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Effect of *Clerodendrum infortunatum* L. Leaf Extracts on the Growth and Development of Maize (*Zea mays* L.)

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ABSTRACT

The allelopathic effects of *C. infortunatum* leaf extracts were assessed to determine their impact on maize growth and development. Extracts were prepared at varying concentrations: C_0 (0%), C_{20} (20%), C_{40} (40%), C_{60} (60%), C_{80} (80%) and C_{100} (100%). Results showed that *C. infortunatum* extracts generally promoted growth across all measured parameters. However, at the highest concentration (C_{100}), inhibitory effects were noted in laboratory bioassays, specifically affecting shoot length and fresh and dry biomass weight. Despite this, significant stimulatory effects ($p<0.05$) were observed in all growth parameters during the polypot experiment. The inhibition at higher conc perhaps attributed to the existence of phenolic compounds for example 4-(1-methylpropyl) phenol as well as 3,5-bis (1,1-dimethylethyl) phenol in leaf extracts. This study aimed to evaluate the compatibility of *C. infortunatum* allelopathic effects with agricultural crops in various land use systems in the region.

Keywords *Clerodendrum infortunatum*, Aqueous extracts, Inhibitory, Stimulatory.

INTRODUCTION

A biological phenomenon known as allelopathy occurs when chemicals produced by one organism affect the survival, growth, as well as reproduction of other organisms. These substances, also referred to as allelochemicals, may affect target species positively or negatively. In plants, allelopathy represents an interaction between donor and recipient plants that can be either beneficial or detrimental (Cheng and Cheng 2015). Effective use of allelopathic plants' stimulatory or inhibitory properties in cultivation methods is essential for managing plant growth, preventing allelopathic auto-toxicity and promoting sustainable agricultural practices. Allelopathic activity can vary across different parts of the plant—for example leaf litter, leaves, stems, flowers, roots, bark, along with soil—throughout a growing season.

Allelochemicals can be discharged into the surroundings through root exudation, volatilization, plant decomposition, as well as leaching. Allelochemicals are found in all organs of the plant, which includes rhizomes, flowers, roots, fruits, stems, leaves, as well as seeds. The quantity and release mechanisms

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of these chemicals differ among species (Li *et al.* 2010). Understanding these allelopathic processes in various contexts is crucial for managing plant interactions, including controlling weeds in agriculture and invasive species in natural settings. Secondary metabolites from plants have the potential to prevent neighboring species' germination along with growth (Inderjit and Duke 2003).

Native to tropical regions of Asia, which includes India, Myanmar, Bangladesh, Thailand, Sri Lanka, Pakistan, the Andaman Islands, along Malaysia, *Clerodendrum infortunatum* is a perennial shrub belonging to the Lamiaceae family. This shrub, which can grow up to 4 meters high with circular leaves up to 15 cm in diameter, is prevalent in the West Garo Hills of Meghalaya and can interfere with the growth of agricultural crops by competing for nutrients and moisture. Despite its role as a weed, *C. infortunatum* is valued in traditional medicine for treating various ailments. The potential removal of this plant from its natural habitat could threaten its medicinal value. Therefore, this research attempts to evaluate the impact of *Clerodendrum infortunatum* on the growth as well as development of maize, a key agricultural crop in the region.

MATERIALS AND METHODS

Study site

The study had been performed at the Department of Forestry, North Eastern Hill University, Tura Campus (25°33.913' N latitude and 90°14.115' E Longitude). Freshly fallen *Clerodendrum infortunatum* leaves had been collected and let to air dry, then chopped into small pieces and crushed with a grinding machine into a fine powder. Mixing 100 grams of the powdered leaves with 1 liter of distilled water leaf extracts had been prepared. The extract obtained had been passed through Whatman filter paper No. 1 for filtration. Concentrations of 20%, 40%, 60%, 80%, as well as 100% had been achieved by utilizing distilled water to make the stock solution diluted. In laboratory bioassays, distilled water was utilized as the control, where as tap water had been employed as the control in pot experiments. Until they were needed for further application, the extracts had been kept in a refrigerator at 4°C. The experiment consisted of six treatments (C_{40} (40%), C_{20} (20%), C_{60} (60%), C_0 (0%), C_{80} (80%), & C_{100} (100%)) along with distilled/tap water controls, each with three replicates.

Laboratory bioassay

The crop seeds were initially sterilized using a 1% sodium hypochlorite solution to prevent any fungal contamination. Subsequently, they were thoroughly rinsed with running tap water. Petridishes (10 cm in diameter) had been sterilized in an oven at 60°C for 24 hrs. Whatman filter paper No. 1 was used to line each Petri dish, and 1 mL of extract was added on alternate days at different concentrations. Twenty maize seeds were placed on the wet filter paper as well as incubated in the laboratory at 25±2°C. Following germination, measurements and records have been taken of the test crops' fresh and dry weights, as well as their shoot and root lengths. Fresh shoots along with roots had been wrapped in aluminum foil as well as dried at 60°C for 24 hrs in an oven to get their dry weight. The inhibition percentage or stimulation relative to the control for both bioassays and pot experiments had been calculated utilizing the following equation (Surendra and Pota 1978).

$$I=100-(E_2\times100/E_1)$$

In which inhibition or stimulation percentage is represented by I, the response of the control is represented by E_1, along the response of the treatments is represented by E_2.

The elongation ratio of roots and shoots was calculated as follows (Rho and Kil 1986).

$$R=(T/Tr)\times100$$

In which the relative elongation ratio is represented by R, the elongation ratio of the treatment crop is represented by T, as well as the elongation ratio of the control crop is represented by T_r.

Pot experiments

Five sterilized crop seeds were planted 2-3 cm deep in polypot (26 cm × 20 cm) filled with 8 kg of soil and

Table 1. Photochemical compounds identified in methanol extract of *Clerodendrum infortunatum* leaves. RT=Retention time, MW=Molecular weight.

Sl. No.	RT	Name of the compound	Molecular formula	MW	Peak area (%)
1	3.254	Methyl pyruvate dimethyl acetal	$C_6H_{12}0_4$	148.15	0.237
2	8.134	0-Anisic acid, 3-chloroprop-2-enyl ester	$C_{11}H_{11}ClO_3$	226.65	22.570
3	8.325	Tridecane	$C_{13}H_{28}$	184.36	1.033
4	8.503	Silane, ethyl dimethyl phenyl	$C_{10}H_{16}Si$	164.31	33.032
5	10.167	Pentadecane	$C_{15}H_{32}$	212.41	2.744
6	10.208	Dodecane, 4,6-dimethyl	$C_{14}H_{30}$	198.39	2.678
7	11.236	Phenol, 4-(1-methylpropyl)-	$C_{10}H_{14}O$	150.22	1.492
8	11.364	Azacyclohexane, 3-methylamino-1-methyl	$C_7H_{16}N_2$	128.22	0.160
9	11423	Dodecane, 2,6,11-trimethyl	$C_{15}H_{32}$	212.41	1.199
10	15.608	Heptadecane	$C_{17}H_{36}$	240.5	1.541
11	15.706	Octadecane, 1-iodo-	$C_{18}H_{37}I$	380.4	3.592
12	16.228	Phenol, 3,5-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	206.32	3.789
13	22.436	Methyl 6,6,8,8-tetramethyl-3=oxo-2,5,7,9-tetraoxa-6,8-disilaundecan-11-oate	$C_{10}H_{22}O_7Si$	282.08	6.135
14	28.187	Hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	2.073
15	32.095	Phytol	$C_{20}H_{40}O$	296.5	0.646
16	46.211	Retinoic acid	$C_{20}H_{28}O_2$	300.43	1.109
17	48.131	2-Propenoic acid,2-methyl-,1,2-ethanediylbis (oxy-2,1-ethanediyl) ester	$C_{14}H_{22}O_2$	286.32	4.263
18	49.013	4a (2H)-Naphthalenol, 2-bromo-4,4-dichloro	$C_{10}H_{13}BrCl_2$	286.03	7.614
19	49.596	1-Cyclohexanol, 2-(1-(phenylsulfonyl) methylidene	$C_{13}H_{16}O_3S$	252.26	4.093

placed in a greenhouse maintained at 29±2°C. One seedling per pot remained after thinning at the two-leaf stage. The polypot had been irrigated with 150 mL of either the extract or plain water, depending on the treatment, every other day for six months. Following this period, the seedlings had been gently taken out of the polypot, their branch and root lengths, as well as their fresh weights, were measured and excess soil was cleaned off with tap water. Aluminum foil had been employed to cover the plants along with it dried them for 24 hrs at 60°C to determine their dry weight. Observed parameters in both the bioassay and pot experiments included dry weight, fresh weight, root length, as well as shoot length.

GC-MS analysis of *Clerodendrum infortunatum* leaf

The GC-MS technique had been utilized to analyze and determine the phytochemicals existing in the extract. Table 1 is a list of the phytochemical components found in the methanol extract, on the basis of the findings reported by Uddin *et al.* (2015). This table highlights compounds that may have potential effects on the growth of the test crop, either inhibiting or stimulating it.

Experimental design and statistical analysis

The experiment utilized CRD (Completely Randomized Design), as well as the data had been investigated utilizing ANOVA (one-way analysis of variance). Following the ANOVA results, the Least Significant Difference (LSD) at the 5% level of significance had been computed to distinguish among the means. Statistical analyses had been performed utilizing MS Excel.

RESULTS AND DISCUSSION

In the laboratory bioassay experiment, dry root weight, root length, as well as fresh root weight ex-

Table 2. Effect of *Clerodendrum infortunatum* leaf extracts on the growth and development of maize (Laboratory Bioassay). Effect of *Clerodendrum infortunatum* of laboratory bioassay leaf extracts on the growth and development of maize (RL: Root length, SL: Shoot length, FWS: Fresh weight shoot, FWR:Fresh weight root, DRS: Dry weight shoot, DWR: Dry weight root). Value presented are means±SE (Standard error). Superscripts (a,b,ab) indicates significant variation between extract concentrations. Values in the parenthesis indicate percentage inhibition (–) or else stimulation (+) relative to control.

Treatments	Root length (cm)	Shoot length (cm)	Fresh wt root (g)	Fresh wt shoot (g)	Dry wt root (g)	Dry wt shoot (g)
T_0 (Control)	$10.87^{a}\pm0.63$	$14.89^{b}\pm1.04$	$2.01^{a}\pm0.45$	$4.11^{ab}\pm0.13$	$0.87^{a}\pm0.03$	$1.55^{ab}\pm0.01$
T_1 (20%)	$9.87^{a}\pm2.01$	$15.81^{b}\pm4.51$	$1.91^{a}\pm0.28$	$4.00^{ab}\pm1.30$	$0.87^{a}\pm0.07$	$1.99^{a}\pm0.07$
	(-9.2)	(+ 6.17)	(-4.98)	(-2.68)	(+0)	(+28.38)
T_2 (40%)	$13.70^{a}\pm0.66$	$17.25^{b}\pm2.01$	$2.38^{a}\pm0.27$	$4.21^{ab}\pm0.65$	$0.88^{a}\pm0.11$	$1.68^{ab}\pm0.25$
	(+26.03)	(+15.84)	(+18.4)	(+2.43)	(+1.14)	(+8.38)
T_3 (60%)	$14.01^{a}\pm2.13$	$18.46^{b}\pm4.17$	$2.83^{a}\pm0.77$	$5.02^{b}\pm1.27$	$1.16^{ab}\pm0.16$	$1.56^{ab}\pm0.50$
	(+28.88)	(+23.97)	(+40.79)	(+22.14)	(+33.33)	(+0.64)
T_4 (80%)	$12.01^{a}\pm1.15$	$12.85^{b}\pm2.57$	$3.07^{a}\pm0.43$	$3.74^{ab}\pm0.74$	$1.33^{b}\pm0.19$	$0.99^{ab}\pm0.73$
	(+10.48)	(-13.71)	(+52.73)	(-9.01)	(+52.87)	(-36.13)
T_5 (100%)	$11.30^{a}\pm2.64$	$3.85^{a}\pm0.05$	$2.78^{a}\pm0.44$	$1.37^{a}\pm0.05$	$1.28^{b}\pm0.74$	$0.64^{a}\pm0.08$
	(+3.95)	(-74.15)	(+38.3)	(-66.67)	(+47.12)	(-58.71)

hibited a stimulatory effect in response to increasing concentrations of the extracts. As the concentration of the extracts increased, these values also rose. Among the treatments, C_{60} demonstrated the most significant stimulatory effect in contrast to the C_0 (control). The highest length of the shoot (18.46 cm) as well as the root length (14.01 cm) had been recorded in the C_{60} treatment, while the lowest were found in C_{20}. The highest fresh weights (2.83 g and 5.02 g) were observed in C_{60} and C_{80}, respectively, while the highest dry biomass (1.33 g and 1.99 g) was found in C_{80} and C_{20}, respectively. The extract inhibited the length of the shoots as well as the fresh and dried biomass of the shoots at its highest conc (C_{100}). Nonetheless, the stimulatory effects observed in the other parameters had been statistically significant ($p<0.05$) (Table 2).

In the polypot experiment, root and shoot lengths increased with higher extract concentrations. The most significant ($p<0.05$) length of the root (55 cm) along with length of the shoot (64.33 cm) had been observed with C_{80} & C_{60}, while the shortest root (25 cm) and shoot (12.66 cm) lengths were recorded with C_{60} and C_0, respectively. C_{100} had the highest fresh

Table 3. Effect of *Clerodendrum infortunatum* leaf extracts on the growth and development of maize (Polypot experiment). Effect of *Clerodendrum infortunatum* of polypot experiment leaf extracts on the growth and development of maize (RL: Root length, SL: Shoot length, FWS: Fresh weight shoot, FWR: Fresh weight root, DRS: Dry weight shoot, DWR: Dry weight root).Values represented are means ±SE (Standard error). Superscripts (a,b,c,d,ab,bc,cd) indicates significant variation between extract concentrations. Values in the parenthesis indicate percentage inhibition (–) or stimulation (+) relative to control.

Treatments	Root length (cm)	Shoot length (cm)	Fresh wt root (g)	Fresh wt shoot (g)	Dry wt root (g)	Dry wt shoot (g)
T_0 (Control)	$28.00^{ab}\pm2.08$	$12.66^{a}\pm2.66$	$0.91^{a}\pm0.49$	$3.99^{a}\pm1.64$	$0.30^{a}\pm0.11$	$1.57^{a}\pm0.93$
T_1 (20%)	$39.00^{ab}\pm4.58$	$35.00^{b}\pm7.63$	$2.26^{ab}\pm0.54$	$17.62^{b}\pm1.57$	$0.76^{a}\pm0.09$	$7.21^{a}\pm1.73$
	(+39.28)	(+176.46)	(+148.35)	(+341.6)	(+153.33)	(+359.23)
T_2 (40%)	$42.33^{bc}\pm3.92$	$50.33^{c}\pm2.90$	$6.54^{c}\pm0.53$	$31.54^{c}\pm1.99$	$4.00^{ab}\pm0.66$	$18.94^{b}\pm1.54$
	(+51.17)	(+297.55)	(+618.68)	(+690.47)	(+1233.33)	(+1106.33)
T_3 (60%)	$25.00^{a}\pm8.66$	$41.66^{bc}\pm4.40$	$5.20^{bc}\pm1.17$	$26.46^{bc}\pm2.87$	$2.07^{a}\pm1.08$	$3.33^{a}\pm1.51$
	(-10.28)	(+229.06)	(+471.42)	(+563.15)	(+590)	(+112.1)
T_4 (80%)	$55.00^{c}\pm2.88$	$64.33^{d}\pm2.33$	$4.80^{bc}\pm0.59$	$30.27^{c}\pm5.64$	$2.07^{a}\pm0.50$	$21.08^{b}\pm3.51$
	(+96.42)	(+405.52)	(+427.47)	(+658.64)	(+590)	(+1242.67)
T_5 (100%)	$39.00^{ab}\pm3.78$	$53.66^{cd}\pm1.85$	$14.90^{d}\pm2.46$	$48.76^{d}\pm4.46$	$6.12^{b}\pm2.40$	$35.49^{c}\pm5.18$
	(+39.28)	(+323.85)	(+1537.36)	(+1122.05)	(+1940)	(+2160.5)

weights for both the root (14.90 g) and the shoot (48.76 g), whereas C_0 had the lowest. Significant ($p<0.05$) stimulatory effects were noted for all parameters studied in the polypot experiment (Table 3).

The direct exposure of seeds to these chemicals may be the cause of the inhibitory effects observed at higher conc of the extracts. This observation is consistent with other studies, which reported that aqueous leaf extracts of *Citrus reticulata* inhibited root and shoot growth in soybean, maize, paddy, chilli, and okra (Sahoo *et al.* 2015). Similarly, it was found that extracts of *Trevesia palmate* and *Excoecaria agallocha* reduced root and shoot length, dry matter, germination, vigour index as well as suppressed the shoot as well as root growth of soybean, maize as well as rice (Lalremsang *et al.* 2017a, Desai and Gaikwad 2015). The study's observed inhibitory effects may be connected to the existence of phenolic compounds for example 3,5-bis (1,1-dimethylethyl) phenol, along with 4-(1-methylpropyl) phenol which are known to disrupt plant growth (Table 1). It has also been observed that phenol 2,4-bis (1,1-dimethylethyl) found in leaf extracts of *Dicranopteris dichotoma* inhibits the growth of *Eupatorium catarium* as well as *Bidens pilosa* (Gul *et al*. 2019, Lalremsang *et al*. 2020b). Additionally, Qin *et al.* (2012) demonstrated that Phenol 2,4-Bis (1,1-Dimethylethyl) had inhibitory effects on rice, lettuce and barnyard grass.

The stimulating impact on the length of maize shoot as well as root in the polypot experiment aligns with findings who reported that leaf extracts of *Acacia auriculiformis* and *Flemingia semialata* enhanced shoot length and dry biomass in rice and maize (Vijayan, 2015, Lalremsang *et al.* 2020b). Similar to the outcome of Lalremsang *et al.* (2017c) who discovered that *Schima wallichii's* leaf extracts, as well as *Mesua ferrea*, positively influenced shoot as well as root length along with fresh as well as dry biomass in a polypot experiment, the observed stimulatory effects on maize growth parameters are similar.

CONCLUSION

On maize seedling growth, the allelochemicals found in *C. infortunatum* leaf extracts exhibited both inhibitory along stimulatory impact. These effects differed between the laboratory and polypot experiments. The stimulatory responses observed suggest that the presence of *C. infortunatum* in agricultural fields may not negatively impact maize yield. Nonetheless, further investigation is required to examine the impacts of specific chemical compounds on crop growth as well as development, to better understand their impact across various agricultural systems.

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