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Effects of *Cnidoscolus aconitifolius* Leaf Extract on the Growth of Maize

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ABSTRACT

The growth and yield of maize (*Zea mays* L.) are constrained by abiotic and biotic stresses, necessitating sustainable approaches to improve crop performance. *Cnidoscolus aconitifolius* leaf extract, rich in bioactive phytochemicals, has potential as a natural biostimulant for enhancing maize growth. This study aimed to evaluate the effects of *C. aconitifolius* leaf extract on the growth of maize. The experiment was conducted using a two-factor Completely Randomized Design (CRD), with solvent type (aqueous, ethanol, methanol, and butanol) and extract concentration (0, 25, 50, and 100 mg/L) as the experimental factors. The results showed that maize treated with *C. aconitifolius* leaf extract prepared using 70% ethanol at 50 mg/L exhibited the highest shoot fresh weight (455.0 g), shoot dry weight (319.1 g), and 100-grain weight (40.2 g). The 70% ethanol extract applied at 25 mg/L produced the greatest leaf number (14) and root fresh weight (202.5 g). In contrast, the highest plant height (199.4 cm) was recorded in plants treated with the 70% methanol extract at 50 mg/L. The greatest root dry weight (102.5 g) was observed in the control treatment, whereas the highest total number of grains per cob (563.7) was obtained with the aqueous extract applied at 25 mg/L.

Keywords: biostimulant, chaya, maize, plant extract

INTRODUCTION

Maize (*Zea mays* L.) is a key component of global food, feed, and industrial sectors. In Indonesia, *Z. mays* serves as a major carbohydrate source after rice and as a primary raw material for livestock feed, processed food products, and bioenergy production (Masters *et al*. 2016, Revilla *et al*. 2022). The increasing demand for *Z. mays* in both food and non-food industries necessitates continuous improvements in its productivity and sustainability (Erenstein *et al*. 2022). However, the growth and yield of *Z. mays* are frequently limited by various abiotic and biotic stresses, including nutrient deficiencies, drought, and temperature fluctuations (Njeru *et al*. 2023). Conventional agricultural practices, such as intensive fertilizer and pesticide use, are not environmentally sustainable (Mahmood *et al*. 2016). Therefore, the application of biostimulants provides a sustainable approach to improve plant performance and enhance resilience under suboptimal environmental conditions.

Biostimulants comprise a group of natural or synthetic compounds that act to improve plant growth performance, physiological functions, and overall productivity (Ummah *et al*. 2017). These substances may be administered through seed treatment, foliar application, or incorporation into the growth substrate. As noted by Nardi *et al*. (2016), biostimulants are effective even when applied in low doses; they are able to regulate key metabolic activities within plants. This is in line with the report of Noli *et al*. (2024) that organic molecules contained in biostimulants have a direct effect on several metabolic processes in plants. Biostimulant sources can be derived from organic materials such as seaweed and plant extracts.

Extracts derived from plant organs contain various bioactive compounds that provide numerous benefits, including their function as biostimulants. Ibrahim (2022) explained that plant metabolite compounds mainly consist of amino acids, low–molecular–weight polypeptides, vitamins, enzymes, and hormones (auxins, cytokinins, and gibberellins) that regulate plant growth and metabolism. Furthermore, Aulya *et al*. (2018) reported that the crude extract of *Gleichenia linearis* leaves could enhance the growth and yield of *Z. mays*. Another study by Aulya (2020) showed that *Acacia nilotica* L. leaf extract at a concentration of 75% increased the germination percentage of mung beans up to 100%.

Based on previous studies, numerous plant species have demonstrated considerable potential as natural sources of biostimulants. However, many of them remain unexplored, including *C. aconitifolius*, a plant

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widely recognized for its nutritional and medicinal value (Kuri-García & Guzmán 2017, Panghal *et al*. 2021). *C. aconitifolius* contains a diverse range of bioactive compounds, including flavonoids, saponins, tannins, and alkaloids, which have the potential to be natural biostimulants by stimulating growth and improving crop performance under various environmental conditions (Padilla-Camberos *et al*. 2021, Silalahi 2021). Therefore, this study aimed to investigate the effects of *C. aconitifolius* leaf extract as a natural biostimulant on the growth of *Zea mays* L.

METHODS

Experimental Design and Treatments

The experiment was carried out in the Plant Physiology Laboratory and greenhouse facilities of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, Padang. The research employed a factorial Completely Randomized Design (CRD) consisting of two factors with three replications. The first factor was solvent type: (A1) water, (A2) 70% ethanol, (A3) 70% methanol, and (A4) 70% butanol. The second factor was extract concentration: (B0) control, (B1) 25 mg/L, (B2) 50 mg/L, and (B3) 100 mg/L. Each replication comprised 16 pots, resulting in a total of 48 pots for the entire experiment.

Preparation and Chemical Analysis of Crude Plant Extracts

Crude extracts of *C. aconitifolius* leaves were prepared using the maceration technique with 70% methanol, 70% ethanol, and 70% butanol as organic solvents. The maceration process was carried out for 72 hours (3 × 24 h) at room temperature with occasional stirring. For the aqueous extract, the extraction was performed by boiling the plant material in distilled water (1:24 w/v) for 1 hour. Phytochemical constituents of *C. aconitifolius* leaves extracted with different solvents were identified using Gas Chromatography–Mass Spectrometry (GC–MS), and the results are presented in Table 1a and Table 1b.

Test Crop and Planting

The planting medium consisted of plantation soil mixed with compost at a 4:1 volume ratio, placed in polybags measuring 50 × 60 cm with a soil capacity of 8 kg per pot. One *Z. mays* seed was planted per polybag at a depth of 3–5 cm, and the spacing between polybags was maintained at 25 × 75 cm. One week after planting, weak seedlings were removed to ensure uniform growth and development of the healthy plants. Fertilizer application was carried out before sowing using urea (1.05 g per polybag) and muriate of potash (MoP; 0.53 g per polybag). No additional fertilizer was applied during the experimental period.

Table 1a Phytochemical composition of *C. aconitifolius* leaves extracted using different solvents (methanol)

a. GC–MS analysis of the aqueous extract of *C. aconitifolius*					
Peak	Retention time (RT)	% Area	Chemical content	Class of compounds	Molecular formula
2	13.585	44.72	Phenol, 4-(2-aminoethyl)-	Tyramine (Tyrosine) Phenolic	$C_8H_{11}NO$
5	16.166	11.55	L-Tryptophan, N-[(phenyl methoxy) carbonyl	Aromatic (Serotonin) alkaloid	$C_{19}H_{18}N_2O_4$
8	16.672	8.12	Neophytadiene	Alkene (Diterpene)	$C_{20}H_{38}$
6	16.585	6.16	Cyclo(L-prolyl-L-valine)	Cycloalkenes	$C_{12}H_{24}$
10	16.995	6.15	Neophytadiene	Alkene (Diterpene)	$C_{20}H_{38}$
12	18.791	4.35	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Fatty acid Linolenic acid	$C_{18}H_{30}O_2$
7	16.622	3.23	3,7,11,15 Tetramethylhexadec-2-ene	Alkenes	$C_{20}H_{40}O$
b. GC–MS analysis of the methanol aqueous extract of *C. aconitifolius*					
Peak	Retention Time (RT)	% Area	Chemical content	Class of compounds	Molecular formula
5	15.470	26.91	3-Deoxy-d-mannoic lactone	Dicarboxylic acids (glutarates)	$C_6H_{10}O_5$
4	15.355	20.35	3-Deoxy-d-mannoic lactone	Dicarboxylic acids (glutarates)	$C_6H_{10}O_5$
9	16.995	9.19	Neophytadiene	Alkene (diterpene)	$C_{20}H_{38}$
7	16.714	7.36	3,7,11,15-Tetramethylhexadee-2-ene	Phytol (terpene)	$C_{20}H_{40}O$
3	15.205	7.17	2,5-Monoformal-l-rhamnitol	Carboxylic acid (quinic acid)	$C_7H_{12}O_6$
8	16.852	7.07	Neophytadiene	Alkene (diterpene)	$C_{20}H_{38}$
6	16.622	5.77	3,7,11,15-Tetramethylhexadee-2-ene	Phytol (terpene)	$C_{20}H_{40}O$

Table 1b Phytochemical composition of *C. aconitifolius* leaves extracted using different solvents (ethanol, butanol)

c. GC–MS analysis of the ethanol extract of *C. aconitifolius*					
Peak	Retention Time (RT)	% Area	Chemical content	Class of compounds	Molecular formula
3	15.775	82.35	Glycero-d-galactose-heptose	Monosaccharide (heptose)	$C_7H_{14}O_7$
2	15.547	14.98	3-Buten-2-one, 4-(2,5,5-trimethyl-3-oxatric	Acrylic acid (acrylates)	$C_7H_{12}O_2$
1	10.737	1.24	Benzofuran, 2,3-dihydro-	Coumarin (phenolic)	C_8H_8O
5	17.551	0.66	Octadecanoic acid	Fatty Acid (stearic acid)	$C_{18}H_{36}O_2$
4	16.289	0.34	Loliolide	Fatty acid ester	$C_{11}H_{16}O_3$
7	18.791	0.25	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Fatty acid (linolenic acid)	$C_{18}H_{30}O_2$
6	18.614	0.19	Neophytadiene	Alkene (diterpene)	$C_{20}H_{38}$
d. GC–MS analysis of the butanol extract of *C. aconitifolius*					
Peak	Retention Time (RT)	% Area	Chemical content	Class of compounds	Molecular formula
13	28.780	19.88	Cholesta-5,22-dien-3-ol, (3.beta.)-	Micronutrients	$C_{27}H_{44}O$
7	17.121	18.54	Methyl 6-O-[1-methylpropyl]-.beta.-d-gala	Amphetamine (Amine)	$C_{10}H_{15}NO$
11	25.407	18.07	2-Methylheptacosane	Alkane (Octacosane)	$C_{28}H_{58}$
6	16.999	16.01	Neophytadiene	Alkene (Diterpene)	$C_{20}H_{38}$
12	28.048	8.29	2-Methylheptacosane	Alkane (Octacosane)	$C_{28}H_{58}$
5	16.854	6.66	Neophytadiene	Alkene (Diterpene)	$C_{20}H_{38}$
4	16.676	4.21	Neophytadiene	Alkene (Diterpene)	$C_{20}H_{38}$

Extract Application

The extracts were initially diluted in dimethyl sulfoxide (DMSO) (100 mg/L) and then mixed with 1 L of water. Approximately 25 mL of the resulting solution was applied to the leaves of *Z. mays* two weeks after planting. Foliar applications were performed once per week in the morning, when relative humidity is typically high (Kalaivanan *et al*. 2012).

Maintenance and Observation Procedures

The plants were cultivated under greenhouse conditions throughout the experiment. Watering was performed twice daily, in the morning and afternoon, to maintain adequate soil moisture. Weeds were removed manually to minimize competition for water, nutrients, and light. No pesticides were applied during the study to avoid potential interference with the effects of the biostimulant treatments.

Measurement of Growth and Yield Parameters

Measurements were initiated two weeks after planting (2 WAP). Growth variables, including plant height and leaf number, were assessed weekly until the end of the vegetative phase, indicated by tassel appearance. At harvest (49 days after planting, DAP), data were collected on shoot and root fresh and dry biomass, plant height, leaf number, number of 100 grains, and number of grains per cob.

Plant height: determined weekly by measuring the distance from the soil surface to the tip of the highest leaf using a measuring ruler (cm scale). Leaf number: Counted weekly, considering only fully expanded leaves per plant. Fresh and dry weights: At harvest, plants were separated into leaves, stems, and roots. Fresh weights were recorded immediately, after which the samples were oven-dried at 70 °C for 48 h until a constant weight was achieved to determine dry weights. Weight of 100 seeds: One hundred grains from each treatment were counted using a seed counter and weighed using an analytical balance with a precision of 0.1 g. Number of grains per cob: After shelling, the grains from each cob were counted manually.

Data Analysis

All data were analyzed using Analysis of Variance (ANOVA) for a factorial Completely Randomized Design (CRD). When significant differences were detected, mean separation was performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Data were analyzed using the SPSS software version 24.

RESULTS AND DISCUSSION

Effect of *C. aconitifolius* Extract on Maize Growth

The effects of *C. aconitifolius* leaf extract prepared using different solvents and applied at various concentration levels are presented in Table 2a and Table 2b. No significant interaction between solvent type and extract concentration was observed for plant height ($p > 0.05$). The highest plant height was numerically recorded in plants treated with the 70% methanol extract at 50 mg/L (199.4 cm), whereas the lowest value was observed in the 70% butanol extract at 100 mg/L (131.9 cm). The main effect of solvent was significant, with the 70% methanol extract producing the greatest average plant height (191.4 cm), significantly higher than the aqueous (150.7 cm) and 70% butanol (154.4 cm) extracts. In contrast, extract

concentration had no significant effect on plant height. Although plants treated with 25 mg/L exhibited the highest average height (171.1 cm), followed by the control (171.2 cm), 50 mg/L (166.8 cm), and 100 mg/L (158.2 cm).

The highest leaf number (14.0) was recorded in maize plants treated with the 70% ethanol extract at 25 mg/L, which differed significantly from the 70% butanol extract at 50 and 100 mg/L, producing 11.7 and 11.3 leaves, respectively. Most other treatment combinations exhibited intermediate values and did not differ significantly. The main effect of the solvent was also significant. Averaged across concentrations, the 70% ethanol extract produced the highest leaf number (13.3), significantly different from the 70% butanol extract (12.2), while the aqueous and 70% methanol extracts showed intermediate values and did not differ significantly from either treatment. In contrast, extract concentration had no significant effect on leaf number, with average values ranging from 12.4 to 13.0 leaves across treatments.

A significant interaction between solvent type and extract concentration was observed for shoot fresh weight. The highest shoot fresh weight was recorded in plants treated with the aqueous extract at 25 mg/L and the 70% ethanol extract at 50 mg/L, both yielding 455.8 g. These values were significantly higher than 70% butanol extract at 100 mg/L (295.8 g), which produced the lowest shoot fresh weight. Most other treatment combinations showed intermediate values and were not significantly different from either group. Averaged across concentrations, shoot fresh weight ranged from 376.6 g in the 70% butanol extract to 432.5 g in the 70% ethanol extract. Similarly, average shoot fresh weight across concentrations varied from 385.8 to 435.8 g, with no significant differences among concentration levels.

Root fresh weight was not significantly affected by the interaction between solvent type and extract concentration ($p > 0.05$). Likewise, the main effect of

Table 2a Effect of *C. aconitifolius* leaf extract with different solvents and levels of concentration on plant height, leaf number, and fresh weight of shoot and root of *Zea mays*

Solvent	Level of concentration				Average
	Control	25	50	100	
			Plant height (cm)		
Aqueous	145.8±44.7^{a}	155.1±33.8^{a}	156.0±48.0^{a}	146.0±70.7^{a}	150.7±43.9^{A}
70% Ethanol	181.1±46.2^{a}	166.5±45.7^{a}	176.7±38.4^{a}	159.2±25.9^{a}	170.9±35.2^{AB}
70% Methanol	181.8 ± 9.4^{a}	188.7±10.0^{a}	199.4±23.6^{a}	195.8±19.4^{a}	191.4±15.9^{B}
70% Butanol	176.1±22.1^{a}	174.2±18.6^{a}	135.3±43.0^{a}	131.9±31.5^{a}	154.4±33.7^{A}
Average	171.2±33.7^{A}	171.1±28.8^{A}	166.8±41.7^{A}	158.2±43.5^{A}	
			Leaf number		
Aqueous	12.3±0.6^{ab}	13.0±1.0^{ab}	12.7±2.5^{ab}	12.7±1.5^{ab}	12.7 ± 1.4^{AB}
70% Ethanol	13.3±0.6^{ab}	14.0±1.0^{b}	13.3±0.6^{ab}	12.7±1.1^{ab}	13.3±0.9^{B}
70% Methanol	12.3±0.6^{ab}	12.3±0.6^{ab}	13.0±1.0^{ab}	13.0±1.0^{ab}	12.2±0.8^{AB}
70% Butanol	13.0±1.0^{ab}	12.6±0.6^{ab}	11.7±0.6^{a}	11.3±1.5^{a}	12.2±1.1^{A}
Average	12.7±0.7^{A}	13.0±0.9^{A}	12.7±1.4^{A}	12.4±1.3^{A}	
			Fresh weight of shoot (g)		
Aqueous	392.5±45.8^{ab}	455.8±70.2^{b}	402.5±157.2^{ab}	369.1±94.5^{ab}	405.0±92.1^{A}
70% Ethanol	432.5±40.0^{ab}	402.5±20.0^{ab}	455.8±50.3^{b}	439.1±35.1^{ab}	432.5±38.1^{A}
70% Methanol	409.1±30.5^{ab}	432.5±65.6^{ab}	369.1±107.8^{ab}	439.1±56.8^{ab}	412.5±66.9^{A}
70% Butanol	402.5±91.6^{ab}	452.5±80.0^{b}	355.8±87.4^{ab}	295.8±90.2^{a}	376.6±96.0^{A}
Average	409.1±51.0^{A}	435.8±58.4^{A}	395.8±100.4^{A}	385.8±88.0^{A}	
			Fresh weight of root (g)		
Aqueous	185.8±41.6^{a}	132.5±55.7^{a}	112.5±90.0^{a}	135.8±94.5^{a}	141.6±69.1^{A}
70% Ethanol	189.1±100.7^{a}	202.5±111.4^{a}	102.5±10.0^{a}	182.5±26.4^{a}	169.1±76.9^{A}
70% Methanol	149.1±41.6^{a}	149.1±68.1^{a}	142.5±34.6^{a}	162.5±20.0^{a}	150.8±38.8^{A}
70% Butanol	189.1±95.0^{a}	165.8±15.3^{a}	89.1±5.8^{a}	115.8±92.4^{a}	140.0±70.3^{A}
Average	178.3±66.5^{B}	162.5±66.6^{AB}	111.6 ± 46.2^{A}	149.1±63.8^{AB}	

Table 2b Effect of *C. aconitifolius* leaf extract with different solvents and levels of concentration on dry weight of shoot and root of *Zea mays*

Solvent	Level of concentration				Average
	Control	25	50	100	
	Dry weight of shoot (g)				
Aqueous 70%	179.1±40.4^{ab}	259.1±105.0^{ab}	225.8±100.2^{ab}	165.8±47.2^{a}	207.5±77.7^{A}
Ethanol 70%	229.1±40.4^{ab}	285.8±40.4^{ab}	319.1±72.3^{b}	232.5±17.3^{ab}	266.7±56.1^{A}
Methanol 70%	275.8±83.3^{ab}	259.1± 51.3^{ab}	262.5±91.6^{ab}	219.1±47.2^{ab}	254.1±71.2^{A}
Butanol	255.8±150.1^{ab}	195.8±41.6^{ab}	262.5±91.6^{ab}	219.1±47.2^{ab}	233.3±84.6^{A}
Average	235.0±85.9^{A}	250.0±65.5^{A}	267.5±83.9^{A}	209.1±53.8^{A}	
	Dry weight of root (g)				
Aqueous 70%	102.5±36.0^{b}	55.8±41.6^{ab}	32.5±30.0^{a}	29.1±30.5^{a}	55.0±42.7^{A}
Ethanol 70%	59.1±72.3^{ab}	45.8±32.1^{ab}	39.1±20.8^{a}	39.1±5.8^{a}	45.8±36.0^{A}
Methanol 70%	39.1±5.8^{a}	29.1±23.1^{a}	29.1±5.8^{a}	62.5±17.3^{ab}	40.0±19.1^{A}
Butanol	32.5±26.4^{a}	55.8±23.1^{ab}	12.5±10.0^{a}	29.1±46.2^{a}	32.5±29.8^{A}
Average	58.3±46.2^{B}	46.6±28.7^{AB}	28.3±19.3^{A}	40.0±28.6^{AB}	

Note: In a column and row, within treatment, the same letter(s) indicate do not differ significantly (a, b, ab) according to DNMRT ($p < 0.05$). ns: nonsignificant

solvent type was not significant, although plants treated with the 70% ethanol extract exhibited the highest average root fresh weight (169.1 g), followed by the 70% methanol (150.8 g), aqueous (141.6 g), and 70% butanol (140.0 g) extracts. In contrast, extract concentration significantly influenced root fresh weight. The control treatment produced the highest average root fresh weight (178.3 g), which was significantly greater than that obtained at 50 mg/L (111.6 g). The 25 mg/L (162.5 g) and 100 mg/L (149.1 g) treatments showed intermediate values and did not differ significantly from either the control or 50 mg/L treatments. The type of extraction solvent had no significant effect on plant growth and yield parameters; however, the 70% ethanol extract generally resulted in the highest numerical values.

A significant interaction between solvent type and extract concentration was observed for shoot dry weight. The highest shoot dry weight was recorded in plants treated with the 70% ethanol extract at 50 mg/L (319.1 g), whereas the lowest value was obtained with the aqueous extract at 100 mg/L (165.8 g). However, most treatment combinations exhibited intermediate values and did not differ significantly from either of these treatments. The main effects of solvent type and extract concentration were not significant.

A significant interaction between solvent type and extract concentration was observed for root dry weight. The highest root dry weight was recorded in the aqueous control treatment (102.5 g), which was significantly greater than the other treatments. The main effect of solvent type was not significant, with average root dry weight ranging from 32.5 to 55.0 g across solvent treatments. In contrast, concentration significantly affected root dry weight. Averaged across solvents, the control treatment produced the highest root dry weight (58.3 g), significantly exceeding the 50 mg/L treatment (28.3 g), while the 25 mg/L (46.6 g) and 100 mg/L (40.0 g) treatments showed intermediate values and were not significantly different.

The effect of biostimulants in promoting plant growth depends on several factors, including the solvent used for extraction, the concentration applied to plants, and the interaction between these factors (Navarro-López *et al*. 2023, Li *et al*. 2024). This study demonstrated that the application of *C. aconitifolius* leaf extract as a biostimulant produced a significant effect on the growth of *Z. mays*, particularly in enhancing leaf production. Comparable results were reported by Adisti (2022), who observed that *Centella asiatica* extracts prepared with methanol and ethanol were the most effective in promoting the growth of pagoda mustard. Similarly, Ashour *et al*. (2022) found that ethanol-based seaweed extracts enhanced vegetative growth in strawberry plants.

The increase in leaf number observed in *Z. mays* treated with ethanol-extracted *C. aconitifolius* may be related to the presence of terpenoid constituents, particularly gibberellins identified through phytochemical analysis of the extract (Auezov *et al*. 2024). According to Lin *et al*. (2016), terpenoids comprise various classes, including diterpenoids with 20 carbon atoms such as gibberellins, which are key regulators of plant cell division and expansion. Gibberellins function as essential hormonal signals governing leaf initiation and development by stimulating both mitotic activity and cellular elongation. Leaf development originates at the lateral regions of the shoot apical meristem (SAM), where intense cell division establishes the early primordium structure,

followed by substantial cell enlargement that ultimately determines final leaf morphology (Ritonga *et al*. 2023).

Ethanol was identified as the most effective solvent for enhancing shoot fresh weight in *Z. mays*. These outcomes indicate that *C. aconitifolius* extract may improve key metabolic processes in plants, including cellular division and photosynthetic activity, thereby contributing to better growth performance (Werdiningsih & Fitria 2024). Plant metabolic capacity, particularly carbohydrate metabolism driven by photosynthesis, is directly reflected in the accumulation of fresh and dry biomass (Li *et al*. 2017). The energy assimilated through photosynthesis is converted into organic molecules that sustain tissue formation and structural development (Yamori & Shikanai 2016). Enhanced chlorophyll levels have also been reported to positively influence photosynthetic efficiency, ultimately increasing dry matter production (Li *et al*. 2018).

The improvements in vegetative parameters, particularly the increase in leaf production and shoot biomass, are closely linked to the plant's capacity to support reproductive processes and determine final yield. An expanded leaf canopy enhances photosynthetic performance and assimilate synthesis, supplying the energy and carbon frameworks required for kernel development during the reproductive stage. Therefore, the stimulatory influence of *C. aconitifolius* extract on leaf growth and chlorophyll accumulation is likely to promote more effective assimilate movement toward developing ears and kernels. This functional connection between vegetative vigor and reproductive productivity emphasizes the potential of *C. aconitifolius* leaf extract to improve not only early-stage growth but also overall *Z. mays* yield through enhanced source–sink efficiency.

Effect of *C. aconitifolius* Extract on Maize Yield

The effects of *C. aconitifolius* leaf extract, prepared using different solvents and applied at various concentrations, on *Z. mays* yield are presented in Table 3. A significant interaction between solvent type and extract concentration was observed for 100-grain weight. The highest 100-grain weight was recorded in plants treated with the 70% ethanol extract at 50 mg/L (40.2 g), whereas the lowest value was observed in the aqueous extract at 100 mg/L (29.0 g). Most treatment combinations exhibited intermediate values and were not significantly different. The main effect of solvent type was significant. Averaged across concentrations, plants treated with the 70% ethanol extract produced the highest 100-grain weight (36.6 g), followed by the 70% methanol (35.8 g) and 70% butanol (34.3 g) extracts, all of which were significantly greater than the aqueous extract (31.5 g). In contrast, extract concentration did not significantly affect 100-grain weight, with average values ranging from 33.7 to 35.1 g across concentration levels.

A significant interaction between solvent type and extract concentration was observed for the total number of grains per cob. The highest number of grains per cob was recorded in plants treated with the aqueous extract at 25 mg/L (563.7 grains), whereas the lowest value was obtained with the aqueous extract at 50 mg/L (298.7 grains). Most of the remaining treatment combinations exhibited intermediate values and did not differ significantly from either of these treatments. The main effect of solvent type was also significant. Averaged across concentrations, the 70% methanol extract produced the highest total number of grains per cob (480.1 grains), significantly exceeding the aqueous extract (401.6 grains). The 70% ethanol (446.8 grains) and 70% butanol (441.1 grains) extracts showed intermediate values and did not differ significantly from either treatment. Extract concentration significantly influenced grain number. The 25 mg/L treatment resulted in the highest average number of grains per cob (505.1 grains), significantly exceeding the 100 mg/L treatment (398.2 grains). The control (448.4 grains) and 50 mg/L (417.9 grains) treatments exhibited intermediate values and were statistically comparable to both groups.

Table 3 Effect of *C. aconitifolius* leaf extract with different solvents and concentrations to the weight of 100 grains and total number of grains per cob in *Z. mays*

Solvent	Level of concentration				Average
	Control	25	50	100	
	Weight of 100 grains				
Aqueous	31.8 ± 4.9^{bc}	35.3 ± 4.0^{abcd}	29.0 ± 0.9^{ab}	29.0 ± 8.5^{a}	31.5 ± 5.2^{A}
70% Ethanol	36.6 ± 1.9^{cd}	34.4 ± 0.7^{abcd}	40.2 ± 1.6^{d}	35.3 ± 2.1^{abcd}	36.6 ± 2.7^{B}
70% Methanol	34.8 ± 1^{abcd}	36.2 ± 1.8^{bcd}	35.6 ± 1.0^{abcd}	36.7 ± 3.6^{cd}	35.8 ± 2.0^{B}
70% Butanol	36.0 ± 3.5^{bcd}	34.5 ± 2.3^{bc}	33.1 ± 3.3^{abc}	33.7 ± 3.8^{abcd}	34.3 ± 3.0^{B}
Average	34.8 ± 3.3^{A}	35.1 ± 2.3^{A}	34.6 ± 4.3^{A}	33.7 ± 5.3^{A}	
	Total number of grains per cob				
Aqueous	400.3 ± 77.7^{abcd}	563.7 ± 14.8^{d}	298.7 ± 161.8^{a}	343.7 ± 212.1^{ab}	401.6 ± 158.3^{A}
70% Ethanol	467.6 ± 45.0^{bcd}	424.3 ± 6.6^{abcd}	440.0 ± 28.3^{abcd}	455.3 ± 7.0^{abcd}	446.8 ± 28.6^{AB}
70% Methanol	438.3 ± 63.3^{abcd}	520.7 ± 4.0^{cd}	486.0 ± 20.1^{abcd}	475.3 ± 17.2^{bcd}	480.1 ± 42.4^{B}
70% Butanol	487.3 ± 11.7^{bcd}	511.7 ± 31.3^{cd}	447.0 ± 15.7^{bcd}	318.3 ± 53.5^{abc}	441.1 ± 82.6^{AB}
Average	448.4 ± 37.8^{AB}	505.1 ± 58.4^{B}	417.9 ± 82.0^{AB}	398.2 ± 78.7^{A}	

Note: In a column and row, within treatment, the same letter(s) indicate do not differ significantly according to DNMRT ($p < 0.05$)

This study demonstrated that variations in solvent type and extract concentration of *C. aconitifolius* significantly influenced the yield attributes of *Z. mays*, particularly 100-grain weight. The observed improvement may be associated with the ability of ethanol-derived extracts to promote leaf development and enhance photosynthetic efficiency. As leaves serve as the primary source of photoassimilate production, enhanced leaf function can increase the availability of assimilates for grain filling, thereby contributing to greater grain weight and improved yield performance.

In contrast, the aqueous extract at 25 mg/L produced the highest total number of grains per cob. These findings suggest that aqueous extracts may be more effective in increasing grain number, whereas ethanol-derived extracts tend to enhance individual grain weight. Consequently, aqueous extracts may contribute to promoting yield through greater grain production, while ethanol extracts improve yield by increasing grain biomass.

The positive association between assimilate allocation and 100-grain weight highlights the importance of an adequate photosynthate supply during kernel development. Proper ear development and efficient grain filling support the formation of larger seeds (Smitchger & Weeden 2018), whereas limitations in kernel development during this stage may reduce final grain weight (Wang *et al*. 2022). During the reproductive phase, a substantial proportion of assimilates is translocated to developing kernels, promoting biomass accumulation and ultimately enhancing yield potential (Zhang *et al*. 2015).

These results demonstrate that solvent polarity and extract concentration are important factors influencing the physiological responses of *Z. mays* to *C. aconitifolius* leaf extract (Purkait *et al*. 2024). Ethanol-derived extracts, which may contain higher concentrations of bioactive compounds, could enhance photosynthetic activity and promote more efficient assimilate translocation to developing kernels during the grain-filling period (Emanet & Ciofani 2023). This response may explain the greater 100-grain weight observed in maize treated with the 70% ethanol extract, particularly at 50 mg/L. In contrast, the aqueous extract at 25 mg/L produced the highest total number of grains per cob, indicating that different solvent systems may influence distinct yield components. While ethanol extracts tended to increase individual grain weight through enhanced assimilate accumulation, aqueous extracts appeared to favor greater grain production.

CONCLUSION

The findings of this study indicate that *C. aconitifolius* leaf extract has potential as a natural biostimulant to enhance the growth and yield performance of *Z. mays*. Ethanol-based extracts applied at 25–50 mg/L resulted in the highest growth and yield parameter, reflected in increased leaf formation, greater shoot biomass accumulation, and higher grain output per plant. Further investigations should focus on identifying the active phytochemical compounds responsible for these effects, optimizing application rates and timing, and evaluating the long-term influence of *C. aconitifolius* extract on soil health and nutrient dynamics.

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