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Effect of Allelopathy from Methanolic Extract of Broadleaf Weeds (*Ageratum conyzoides* and Borreria alata) on the Viability of Soybean Seeds (*Glycine max* L.)

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Abstract

The experiment aimed to investigate the allelopathic effects of active compounds present in broadleaf weeds *Ageratum conyzoides* and *Borreria alata*. The study also sought to assess the viability of various seed varieties grown using allelopathic extracts from these weeds, varying in concentrations of tannins and phenols. The research findings revealed that methanol extracts exhibited potent allelopathic properties, inhibiting germination, radicle and hypocotyl growth, as well as reducing the germination rate of soybean seeds. *B. alata* weed extracts demonstrated lower allelopathy than *A. conyzoides*, suggesting a possible alteration in the active compounds (allelochemicals) present due to a reforming process. Extracts from both *A. conyzoides* and *B. alata* weeds inhibited root length, hypocotyl growth, and germination across a range of concentrations, from low (10% v/v) to high (30% v/v).

Keywords: allelopathy, phenols, sprouts, tannins, viability

Introduction

Allelopathy refers to the release of chemical compounds by weeds that are toxic and disrupt the growth of surrounding plants (Andika et al., 2020; Junaedi et al., 2006; Tanjung et al., 2023). Some weeds compete with plants by secreting compounds and toxic substances from their roots (root exudates or leachates) or from decaying vegetative parts. Allelopathy, as a competitive strategy, involves the release of substances that poison other plants. Weeds exhibiting allelopathy possess a stronger competitive ability, hindering the growth of main plants and reducing yields (Kilkoda, 2015; Nugroho et al., 2022; Prasetia et al., 2022). In general, the compounds released belong to the phenol group, specifically carbolic acid or benzenol. These substances are colorless crystalline compounds with a characteristic odor, characterized by the chemical formula C6H5OH. The structure of these compounds includes a hydroxyl group (-OH) bonded to a phenyl ring (Aldywaridha et al., 2021; Lehninger, 2000; Prasetia et al., 2022).

Allelopathy constitutes a category of secondary metabolites that can be categorized into three groups: gases released by above-ground plant parts, exudates from roots, and exudates from above-ground plant parts. These allelopathic compounds, namely phenolics, terpenoids, and alkaloids, are volatile in nature. They possess the ability to repel insects or inhibit the growth of competing plants (Moenandir, 1993; Tanjung et al., 2023; Windartianto et al., 2018). Chemical compounds with allelopathic potential are present in various plant tissues, including leaves, stems, roots, rhizomes, tubers, flowers, fruits, and seeds (Kilkoda et al., 2015; Rahayu et al., 2021; Satroutomo and Sutikno, 1992).

Natural allelopathy can be classified into two types: (1) true allelopathy and (2) functional allelopathy. True allelopathy refers to the release of original toxic compounds from plants into the surrounding environment. In contrast, functional allelopathy involves the release of harmful chemical compounds by plants into the surrounding environment after being modified by soil microbes. Chemical compounds with allelopathic potential are present in various plant tissues, such as leaves stems, roots, rhizomes, fruits, and seeds (Junaedi et al., 2006; Satroutomo and Sutikno, 1992).

Plants can compete with each other in biochemical interactions. Some plants emit toxic compounds into the surrounding environment and can interfere with

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the growth of nearby plants. Biochemical interactions between weeds and plantings include causing disturbances in seed germination, sprouts becoming abnormal such as elongated growth, stunted roots, changes in the arrangement of root cells, and so on (Hafsah et al., 2012; Nugroho et al., 2022; Windartianto et al., 2018). *Ageratum conyzoides,* and *Borreria alata* are broadleaf weeds that are often found in soybean planting areas (Anistia et al., 2022; Kilkoda, 2015; Kilkoda et al., 2015; Wardani et al., 2018). Both of these weeds have high competitiveness because they can inhibit seed germination so that they can cause losses in crop cultivation (Dewi et al., 2017; Kilkoda, 2015; Kilkoda et al.n 2015; Rahayu et al., 2021).

The inhibition caused by the allelopathic effects of *Ageratum conyzoides* and *Borreria alata* on the germination of soybean with varying seed sizes has not been previously documented. The extent of allelopathic inhibition by each weed, in terms of suppressing germination, is likely to vary based on the allelopathic content of the plant variety and the size of its seeds. Consequently, this research aims to investigate the impact of methanol extracts from *A. conyzoides* and *B. alata* weeds on the germination process of multiple soybean varieties with diverse seed sizes.

Material and Methods

Analysis of phenol and tannin levels of methanolic extract from *Ageratum conyzoides* and *Borreria alata* was carried out at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Pattimura University. Soybean seed germination tests were conducted at the Laboratory of Physiology and Plant Analysis, Faculty of Agriculture, University of Pattimura, Poka Ambon, Maluku in September 2022. The materials used to test the germination were small soybean seeds (“Gepak Kuning” variety), medium size seeds (“Gema” variety), and large seeds (“Grobogan” variety).

The materials used technical-grade methanol, gallic acid, tannic acid, Folin-Ciocâlteu reagent, Folin-Denis reagent, saturated Na_2CO_3. The equipment used for analyzing phenol and tannin levels is a stainless-steel pan, glass jar, magnetic stirrer, beaker, aluminum foil, thermometer, pH meter, Bunsen burner, measuring cup, filter, analytical balance, dropper pipette, titration flask, filter paper, gas chromatography, and Shimadzu UV-1201 UV-Vis spectrophotometer. The germination test used 20-cm Petri dishes, an Ohaus Pioneer digital scale (0.0001-200 g), a digital caliper, and a ruler.

Phenol and Tannin Content Analysis

Quantification of phenol and tannin of the weed extracts used the spectrophotometric method (AOAC, 2000). Soybean germination and seed viability test was conducted using a completely randomized design in factorial form with three replications. The first factor was the concentration of weed extract (*Ageratum conyzoides* + *Borreria alata*), which consisted of four levels: 0 %v/v (control), 10 %v/v, 20 %v/v, 30 %v/v. The second factor was soybean seed size which consisted of three groups: small seed size (“Gepak Kuning” variety; 100 seed weight 6.82 g); medium seed size (“Gema” variety; 100 seed weight =12 g); large seed size (“Grobogan” variety; 100 seed weight= 17.8 g). Each treatment factor was combined so that overall, there were 36 experimental units.

Preparation of Weed Extract

The preparation of Ageratum conyzoides and Borreria alata weed extracts was carried out using the methanol extraction method. Five grams each of fresh leaves of *Ageratum conyzoides* and *Borreria alata* were mashed and extracted with methanol by maceration method for 3 x 24 hours. The maceration was repeated followed by filtering using a rotary evaporator to produce clean methanol extract (Halimatussakdiah et al., 2018). The methanol extract obtained was then prepared in several concentration levels to test the viability of several varieties of soybean seeds at a later stage.

Determination of the phenolic content of *Ageratum conyzoides* and *Borreria alata* leaf extracts was measured with a spectrophotometer using Folin-Ciocâlteu reagent and gallic acid as a comparison. The principle of this method is the formation of a blue complex compound due to the reaction between the phenolic compounds in the sample and the Folin-Ciocâlteu reagent in an alkaline condition, which can be measured with a visible spectrophotometer, then equated with gallic acid (Hapsaria et al., 2018; Puspaningrum et al., 2022). Determination of phenol content begins with determining the maximum wavelength of gallic acid at a wavelength of 400-800 nm using UV-Vis spectrophotometry. Determination of the maximum wavelength aims to determine the wavelength required for the gallic acid solution to achieve maximum absorption. The gallic acid solution varied in concentration, namely 0, 0.1, 0.2, 0.4, 0.8, and 1.6 $mg.g^{-1}$ then the absorbance was measured. The results of measuring the absorbance of a standard solution of gallic acid using UV-Vis spectrophotometer were carried out at the maximum wavelength. The results were then substituted into the regression equation obtained on the calibration

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curve to obtain the phenol content of the extracts of the two weeds.

Determination of the tannin content of *Ageratum conyzoides* and *Borreria alata* weed extracts begins with making a standard solution of tannic acid using Folin-Denis reagent. The Folin-Denis reagent acts as an oxidizer, where the oxidized tannin will change the phospholybdate in Folin-Denisinto blue phospholybdenim which can absorb light in the ultraviolet visible wavelength region (Pratiwi and Sughita, 2020). The tannic acid standard curve was prepared using concentrations of 0, 10, 20, 40, 80, 160 mg.L^{-1} to which Folin-Denisreagent and Na_2CO_3 solution were added. Determination of tannin content was carried out by measuring the absorbance value of the methanol extract of *A. conyzoides* and B. alata weeds using a spectrophotometer at the maximum wavelength. The results were then substituted into the regression equation obtained on the calibration curve to obtain the tannin content of the extracts of the two weed species.

Soybean Seed Viability Testing

Testing the viability of soybean seeds used four concentration levels of weed extracts (*A. conyzoides* and *B. alata*), where the concentration of 0 (control) was distilled water. The concentration of the weed extract was used to test three soybean varieties based on their seed size criteria. Testing the viability of soybean seeds begins by preparing 36 petri dishes, and each petri dish is padded with two pieces of cotton fiber. The cotton fiber is then moistened with weed extract according to the treatment concentration. Before the seeds are placed on the cotton in a petri dish, the seeds were first soaked in weed extract according to the concentration used. Four seeds were then put in each petri dish that has been treated (extracts with various concentrations). Measurements of seed viability began with the appearance of the radicle and hypocotyl, which was carried out daily for seven days.

Data Analysis

The data from the phytochemical analysis of the allelopathic content of *Ageratum conyzoides* and *Borreria alata* were presented descriptively, while the data from the soybean seed viability test used analysis of variance (F test) at the 5% level. If a treatment significantly affects the observed response, then data analysis is continued with the Duncan Multiple Range Test (DMRT) (Mattjik and Sumertajaya, 2013). Research data was analyzed using STAR 2.0.1 software.

Result and Discussion

Phenol Content of the Weed Extracts

The maximum wavelength of gallic acid to quantify phenol was 760 nm (Table 1). The test results showed that the absorbance value increased to a maximum of 1,608 at a gallic acid concentration of 1.6 mg. g^{-1}. The gallic acid regression equation obtained is y=1.0055x-0.000, and the correlation coefficient (r) is 0.9992 (Figure 1). The results of substitution into the regression equation obtained phenolic levels of both weed extracts (Table 2). The analysis showed that the phenol content of *A. conyzoides* was 1.61%, while the phenol content of B. alata was 1.25%.

Tannin Content of the Weed Extracts

The maximum absorption wavelength for tannic acid was 760 nm. The maximum absorbance value obtained at the maximum wavelength was 1.184 at a tannic acid concentration of 160 mg.L^{-1} (Table 3). The standard curve equation for tannic acid had a good R-value (r=0.9975) with the line equation y = 0.0072x + 0.036 (Figure 2).

The absorbance values of the methanol extracts of *Ageratum conyzoides* and *Borreria alata* weeds at 760 nm showed the tannin levels of both weed extracts.

Table 1. The standard concentrations of gallic acid

The concentration of gallic acid (mg.g^{-1})	Absorbance	Regression equation
0.0	0.000	1.005x-0.000
0.1	0.076	
0.2	0.229	
0.4	0.398	
0.8	0.801	
1.6	1.608	

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Table 2. Phenol content of *Ageratum conzoides* and *Boreria alata* using the UV-Vis spectrophotometry method

Weed species	Initial concentration	Initial concentration ($mg.g^{-1}$)	Sample absorption	Phenol concentration ($mg.g^{-1}$)	The average phenol concentration ($mg.g^{-1}$)	Phenol content (%)
Ageratum conzoides	51mg.1.4058g^{-1}	36.2783	0.578	0.58	0.58	1.61
			0.589	0.59		
			0.590	0.59		
Borreria alata	51.1mg.1.462g^{-1}	34.9521	0.432	0.43	0.44	1.25

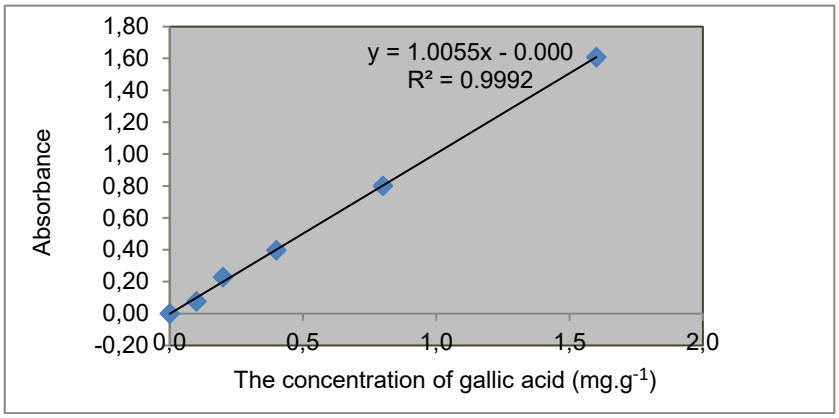

Figure 1. The absorbance of the gallic acid standard curve

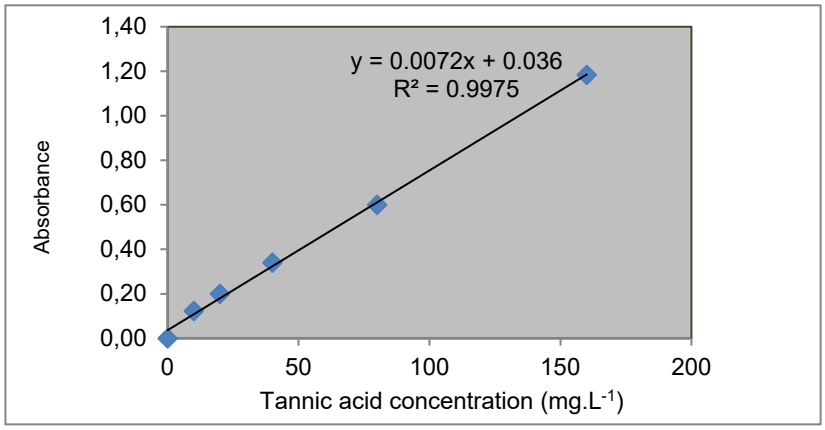

Figure 2. Absorbance standard curve of tannic acid

The results of the substitution of the regression curve obtained showed that the tannin content of the weed *A. conyzoides* was 1.16%, while the phenol content of B. alata was 0.98% (Table 4).

Weed Extract Test on Soybean Seed Viability

The statistical analysis results of all germination variables showed no interaction between weed extract treatment and soybean seed size. The results of the single factor variance of seed size showed a significant effect on the fresh weight of sprouts and radicle length. At the same time, seed size did not affect the number of seeds germinated, the nodes' dry weight, and the hypocotyl's height. The single factor of weed extract showed a significant effect on all observed variables (Table 5).

The Duncan's Multiple Range (DMRT) test results showed that the variables for the number of germinated seeds, dry weight of sprouts, and hypocotyl length did not differ between the three varieties. The only differences were in the shoots' fresh weight and the radicle's length. The "Grobogan" variety had a heavier fresh weight of sprouts (10.78 g), while the "Gepak Kuning" and "Gema" varieties had sprout weights of 7.67 g and 8.58 g, respectively. The most extended radicle length is also found in the "Grobogan" variety,

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Table 3. The standard concentrations of tannic acid

Tannic acid concentration ($mg.L^{-1}$)	Absorbance	Regression equation
0	0.000	0.007x-0.036
10	0.123	
20	0.200	
40	0.340	
80	0.600	
160	1.184	

Table 4. The tannin content of *Ageratum conzoides* and *Boreria alata* using UV-Vis spectrophotometry.

Weed species	Initial concentration	Initial concentration ($mg.g^{-1}$)	Sample absorption	Tannin concentration ($mg.g^{-1}$)	The average tannin concentration ($mg.g^{-1}$)	Tannin content (%)
Ageratum conzoides	0.5018/50ml	10036	0.866	118.57	116.48	1.16
			0.850	116.29		
			0.838	114.57		
Borreria alata	0.5108/50ml	10216	0.750	102.00	99.95	0.98
			0.732	99.43		
			0.725	98.43		

with a radicle length of 5.05 cm, which is significantly different from the radicle length of the “Gepak Kuning” variety and the “Gema” variety. The shortest radicle length was found in the seeds of the “Gepak Kuning” variety (4.37 cm), but it was not significantly different from the “Gema” seeds (4.62 cm).

The difference in the soybean seed sizes did not affect the number of seeds germinated, the sprouts’ dry weight, and the hypocotyl length. However, the amount of inhibition was more influenced by the concentration of allelopathy. The height of the radicle and the fresh weight of the sprouts differed, but they were still inhibited at higher concentrations of weed extract.

Weed allelopathy, which contains phenols and tannins, can inhibit seed germination because the phenolic and tannin compounds absorbed into the seeds will inhibit the metabolism of the endosperm overhaul (Puspaningrum et al., 2022; Sumi et al., 2018). Germination begins after imbibition, which stimulates the activity of germination hormones and enzymes. The inclusion of phenolic and tannin compounds will result in damage to the catalytic power of the germination enzymes, especially those related to the breakdown of carbohydrates (Mohammad et al., 2022; Rahayu et al., 2021).

Sprout growth inhibition is influenced by many factors, including obstacles to the mobilization of nutrients resulting from the breakdown of the endosperm to the institution. The high protein content reflects an indication of mobilization barriers. The protein content indicates the rapid activity of germination enzymes in overhauling the seed endosperm. Accumulation of soluble protein accompanied by symptoms of inhibition of sprout growth can show inhibition of metabolite mobilization from the endosperm to the institution so that the development of the sprouts is also hampered (Rahayu et al., 2021; Sadjad, 2014).

Another possible cause of the stunted growth of sprouts is the disturbance of hormone activities due to allelochemical substances in the extract; Einhellig (1995) confirmed that coumarin, cinnamic acid, and their derivatives can inhibit the transport of the hormone gibberellin. The inclusion of phenolic compounds can increase the decarboxylation of IAA so that IAA becomes inactive, and its growth is stunted (Andriyani et al., 2010; Hapsaria et al., 2018; Puspaningrum et al., 2022). The mechanism of germination inhibition by tannins can occur in two ways, namely (1) tannins act as protein or enzyme inhibitors that specifically recognize GA; (2) tannins bind to GA3 so that GA becomes inactive (Murrinie et al., 2021).

The effect of allelopathy on germination may have occurred during water intake, as the water has been mixed with weed extracts containing inhibitory substances. The uptake of weed extract will disturb

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Table 5. Effect of weed methanol extract treatment and soybean seed size on seed viability.

Treatments	Number of germinated seeds	Sprout fresh weight (g)	Sprout dry weight (g)	Hypocotyl length (cm)	Radicle length (cm)
Seed size					
Small seeds (“Gepak Kuning”)	7.75 a	7.67 c	4.76 a	2.95 a	4.37 b
Medium seeds (“Gema”)	7.50 a	8.59 b	4.86 a	2.96 a	4.62 b
Large seeds (“Grobogan”)	7.91 a	10.78 a	5.04 a	3.02 a	5.05 a
F test	ns	*	ns	ns	**
Weed extract concentration (%v/v)					
Control	9.77 a	12.05 a	8.55 a	4.66 a	6.70 a
10	7.44 b	7.99 b	3.67 b	2.38 b	4.27 b
20	6.88 b	8.12 b	3.68 b	2.38 b	3.91 b
30	6.77 b	7.89 b	3.64 b	2.49 b	3.83 b
F test	**	*	*	*	*
Interaction (α≥0.05)	ns	ns	ns	ns	ns

Note: Values followed by the same letter in the same column are not significantly different using the DMRT test at α of 5%.
**: significantly different at α by 1%; ns= not significantly different.

the activity of the enzymes that eventually disrupts the formation of α-amylase from gibberellin acid (GA), hence inhibited seed germination (Murrinie et al., 2021). Enzymes function as catalysts in metabolic reactions in seeds, including promoting mitosis, cell division, and cell elongation (Nainggolan et al., 2022; Rahayu et al., 2021; Un et al., 2018).

The inhibition of seed germination can occur due to barriers that reduce water imbibition. Diffusion may also be hindered by differences in water potential inside and outside the cell (Andriyani et al., 2010; Lumbangaol, 2020; Pratiwi and Sughita, 2020). Additionally, the type of weed extract can affect germination by inhibiting nutrient transport and assimilation (Murrinie et al., 2021). According to Sadjad (2014), the presence of H_2O and O_2 leads to the reactivation of reserve materials, initiating complex biochemical activities. Under favorable conditions, seed absorption of water triggers various activities. Protoplasm undergoes rehydration, enzymes become active, starches are broken down into sugars, fats transform into soluble substances, and proteins convert into amino acids. These available materials provide energy for respiration, enable the translocation of food substances to the embryo, and initiate seed growth (Anistia et al., 2022; Tjitrosoedirdjo et al., 1984).

The concentration of weed methanolic extract in the study showed a significant effect on all growth variables, including the number of seeds germinated, the fresh weight of the sprouts, the dry weight of the nodes, the length of the hypocotyl and the length of the radicle. DMRT test results showed that the control treatment (without the addition of weed extract) had the highest value for all observation variables and significantly differed from the administration of weed extract 10 %v/v, 20 %v/v, and 30 %v/v. The 10 %v/v weed extract resulted in more seeds germinating (7.44 grains) and longer radicle length (4.27 cm), but not significantly different from the 20 %v/v and 30 %v/v weed extract concentrations. Weed extract 20 %v/v resulted in heavier wet sprout weight (8.12 g) and heavier sprout dry weight (3.68 g), but not significantly different from weed extract concentrations of 10 %v/v and 30 %v/v. The 30 %v/v weed extract only obtained a longer hypocotyl length (2.49 cm) and was not substantially different from the 10 %v/v and 20 %v/v weed extract concentrations. Our results were supported by previous research conducted by Anistia et al. (2022), which reported that the average length of the radicle, hypocotyl, and plumule of sprouts was hampered due to the concentration of weed extract at various concentration levels. According to Sumi et al. (2018), the phenolic compounds in the weed extracts cause the sprouts to become short and thin, and over time they will die. Research by Abdul Karim Kilkoda (2015) and Wardani et al. (2018) reported that dry extracts of the weeds *Borreria alata* and *Ageratum conyzoides* tend to inhibit soybean growth and production, and increasing the extract concentration tends to increase the inhibitory effect on soybean growth and yield.

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Our study revealed that weed extracts from Aconyzoides (*A. conyzoides*) and *B. alata*, ranging from concentrations of 10% v/v to 30% v/v, had a uniform adverse impact on all growth parameters during seed germination. Treatment with both types of weed extracts (A. conyzoides and B. alata) hindered radicle length and germination across low (10% v/v) to high concentrations (30% v/v). Einhellig (1995) emphasized that tannins can hinder the activity of germination enzymes such as cellulase, polygalacturonase, proteinase, dehydrogenase, and decarboxylase. Barriers to germination can also result from disruptions in the mitotic process within the embryo. Phenolic compounds and their derivatives, including coumarin, cinnamic acid, and benzoic acid, can impact several vital processes such as cell division, mineral absorption, water balance, respiration, photosynthesis, protein synthesis, chlorophyll, and phytohormones. Phenol compounds disturb mitosis by damaging spindle threads during metaphase. Einhellig (1995) stated that allelopathic compounds can suppress the activity of several enzymes along the respiratory pathway, such as decarboxylase and dehydrogenase.

Conclusion

Ageratum conyzoides and *Borreria alata* weed extracts, ranging from 10% v/v to 0% v/v concentrations, exhibited negative effects on all growth parameters of soybean germination. Surprisingly, variations in soybean seed size did not impact the germination process. Instead, the primary factor influencing soybean germination was the concentration of allelopathic treatments. Based on these findings, it is advisable to implement effective control measures to manage these two weed species within soybean cultivation systems, given the significant allelopathic effects they can exert.

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