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Research Article

Haricot bean (*Phaseolus vulgaris* L.) genotypes at germination stage under the effect of allelopatic leaf extract of Lantana (*Lantana camara* L.)

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ABSTRACT

Background: Allelopathy is considered as an effective and environmentally friendly method to control the weeds and consequently enhances crop production. *Lantana camara* L. is a major exotic weed spreading rapidly in wastelands and agricultural fields. The ability of this weed to germinate fast and to inhibit the growth of other neighboring plants smoothest way for its quicker growth in an ecosystem.
Methods: This study was carried out at Bule Hora University in the biology laboratory to assess haricot bean (*Phaseolus vulgaris* L.) genotypes at germination stage under the effects of allelochemical leaf extract from *Lantana camara* L. at different concentration levels. *L. camara* leaf and seven genotypes of haricot bean responsible for this study were collected from the local farmer's farm land in Bule Hora Woreda with their local name. Experiment was carried out with completely randomized block design with three replications in petridishes where three seeds of haricot bean were planted in each 84 petridishes and 0 gram/100 millileter (control), 2 gram/75 millileter (treatment 1), 2 gram/50 millileter (treatment 2) and 2 gram/25 millileter (treatment 3) grinded powder of *L. camara* L.: distil water were applied to petridishes daily. Germination parameters like germination day, number of germinated seeds, plumule length, radicle length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, germination percentage, germination index and inhibition or stimulation were collected.
Results: The result showed that there were variation responses of haricot bean genotypes parameters to each concentration levels of *L. camara* leaf extracts. Germination day, plumule length and radicle length were significantly influenced by *L. camara* leaf extract. The values of germination day, plumule length, shoot fresh weight and root fresh weight were decreased with the increasing of concentration levels. From the all genotypes Nasir was highly influenced in days to germination which shifted from 7.67 to 10.33 days. The correlation between concentration and all parameters were also seen that concentration and parameters plumule length (-0.428), shoot fresh weight (-0.015), root fresh weight (-0.237), shoot dry weight (-0.074) and root dry weight (-0.144) showed negatively correlated with concentration level. It can be concluded that allelopathic leaf extracts of lantana can inhibit germination parameters of some haricot bean genotypes which should take attention by breeders and farmers to avoid such weed from the crops.
Conclusions: Generally it can be concluded that concentrated aqueous leaf extract of *L. camara* L. weed species inhibited germination day, plumule length, shoot fresh weight and germination index of haricot bean (*p. vulgaris* L). High concentration levels of this chemical has high influential power on the germination parameters of this crop.

Keywords: *L. camara L.*, *Phaseolus vulgaris*, Concentration, Haricot bean

INTRODUCTION

Allelopathy of plants are considered to be safe and beneficial to environment and mankind, unlike synthetic chemical herbicides widely used which may pollute water and soil in crop ecosystems.[1] It refers to the direct or indirect chemical effects of one plant on the germination, growth, or development of neighbouring plant which can be regarded as a component of biological control in which plants are used to reduce the vigour and development of other plants. Many of these compounds are phytotoxic and have potential as herbicides or as templates for new herbicides classes. These allelochemicals offer great potential for pesticides because they are free from problems associated with present pesticides. *Lantana camara* L. is among the top ten aggressive weeds on earth. [2] The different parts of this weed contain allelochemicals which can interfere with seed germination and early growth of many plant species.[2] It has influence one plant upon another plant growing in its area by the release of certain metabolic toxic products in the environment. This study was carried out to see the influence of allelochemical leaf extracts of this species on agronomic crop, haricot bean (*Phaseolus vulgaris* L). Haricot bean (17%) is one of the cash crops next to faba beans (36%) accounts for the greatest portion of production in the world.[3] It is considered as the main cash crop and protein source for farmers in many low lands and mid altitude zones of Ethiopia.[4] The two major bean producing regions are Oromia and Southern Nations, Nationalities and People's Region (SNNPR), which produce 70 and 60 thousand tones, respectively. These two regions make up 85% of the production.[5] It is a principal food crop particularly in Southern and Eastern part of Ethiopia, where it is widely intercropped with maize and sorghum, respectively, to supplement farmer's income.[6] Any study that carried out on the ecological interference of *L. camara* L. on the growth and establishment of native plants, especially on agronomic crops such as haricot bean, is all most no in Ethiopia. Hence, it was thought worthwhile to evaluate the influence of aqueous extracts of leaf of the weed *L. camara* L. on germination parameters of haricot bean (*P. vulgaris* L.) which is one of the important legume crops with the objective to assess haricot bean (*P. vulgaris* L.) genotypes at germination stage under the effects of allelochemical leaf extract from *L. camara* L. at different concentration levels.

METHODS

Description of the study area

This study was conducted at Bule Hora University in the Biology Laboratory which is located on 467 Km from Addis Ababa to south on the road of Moyale and its geographical extent ranges from 08045'15" to 08046'45" north latitude and form 38046'45" to 39001'00" east longitude. It has an altitude 1850 m a.s.l and hot to warm sub-humid climate.

Collection of plant materials

Fresh leaves of *L. camara* L. was collected from different farmers' farm land and adjacent grazing lands in the Bule Hora Woreda, the area where is covered by *L. camara* species. The plant materials were chopped into small pieces with cutter. The materials were dried for about three days in oven dry. The well dried plant samples were gently ground and stored in a plastic container. Similarly dried seeds of seven haricot bean genotypes (Boya, Nasir, Sosiad, White, Black, Kenya and Canada) were collected from different kebeles of local farmers of Bule Hora Woreda with its local name and brought to Bule Hora University biology laboratory.

Preparation of extract from L. camara L.

The ground powder of *L. camara* L was diluted with distilled water to prepare different concentrations. 2 gram of *L. camara* powder was diluted in 100 ml; 75 ml, 50 ml and 25 ml of distil water to generate 0 ml, 25 ml, 50 ml and 75 ml concentrations of *L. camara* L. respectively. Then, it was filtered by filter paper and ready for use.

Sowing of seeds

Eighty four petri dishes were washed with detergent using hot water as protective measure against pathogens and the experiment was laid out in completely randomized block design (CRBD) with three replications. Haricot bean seeds were also cleaned manually after physical purity is checked. Then the seeds were soaked in cold water for 12 hours. Three seeds of each haricot bean genotypes were sown with a 3 cm diameter in each petri dish containing a filter paper. 4 ml of solution was applied to petri dishes and control treatment was received 4 ml of distilled water. The petri dishes were kept at room temperature ($30^{o}C\pm4$) throughout the study and both treated and control petri dishes were kept moist continuously by adding solution and distilled water for treatment and control respectively whenever needed.

Data collection

Germination day

Each petri dish was checked and recorded daily for days to germination after sowing to 9^{th} day.

Number of germinated seed

Numbers of germinated seeds were counted on the 9^{th} day after sowing.

Radicle length

Each of the length of the main radicle of the control and treated plants was measured in unit of centimetre.

Plumule length

Each of the length of the plumules of control and treated plants was measured in unit of centimetre.

Germination percentage

It was calculated using the following formula:

$$\text{Germination } \% = \frac{\text{number of germinated seed}}{\text{Total number of seed}} \text{x } 100$$

Germination index (GI)

It was calculated according to equation of Karim. [7]

$$\text{GI} = \frac{\text{Germination percentage in each treatment}}{\text{Germination percentage in the control}} x100$$

Inhibition (-) or stimulation (+) percent

It was calculated following the formula given below:

$$IOS(\%) = (\text{germinated seed in in treatment} - \text{germinated seed in control})/ (\text{germinated seed in control}) \ X\ 100$$

Shoot and root weight (fresh and dry weight)

Each shoot and roots were cut off separately and measured by electronic balance and then dried in oven for dry weight measurement.

Data analysis

Data analysis was carried out using SPSS and SAS (version 9.0) statistical software (SAS Institute Inc., USA) where two way analysis of variance (ANOVA) will be done. Whenever treatment differences are significant, means will be separated by using the least significant difference (LSD) at 0.05 tests.

RESULTS

The following Table 1 shows the whole analysis of variances where there were significance differences between different concentrations levels of *L. camara* L. leaf extract on germination parameters of haricot bean (*Phaseolus vulgaris* L.) genotypes. Germination day, plumule length and radicle length were significantly influenced by *L. camara* leaf extract

As it has been shown in the Table 2, germination day increases with the increasing of the concentration level of the leaf extract of *L. camara* L. It was increased from control (9.14) to treatment three (10.43) which indicates that high amount of allelochemical in the leaf extracts of *L. camara* L. affect the germination day by shifting it forward when compared to the control. Plumules length, shoot fresh weight and root fresh weight were decreased with the increasing of leaf extract concentration of *L. camara* L. means that they were highly influenced more than the other parameters by this chemical. The inhibitory effect was strictly concentration dependent.

There were variation between different genotypes of haricot beans (*P. vulgaris* L.) as shown Figure 1 where some of them inhibited and some of them with stand in their germination parameters under the effect of *L. camara* L. leaf extract. Genotypes Sosiad and Boya showed high and uniform from control to treatment three in days to germination but Nasir was highly affected as the concentration increases which shifted germination day from 7.67 to 10.33. All genotypes were decreased with their plumules length but genotype Boya showed to has high in radicle length, shoot fresh weight and root dry weight at treatment two with the value of (12.52, 13.10 and 0.4) respectively. Canada was highly influenced at treatment one (-33.33), two (-22.33) and three (-33.33) which their graph was down ward to negative direction while Boya was highly (4377.60) stimulated at treatment two.

Table 1: Analysis of variance showing each parameter of haricot bean (*Phaseolus vulgaris* L.) genotypes under the influence leaf extracts of *Lantana camara* L.

	Parameters									
	GD	**NGS**	**PL**	**RL**	**SHFW**	**SHDW**	**RDW**	**GP**	**GI**	**IOS**
Mean	9.63	2.56	3.50	6.77	7.65	2.23	0.20	86.11	83.33	178.24
Df	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Std. Devi	1.39	0.62	2.58	2.14	2.25	0.86	0.08	20.20	21.81	825.98
Variance	1.92	0.38	6.65	4.57	5.05	0.74	0.01	408.13	475.54	682242.22
Sum of Squa	51.856*	10.21	179.414*	123.299*	136.31	20.04	0.17	11019.59	12839.61	18400000
Mean Square	3.01	0.21	17.56	13.17	2.56	0.09	0.01	263.08	925.90	643574.28
Sum	269.65	71.68	97.98	189.51	214.10	62.50	5.50	2411.10	2333.34	4990.68

Where, GD: Germination Day, NGS: Number of Germinated Seed, PL: Plumule Length, RL: Root Length, SHFW: Shoot Fresh Weight, SHDW: Shoot Dry Weight, RDW: Root Dry Weight, GP: Germination Percentage, GI: Germination Index, IOS: Inhibition or Stimulation.

Table 2: Effects of leaf extract concentration from *L. camara* on germination day, plumule length, shoot fresh weight and root fresh weight of haricot bean (*Phaseolus vulgaris* L.) germination.

Parameters				
Treatments	GD	PL (cm)	SHFW (g)	RFW (g)
Control (0 g/100 ML)	9.14 ± 1.73	5.63 ±3.35	7.94 ±1.79	2.07 ±1.30
Treatment 1 (2 g/75 ML)	9.91 ± 1.46	2.62 ±2.17	6.94 ±3.02	1.43 ±0.39
Treatment 2 (2 g/50 ML)	9.05 ±1.06	3.73 ±1.90	8.31 ±2.74	1.93 ±0.93
Treatment 3 (2 g/25 ML)	10.43 ±0.94	2.03 ±1.25	7.39 ±1.28	1.31 ±0.24

Where, GD: Germination Day, PL: Plumule Length, SHFW: Shoot Fresh Weight, Root RFW: Fresh Weight.

Table 3: correlation analysis of the parameters of haricot bean genotypes under the effect of leaf extracts of *Lantana camara* L.

	CONC	NGS	GD	PL	RL	SHFW	RFW	SHDW	RDW	GP	GI	IOS
CONC	1											
NGS	0.168	1										
GD	0.246	-.709**	1									
PL	-.428*	.550**	-.865**	1								
RL	0.167	.410*	-.406*	0.205	1							
SHFW	-0.015	-0.229	-0.074	-0.004	0.372	1						
RFW	-0.237	0.232	-.394*	0.373	.648**	.566**	1					
SHDW	-0.074	-.663**	.662**	-.685**	-0.176	.383*	0.043	1				
RDW	-0.144	0.194	-.429*	.397*	.574**	.575**	.879**	0.034	1			
GP	0.224	.979**	-.670**	.519**	.421*	-0.235	0.228	-.647**	0.199	1		
GI	-0.273	0.077	-0.307	.528**	-0.363	-0.346	-0.076	-.469*	0.036	0.088	1	
IOS	0.1	0.146	-0.209	-0.012	.515**	.479**	.404*	0.183	.487**	0.137	-.594**	

*Correlation is significant at the 0.05 level, **Correlation is significant at the 0.01 level.

Where, CONC: Concentration, NGS: Number of Germinated Seed, GS: Germinated Seed, PL: Plumule Length, RL: Root Length, SHFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SHDW: Shoot Dry Weight, RDW: Root Dry Weight, GP: Germination Percentage, GI: Germination Index, IOS: Inhibition(-) or Stimulation(+).

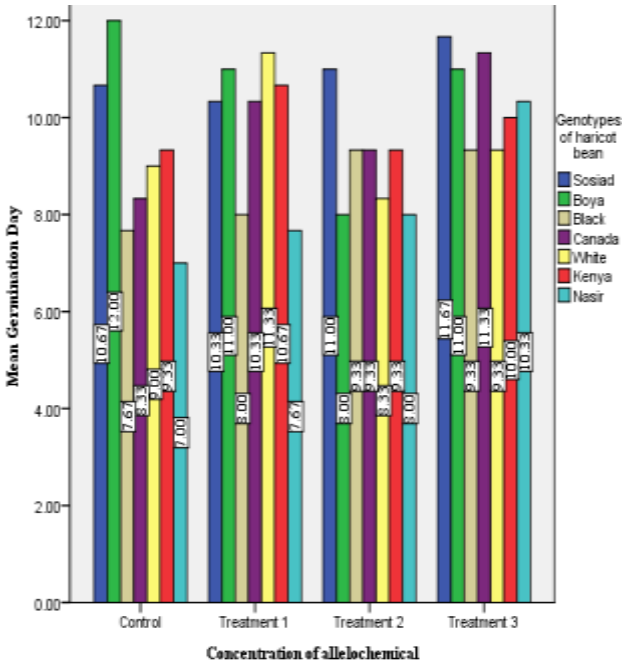

Figure 1a: Mean values of germination day in seven genotypes of haricot bean (*Phaseolus vulgaris* L.) at different concentration levels of *L. camara* L leaf extracts.

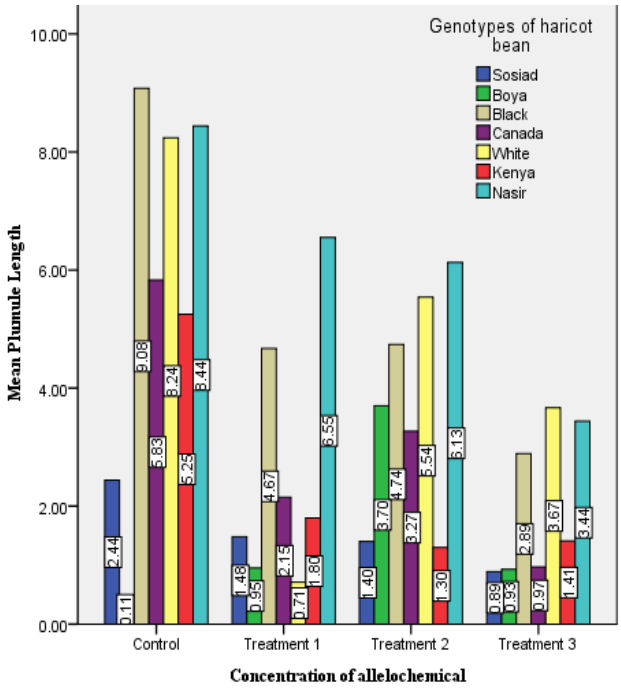

Figure 1b: Mean values of plumule length in seven genotypes of haricot bean (*Phaseolus vulgaris* L.) at different concentration levels of *L. camara* L leaf extracts.

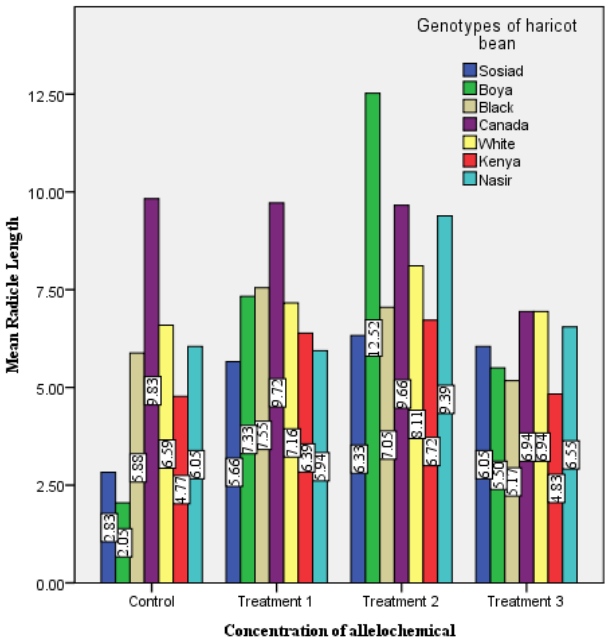

Figure 1c: Mean values of radicle length in seven genotypes of haricot bean (*Phaseolus vulgaris* L.) at different concentration levels of *L. camara* L leaf extracts.

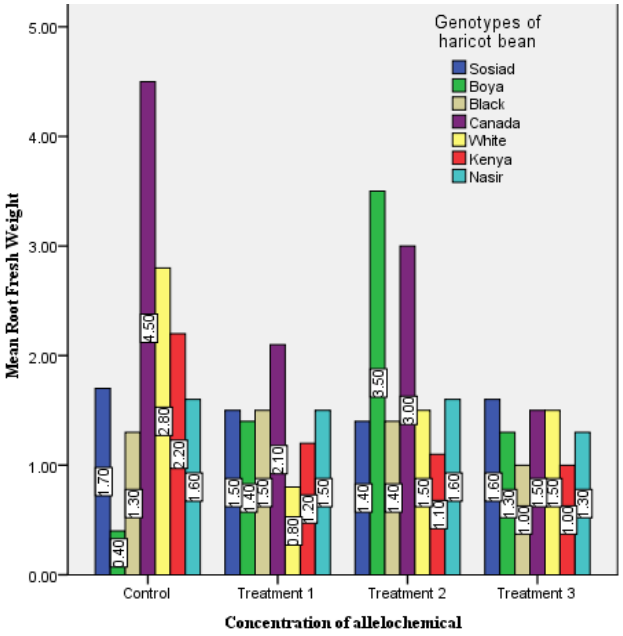

Figure 1e: Mean values of root fresh weight in seven genotypes of haricot bean (*Phaseolus vulgaris* L.) at different concentration levels of *L. camara* L leaf extracts.

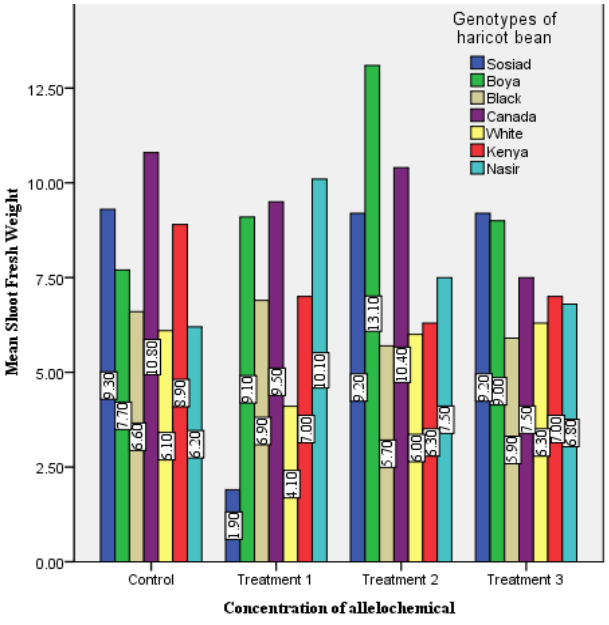

Figure 1d: Mean values of shoot fresh weight in seven genotypes of haricot bean (*Phaseolus vulgaris* L.) at different concentration levels of *L. camara* L leaf extracts.

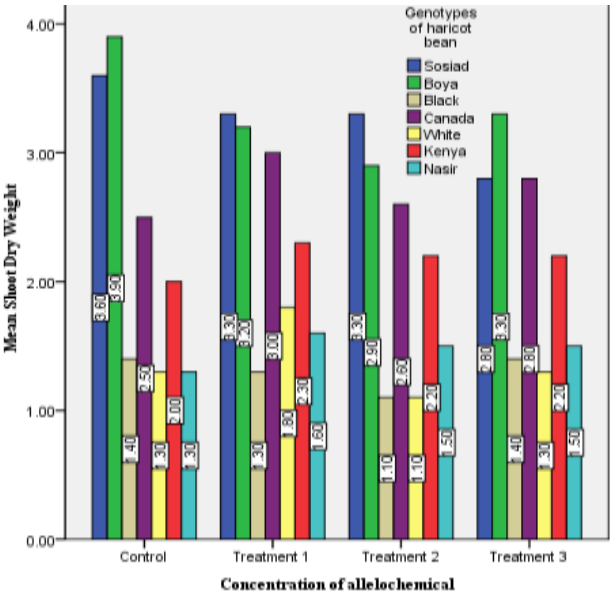

Figure 1f: Mean values of shoot dry weight in seven genotypes of haricot bean (*Phaseolus vulgaris* L.) at different concentration levels of *L. camara* L leaf extracts.

Table 3 shows correlation within parameters of haricot bean and between *L. camara* leaf extract concentrations where concentration was significantly negative (-0.428*) correlated with plumule length and non-significant negative correlated with shoot fresh weight (-0.015), root fresh weight (-0.237), shoot dry weight (-0.074), root dry weight (-0.144) and germination index (-0.273).

DISCUSSION

Germination day increases with the increasing of the concentration level of the leaf extract of *L. camara* L. which indicates that high amount of allelochemical in the leaf extracts of *L. camara* L affect the germination day by shifting it forward when compared to the control as in Table 2. The result was similar with the result of central

statistical authority which says that the leaf extract of *L. camara* L. hindered the germination significantly in all the receptor crops compared to the control treatment.[5] Decrement of plumules length, shoot fresh weight and root fresh weight with the increasing of leaf extract concentration of *L. camara* L were indicating that they were highly influenced more than the other parameters by this chemical which in line with the reports of Cruz-Ortega et al says that, as aqueous extract of *L. camara* L induced the greatest inhibition in bean and tomato radicle growth with 41% and 81%, respectively. Similarly the work of Arpana supports this result which says leaf extract showed pronounced inhibition of shoot length, root length, leaf area; fresh and dry weight of the *Parthenium hysterophorus*.[9] The inhibitory effect was strictly concentration dependent. There were variation between different genotypes of haricot beans (*P. vulgaris* L.) where some of them inhibited and some of them with stand in their germination parameters under the effect of *L. camara* L. leaf extract as in Figure 1.

Genotypes of Sosiad and Boya showed high and uniform from control to treatment three in days to germination but Nasir was highly affected as the concentration increases. All genotypes were decreased with their plumules length but genotype Boya showed to has high in radicle length, shoot fresh weight and root dry weight at treatment two. Canada was highly influenced at treatment one, two and three which their graph was down ward to negative direction while Boya was highly stimulated at treatment two. This shows that allelochemical concentration in the leaf extract of *L. camara* has invading property of haricot bean genotypes. Similarly the study of Arpana supports this result which says as root length, fresh and dry weight had inhibited was strictly concentration dependent in another crops.[9] Correlation within parameters of haricot bean and between *L. camara* leaf extract concentrations where concentration was significantly negative correlated with plumule length and non-significant negative correlated with shoot fresh weight, root fresh weight, shoot dry weight, root dry weight and germination index as in Table 3. This shows that the parameters were highly influenced by the allelochemical concentration found in the leaf of *L. camara* L.

CONCLUSION

Generally the present study was done on Haricot Bean (*P. vulgaris* L.) Genotypes at Germination stage under the effect of allelopatic leaf extract of lantana (*L. camara* L.) with the general objective to assess haricot bean genotypes at germination stage under the effects of allelochemical extract from *L. camara* L. at different concentration levels. The result showed that concentrated aqueous leaf extract of *L. camara* L. weed species inhibited germination day, plumule length, shoot fresh weight and germination index of haricot bean (*P. vulgaris* L.). High concentration levels of this chemical has high influential power on the germination parameters of this crop.

Recommendations

Keeping the above in view, it can be recommended that seeds of haricot bean (*P. vulgaris* L.) should not be planted close to *L. camara* L. due to adverse effect on their growth. And also since this experiment was carried out only in petridish and on aqueous extract of leaf, another study which can show detail of *L. camara* L. weed influence on haricot bean (*P. vulgaris* L.) genotypes is kept back.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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