

Allelopathic Effect of (*Casimiroa edulis* Llave et Lex.) Leaf Extracts on Germination and Seedling Growth of Maize (*Zea mays* L) and Sorghum (*Sorghum bicolor* (L) Moench)

Sintayehu Worku

Haramaya University

Meseret Chimdesa

Haramaya University

Zekeria Yusuf

zakoyusuf@yahoo.com

Haramaya University

Research Article

Keywords: Allelopathic index, Correlation coefficient, Germination inhibition rate, Relative length of plumule, germination speed, relative length of radical, Mean germination rate, Principal component

Posted Date: September 5th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4819413/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

The changes over time of both composition and quantity of allelochemicals can either increase or decrease the phytotoxicity of decomposing plant litter. The present study was undertaken to investigate the allelopathic effects of leaf extracts of *C. edulis* on sorghum and maize seed germinations. The aqueous extract of white sapota (*Casimiroa edulis* Llave & Lex) leaf samples were used as a source of allelochemicals. Data collection involved the germination and seedling growth parameters including Germination rate (GR); germination inhibition rate (GIR); MGT: mean germination time (MGT); germination index (GI); Relative length of plumule (RLP); germination speed (v); relative length of radicle (RLR); plumule inhibition rate (PIR); radicle inhibition rate (RIR); allelopathic index (AI). The result indicated that highest concentration of the allelopathic extract (20 mg/L) has demonstrated significantly the highest germination inhibition rate (56.67%) for sorghum; GIR (36.67%) and MGT (3.90 days) for maize seeds, while the highest PIR (76.05%) and RIR (89.60%) for sorghum, as PIR (56.79%), RLR (80.09%) for maize seeds. The allelopathic intensity was found to be the highest (50%) for sorghum, and (36.67%) for maize seed germination. For sorghum seed germination, the first principal component (PC1) has got high positive loads from germination rate (0.36), RLP(0.36), AI(0.36), RLR (0.35), and GI (0.31). The PC2 has got the highest positive load (0.67) from germination speed, but high negative load from MGT(-0.68). In allelopathic effect on maize seed germination, PC1 has got highest scores from AI (0.51), and GI (0.51), but highest negative scores from relative length of plumule (-0.51). It can be concluded that the highest negative scores in PCs which is GIR indicate direct relationship between the negative allelopathic index and negative score factors. However, the highest positive scores in PCs indicate positive AI in both sorghum and maize seed germinations.

1. INTRODUCTION

Casimiroa edulis Llave & Lex is a fruit tree belonging to the family Rutaceae, native to eastern Mexico and Central America.^{1,2} Family Rutaceae; is a small family made up of cultivated fruiting trees and medicinal herbs frequently called the citrus family, which has great economic importance because of its several edible fruits.³ *C. edulis* is a non-citrus fruit that belongs to this family, it is commonly known as Zapote blanco or white sapota. *C. edulis* is widely consumed in different parts of the world for its valuable fruit,⁽⁴⁾ as it is a rich source of sugar, protein, ascorbic acid, phenols, carotenoids, polyunsaturated fatty acids, and minerals like Fe, Cu, Zn, Ca, and K, as traditional medicinal plants.⁵

Allelopathic effects are due to inhibitory or stimulatory phytochemicals produced by one plant against others that are released directly or indirectly into the environment through root exudation, leaching, volatilization, and residual decomposition of the donor plant.^{6,7} Most allelochemicals are secondary plant metabolites, including alkaloids, phenolics, flavonoids, terpenoids, and glucosinolates.⁸ Allelochemicals are produced by plants as end products, by-products, and metabolites and are contained in the stem, leaves, roots, flowers, inflorescence, fruits, and seeds of the plants. Among these plant parts, leaves seem to be the most consistent producers of these allelochemicals. Allelochemicals may escape from a plant as volatilization, leaching, exudation, decomposition.^{6,8}

Allelopathic effects can be used for controlling weeds, improving crop yield, lessening our dependence on synthetic pesticides, and recovering the biological environment.^{9,10} Allelopathic activity is often the combination and interaction of many allelochemicals which appear to work in an additive or synergistic manner to reduce weed germination and growth.¹¹ Allelopathic activities can be selective, species-specific, and concentration-dependent.¹² Allelochemicals have been reported to affect neighboring plant individuals,^{13,14} and soil microbial communities, including bacteria, nematodes.¹⁵ Allelochemicals can be either actively released by plants¹⁶ or passively produced during the decomposition process of both above- and below-ground plant residues.¹⁷ In East Hararghe region, Ethiopia, *C. edulis*

is commonly grown in crop land and gardens for cultivation of cereals like maize and sorghum, but its allelopathic effect on seed germination and early seedling growth of these crops has not been investigated. This study is, therefore, undertaken to investigate the allelopathic effects of leaf extracts of *C. edulis* on maize and sorghum seed germination.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

The experiment was conducted at the Botany laboratory in the School of Biological Sciences and Biotechnology, Haramaya University, Ethiopia. The white sapota (*Casimiroa edulis*) leaf samples were collected from a farmer's field in Erer district, Harari regional state, Ethiopia. Voucher specimens were deposited in Haramaya University Herbarium, and the *Casimiroa edulis* leaf samples were identified by Mr. Abdulkadir Hassen." 500gram seed samples of *Afrankalo* (maize variety) and *Muyra* 1 (sorghum variety) were obtained from Raare Research Station, Haramaya University. The leaf samples of *C. Edulis* were initially washed with distilled water, and hot air dried in a Mild Steel Hot Air Dryer (Nikul Pharma Equipment, India) at 60°C. The leaf samples were then powdered using mortar and pestle. 100gm powder was mixed with 500 mL of solvent (distilled water) and left on the bench in the laboratory for 24 hours with intermittent stirring using a glass rod. After 24 hours of soaking, the extract was filtered under suction using Whatmann no.1 filter paper, and the solvent in the filtrate was evaporated under reduced pressure using a rotary evaporator (Fisher Scientific, UK) at 100°C to remove the solvent and the dried extract was then stored in the refrigerator until bioassay. The experiment was replicated three times. For bioassay, the dried extract was reconstituted in distilled water to get 10, 15, and 20 mg/L extract concentrations. For example, to prepare 10mg/L of the crude extract, 10g of the crude extract was dissolved in a liter of water.

2.2. Bioassays in the Laboratory

2.2.1. Sterilization of Test Plants (Seeds)

The experiment was carried out following the internationally accepted guide for seed testing.¹⁸ For this purpose, 420 healthy seeds of maize and sorghum cultivars were surface sterilized in 1:20 dilution of commercial sodium hypochlorite (NaClO₂) for 10 min and rinsed with distilled water a number of times, and then, the seeds were allowed to soak for 2 hours on moistened paper towels.

2.2.2. Seed Germination and Seedling Growth Bioassays

Sterile filter papers (Whatman No. 1) containing 10 seeds per plate were placed in each sterilized 9 cm Petri plate in a completely randomized design in three replications. Extract (10mL) of each concentration was added to the filter paper in Petri plates. Sterile distilled water of equal volume was used as a negative control. The experimental samples were incubated for days at grown at 25°C to complete germination. Petri dishes were made to get more or less the same amount and throughout the incubation period and moved around to avoid the position effect. Moisture in the Petri dishes was maintained by adding 2 mL of the extract or distilled water every 2 days. After days of incubation, percent germination and other germination and growth parameters were measured for all 10 seed per plate.

2.3. Data collection

(i) Germination rate (GR): the germination rate was calculated according to the formula given by Come.¹⁹

$$\text{GRc (\%)} = \frac{N_{gc}}{N_s} \times 100$$

$$GRt (\%) = \frac{N_{gt}}{N_s} \times 100$$

Where GRc: germination rate of control plants; GRt: germination rate of treatment plants; Ngc: number of germinated seeds in a control sample; Ng: Number of germinated seeds in the treated sample; Ns: total number of seeds sown.

(ii) Germination inhibition rate: according to Ben Khettou²⁰ and Alemayehu et al²¹, the inhibition rate (GIR) is calculated by the following formula:

$$GIRc (\%) = 100 - GRc$$

$$GIRt (\%) = 100 - GRt$$

Where GIRc: germination inhibition rate of the control; GIRt: germination inhibition rate of treatment sample.

(iii) Germination index: the germination index (GI) is a quantitative expression of germination that relates to the daily germination rate at the maximum value of noted germination²², it is calculated by the following equation:

$$GI = N_1 + \frac{N_2 - N_1}{2} + \frac{N_3 - N_2}{3} + \dots + \frac{N_n - N_{n-1}}{n}$$

Where Nn: Percentage of germination on the nth day

(iv) Mean Germination Time (MGT): calculated as described by Ranal et al⁽²³⁾:

$$MGT = \frac{\sum_{i=1}^k n_i t_i}{\sum_{i=1}^k n_i}$$

Where n_i and t_i are consecutively the numbers of newly germinated seeds in the last time and the time from the beginning of the experiment to the last observation; k: last time of germination.

(v) Allelopathic index (AI) was calculated to reflect the intensity of the allelopathic effect according to the work of Luo et al⁽²⁴⁾ as follows:

$$AI (\%) = \left[\frac{(GRt - GRc)}{GRc} \right] \times 100$$

Where GRt is the germination rate of treatment extract; GRc is the germination rate of the control (given sterile distilled water). AI < 0 reflects a negative allelopathic effect of the extracts on seed germination, and an AI > 0 value indicates a promoting effect on the germination, while the absolute value of AI indicates the allelopathic intensity.

(vi) Kinetics of germination: according to Come⁽¹⁹⁾ it is the variation of the germination rate of seeds as a function of time.

Germination speed (v): the germination speed is calculated by the following formula proposed by Come¹⁹:

$$V = \frac{N_1 + N_2 + N_3 + \dots + N_n}{N_1 T_1 + N_2 T_2 + N_3 T_3 + \dots + N_n T_n}$$

Where V: Speed of germination; N: The number of seeds germinated at time T.

The relative length of radicles (RLR)

the relative length of radicles is calculated according to the formula given by Rho and Kil²⁵

$$RLR = \frac{L_r}{L_c} \times 100$$

Where RLR: Relative length of radicle; L_r: Average length of radicles of treated plants; L_c: Average length of radicle of control plants.

The relative length of plumule (RLP)

according to Rho and Kil ²⁵ this parameter is calculated by the following formula

$$RLP = \frac{L_p}{L_c} \times 100$$

Where RLP: Relative length of plumule; L_p: Average length of plumules of treated plants; L_c: Average length of plumule of control plants.

The growth inhibition Rate

this parameter is calculated according to the formula given by Abiyu and Nagappan ⁽²⁶⁾

PIR (%) = 100-RLP, for inhibition of plumule length;

RIR (%) = 100-RLR, for inhibition of radicle elongation.

Where PIR: plumule inhibition rate; RIR: radicle inhibition rate.

2.4. Statistical Analysis

Data were subjected to a one-way analysis of variance by using SAS version 9.2. Mean separation was done and statistical significance was determined at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Allelopathic effect of white sapota (*C. edulis* L1ave& Lex) leaf aqueous extract on germination of sorghum and maize seeds

The allelopathic effects of *C. edulis* leaf extract on germination of sorghum and maize seeds (Table 1) were assessed using germination parameters like germination rate (GR), germination inhibition rate (GIR), germination speed, mean germination time (MGT), and germination index (GI). For both bioassayed plants the control group has recorded significantly the highest GR (93.33 for sorghum, and 100% for maize); germination speed (0.41 U/day for sorghum, and 0.70 U/day for maize); GI (1.58% for sorghum, and and 3.56% for maize). On the otherhand, the maximum concentration of the extract has demonstrated significantly the highest GIR (56.67% for sorghum, and 36.67% for maize); MGT (3.64 for sorghum, and 3.90 for maize) indicating that GIR and MGT of the extracts increased with increasing extracts concentrations. Thus, MGT and GIR can be recommended as the best indicators of allelopathic effect of *C. edulis* leaf extracts on seed germination.

Table 1
The allelopathic effect of *C. edulis* leaf extract on germination of sorghum and maize seeds

Source	Allelopathic extract (mg/L)	GR (%)	GIR (%)	Germination speed (U/day)	MGT (day)	GI (%)
Sorghum	0	93.33 ± 0.02 ^a	6.67 ± 0.004 ^d	0.41 ± 0.006 ^a	2.48 ± 0.002 ^c	1.58 ± 0.002 ^a
	10	76.67 ± 0.03 ^b	23.33 ± 0.01 ^c	0.27 ± 0.003 ^b	3.31 ± 0.001 ^b	1.13 ± 0.004 ^{ab}
	15	60.00 ± 0.01 ^c	40.00 ± 0.04 ^b	0.31 ± 0.007 ^{ab}	3.36 ± 0.002 ^b	0.73 ± 0.006 ^{bc}
	20	43.33 ± 0.03 ^d	56.67 ± 0.02 ^a	0.29 ± 0.003 ^b	3.64 ± 0.003 ^a	0.37 ± 0.001 ^c
Maize	0	100.00 ± 0.01 ^a	0.00 ± 0.00 ^d	0.70 ± 0.002 ^a	1.43 ± 0.003 ^d	3.56 ± 0.004 ^a
	10	96.67 ± 0.03 ^a	3.33 ± 0.003 ^c	0.47 ± 0.002 ^b	2.14 ± 0.002 ^c	2.35 ± 0.006 ^b
	15	86.67 ± 0.05 ^b	13.33 ± 0.01 ^b	0.33 ± 0.006 ^c	3.01 ± 0.001 ^b	1.21 ± 0.003 ^c
	20	63.33 ± 0.06 ^c	36.67 ± 0.04 ^a	0.26 ± 0.001 ^d	3.90 ± 0.005 ^a	0.69 ± 0.001 ^d

Means followed by the same letter within a column were not significantly different at 0.05 probability level based on the Least Significant difference (LSD) t-test. a,b, c & d: significance within the column. GR: Germination rate; GIR: germination inhibition rate; MGT: mean germination time; GI: germination index.

3.2. Effect of *C. edulis* aqueous leaf extract on seedling growth of sorghum and maize

In concomitant with seed germination, the initial growth of seedlings appeared to show a similar trend to the allelochemicals from *C. edulis*. The allelopathic effects of *C. edulis* leaf extract on plumule and radicle growth are shown in Table 2. The allelopathic effect of *C. edulis* leaf extract on seedling growth of both bioassayed plants including sorghum and maize varieties as in Table 2. The maximum RLP (50.20%), RLR (24.80%) were recorded for sorghum seed germination; RLP (66.15%), RLR (30.81%) for maize were recorded with the control group. As the least RLP (23.95%), RLR (10.38%) were recorded for sorghum seed germination; RLP (43.21%), RLR (19.92%) for maize were recorded with maximum extract concentration (20 mg/L). The reduction in the relative growth of both plumule and radicle with increase in concentration of the extract might indicate the growth inhibitory effect of allelochemicals in the crude leaf extract of *C. edulis*.

The highest PIR (76.05%), RIR (89.62%), negative allelopathic index (-50.00) or allelopathic intensity (ΣAI) (50.00) for sorghum; PIR (56.79%), RIR (80.09%), allelopathic intensity (ΣAI) (-36.67) for maize were recorded with the maximum concentration of the allelopathic extract (20mg/L) indicating that PIR, RIR and ΣAI as the best parameters to measure the negative allelopathic effect on seed germination and seedling growth of crop plants.

Table 2
The effect of allelopathic extract from *C. edulis* leaf on plumule and radicle growth

Source	Extract concentration (mg/L)	RLP (%)	RLR (%)	PIR (%)	RIR (%)	AI (AI)
Sorghum	10	50.20 ± 0.03 ^a	24.80 ± 0.01 ^a	49.80 ± 0.05 ^c	75.20 ± 0.04 ^c	-11.11c (11.11) ± 0.03
	15	36.27 ± 0.01 ^b	18.60 ± 0.02 ^b	63.74 ± 0.03 ^b	81.40 ± 0.02 ^b	-31.02b (31.02) ± 0.01
	20	23.95 ± 0.03 ^c	10.38 ± 0.05 ^c	76.05 ± 0.06 ^a	89.62 ± 0.01 ^a	-50.00a (50.00) ± 0.02
Maize	10	66.15 ± 0.04 ^a	30.81 ± 0.02 ^a	33.85 ± 0.04 ^c	69.19 ± 0.02 ^c	-3.33 (3.33) ± 0.002 ^c
	15	53.16 ± 0.02 ^b	26.68 ± 0.04 ^b	46.84 ± 0.02 ^b	73.32 ± 0.01 ^b	-13.33 (13.33) ± 0.02 ^b
	20	43.21 ± 0.03 ^c	19.92 ± 0.03 ^c	56.79 ± 0.01 ^a	80.09 ± 0.04 ^a	-36.67(36.67) ± 0.01 ^a

Means followed by the same letter within a column were not significantly different at 0.05 probability level based on the Least Significant difference (LSD) t-test. a,b, c & d: significance within the column. RLP: the relative length of the plumule; RLR: the relative length of the radicle; PIR: plumule inhibition rate; RIR: radicle inhibition rate; AI: allelopathic index.

3.3. Allelopathic index for sorghum and maize seed germination

The allelopathic index (AI) for sorghum seed germination (Fig. 1) and maize seed germination (Fig. 2) demonstrated negative allelopathic activity as the concentration of the allelopathic extract from *C. edulis* leaf increased from 10 to 20mg/L. The allelopathic intensity was found to be the highest (50%) for sorghum, and (36.67%) for maize seed germination. The large negative allelopathic activity of *C. adulis* might contribute to the retardation of growth and yield loss of crop plants near *C. adulis* fruit tree crops.

3.4. Association among germination parameters and allelopathic index of *C. edulis* leaf extract

Pearson's correlation coefficient (Tables 3 & 4) and the principal component analysis (Table 5) were used to assess the association of seed germination parameters and allelopathic index of *C. edulis* leaf aqueous extract. The negative allelopathic index (AI) for sorghum seed germination (Table 3) was significant and negatively correlated with germination inhibition rate (-0.974), plumule inhibition rate (-0.942), and radicle inhibition rate (-0.913) indicating that the negative allelopathic activity increases with increasing germination inhibition rates. However, the allelopathic index was found to be significant and positively correlated with germination rate (0.974), germination index (0.813), relative length of plumule (0.942), and relative length of radicle (0.913) indicating that the negative allelopathic activity decreases with increasing rate of germination, germination index, plumule and radicle elongations during sorghum seed germination. Thus, germination inhibition rate (GIR), plumule inhibition rate (PIR), and radicle inhibition rate (RIR) were found to be the best parameters or traits that can be used as indicators of allelopathic activity during sorghum seed germination.

Table 3
Pearson's correlation coefficient of allelopathic index with germination parameters for sorghum

	GR	GIR	V	MGT	GI	RLP	RLR	PIR	RIR	AI
GR	1	-1.00**	-0.409	0.358	0.827**	0.930**	0.888**	-0.930**	-0.888**	0.974**
GIR		1	0.409	-0.358	-0.827**	-0.93	-0.888**	0.930**	0.888**	-0.974**
V			1	-0.994**	-0.037	-0.125	-0.063	0.125	0.064	-0.279
MGT				1	-0.03	0.065	0.02	-0.065	-0.02	0.229
GI					1	0.836**	0.740*	-0.836**	-0.740*	0.813**
RLP						1	0.977**	-1.00**	-0.977**	0.942**
RLR							1	-0.977**	-1.00**	0.913**
PIR								1	0.977**	-0.942**
RIR									1	-0.913**
AI										1

*significant at $P < 0.05$; **significant at $P < 0.01$. GR: Germination rate; GIR: germination inhibition rate; MGT: mean germination time; GI: germination index; RLP: relative length of plumule; RLR: relative length of radicle; PIR: plumule inhibition rate; RIR: radicle inhibition rate; AI: allelopathic index.

The negative allelopathic index (AI) for maize seed germination (Table 4) was significant and negatively correlated with germination speed (-0.872), mean germination time (-0.870), relative length of plumule (-1.00) and plumule inhibition rate (-0.930) indicating that the negative allelopathic activity increases with increasing germination speed, mean germination time, relative length of plumule and plumule inhibition rate. By contrast, the allelopathic index was found to be significant and positively correlated with germination rate (0.872), germination inhibition rate (0.870), germination index (1.00), relative length of radicle (0.883) and radicle inhibition rate (0.872) indicating that the negative allelopathic activity decreases with increasing rate of germination rate, germination inhibition rate, germination index, relative length of radicle and radicle inhibition rate while it decreases with increasing rate of germination rate, germination inhibition rate, germination index, relative length of radicle and radicle inhibition rate in maize seed germination.

Table 4
Correlation coefficient of allelopathic index with germination parameters for maize

	GR	GIR	V	MGT	GI	RLP	RLR	PIR	RIR	AI
GR	1	0.934**	-1.00**	-0.934**	0.872**	-0.872**	0.974**	-0.974**	0.937**	0.872**
GIR		1	-0.934**	-1.00**	0.870**	-0.870**	0.908**	-0.935**	0.847**	0.870**
V			1	0.934**	-0.872**	0.872**	-0.974**	0.974**	-0.937**	-0.872**
MGT				1	-0.870**	0.870**	-0.908**	0.935**	-0.847**	-0.870**
GI					1	-1.00**	0.883**	-0.930**	0.872**	1.00**
RLp						1	-0.883**	0.930**	-0.872**	-1.00**
RLr							1	-0.988**	0.984**	0.883**
PIR								1	-0.964**	-0.930**
RIR									1	0.872**
AI										1

*significant at $P < 0.05$; **significant at $P < 0.01$. GR: Germination rate; GIR: germination inhibition rate; MGT: mean germination time; GI: germination index; RLP: relative length of plumule; RLR: relative length of radicle; PIR: plumule inhibition rate; RIR: radicle inhibition rate; AI: allelopathic index.

Even though the correlation coefficient indicated the existence of association among traits. It doesn't indicate the explicit direct and indirect relationships among them. Therefore, the principal component analysis (PCA) was used to identify the most discriminant direct and indirect relationship among traits for measuring the allelopathic index of the allelopathic plant extract (Table 5). Eigenvalues corresponding to the sum of squares of variances greater than one being responsible for the explanation of the majority of the variances were considered for interpretation of the result. In PCA of sorghum, the first two PCs (with eigenvalue > 1) account for 96% of the variances while in maize the first three PCs (with eigenvalue > 1) account for about (100%) of the variations hence the first two PCs were used in sorghum while in maize the first three PCs were considered for the interpretation of the result.^{21,35}

For sorghum seed germination, the first principal component (PC1) has high positive loads from germination rate (0.36), relative length of plumule (0.36), allelopathic index (0.36), relative length of root (0.35), and germination index (0.31). However, in PC1 the highest negative component loads were recorded by germination inhibition rate (-0.36), plumule inhibition rate (-0.36), and radicle inhibition rate (-0.35). The second PC has got the highest positive load (0.67) from germination speed, but a high negative load from mean germination time (-0.68). The negative loads indicate that the negative allelopathic activity increases with increasing germination, plumule, and radicle inhibition rates. As the positive loads indicate that the positive allelopathic index increases with increment of germination rate, relative length of plumule, allelopathic index, relative length of root, and germination index.

Table 5
The principal component analysis (PCA) for the allelopathic activity of *C. edulis* leaf aqueous extract on germination of sorghum and maize seeds

Parameters	Sorghum		Maize		
	PC1	PC2	PC1	PC2	PC3
Eigenvalue	7.46	2.09	937.41	43.55	4.90
Difference	5.37	1.73	893.86	38.66	4.83
Proportion	0.75	0.21	0.95	0.04	0.01
Cumulative	0.75	0.96	0.95	0.995	1.00
GR	0.36	-0.11	0.30	-0.58	0.26
GIR	-0.36	0.11	0.15	-0.22	-0.66
V	-0.10	0.67	-0.30	0.58	-0.26
MGT	0.08	-0.68	-0.15	0.22	0.66
GI	0.31	0.14	0.51	0.27	0.02
RLP	0.36	0.10	-0.51	-0.27	-0.02
RLR	0.35	0.13	0.003	-0.004	0.003
PIR	-0.36	-0.10	-0.03	0.03	-0.01
RIR	-0.35	-0.13	0.02	-0.03	0.06
AI	0.36	-0.02	0.51	0.28	0.02

GR: Germination rate; GIR: germination inhibition rate; MGT: mean germination time; GI: germination index; RLP: relative length of plumule; RLR: relative length of radicle; PIR: plumule inhibition rate; RIR: radicle inhibition rate; AI: allelopathic index.

In the allelopathic effect on maize seed germination, PC1 has the highest scores from the allelopathic index (0.51), and germination index (0.51), but the highest negative scores from the relative length of plumule (-0.51). Similarly, PC2 has high positive scores from germination speed (0.58), AI (0.28), and germination index (0.27) while negative scores from GR (-0.58) and GI (-0.27). The third principal component (PC3) has a high positive score from MGT (0.66) but the highest negative score from GIR (-0.66). Therefore, the highest negative score in PCs which is GIR indicates a direct relationship between the negative allelopathic index and negative score factors. However, the highest positive scores in PCs indicate an inverse relationship between allelopathic index and positive score factors.

4. Discussion

The maximum concentration of the extract has demonstrated significantly the highest GIR; MGT indicating that GIR and MGT of the extracts increased with increasing extracts concentrations. Thus, MGT and GIR can be recommended as the best indicators of allelopathic effect of *C. edulis* leaf extracts on seed germination of both sorghum and maize. Similar finding was reported by Al-Amin ⁽²⁷⁾ who reported leaf extracts of *Anthocephalus cadamba* were significantly inhibited the germination and seedling growth of pulses. The inhibition of seed germination is generally attributed to

the change in pH, presence of phenolic content in plant extracts and due to alterations in the enzymatic activity of seeds.^{28–30}

On the other hand, germination inhibition was the least for the distilled water control group and increased progressively with increasing extract concentration. There was also a negative allelopathic effect on germination speed for both sorghum and maize seeds. That is compared to the control, the germination speed of seeds treated with extract decreased progressively with increasing extract concentration (Table 1). In contrast to germination speed, the mean germination time was significantly lower under control treatment when compared to extract-treated seeds of sorghum and maize, and delay in germination increased with increasing extract concentrations. Previous work on the allelopathic influence of *Eucalyptus* spp. has significantly inhibited the germination speed, radical and plumule length of crops with increased concentrations of leaf extracts.^{31,32}

Compared to the control, plumule and radicle lengths of both sorghum and maize were significantly ($P < 0.05$) lower than extract-treated seeds, and plumule and radicle growth inhibitory effect significantly ($P < 0.05$) increased with increasing extract concentration (Table 2). This finding was in accordance with Gebreyohanne et al.⁽³³⁾ who reported that leaf extracts of *Lantana* had negative effects on *L. sativum*'s seed germination and early seedling growth. Inhibitory effects of allelochemicals at the germination and early seedling growth stage may predispose crops to other abiotic and biotic stresses and hence reduce their productivity.³⁴

The present study has investigated allelopathic effect of *C. edulis* aqueous leaf extract on germination of sorghum and maize seeds, and identified allelopathic intensity and its quantification during seed germination. However, studies are required on the identification of the major phytotoxicity of *C. edulis* leaf extract using chromatographic techniques; the effect of aerobic and anaerobic decomposition on allelopathic activity of allelopathic plants; the allelopathic effect on growth, yield and nutritional quality of the crop plant.

Conclusion

The large negative allelopathic activity of *C. adulis* might contribute to the retardation of growth and yield loss of crop plants near *C. adulis* fruit tree crop. The germination inhibition rate (GIR), plumule inhibition rate (PIR), and radicle inhibition rate (RIR) were found to be the best traits that can be used as indicators of allelopathic activity during sorghum seed germination. By contrast for maize seed germination, the negative allelopathic activity increases with increasing germination speed, mean germination time, relative length of plumule, and plumule inhibition rate. The highest negative score in PCAs which is germination inhibition rate indicates a direct relationship between the negative allelopathic index and negative score factors. However, the highest positive scores in PCs indicate an inverse relationship between negative allelopathic index and positive score factors for both sorghum and maize seed germinations.

Declarations

Authors' contribution

Zekeria Yusuf: initiation and design of the study, Lab experiment, data analysis; Sintayehu Worku: Lab experiment, data collection, and write-up of the document; Meseret Chimdesa: Analysis and interpretation of data. All authors contributed to drafting the article and revising it critically for important intellectual content.

Ethics approval and consent to Participate

The authors confirm that all methods were carried out by relevant guidelines and regulations of Haramaya University Ethical Committee. The experimental protocols were approved by the Haramaya University Research Office. The collection of the plants used in the study complies with local or national guidelines with no need for further affirmation. All the guidelines were followed as per the University research ethics for collection, characterisation and documentation of landraces or germplasm accessions.

Consent for Publication

Not applicable

Acknowledgements

The authors are grateful to Haramaya University Research Office for their financial support and Laboratory facility.

Human and Animal Rights

No humans and animals were used in this study

Research Involving Plants

The plant species used in this study are not en-dangered.

Funding

This project was funded by a Haramaya University Research grant, under project code: HURG_2021_06_01_90

Data Availability

The data supporting the findings of this study will be available on request from the corresponding author.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

References

1. Aiko I, Shamon LA, Boyang YU, Greenwood EM, Lee SK, Breenen RB, Mehta RG, Farnsworth NR, Fong HS, Pezzuto J, Kinghorn D. Antimutagenic constituents of *Casimiroa edulis* with potential cancer chemopreventiv activity. *J Agric Food Chem* 1998; 46: 3509–3516
2. Elkady WM, Ibrahim EA, Gonaïd MH, El-Baz FK. Chemical Profile and Biological Activity of Casimiroa Edulis Non-Edible Fruit`s Parts. *Adv Pharm Bull*, 2017; 7(4), 655-660. doi: 10.15171/apb.2017.079 <http://apb.tbzmed.ac.ir>
3. Pollio A, De Natale A, Appetiti E, Aliotta G, Touwaide A. Continuity and change in the Mediterranean medical tradition: *Ruta* spp. (Rutaceae) in Hippocratic medicine and present practices. *J Ethnopharmacol*, 2008; 116(3): 469-82. doi: 10.1016/j.jep.2007.12.013
4. Romero ML, Escobar LI, Lozoya X, Eniquez RG. High-performance liquid chromatographic study of *Casimiroa edulis*: I. Determination of imidazole derivatives and rutin in aqueous and organic extracts. *J Chromatogr A* 1983; 281:245-51.
5. Moo-Huchin VM, Estrada-Mota I, Estrada-Leon R, et al. Determination of some physicochemical characteristics, bioactive compounds, and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food Chem*, 2014; 152:508-15.

6. Chon SU, Nelson CJ. 'Allelopathy in Compositae plants. A review, *Agron. Sustain. Dev.* 2010; 30, 349 – 358.
7. Cheng F, Cheng Z. 'Research Progress on the Use of Plant Allelopathy in Agriculture and the Physiological and Ecological Mechanisms of Allelopathy, *Front. Plant Sci.* 2015; 6: 1020 – 1035.
8. Amb MK, Ahluwalia AS. 'Allelopathy: Potential Role to Achieve New Milestones in Rice Cultivation', *Rice Sci.* 2016; 23, 165 – 183.
9. Sodaiezadeh H, Hosseini Z. Allelopathy an environmentally friendly method for weed control. *In Tech Open*; 2012.
10. Aslam Z, Zaman Q, Ihsan MZ, Syed S, Fujii Y, Khaliq A, et al. Efficacy of rice straw extracts in controlling weeds. *Pakistan J Weed Sci Res.* 2016; 22(2):197-210.
11. Ihsan MZ, Khaliq A, Mahmood A, Naeem N, El Nakhlawy F, Alghabari A. Field evaluation of allelopathic plant extracts alongside herbicides on weed management indices and weed–crop regression analysis in maize. *Weed Biol Manage.* 2015; 15(2):78-86.
12. Duke SO, Baerson SR, Rimando AM, Pan Z, Dayan FE, Belz RG. Biocontrol of weeds with allelopathy: conventional and transgenic approaches. In *Novel biotechnologies for biocontrol agent enhancement and management*. Springer, Dordrecht. 2007; 75- 85.
13. Schenk HJ, Callaway RM, Mahall BE. Spatial root segregation: are plants territorial? *Advances in Ecological Research* 1999; 28: 146–180.
14. Vivanco JM, Bais HP, Stermitz FR, Thelen GC, & Callaway RM. Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters*, 2004; 7, 285– 292.
15. Shaukat SS, Siddiqui IA, Khan GH, Zaki MJ. Nematicidal and allelopathic potential of *Argemone mexicana*, a tropical weed. *Plant and Soil* 2002; 245: 239–247.
16. Bais HP, Royal V, Gilroy S, Callaway MR, Vivanco JM. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* 2003: 1377–1380.
17. Blum U, Shafer SR, Lehman ME. Evidence for inhibitory allelopathic interactions involving phenolic acids in field soils: concepts vs an experimental model. *Critical Reviews in Plant Science*, 1999; 18: 673–693.
18. International Seed Testing Association (ISTA), *International Rules for Seed Testing*, International Seed Testing Association, Bassersdorf, Switzerland, 2021.
19. Come D. Les obstacles à la germination (Monographie et physiologie végétale n 6). Éd. Masson et Cie (Paris), 1970; pp:14, 24 and 27.
20. Ben Khettou H. Contribution à l'étude de l'aptitude à la germination des graines d'*Agrania spinosa* L. (SAPOTACEAE) dans la région d'Ouargla. Mémoire ing. éco., univ. Ouargla, 2010.
21. Alemayehu Y, Chimdesa M, Yusuf Z. "Allelopathic Effects of *Lantana camara* L. Leaf Aqueous Extracts on Germination and Seedling Growth of *Capsicum annum* L and *Daucus carota* L." , *Scientifica*, 2024; Vol 2024, Article ID 9557081, 9 pages.
22. Throneberry GO, and Smith FG. Relation of respiratory and enzymatic activity to corn seed viability. *Plant Physiology* 1955; 30. 337-343.
23. Ranal MA, Santana DG, Ferreira WR, Mendes Rodrigues C. Calculating germination measurements and organizing spreadsheets. *Revista Brasileira de Botânica*, 2009; v.32, n.4, p.849-855. <http://www.scielo.br/pdf/rbb/v32n4/a22v32n4.pdf>
24. Luo Y, Zhao X, Li Y, and Wang T. "Effects of foliage litter of a pioneer shrub (*Artemisia philodendron*) on germination from the soil seed bank in a semi-arid sandy grassland in China," *Journal of Plant Research*, 2017; vol. 130, no. 6, pp. 1013–1021.

25. Rho BJ, Kil BS. Influence of phytotoxication from *Pinus rigida* on the selected plants. Journal of Natural Science, 1986; 5. 19-27.
26. Abiyu A, Teketay D, Gratzner G, Shete M. Tree planting by smallholder farmers in the upper catchment of Lake Tana Watershed, Northwest Ethiopia. Small-scale For 2016; 15(2):199–212. <https://doi.org/10.1007/s11842-015-9317-7>
27. Al-Amin Sk. Md. I. Allelopathic effects of *Anthocephalus cadamba* on germination and growth behavior of some pulses. Flora and fauna. 2021; 27(2): 209-216.
28. Conway WC, Smith LM, Bergan JF. Potential allelopathic interference by the exotic Chinese tallow tree (*Sapium sebiferum*). American Midland Naturalist. 2002; 148 : 43- 53.
29. Sisodia S, Siddiqui MB. Allelopathic potential of rhizosphere soil of *Croton bonplandianum* on growth and establishment of some crop and weed plants. African Journal of Agricultural Research. 2009; 4(5): 461-467.
30. Gulzar A, Siddiqui MB. Evaluation of allelopathic effect of *Eclipta alba* (L.) Hask on biochemical activity of *Amaranthus spinosus* L., *Cassia tora* L. and *Cassia sophera* L. African Journal of Environmental Science and Technology. 2014; 8(1): 1-5.
31. Zhang C, Fu S. Allelopathic effects of leaf litter and live roots exudates of *Eucalyptus* species on crops. Allelopathy Journal. 2010; 26(1) : 91-100.
32. Saberi M, Davari A, Tarnian F, Shahreki M, Shahreki E. Allelopathic effects of *Eucalyptus camaldulensis* on seed germination and initial growth of four range species. Annals of Biological Research. 2013; 4(1) :152-159.
33. Gebreyohannes L, Egigu MC, Manikandan M, Sasikumar JM. Allelopathic Potential of *Lantana camara* L. Leaf extracts and soils invaded by it on the growth performance of *Lepidium sativum* L. The Scientific world Journal. Vol 2023, Article ID 6663686, 6 pages, 2023.
34. Akey WC. Competition and Crop Loss Due to Velvet leaf. Section of Plant Biology, University of California Davis, Davis, CA, USA, 2023.
35. Sileshi T, Yusuf Z, Desta M. Enzymatic Properties of Red Beet (*Beta vulgaris* L.) Leaf, Root Pulp, and Peel. Recent Pat Biotechnol. 2023; 17(4):395-404. doi: 10.2174/1872208317666230201091358. PMID: 36722474.

Figures

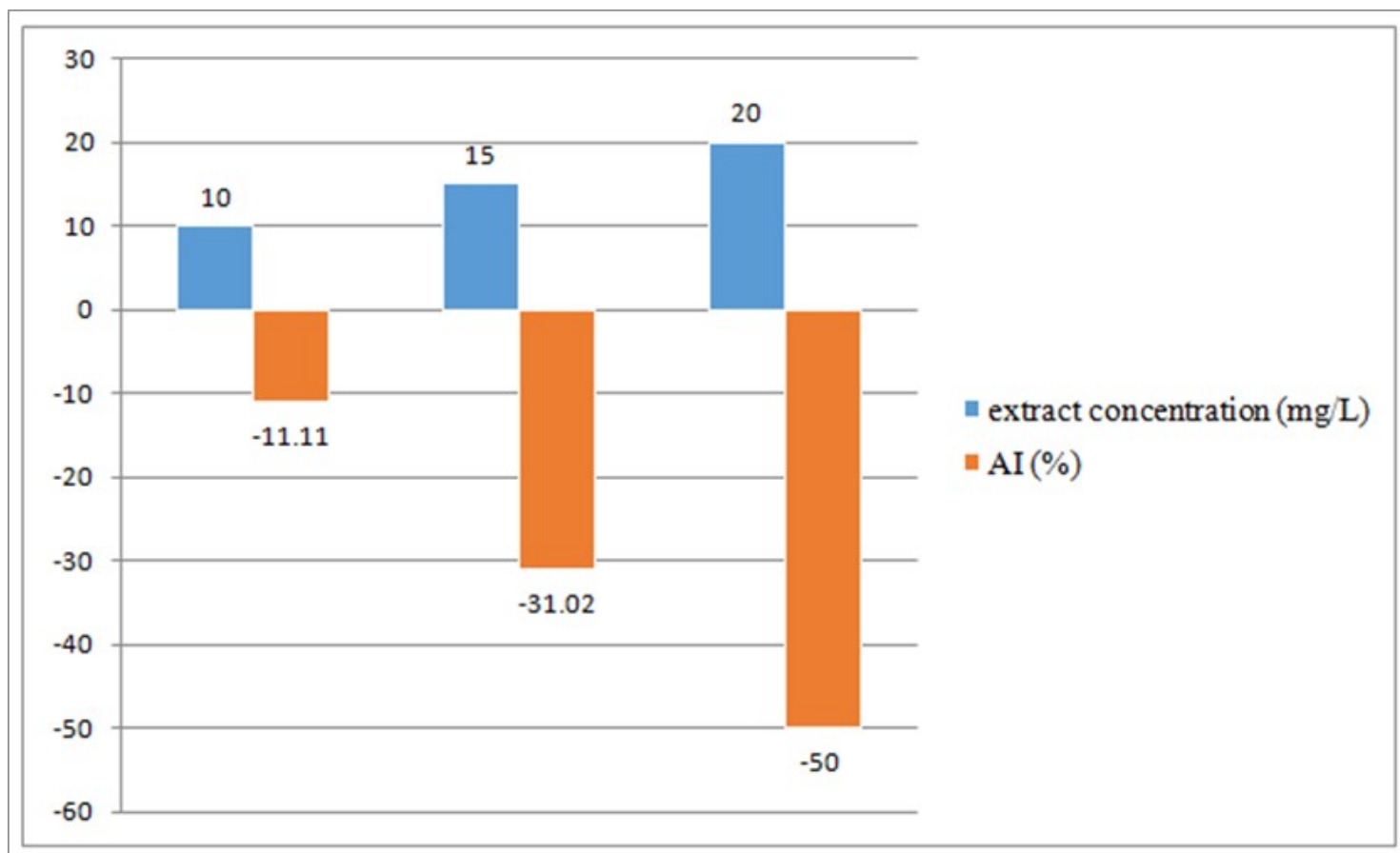


Figure 1

Allelopathic index for sorghum seed germination

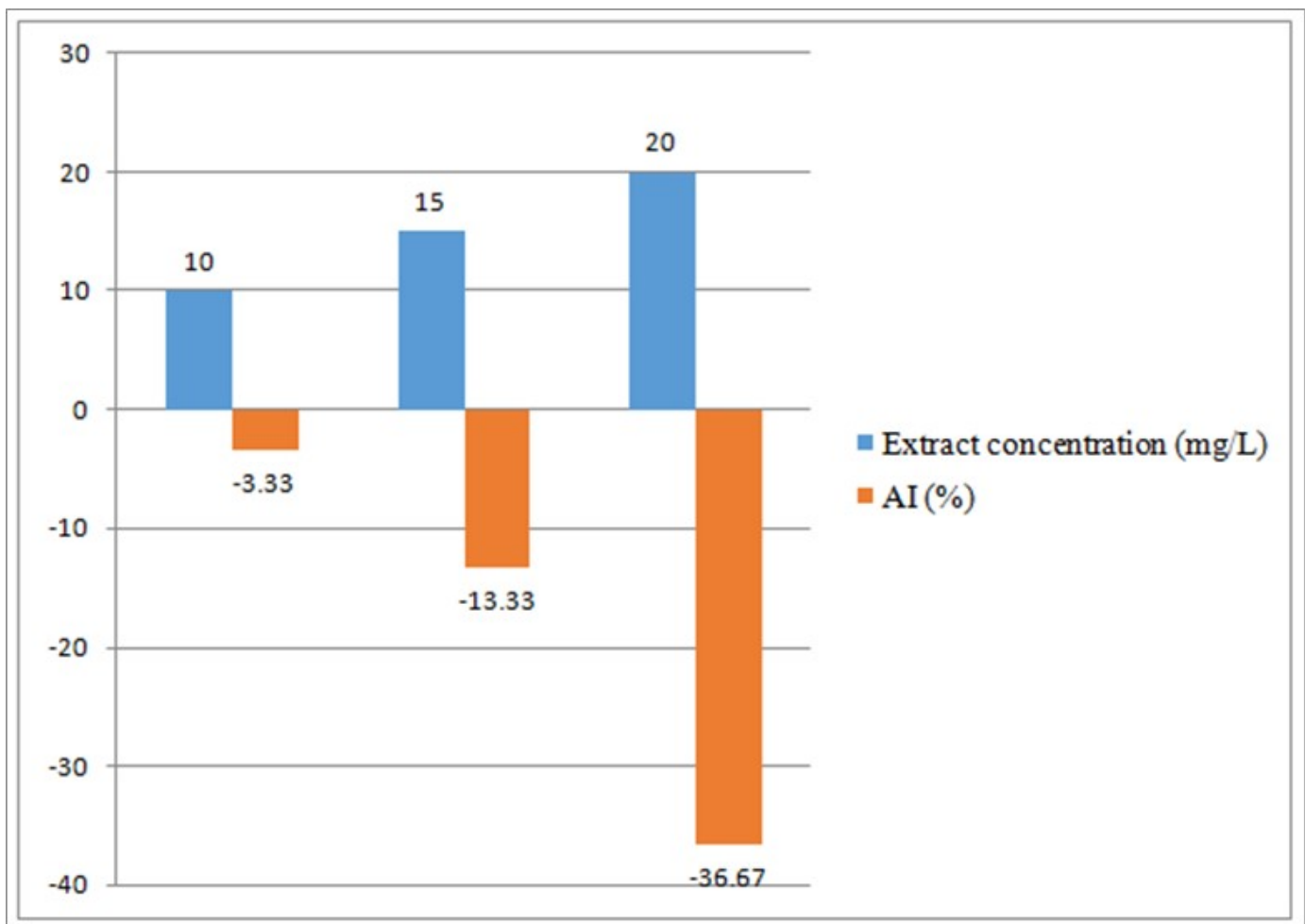


Figure 2

Allelopathic index for maize seed germination