bc}	78.1_{b}	3.5_{cd}	8.2_{b}	13.3_{bc}
Soybean	76.9_{b}	4.9_{bc}	13.2_{a}	14.7_{bc}	61.9_{c}	4.1_{cd}	6.2_{bc}	12.5_{cd}
Groundnut	85.0_{b}	3.8_{cd}	4.7_{c}	12.6_{cd}	36.8_{e}	3.7_{cd}	8.2_{b}	15.6_{bc}
Ethanol								
Lablab	41.3_{e}	7.5_{a}	0.2_{e}	10.5_{d}	37.5_{e}	7.6_{a}	0.5_{e}	14.2_{bc}
Bean	65.0_{c}	4.6_{cd}	1.5_{de}	12.5_{cd}	68.8_{c}	6.4_{ab}	1.0_{e}	12.3_{cd}
Soybean	76.3_{bc}	7.1_{a}	1.3_{de}	13.9_{bc}	38.1_{e}	7.8_{a}	2.0_{de}	12.1_{cd}
Groundnut	48.1_{de}	6.9_{a}	2.1_{de}	16.2_{ab}	61.3_{cd}	7.4_{a}	1.2_{ef}	12.9_{cd}
Control	99.4_{a}	3.0_{d}	12.6_{a}	18.6_{a}				
Mean	62.6	5.34	4.6	14.2				
LSD ($p \leq 0.05$)	12.4	0.96	2.4	2.4				
CV (%)	13.9	12.8	36.7	11.8				

G.P- Germination percentage; G.I- Germination index; MGT- Mean germination time; S.L- shoots length; DW- Dry weight. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$)

Table 2. Percentage germination, mean germination time, shoot length and dry weight of bean seeds treated with different legume extracts in the repeat experiment

Legume extracts	Fresh				Compost			
	GP	MGT (Days)	S.L	DW	GP	MGT (Days)	S.L	DW
Water								
Lablab	77.0_{b}	3.2_{e}	6.4_{bc}	15.4_{b}	80.5_{b}	4.3_{d}	3.3_{d}	16.1_{b}
Bean	88.0_{ab}	3.7_{e}	4.7_{c}	17.3_{a}	90.5_{ab}	3.7_{e}	8.2_{b}	15.7_{b}
Soybean	88.5_{ab}	4.7_{d}	5.2_{c}	16.8_{ab}	90.0_{ab}	3.9_{e}	12.6_{a}	15.8_{b}
Groundnut	84.5_{b}	4.5_{d}	4.5_{cd}	16.3_{a}	81.0_{b}	3.7_{e}	12.6_{a}	18.6_{a}
Ethanol								
Lablab	46.5_{d}	6.8_{b}	0.2_{e}	17.3_{a}	84.0_{b}	5.5_{c}	2.9_{de}	15.7_{b}
Bean	62.0_{c}	7.8_{a}	1.6_{e}	16.6_{a}	64.5_{c}	7.1_{ab}	1.0_{e}	16.3_{b}
Soybean	61.5_{c}	6.8_{b}	1.3_{e}	17.8_{a}	79.5_{b}	6.4_{bc}	2.0_{e}	16.1_{b}
Groundnut	63.0_{c}	7.3_{ab}	2.1_{e}	18.9_{a}	65.5_{c}	7.9_{a}	1.2_{e}	15.8_{b}
Control	99.5_{a}	3.2_{e}	12.6_{a}	15.2_{b}				
Mean	76.8	5.3	4.9	16.6				
LSD ($p \leq 0.05$)	13.8	0.7	1.9	2.1				
CV (%)	12.6	9.4	27.7	9.0				

G.P- Germination percentage; G.I- Germination index; MGT- Mean germination time; S.L- shoots length; DW- Dry weight. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$)

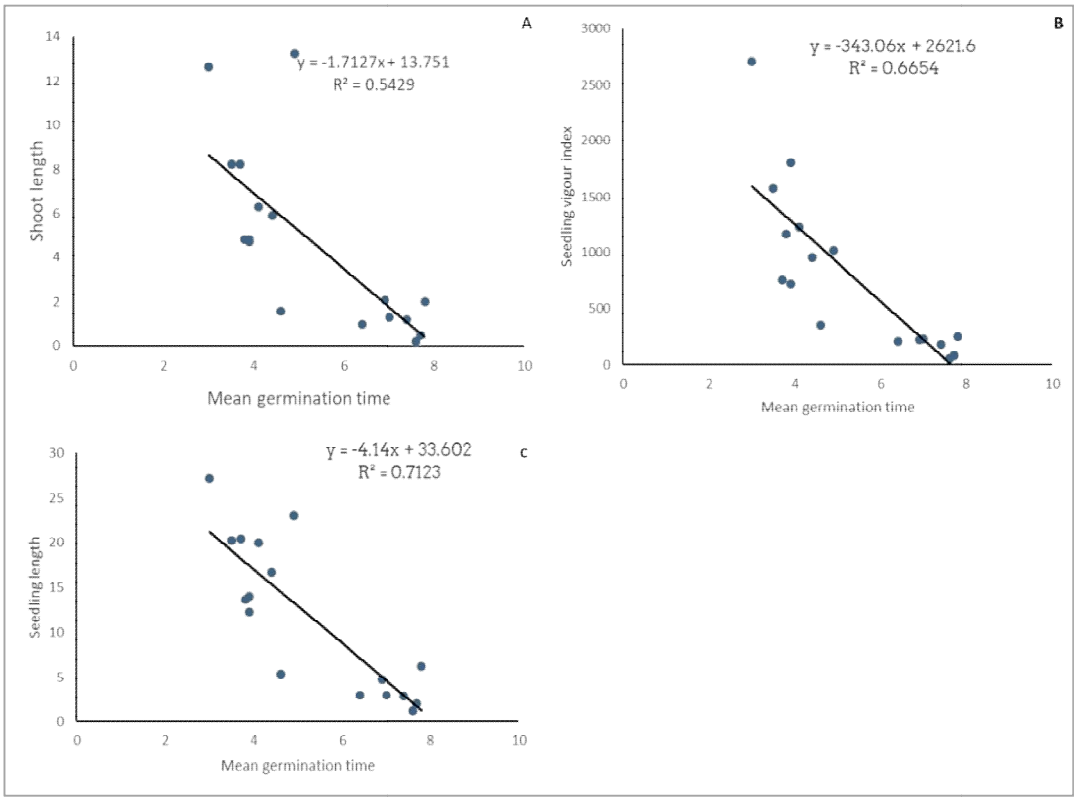

Fig. 1. Relationship among mean germination time, and shoot length, seedling vigour index, and seedling length

Table 3. Percentage inhibition or stimulation by extracts of different legumes on mycelial growth of different fungi in the initial experiment

Legume extracts	Fresh extracts				Compost extracts			
Experiment one	**Pyth**	**Fus**	**Tricho**	**Asperg**	**Pyth**	**Fus**	**Tricho**	**Asperg**
Water								
Lablab	11.2_{c}	12.2_{b}	0.8_{d}	28.8_{b}	-2.3_{d}	8.9_{bc}	-19.2_{e}	22.6_{b}
Bean	3.5_{c}	32.2_{a}	-8.9_{d}	17.6_{bc}	5.8_{c}	8.9_{bc}	-12.6_{e}	32.6_{ab}
Soybean	-2.7_{d}	44.4_{a}	1.4_{d}	23.8_{b}	-1.4_{d}	-1.1_{c}	-0.1_{d}	25.1_{b}
Groundnut	-11.7_{d}	46.7_{a}	-28.0_{e}	16.4_{b}	-4.1_{d}	3.3_{c}	-3.0_{d}	28.8_{b}
Ethanol								
Lablab	13.4_{c}	33.3_{a}	37.5_{c}	43.8_{a}	-2.7_{d}	0.0_{c}	-8.9_{d}	3.9_{c}
Bean	54.2_{a}	38.9_{a}	53.6_{b}	46.3_{a}	69.9_{a}	8.9_{bc}	4.3_{d}	31.3_{ab}
Soybean	69.9_{a}	20.0_{ab}	56.6_{b}	43.8_{a}	48.0_{b}	1.1_{c}	-13.3_{e}	20.1_{b}
Groundnut	69.0_{a}	36.7_{a}	75.0_{a}	41.3_{a}	39.0_{b}	12.2_{c}	3.6_{d}	33.8_{ab}
Mean	22.4	20.4	8.6	28.8				
LSD ($p \leq 0.05$)	10.7	15.2	10.6	17.6				

Means followed by different letter(s) within each column are significantly different at p ≤0.05; Pyth- Pythium; Fus- Fusarium; Tricho- Trichoderma; Asperg – Aspergillus. – denotes stimulation

mycelial growth were observed with groundnut, soybean and bean fresh extracts while absolute inhibition was observed with ethanol based extracts from groundnut, soybean, beans and lablab respectively while in the second season, near complete inhibition were observed with beans, lablab and soybean extracts.

Table 4. Percentage inhibition or stimulation by extracts of different legumes on mycelial growth of different fungi in the repeat experiment

Legume extracts	Fresh extracts				Compost extracts			
Experiment two	***Pyth***	***Fus***	***Tricho***	***Asperg***	***Pyth***	***Fus***	***Tricho***	***Asperg***
Lablab	2.7_{c}	8.4_{a}	8.2_{ab}	11.5_{ab}	-50.6_{e}	-1.6_{b}	-4.7_{b}	-3.1_{b}
Bean	5.6_{c}	14.2_{a}	-2.8_{b}	3.1_{b}	-25.9_{d}	-11.6_{b}	-0.1_{b}	2.1_{a}
Soybean	6.7_{c}	17.0_{a}	-0.1_{b}	1.0_{b}	3.8_{c}	-14.4_{b}	33.9_{a}	-4.2_{b}
Groundnut	-7.4_{c}	22.7_{a}	-12.0_{c}	4.2_{b}	-4.9_{c}	-7.3_{b}	41.3_{a}	-8.3_{b}
Ethanol								
Lablab	26.4_{b}	4.1_{a}	21.0_{ab}	13.5_{a}	-6.5_{c}	7.0_{a}	-2.8_{b}	-3.1_{ab}
Bean	39.3_{b}	11.3_{a}	26.5_{ab}	15.6_{a}	62.9_{a}	-5.9_{b}	6.3_{b}	5.2_{b}
Soybean	53.3_{a}	19.9_{a}	-9.3_{b}	13.5_{a}	34.8_{b}	-8.7_{b}	-4.7_{b}	-7.3_{ab}
Groundnut	48.3_{a}	19.9_{a}	3.6_{b}	15.6_{a}	32.5_{b}	4.1_{a}	16.4_{ab}	2.1_{a}
Mean	13.8	5.0	7.5	3.8				
LSD ($p \leq 0.05$)	14.4	12.4	10.4	17.4				

Means followed by different letter(s) within each column are significantly different at $p \leq 0.05$; Pyth- Pythium; Fus- Fusarium; Tricho- Trichoderma; Asperg – Aspergillus. – denotes stimulation

Table 5. Percentage spore germination, germtube growth of *F. oxysporum* treated with different legume extracts in the initial experiment

Legume extracts	Fresh extracts				Compost extracts			
	Experiment one							
	GP	**GL**	**Germtube number**	**PI**	**GP**	**GL**	**Germtube number**	**PI**
Water								
Lablab	84.0_{a}	1.84_{a}	2.8_{a}	13.9_{c}	55.6_{a}	1.4_{abc}	2.1_{ab}	48.6_{b}
Bean	54.6_{a}	0.67_{b}	1.9_{ab}	77.8_{ab}	61.1_{a}	0.9_{abcd}	1.9_{ab}	62.5_{b}
Soybean	71.8_{a}	0.82_{b}	2.8_{a}	48.6_{b}	31.5_{ab}	0.8_{abcd}	0.5_{b}	73.6_{ab}
Groundnut	50.4_{a}	0.62_{b}	2.7_{a}	63.9_{b}	45.3_{a}	0.4_{cd}	1.3_{ab}	68.1_{ab}
Ethanol								
Lablab	2.2_{b}	0.27_{bc}	0.22_{c}	98.6_{a}	22.1_{ab}	0.5_{cd}	1.1_{ab}	90.3_{a}
Bean	5.9_{b}	0.30_{bc}	0.44_{bc}	95.8_{a}	13.3_{b}	0.2_{d}	0.7_{b}	91.7_{a}
Soybean	8.7_{b}	0.27_{bc}	0.77_{bc}	94.4_{a}	59.5_{a}	0.3_{cd}	3.4_{a}	48.6_{ab}
Groundnut	10.0_{b}	0.22_{c}	0.44_{bc}	94.4_{a}	16.7_{ab}	0.3_{cd}	1.1_{ab}	93.1_{a}
Control	73.3_{a}	1.7_{a}	2.5_{a}	0.0_{c}				
Mean	39.2	0.67	1.57	68.5				
LSD ($p = 0.05$)	47.1	0.61	1.41	31.0				
CV (%)	72.3	54.6	54.2	27.3				

G.P- Germination Percentage, G.L- Germtube length, P.I- Percent inhibition. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test ($P \leq 0.05$)

3.3 Effect of Legume Compost Extracts on Spore Germination

The extracts had variable effects on spore germination of *F. oxysporum* (Table 5). In the first experiment, aqueous extract of lablab resulted in the highest spore germination percentage at (84%), followed by aqueous fresh soybean extract at (72%). However, in the second experiment, spores treated with aqueous extracts from beans had the highest spore germination (80%) followed by spores treated with aqueous lablab extracts (71%) (Table 6). This was significantly ($P \leq 0.05$) higher than the other extracts. The least spore percentage germination was observed with fresh ethanol lablab and soybean extracts respectively in both experiments. After 24 hours of incubation, the longest germ tube formation (1.8 µm) was recorded in samples treated with fresh aqueous lablab extracts followed by the lablab compost aqueous extracts with 1.4 µm in the first experiment. However, in the second experiment, samples treated with fresh aqueous bean extracts had the longest germ tube length (1.9 µm) followed by samples treated with lablab

aqueous extracts. The least germ tube length ranging from 0.20 µm to 0.33 µm was recorded in samples treated with bean compost, soybean compost ethanol extracts, and lablab ethanol fresh extracts.

In the first experiment, the maximum number of germ tubes per spore was recorded in samples treated with soybean compost ethanol extracts followed by those treated with lablab and soybean aqueous fresh extracts. In the second experiment, the maximum number of germtubes was recorded in the control followed by those treated with fresh aqueous extracts from beans, and ethanol based compost extracts from soybean and groundnuts. In both experiments, the minimum number of germ tube per spore was recorded in samples treated with ethanol lablab fresh extract, followed by bean fresh extract, and soybean aqueous extracts. High inhibition (98%) was recorded in spores treated with ethanol

Table 6. Percentage spore germination, germtube growth of *F. oxysporum* treated with different legume extracts in the repeat experiment

Legume extracts	Fresh extracts				Compost extracts			
	Experiment two							
	GP	GL	Germtube number	PI	GP	GL	Germtube number	PI
Water								
Lablab	71.1_{ab}	1.6_{a}	1.7_{a}	41.8_{b}	51.4_{bc}	0.7_{c}	1.6_{a}	78.6_{b}
Bean	80.4_{a}	1.9_{a}	2.7_{a}	71.4_{ab}	39.4_{c}	0.8_{c}	1.0_{ab}	87.8_{a}
Soybean	43.2_{bc}	0.9_{b}	1.9_{a}	78.6_{ab}	60.0_{b}	0.4_{d}	1.2_{b}	85.7_{a}
Groundnut	81.9_{a}	1.8_{a}	2.1_{a}	63.3_{b}	34.5_{c}	0.4_{d}	1.1_{b}	90.8_{a}
Ethanol								
Lablab	3.0_{d}	0.1_{d}	0.2_{c}	97.9_{a}	53.6_{bc}	0.9_{b}	1.9_{a}	70.4_{b}
Bean	25.5_{cd}	0.5_{c}	1.0_{b}	82.6_{a}	50.1_{bc}	0.9_{b}	1.9_{a}	69.4_{b}
Soybean	1.4_{d}	0.1_{d}	0.3_{c}	97.9_{a}	57.8_{bc}	1.4_{ab}	2.2_{a}	76.5_{b}
Groundnut	0.0_{d}	0.0_{d}	0.0_{c}	100.0a	46.5_{bc}	1.0_{b}	2.2_{a}	50.0_{c}
Control	93.7_{a}	1.6_{a}	2.78_{a}	0.0_{c}				
Mean	46.7	0.8	1.5	73.1				
LSD (p=0.05)	28.9	0.5	0.9	19.6				
CV (%)	37.3	35.7	39.4	16.4				

G.P- Germination Percentage; G.L- Germtube length; P.I- Percent inhibition. Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05)

Table 7. Percentage inhibition by fresh and compost legume extracts on bacteria growth

Legume extracts	Experiment one			Experiment two		
	Fresh	Compost	Mean	Fresh	Compost	Mean
Water						
Lablab	7.2_{d}	0.0_{f}	3.6_{bc}	2.0_{c}	0.2_{f}	1.1_{c}
Beans	5.8_{d}	0.0_{f}	2.9_{c}	6.6_{b}	0.6_{ef}	3.6_{b}
Soybean	5.9_{d}	1.6_{ef}	3.8_{bc}	4.9_{b}	1.1_{ef}	3.0_{bc}
Groundnut	5.1_{d}	2.3_{ef}	3.7_{bc}	4.9_{b}	1.9_{ef}	3.4_{b}
Ethanol						
Lablab	16.5_{ab}	3.7_{e}	10.1_{a}	11.5_{a}	2.9_{e}	7.2_{a}
Beans	10.6_{c}	1.8_{e}	6.2_{b}	12.5_{a}	2.8_{e}	7.7_{a}
Soybean	17.8_{a}	3.9_{e}	10.9_{a}	11.5_{a}	2.4_{e}	6.9_{a}
Groundnut	14.8_{b}	5.9_{d}	10.4_{a}	14.3_{a}	4.1_{b}	9.2_{a}
Mean	5.1		6.5	4.2		5.3
LSD (p ≤ 0.05)	2.9		2.9	1.7		2.4
CV (%)	40.5		12	30.1		8.1

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05)

lablab extracts. This was significantly (P≤0.05) higher than other extracts. In both experiments, ethanol extracts of soybean, groundnut and beans had similar inhibitory effects on spore germination, with percentage inhibitions of above 90%. In both experiments increased spore elongation was observed with water extracts of lablab (-13%) and (-41%) respectively. Ethanol extracts were relatively inhibitive on germinating spores than water extracts of the legume plants. Longer germtube lengths were observed under the aqueous lablab extracts while shorter germ tubes were observed under the ethanol extracts. This was noted particularly with lablab and soybean ethanol extracts.

3.4 Effects of Legume Extracts on Bacterial Growth

All the extracts showed varying degrees of antibacterial activities against *Bacillus* spp. The results of the inhibition zone diameter and percentage growth inhibition in Table 7 shows the antibacterial activity of legumes aqueous and ethanol extracts against bacteria. Results indicate that, the effect of the tested extract showed variable inhibition zones ranging from 0.1 mm to 14.5 mm. Soybean (14.6 mm), and groundnut (14.5 mm) ethanol extracts showed the highest zone of inhibition on *Bacillus* spp. lablab and beans extracts had the lowest antibacterial activity against *Bacillus* spp. In general, fresh ethanol based extracts had the greatest growth inhibition on *Bacillus* spp growth when compared with other extracts.

4. DISCUSSION

The results indicate that ethanol legume extracts displayed adverse effects on seed emergence and germination. This suggests that ethanol is effective in extracting substances due to high polarity and good solubility [24]. The presence of extracts potentially reduced seed germination and the seeds that germinated with extracts required more time for mean germination times. The same results were reported by Ayub et al. [7]. However, germination was significantly improved by aqueous extracts except for lablab. Germination percent is a commonly used index to measure the effects of phytotoxic substances on germination and mainly depends on final measurements [25]. However, germination index cannot explain the delay in germination caused by legume extracts.

The difference in seed germination between compost and fresh extracts may suggest that chemical in compost extracts may have degenerated and enhanced germination and emergence. Germination index, seedling vigour index, and mean germination time together with germination percentage were considered in order to understand inhibition in germination. The delay in germination was more pronounced in seeds treated with ethanol extracts, and in lablab aqueous extracts and less pronounced in seeds treated with aqueous extracts of soybean, groundnuts and beans. These results show the inhibitory potential of the legume extracts and type but this is dependent on the extract medium used since ethanol extracts were more efficient in extracting bioactive compounds in the legume plants. The delay in germination and inhibition has been reported by Hussain et al. [26] since early seedling growth is very sensitive to phytotoxins [27]. Results show that the chemicals from legume extracts had severe effect on seedling growth, significantly reducing shoot, secondary root formation and dry weight of bean seedlings. These results are comparable to those reported by [25, 28, 29] where extracts were phytotoxic to seedlings and decreased radicle elongation. There was also great inhibition in the seedling length because after germination the sustenance of the seedlings was done with the extracts. Phytochemicals from green manure are not only inhibitory to germination but also retard seedling growth after germination [30]. Similar result was reported by Terzi, [31] with walnut juice where high inhibition was recorded.

There was close relationship between mean germination time and the shoot length, seedling length and seedling vigour index, with regression analysis ranging from 0.54 to 0.71. Thus, the laboratory assessment of mean germination time was highly predictive of all the growth parameters. This implies that later germinating seeds resulted in smaller seedlings and with greater spread of germination. Similar findings were reported in comparisons of seed lots of maize [32]. The shorter shoot length produced by the seeds having high MGT, may have resulted from the spread in time of germination of seeds [31]. Seed germination involves both biochemical and physiological changes and any interruption to the two processes by chemical substance may result into germination failure and the germination bioassays done explains the effect of exogenously applied material [33]. The implication of these substances is the injurious

effect they impart on the crops that result in reduced and delayed germination ultimately leading to decline in plant stand and yield. In the present study, the vigour index ranged from 2.1 to 5.7 with a mean of 3.6, and since the percentage germination inhibition was high for beans with lablab green manure, legible vigour index was not observed. The results show that the fresh ethanol extracts influenced more vigour loss. Similar results in loss of vigour were reported by [34] where the allelopathic effects of Eucalyptus, Melia, Moringa, and Parthenium were observed on wheat, rice, millet, and sorghum.

The effect of four plant extracts resulted in different levels of antifungal activity against various fungi. Results showed that the aqueous extracts from soybean, groundnuts and bean showed more inhibitory effect against mycelial germination of the tested fungi when compared with the lablab aqueous extracts and control. However, results of the mycelial growth assay suggested that crude ethanol extracts from Lablab and soybean were the most active against fungi *Aspergillus, Pythium* and *Fusarium*. Aqueous crude extracts from lablab stimulated germination of spores and enhanced germtube elongation. Several reports have shown that plant extracts have inhibitory effects against pathogenic fungi [35]. The toxicity observed against these fungi may be due to alteration of cell wall permeability, interference with electron transport, the nutrient absorption, and other metabolic processes of the cell. The result shows that the tested compost extracts had different effects on the fungi tested. Composts prepared from lablab and soybean, groundnut and beans had no relative effects on the growth of these fungi when compared with the control. This may be due to the absence of biologically active antifungal compounds.

The current study shows that fresh ethanol extracts prepared from various legumes had high inhibitory effects against *Fusarium solani*, *Fusarium oxysporum*. The presence and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms [35]. Phytochemical analysis by Torres and Manalo [36] and Balekeri [37]. showed that the fresh leaf extracts of lablab (*Lablab purpureus* L.) contains sugar, alcohols, phenols, steroids, essential oils, alkaloids, tannins, flavonoids, saponins, coumarins, terpenoids pigments, glycosides and anthnanoids. However, the total phenolic contents (TPC) were lower compared with other plants.

The bioactive compounds in extracts may have applied two inhibitory actions on mycelia and spores since they act simultaneously and differently on various targets [38]. Arif et al. [39] suggested enzyme inhibition by oxidized compounds through reaction with sulfhydryl groups, or through non-specific interactions with proteins as main mechanisms responsible for phenol toxicity. The notable fungitoxic ability of the legume extracts suggests that the contents of the plant material are highly soluble in the extracting solvents used [40]. Legume extracts were effective in reducing the radial growth of the pathogens after two days in culture, which decreased as incubation period increased indicating that the efficacy of the active compounds was not persistent in the culture medium or degenerated in toxicity levels after two days of culture.

Different extracts had different effects on spore germination. However, most of the extracts macerated with sterile distilled water, were moderately or slightly inhibitory to spore germination and elongation. The maximum inhibition in spore germination was found in ethanol extracts of all legumes in both experiments. However, fresh lablab aqueous extract was found to stimulate the germination of *F. oxysporum* conidia with increased number of germ tubes per spore. The other plants extracts had intermediary effect. The results show that the legumes have antifungal properties against *F. oxysporum* and the amount of the fungitoxic substances extracted may be significantly increased when different extraction methods are used. Water, acetone, ether, and chloroform are not very effective in extracting the inhibitory substances [41]. Chemical contents differ depending on the type and nature of solvent used in the extraction technique while the sensitivity of the extracts depends on the concentrations and the effectiveness of extracts constituents [18].

In the present study, ethanol extracts of lablab and soybean showed inhibitory activity against *Bacillus* spp. The assessment of the antibacterial activity of several leguminous extracts showed that the highest growth inhibitory effect relative to the diameter of inhibition zones was as a result of lablab ethanol extracts while the least diameter was shown by compost extracts. When inhibition zone size is considered as indicators of antibacterial effectiveness soybean and lablab

crude extracts emerged as the most potent of all plant extracts tested. As lablab crude extract produced the largest inhibition zones compared to the rest. Chemical contents differ depending on the type and nature of solvent used in the extraction technique [23] while the sensitivity of the extracts depends on the concentrations and the effectiveness of extract constituents [23]. Plant extracts possess antibacterial characteristics against pathogenic bacteria since they are hydrophobic and can bond both lipidic layer of the cellular membrane and mitochondria of the bacteria resulting into rupture and the important molecules and ions exit from the cell, leading to the eventual death of the bacteria [42].

The treatment of the seeds with extracts affected germination and seedling length. Aqueous lablab extracts stimulated growth and germination of mycelial spores of *F. oxysporum* while ethanol extracts were more efficient in inhibiting spore and mycelial growth of fungi. The low inhibition effects of aqueous extract were probably as a result of antimicrobial compounds and fungicidal materials being not lipophilic [43].

5. CONCLUSION

The present study shows that poor emergence and establishment of common bean in the field following lablab green manure application is due to a combination of various factors that include stimulation of mycelial and spore growth while inhibiting the population of saprophytic fungi. Phytotoxicity and presence of inhibitory substances in lablab green manure during decomposition affect the root elongation of beans. Further investigation on the identity of the inhibitory substances released during decomposition is needed to determine their significance under field conditions on other crops.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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