Weed Extract of Ageratum conyzoides and Chromolaena odorata to Suppress Weed Growth in the Edamame Cultivation

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Abstract. Allelochemicals are alternatives for reducing chemical herbicide applications. These are plant-based compounds with phytogrowth-inhibitory activities. Allelochemicals can be found in several plant species, including Ageratum conyzoides (Ac) and Chromolaena odorata (Co). This study aimed to investigate the effects of aqueous crude extract of A. conyzoides and C. odorata, known as weed extracts, at various concentrations to suppress weeds in edamame cultivation. The experiment was conducted at the experimental field in Cangapan, Jetis, Bantul, Indonesia and carried out during the period of July to October 2022. This study employed Randomized Complete Block Design (RCBD) model, which consisted of 9 treatments and 3 repetitions, i.e., Ac 15%, Ac 30%, Co 15%, Co 30%, Ac 7.5% + Co 7.5%, Ac 7.5% + Co 15%, Ac 15% + Co 7.5%, Ac 15% + Co 15%, and control. The collected data were analyzed using Analysis of Variance and Tukey's test at the 5% level. This study has shown that weed extract suppresses weed growth with the best application at Ac 15% + Co 15%, with 43.63% efficiency. Weed extract application also shows slight injuries on edamame leaves. However, there is no decreased in chlorophyll content due to the tolerance ability of edamame to allelochemicals.

Keywords**:** weed control, weed extract, A. conyzoides, C. odorata, edamame

1 Introduction

The increasing number of chemical herbicide application has caused several problems to the environment, such as polluting the stream, reducing organic matter in soil, and occurring resistant weed issues [1]. Alternatives to use herbicide that is environmentally friendly has been studied to replace chemical herbicide dependent by farmers [2]. In the recent years, allelochemicals have been considered to be one of the best topics to study its

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phytogrowth-inhibitory activities in order to achieve a sustainable weed management practice.

Allelochemicals define as a secondary metabolite derived from plant that escape into environment as a result of abiotic stress response [3]. It produces several phytotoxic compounds, such as alkaloid, phenols, and terpenes [4]. Plant species that was confirmed to contain allelochemicals and used in several natural herbicide research, including *Ageratum conyzoides, Chromolaena odorata, Imperata cylindrica, Bidens Pilosa, Cyperus rotundus,* and *Euphorbia heterophylla.* In this study, we attempted to investigate the allelochemicals of *A. conyzoides* and *C. odorata* for weed control. Species of *A. conyzoides* and *C. odorata* are easily found in many areas. Moreover, these species were frequent to use in studying allelochemicals as an aqueous crude weed extract.

Billy goat weed (*A. conyzoides)* is a perennial shrub containing allelopathic compounds such as flavonoid, alkaloid, chromene, terpenoid, coumarin, and sterol [5]. This allelopathic effects was reported to suppress *Amaranthus spinosus* growth at concentration of 15% *A. conzyzoides* compared to control [6]. Another study concluded that application of *A. conyzoides* at concentration of 30% showed complete control on *A. spinosus* at 7 days after application [7]. The research of [8] confirmed that the higher concentration of weed extract resulted in higher suppression on weed growth.

Siam weed (*C. odorata)* is a perennial shrub containing allelopathic compounds such as flavonoid, pyrrolizidine, alkaloids [9], monoterpene, and sesquiterpene [10]. The effectiveness of allelochemicals in *C. odorata* as a weed extract was reported to suppress *Mimosa invisa* germination at concentration of 15% compared to the mung bean. The research of [11] reported that combination of weed extract of *C. odorata* and *I.* cylindrica suppressed *A. conyzoides* growth at concentration of 30%.

Edamame is a type of soybean that is introduced from Japan, and it is classified as a vegetable and not a grain crop as in case of mature soybean seeds [12]. In comparison with mature soybean, edamame has a sweat taste, distinctive good flavour, and various nutrients, such as proteins, fiber, folic, vitamin A, C, K, magnesium (Mg), phosphorus (P), manganese (Mn), iron (Fe), potassium (K), calcium (Ca), lipids, carbohydrates, and minerals [13], [14]. As a result, edamame has a higher selling value compared to mature soybean [15]. Moreover, edamame is one of the major Indonesia's exports of vegetable products with the total amount of 6,000-7,000 tons in 2022 [16]. However, the presence of weeds is able to reduce the total yield up to 80% [17]. Therefore, it is necessary to find alternatives to manage weed presence in edamame cultivation.

Previous published studies were limited to single aqueous crude extract application. Currently, there are no data regarding to combination of aqueous crude extract of *A. conyzoides* and *C. odorata.* Therefore, this study set out to assess the effect of single aqueous crude extract of *A. conyzoides* and *C. odorata* and their combination at various concentrations in edamame cultivation.

2 Materials and method

2.1 Design and setting

This study employed Randomized Complete Block Design (RCBD) model, which consisted of 9 treatments with 3 replications per treatment and 35 plants per treatment. The treatment comprised various concentrations of aqueous crude extract of *A. conyzoides* (Ac) and *C.*

odorata (Co) leave parts, namely Ac 15%, Ac 30%, Co 15%, Co 30%, Ac 7.5% + Co 7.5%, Ac 7.5% + Co 15%, Ac 15% + Co 7.5%, Ac 15% + Co 15%, and control.

The study was carried out during the period of July to October 2022 at the experimental field in Cangapan, Jetis, Bantul. The experimental field was located at 7°56'14.9"S latitude and 110°20'35.8"E longitude with regosol soil. The edamame variety "Ryokkoh-75" was sown at 30 cm x 30 cm spacing. The fertilizer dose of 25 kg/ha of urea, 125 kg/ha of SP-36, and 50 kg/ha of KCl were applied to crop at the time of sowing and remaining of 50 kg/ha of urea was applied at 30 days after sowing.

Chlorophyll content in edamame leaves was observed and calculated at 7 days after the first application of weed extracts. Assessment of chlorophyll content was performed at the Integrated Laboratory of Universitas Pembangunan Nasional (UPN) "Veteran" Yogyakarta, Indonesia.

2.2 Sample preparation of plant extracts

The procedure was performed as previously described by [18] with some modifications. Leaves of *A. conyzoides* and *C. odorata* were collected from exploration around the experimental field. The leaves were well cleaned with tap water and air-dried at ambient temperature (27 ℃ ~ 29 ℃) from 09.00 a.m. to 12.00 p.m. The dried leaves were ground by a blender to obtain 500 g of leaves weight. Leaves of *A. conyzoides* and *C. odorata* were separately immersed in tap water for 24 hours with a 1:2 weight/volume ratio. The next step was filtering to remove the plant traces, and diluted with tap water to obtain 7.5, 15, and 30% of aqueous extract concentration. The immersing to filtering process was repeated three times.

Weed extracts were applied with automatic sprayer with spray volume at 400 L/ha. Application of weed extracts were done at 14, 28, and 42 days after sowing.

2.3 Analysis of weed vegetation

The analysis of weed vegetation was carried out before the land was tilled and 49 days after sowing. The analysis was performed by identifying and recording weed species based on characteristic and morphological of each species found in a quadrant (50 x 50 cm). Each species was placed in an envelope and labelled according to its name species.

To determine the weed dry weight or total biomass of oven-dried of weed species, weeds were oved-dried at 80 ℃ for 72 hours or until constant weight. After obtaining constant weight, weeds were weighed using a digital weight scale. The displayed weight number of each weeds species was recorded.

The Summed Dominance Ratio (SDR) values were calculated to find which species dominated the area as previously described by [19] with minor modification through the following formula:

$$\text{Density of species A (D)} = \text{Total number of individuals of species A in observation plot} \quad (1)$$

$$\text{Relative density of species A (RD)} = \frac{\text{Density of species A}}{\text{Density of all species}} \times 100 \quad (2)$$

$$\text{Frequency of species A (F)} = \text{Number of plots where species A is found} \quad (3)$$

$$\text{Relative frequency of species A (RF)} = \frac{\text{Frequency of species A}}{\text{Frequency of all species}} \times 100 \quad (4)$$

$$\text{Dominance of species A (Do)} = \text{Total biomass of oven dried of species A in observation plot} \quad (5)$$

$$\text{Relative dominance of species A (RDo)} = \frac{\text{Dominance of species A}}{\text{Dominance of all species}} \times 100 \quad (6)$$

$$\text{Important values index (IVI)} = \text{RD} + \text{RF} + \text{RDo} \quad (7)$$

$$\text{Summed dominance ratio (SDR)} = \frac{\text{IVI}}{3} \times 100 \quad (8)$$

2.4 Weed control efficiency

The weed control efficiency was calculated at 49 days after sowing as previously described by [20] through the following formula:

$$\text{Weed Control Efficiency (\%)} = \frac{DWC - DWT}{DWC} \times 100 \quad (9)$$

where, *DWC* = Dry matter weight of weed in control plot and *DWT* = Dry matter weight of weed in treated plot.

The efficiency of weed control is characterized in Table 1, based on standard methods of efficacy trials for authorization on herbicides in Hungary by [21].

Table 1. Evaluation of weed control efficiency in nine categories.

Weed Control Efficiency	
98,1 – 100%	Excellent
95,1 – 98%	Very good
90,1 – 95%	Good
82,1 – 90%	Acceptable
70,1 – 82%	Uncertain
50,1 – 70%	Weak
30,1 – 50%	Extremely weak
0,1 – 30%	Bad
0%	Ineffective

2.5 Phytotoxicity of weed extracts on edamame leaves

Phytotoxicity or visual injury on edamame leaves was observed at 7 days after application of weed extract. Visual injury was recorded on percentage leaf damage by visual score in Table 2, including chlorosis, white blotches, and bronzing. Leaves of edamame were observed in all repetitions and treatments based on the rating scale as previously presented by [22] with minor modification:

Table 2. Phytotoxicity rating scale for determining the Percent Phytotoxicity.

Rating	**Percent Phytotoxicity (leaf injury)**	**Verbal Description**
0	0	No evident effects/symptoms

1	1-10	Slight discoloration, distortion, stunting
2	11-20	More severe, but not lasting
3	21-50	Medium severe and lasting
4	>50	Severe necrosis or wilting, shrivelled

The percent phytotoxicity index of edamame leaves were calculated using the following formula [23] with minor modification:

$$\text{Percent Phytotoxicity Index (\%)} = \frac{\sum(n \times v)}{(N + Z)} \times 100 \quad (10)$$

where, n = Number of plant damaged, v = Phytotoxicity rating, N = Total number of plants observed, and Z = Maximum of phytotoxicity rating.

Phytotoxicity effects of weed extracts on edamame leaves were categorized using the European Weed Research Council (EWRC) in Table 3. The categorization was previously described by [24]:

Table 3. European Weed Research Council (EWRC) rating scale for phytotoxicity.

EWRC Score	Crop Tolerance	Damaged Plants (%)
1	No effect	0
2	Very slight effects; some stunting and yellowing just visible	1
3	Slight effects; stunting and yellowing; effects reversible	2
4	Substantial chlorosis and or stunting; most effects probably reversible	5
5	Strong chlorosis/stunting; thinning of stand	10
6	Increasing severity of damage	25
7	Increasing severity of damage	50
8	Increasing severity of damage	75
9	Total loss of plants and yield	100

2.6 Chlorophyll content on edamame leaves

To determine chlorophyll content on edamame leaves, 1 g of edamame leaves were collected from each treatment and each repetition. Leaves of edamame were ground by pestle in a mortal with 50 mL of ethanol 96%, then left for 24 hours. The chlorophyll extract was filtered by filter paper and then poured into 100 mL of volumetric flask. To reach at 100 mL level, ethanol 96% was added into the same volumetric flask.

Chlorophyll content estimation was performed using Spectrophotometer UV-VIS that computed the Optical Density (OD) at two wavelengths. The optical density or absorbance readings were performed at wavelengths of 649 nm and 665 nm. However, calibration procedure for the Spectrophotometer UV-VIS was needed before estimating chlorophyll content. Transmittance value was adjusted at 100% (Absorbance value = 0) using ethanol 96% as the blank solution (buffer). Due to the blank measurement, there should be no absorbance of the buffer recorded, and only the substance of interest values was recorded. Measurement on chlorophyll content was performed 3 times.

To obtained values for substitution in the following formulas, described by [25] using the Wintermans and De Mots equation, the estimation of photosynthetic pigments is expressed as:

$$\text{Chlorophyll a (mg/L)} = (13.7 \times OD665) - (5.76 \times OD649) \quad (11)$$

$$\text{Chlorophyll b (mg/L)} = (25.8 \times OD649) - (7.7 \times OD665) \quad (12)$$

$$\text{Chlorophyll total (mg/L)} = (20 \times OD649) + (6.1 \times OD665) \quad (13)$$

where OD649 and OD665 were measured from 649 nm and 665 nm, respectively.

2.7 Data analysis

The collected data were analyzed using analysis of variance. If an effect of treatment was observed, post hoc testing was performed using Tukey test at a 5% probability level. The analysis was performed using Microsoft Excel 365.

3 Result and discussion

3.1 Analysis of weed vegetation on edamame plantation

Based on the observations regarding to analysis of weed vegetation before tilling at the experimental field, there was 4 species of broadleaf weeds, including *Alternanthera sessilis, Ammannia baccifera, Ipomoea aquatica,* and *Ludwigia erecta*. Moreover, 2 species of grasses weeds were found, such as *Cynodon dactylon* and *Juncus compressus* as shown in Table 4. Through estimating the summed dominance ratio (SDR), population, and total weed dry weight, *I. aquatica* was found to be the most dominant species, resulting 32,58%, 36, and 13,91 g/m^2, respectively.

The species ability to grow and dominate in the particular area is determined by the SDR values. The higher SDR value of a species indicates that the species dominates the area [19], which causes low species diversity [26]. According to a study conducted by [27], concluded that the number of *I. aquatica* remained high especially in agricultural land after flooding treatment. Based on the observation during vegetation analysis, the area had been used to grow paddy rice. Edamame produces high yield where cultivated in regosol, grumusol, and alluvial soils which is the same type of soil used for paddy rice cultivation [28]. However, application of weed extracts with various concentrations resulted a weed shift at the experimental field compared to analysis of weed vegetation before tilling as shown in Table 5 and Table 6.

Table 4. Summed dominance ratio (SDR), populations, and total weed dry weight before tilling.

No.	Species of weed	SDR (%)	Populations of weed	Total weed dry weight (g/m^2)
Broadleaf				
1	*Alternanthera sessilis*	14.48	13	3.59
2	*Ammannia baccifera*	8.30	7	1.60

3	*Ipomoea aquatica*	32.58	36	13.91
4	*Ludwigia erecta*	9.94	4	2.40
Grasses				
1	*Cynodon dactylon*	26.84	22	13.56
2	*Juncus compressus*	7.85	4	2.40
Total		100	86	37.46
Ac (*Ageratum conyzoides);* Co (*Chromolaena odorata*).				

Estimation value of SDR in Table 5 shows that *P. angulata* is the most dominant weed species in treated plot of Ac 15%, Ac 30%, Co 15%, Ac 15% + Co 7.5%, Ac 15% + Co 15%, and untreated plot (control). Other treated plots, such as Co 30% and Ac 7.5% + Co 15% shows that *A. viridis* is the most dominant species among the other species. In Ac 7.5% + Co 7.5% treated plot, *C. dactylon* is the most dominant species.

A community has a high species diversity when it is composed of many different species. The high diversity of weed species compared to the analysis of weed vegetation before tillage was caused by the plant spacing of edamame plants. Due to the wider spacing used in edamame cultivation, nutrient availability, water, sunlight, and growth space are provided for weeds to develop and utilize it [26]. The density of weeds has distinct abilities to compete differently with crops and other weeds based on the morphological and physiological characteristic while reducing the total yield [29]. Besides plant spacing, land preparation, such as manure input and tillage, may stimulus the emergence of weed on the soil surface. In the case of plowed field, weed seeds that are distributed in the soil layer up to 30 cm will disperse on the upper soil layer due to the land preparation to create an optimum condition for plant to grow. Nevertheless, this condition is favourable for weed to germinate as well [30]. As a result, a sudden increase of weed species occurred in the experimental field.

Table 5. Summed dominance ratio (SDR) of weeds at 49 days after sowing (DAS) across the treatments in edamame cultivation

No.	Species of weed	SDR of weeds after weed extract application (%)								
		Ac 15%	Ac 30%	Co 15%	Co 30%	Ac 7.5% + Co 7.5%	Ac 7.5% + Co 15%	Ac 15% + Co 7.5%	Ac 15% + Co 15%	Control
Broadleaf										
1	*Acalypha indica*	-	2.48	-	-	-	-	-	6.06	-
2	*Alternanthera sessilis*	3.28	3.03	5.05	-	3.90	1.80	2.18	-	3.74
3	*Amaranthus viridis*	17.85	17.26	16.19	19.56	13.60	21.25	16.77	16.06	18.05
4	*Atriplex patula*	-	-	3.27	-	-	3.78	-	-	2.61
5	*Chenopodium album*	3.89	-	-	-	3.24	-	-	-	-
6	*Cleome rutidospermae*	6.47	-	3.72	2.77	3.34	-	6.79	-	2.72
7	*Commelina diffusa*	-	-	-	-	-	-	-	2.23	-
8	*Cyanthillium cinereum*	-	-	-	1.92	-	-	-	-	-
9	*Eclipta prostata*	-	4.60	3.36	6.85	-	2.24	2.22	1.98	1.90

10	*Heliotropium indicum*	12.33	10.11	10.14	9.20	12.67	9.35	10.15	16.10	10.55
11	*Ipomea aquatica*	6.66	-	1.85	-	-	5.56	-	-	2.33
12	*Ludwigia erecta*	-	-	-	-	-	-	-	-	2.16
13	*Phyllanthus amarus*	-	7.72	8.78	11.97	6.10	8.44	9.68	5.86	6.26
14	*Physalis angulata*	25.32	21.55	18.00	13.64	16.69	11.03	18.07	19.21	18.72
15	*Portulaca oleracea*	-	-	-	2.39	5.62	2.09	2.18	1.98	-
16	*Spigelmia anthelmia*	-	-	-	-	-	-	-	-	1.78
Grasses										
1	*Cynodon dactylon*	14.23	17.04	12.58	13.82	21.02	17.01	16.95	13.02	14.62
2	*Echinochloa colona*	9.98	16.21	17.05	16.03	13.81	17.44	15.01	17.51	14.56
3	*Digitaria sanguinalis*	-	-	-	1.85	-	-	-	-	-
Total		100	100	100	100	100	100	100	100	100

Ac (*Ageratum conyzoides);* Co (*Chromolaena odorata*).

Based on observation on weed populations in Table 6, *A. viridis* is the highest species at treated plot of Ac 15%, Co 15%, Co 30%, Ac 7.5% + Co 15%, Ac 15% + Co 7.5%, and untreated plot (control). In other treated plots, such as Ac 30%, Ac 7.5% + Co 7.5%, Ac 15% + Co 7.5%, and Ac 15% + Co 15% shows that *C. dactylon* is the highest weed among other weed species. In conclusion, *P. angulata, A. viridis,* and *C. dactylon* are the most common weeds to be found in the experimental field after application of weed extracts.

Allelochemicals in weed extracts play a key role in suppressing weed growth in a plantation that leads to a weed shift at the experimental field. Modification of weed growth and development in response to allelochemicals may reflect alterations in the molecular biology of cells and interrupt the activities of membrane proteins, as well as the intracellular enzymes [31]. By inhibiting plasma membrane H^+-ATPase, mitochondrion, chloroplast electron transport, and altering protein metabolism, allelochemicals may induce abnormalities on cell that are reflected in morphological disturbances and decreased growth rates in the target weeds [32].

Interestingly, the total weed dry weight at 49 DAS shows insignificant differences to application of weed extracts as shown in Table 7, suggesting that concentrations in weed extracts are yet to disrupt the physiological activities of weeds. Further analysis of WCE at 49 DAS shows that application of Ac 15% + Co 15% is 43.63% (extremely weak), resulting in highest suppression to weeds among other treatments. Meanwhile, application

of Ac 15% is 13.89% (bad), resulting in lowest suppression to weeds in edamame cultivation.

Weed responses to an extract depends on the concentration of active compounds in a weed extract [33]. The degree of suppression will be increased when the concentration of the weed extract is raised [34]. In contrast, lower concentration of weed extract may result in lower toxicity which causes weed extract incapable to disrupt physiology activity of the target weeds [35]. As result, the value of WCE will be lower than 82.1% that is categorized as "weak" or even "ineffective" [21].

Table 6. Populations of weeds at 49 days after sowing (DAS) across the treatments in edamame cultivation.

No.	Species of weed	Populations of weed after weed extract application								
		Ac 15%	Ac 30%	Co 15%	Co 30%	Ac 7.5% + Co 7.5%	Ac 7.5% + Co 15%	Ac 15% + Co 7.5%	Ac 15% + Co 15%	Control
Broadleaf										
1	*Acalypha indica*	-	2	-	-	-	-	-	6	-
2	*Alternanthera sessilis*	3	3	4	-	2	1	1	-	6
3	*Amaranthus viridis*	22	25	24	33	23	49	25	20	24
4	*Atriplex patula*	-	-	2	-	-	1	-	-	3
5	*Chenopodium album*	3	-	-	-	1	-	-	-	-
6	*Cleome rutidospermae*	4	-	3	2	1	-	9	-	3
7	*Commelina diffusa*	-	-	-	-	-	-	-	1	-
8	*Cyanthillium cinereum*	-	-	-	1	-	-	-	-	-
9	*Eclipta prostata*	-	2	2	5	-	2	2	1	1
10	*Heliotropium indicum*	11	12	12	8	16	11	12	15	10
11	*Ipomea aquatica*	2	-	1	-	-	6	-	-	1
12	*Ludwigia erecta*	-	-	-	-	-	-	-	-	1
13	*Phyllanthus amarus*	-	7	14	19	8	13	15	-	12
14	*Physalis angulata*	8	8	7	4	7	5	7	7	10
15	*Portulaca oleracea*	-	-	-	2	2	2	1	14	-
16	*Spigelnia anthelnia*	-	-	-	-	-	-	-	1	1
Grasses										
1	*Cynodon dactylon*	15	26	16	21	38	21	27	20	22
2	*Echinochloa colona*	14	16	16	13	15	22	13	11	16

3	*Digitaria sanguinalis*	-	-	-	1	-	-	-	-	-
Total		100	82	101	101	109	113	133	112	96
Ac (*Ageratum conyzoides);* Co (*Chromolaena odorata*).										

Table 7. Means of total weed dry weight at 49 days after sowing (DAS), weed control efficiency (WCE) across the treatments in edamame cultivation.

Weed extract concentration	Total weed dry weight (g/m^2)	WCE (%)
Ac 15%	39.72 a	13.89
Ac 30%	29.55 a	33.03
Co 15%	38.66 a	16.03
Co 30%	34.33 a	28.65
Ac 7.5% + Co 7.5%	33.47 a	28.22
Ac 7.5% + Co 15%	29.79 a	33.61
Ac 15% + Co 7.5%	29.70 a	36.36
Ac 15% + Co 15%	26.30 a	43.63
Control	46.28 a	00.00
Means in the same columns with the same letter are not significantly different. Ac (*Ageratum conyzoides);* Co (*Chromolaena odorata*).		

3.2 Phytotoxicity assessment

Observation on visual injury of weed extracts on edamame is summarized in Table 8. Application of weed extracts shows significantly different at 7 days after the first and second application of weed extracts. However, there was no visual injury found after the last application of weed extracts. At 7 days after the first weed extract applications, Ac 15%, Ac 30%, Co 30%, Ac 7.5% + Co 15%, and Ac 15% + Co 15% produced very slight injury on edamame leaves. Other weed extract concentrations, such as Co 15%, Ac 7.5 + Co 15%, and Ac 15% + Co 7.5% were found safe to edamame.

At 7 days after the second application of weed extracts, Ac 15%, Ac 30%, Co 30%, Ac 7.5% + Co 7.5%, Ac 15% + Co 7.5%, and Ac 15% + Co 15% produced very slight injury while Ac 7.5% + Co 15% produced slight effects on edamame leaves. Application of Co 15% was found to be the only concentration that safe to edamame. However, all treatments were found to cause slight effects after the third application of weed extracts as shown in Table 8.

An increased number of visual injuries on edamame leaves were not found after the third weed extract application due to edamame ability to adapt and create an antibody which modified the toxicity of allelochemicals inside edamame to a non-toxic compound [36]. As a potential novel herbicide, allelochemical-based herbicides selectively suppress plant species while providing low toxicity and environmental safety [37].

Table 8. Means of visual injury on edamame leaves after weed extract application

Weed extract concentration	Percent phytotoxicity (leaf injury) at 7 days after each application			EWRC score for phytotoxicity		
	1A	2A	3A	1A	2A	3A
Ac 15%	0.48 ab	0.71 ab	1.43 a	2	2	3
Ac 30%	0.71 a	0.71 ab	1.67 a	2	2	3
Co 15%	0.00 b	0.00 b	1.19 a	1	1	3

Co 30%	0.48 ab	0.48 ab	1.67 a	2	2	3
Ac 7.5% + Co 7.5%	0.00 b	0.48 ab	1.43 a	1	2	3
Ac 7.5% + Co 15%	0.71 a	1.43 a	2.38 a	2	3	3
Ac 15% + Co 7.5%	0.00 b	0.24 b	1.67 a	1	2	3
Ac 15% + Co 15%	0.24 b	0.71 ab	2.38 a	2	2	3
Control	0.00 b	0.00 b	0.00 a	1	1	1

Means in the same columns with the same letter are not significantly different.

1A=First application of weed extract; 2A=Second application; 3A=Third application.
Ac (*Ageratum conyzoides);* Co (*Chromolaena odorata*).
1=No effect; 2=Very slight effects; 3=Slight effects.

Regardless of the slight effects of phytotoxicity on edamame leaves as summarized in Table 8, Table 9 describes that chlorophyll content shows insignificant differences to the application of weed extracts. In edamame, allelochemicals are relatively tolerant, suggesting that phytotoxicity does not directly affect the number of chlorophylls. Amount of chlorophyll in leaf tissue is influenced by environmental stress and nutrient availability [38]. Chlorophyll is synthesized within chloroplast from a plentiful precursor, namely amino acid glutamate [39], and elements, such as nitrogen, iron, and magnesium. They play a key role in photosynthesis for plant's growth and development [40]. Based on a previous study that demonstrated the effect of aqueous crude extract of *A. conyzoides* on the seed germination of mung bean, aqueous crude extract of *A. conyzoides* showed insignificant differences at 7 days after application. However, shoot height of mung bean was shorter than control based on visual observation, suggesting that allelochemicals inside mung bean continuously suppress plant growth with a slight suppression [2].

Table 9. Means of chlorophyll content on edamame leaves after weed extract application.

Weed extract concentration	**Chlorophyll content (mg/L)**		
	Chlorophyll a	Chlorophyll b	Total chlorophyll
Ac 15%	12.67 a	5,52 a	18,29 a
Ac 30%	14.87 a	5,89 a	20,87 a
Co 15%	15.21 a	6,14 a	21,46 a
Co 30%	13.76 a	5,35 a	19,21 a
Ac 7.5% + Co 7.5%	15.33 a	5,79 a	21,40 a
Ac 7.5% + Co 15%	14.84 a	5,78 a	20,73 a
Ac 15% + Co 7.5%	16.17 a	4,96 a	21,25 a
Ac 15% + Co 15%	14.64 a	5,94 a	20,69 a
Control	16.87 a	7,03 a	24,02 a

Means in the same columns with the same letter are not significantly different.
Ac (*Ageratum conyzoides);* Co (*Chromolaena odorata*).

4 Conclusion

This study demonstrates that weed extracts of *A. conyzoides* and *C. odorata* has suppressed weed growth in edamame cultivation with the best application at Ac 15% + Co 15%. This treatment results efficiency of weed control at 43.63%. In addition, the application of weed extracts shows slight effects of visual injury on edamame leaves. However, there is no decrease in chlorophyll content due to the tolerance of edamame to allelochemicals in the weed extracts.

This finding has extended the knowledge of utilizing allelochemicals of *A. conyzoides* and *C. odorata* as a single and combined extract application, suggesting a potential use of combined aqueous crude extract for weed control. Regarding to the development of weed extract for further use, it will be necessary to consider of using surfactant, such as detergent at 0.3 g, while applying weed extracts. Surfactants will help its spray stick onto weeds instead of falling onto the soil immediately. Moreover, using a polar solvent, such as ethanol 70% instead of tap water, will enhance the toxicity of weed extracts due to its ability to extract phenolic compounds from plant leaves.

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