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Herbicidal Effects of Ethyl Acetate Extracts of Billygoat Weed (*Ageratum conyzoides* L.) on Spiny Amaranth (*Amaranthus spinosus* L.) Growth

Gina Erida [1,*], Nurdin Saidi [2], Hasanuddin Hasanuddin [1] and Syafruddin Syafruddin [1]

[1] Department of Agrotechnology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; hasanuddin@unsyiah.ac.id (H.H.); syafruddin@unsyiah.ac.id (S.S.)
[2] Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia; nurdin@unsyiah.ac.id
* Correspondence: ginaerida@gmail.com; Tel.: +628-12-697-0765

Abstract: This study aimed to evaluate the herbicidal activity of ethyl acetate leaf extract of *Ageratum conyzoides* L. at different subfractions on *Amaranthus spinosus* L. The leaves of *A. conyzoides* were sequentially extracted with *n*-hexane and ethyl acetate respectively and fractionated by chromatography column. The extracts were applied to *A. spinosus* in pot assays at a concentration of 5%, 10% and 15%. We applied A synthetic herbicide (2,4-D at 0.686 kg a.i. ha^{-1}) for positive control and distilled water for negative control. The *A. conyzoides* extracts strongly differed in their effect on weed control, shoot and root dry weight and root length of *A. spinosus*. The most inhibition on *A. spinosus* growth caused by application of ethyl acetate of *A. conyzoides* extracts subfraction A by 10% concentration can cause 100% destruction and subfraction B were 95% which both of them cause strongest death on *A. spinosus* compared with synthetic herbicide (2, 4-D) (23.33%) at 1 Day After Application, while subfraction C and D were not effective. Main constituents identified by GC-MS in subfraction A extract were tetradecanoic acid, ethyl ester (10.26%), precocene II (9.39%), octadecanal (8.23%), 9,12,15-octatadecatrienoic, methyl ester (7.32%), 10-heneicosene (c,t) (5.19%) and neophytadiene (5.09%); in subfraction B were 1-octadecyne (38.57%), phytol (11.24%), di-tert-utylphosphine-d (5.17%) and 1-hexadecine (4.08%); in subfraction C were allobarbital (8.53%), octadecanal (12.69%), and bannamurpanin (26.01%) and octadecanal (30.52%), bannamurpanin (24.06%), 1,8-cineole (15.75%), trans-dodec-5enal (12.28%) and phytol (8.26%) in subfraction D. The ethyl acetate extract subfraction A and B concentration 10% proved the promising control agent against *A. spinosus*.

Keywords: *Ageratum conyzoides*; allelopathy; *Amaranthus spinosus*; bioherbicide; subfraction ethyl acetate; precocene II

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1. Introduction

Weed suppress crop growth and development. They generate yield reductions usually higher than those caused by disease, pests and insects [1] such as in edible starch grains (maize, rice) and legume family (peanut, mung bean, soybean). The main choice in controlling weeds is still the application of synthetic chemical herbicide due to its effectiveness to control the weeds. However, continuous application of such synthetic chemical herbicide tends to create a negative impact on soil [2], weeds resistance to herbicide [3,4], poison to non-targeted organisms, disturb ecology as a whole and leave chemical residues on the environment [5]. It requires effective and efficient control as alternative ways which are environmentally safe. Some efforts have been made to explore the herbicidal potential of plants derived compounds (allelochemicals) [6,7]. Allelopathy refers to the beneficial or harmful effects of one plant on another plant, both crop and weed species, from the release of biochemicals, known as allelopath or allelochemicals, from plant parts by leaching, root

exudation, volatilisation, residue decomposition and other processes in both natural and agricultural systems [8]. Results of study about allelopathic screening of five perennials plants species (*Pinus merkusii* Jungh. et de Vriese, *Acacia mangium* Willd., *Jatropha curcas* L., *Tectona grandis* L.f., *Terminalia catappa* L.) and weed species (*Imperata cylindrica* L., *Ageratum conyzoides* L., *Cyperus rotundus* L., *Chromolaena odorata* L. and *Axonopus compressus* (Swartz) Beauv). against the growth of *Amaranthus spinosus* L. showed that *A. conyzoides* (billygoat; Asteraceae) had the strongest bioherbicidal potential [9,10]. The methanol *A. conyzoides* extract at 20% completely suppressed *A. spinosus* growth 7 days after its application (DAA). This effect was similar to that observed for a synthetic herbicide (2,4-dichlorophenoxyacetic acid) applied at 0.686 kg a.i. ha^{-1} [10]. Main constituents identified by GC-MS in methanol extract of *A. conyzoides* were precocene II (28.52%), ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2h-1-benzopyran-6-yl)-(11.13%), dibutylphthalate (10.64%) and 1-acetonaphthonehone, 2-hydroxy-4-methoxy-(10.46%). *A. conyzoides* (Figure 1) is an annual invasive weed native of tropical America and has now naturalized worldwide, particularly in Southeast Asia including India, China, Japan, Indonesia and Korea [11,12]. In Indonesia, this weed is commonly found in crop fields, yards, roadsides and water edges [13]. Several reports indicate that *A. conyzoides* produces a set of allelochemicals exerting a strong allelopathic effect on crop plants. The phenolics present in its leaf extracts and residues negatively interfere with growth and development of wheat crop [14]. Phenolics release as root exudates and *A. conyzoides* residues suppressed the growth of rice (*Oryza sativa* L.) [15]. Both the volatile oil and the aqueous extract of *A. conyzoides* were allelopathic to many crops including radish, mungbean and ryegrass [16]. According to Harborne (1998), in isolating allelochemical compounds, it is necessary to use certain solvents according to the properties of the desired compound [17]. Differences in solvent polarity result in different amounts and types of allelochemical compounds obtained. Our previous findings, using bio guided fractionation, showed that the extraction of *A. conyzoides* leaves in using the maceration method and fractionated with different polarity solvents consisting of *n*-hexane, ethyl acetate, and methanol showed that ethyl acetate extract of *A. conyzoides* fraction had the strongest post-emergence herbicidal effects on *A. spinosus*. Twenty-one days after application, the ethyl acetate extract was applied at a concentration of 20% to completely controlled the *A. spinosus* similar to 2,4-D at 0.686 kg a.i. ha^{-1}. Main compounds identified by GC-MS in ethyl acetate extract were precocene II (59.22%), neophytadiene 14.94%, methyl linolenate (14.13%) and phytol (8.24%). Based on this research, we conducted the following research to study the potency of herbicides at the subfraction level of ethyl acetate extract on the growth of *A. spinosus* weed.

(**A**) (**B**)

Figure 1. Blooming *Ageratum conyzoides* L. (**A**), and (**B**) Whole aerial Part.

The essential aspect of the bioassay test is to detect the allelopathic action of compounds on target species [18,19]. In this study spiny amaranth (*Amaranthus spinosus* L.) was selected as the target plant, due to its fast uniform germination and sensitivity and its

active competitor with crops [20,21]. This work aimed to test the herbicidal activity of the ethyl acetate leaf extracts of *A. conyzoides* at different subfractions against *A. spinosus*.

2. Materials and Methods

2.1. Place and Time

The study was conducted from April to July 2020 in the Laboratory of Biology, Chemistry and Weed Science, Syiah Kuala University (USK), Province of Aceh-Indonesia and Organic Chemistry Laboratory, Gadjah Mada University (UGM), Yogjakarta-Indonesia. Pot studies in screen house were done in Experimental Farm, Faculty of Agriculture, Syiah Kuala University (USK) (95°22′34, 49° T longitude, 5°34′3, 44° U latitudes), altitude: 3 m above sea level, Annual rainfall: 1.241.5 mm with an average temperature: 27.52 °C.

2.2. Experimental Design

This research applied a completely randomized design (CRD) non-factorial pattern with 14 treatments consisting of ethyl acetate extracts of *A. conyzoides* subfractions A, B, C and D at concentrations 5, 10 and 15, negative control (distilled water) and positive control (2,4-Dat 0.686 kg a.i. ha^{-1}) with 3 replications.

2.3. Preparation of Plant Extract and Seed Source of A. spinosus

A. conyzoides leaves were obtained from Indrapuri district, Aceh Besar. The *A. spinosus* seeds were collected from Meunasah Gle, Sigli, Pidie. Both plants were identified by Mr Suwarno, Botanist. We used 30 kg of dry leaf of *A. conyzoides* to obtain 300 g extract. The amount of *n*-hexane solvent needed was 80 L and the amount of ethyl acetate solvent was 90 L. The *A. conyzoides* leaves were dried for 2-weeks at room temperature and ground. The ground leaves (30 kg) were left for 1 h in 1 L ammonia. Then, they were sequentially extracted 8 times with *n*-hexane, 9 times with ethyl acetate. Each extraction was done with 10 L of solvent and lasted 3 days. At the end of extraction with each solvent, the organic fractions recovered were filtered, combined and evaporated to dryness in a rotary evaporator [17].

2.4. Fractionation

The fractionation of concentrated ethyl acetate extract was carried out using column chromatography. Cotton was put into the bottom column and sand was heated and sieved using a 12 mesh sieve. Then 350 g silica gel was added which had been soaked for about 1×24 h with n-hexane solution, added sand on top of it and poured the extract that had been rotated as much as 100 g. The extract that had been poured was managed to go down slowly and then put into the solvent of n-hexane: ethyl acetate at a ratio of 9:1 by keeping no air bubbles in the static phase. Then the column faucet was slowly opened so that the eluent will flow, the normal droplet was as much as 15 drops per minute. Each fraction released was accommodated in a 100 mL bottle. Each fraction was carried out by TLC with the eluent n-hexane: ethyl acetate (9:1) and the same pattern was joined together [17]. The results of combining were based on the staining pattern on the TLC plate and after that, we obtained four subfractions, namely A, B, C and D. The dry residues of the ethyl acetate extracts were suspended in distilled water to prepare concentrations of 5, 10 and 15%.

2.5. Pot Culture

The soil was collected up to 20 cm depth from Lampakuk Village, Aceh Besar. The soil was dried for 7-days, sieved to remove the plant remains. In each plastic pot (16 cm dia, 13 cm depth) 1.0 kg soil was added. Unsterilized seeds of *A. spinosus* seeds were soaked in water for 2 h and 5 seeds were sown per pot at 2 cm depth on 4 June 2020. Seven days after sowing, thinning was done to keep one healthy plant per pot. After 21 days of sowing (25 June 2020), the plants were foliar sprayed (15 mL per pot) either with water or plant extract as per treatments. The pots were irrigated twice daily with 200 mL tap water.

Growth parameters of *A. spinosus* (weed control (%), dry shoot and root weight, root length) were recorded 7, 14, 21 and 28 days after application (DAA). The weed control (%) of *A. spinosus* was assessed based on 5-observations using 0–100 rating system (Table 1). Dry weights of shoots and roots were recorded after oven drying at 60 °C or 48 h until achieving constant dry weight. The root length was measured after washing with tap water.

Table 1. Rating system used to assess weed control [22].

Effects	Rating	Effects Description
No effect	0	No weed control No crop reduction or injury
Slight	10	Very poor weed control Slight crop discolouration or stunting Poor weed control
	20	Some crop discolouration. Stunting. or stand loss Poor to deficient weed control
	30	Crop injury more pronounced. but not lasting
Moderate	40	Deficient weed control Moderate injury. crop usually recovers
	50	Deficient to moderate weed control Crop injury more lasting. recovery doubtful
	60	Moderate weed control Lasting crop injury no recovery
Severe	70	Weed control somewhat less than satisfactory Heavy crop injury and stand loss
	80	Satisfactory to good weed control Crop nearly destroyed. A few surviving plants
	90	Very good to excellent weed control Only occasional live crop plants left
Complete effect	100	Complete weed destruction Complete crop destruction

2.6. GC-MS Analysis

The characteristics of GC-MS used were Shimadzu brand with type QP2010S, injector temperature 280 °C, injector slipt mode, 1 min sampling time, column temperature 40–270 °C with an initial temperature setting of 40 °C for 5 min, and for 10 min to reach a temperature of 270 °C (23 °C/min) held for 60 min so that the total program time was 88 min, the detector temperature was 280 °C, the temperature interval was 250 °C, the carrier gas was He, the main pressure was 500–900, pressure flow control mode, pressure 10.9 Kpa, total flow 58.8 mL/m, column flow 0.55 mL/m, linear acceleration 26.0 cm/s, cleaning flow 3.0 mL/m, separation ratio 99.8, Rtx-5MS column type, column length of 30.00 m, the thickness of 0.25 µm, the diameter of 0.25 mm, and type of El (Electron Impact) ionization was 70 eV. Compounds were identified based on their retention times and matching of their mass spectra with those of the Willey- NIST library. They were quantified according to their relative areas.

2.7. Data Analysis

All Data were subjected to analysis of variance (F test) and Duncan's new multiple range test at a 5% probability level. The analyses were performed using the SPSS version 16 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Weed Control

The ethyl acetate *A. conyzoides* subfraction A extract concentration of 10% gave a complete effect caused *A. spinosus* mortality (100%), followed by the application of *A. conyzoides* ethyl acetate subfraction B extract with 10% concentration (95% inhibition with severe influence) at 1 DAA (Figure 2). Field observations showed that the reaction immediately occurred after the application of ethyl acetate subfraction A extract, started with the tight surface of leaves, yellowing, dry, curling down and subsequent leaf loss, followed by the stem and all parts of *A. spinosus* turned into brown like burning and finally die within 1 DAA. While the application of 2,4-D cause phytotoxicity 23.33% at 1 DAA and cause 80% inhibition at 14 DAA and die at 21 DAA. These phytotoxic symptoms differed 2,4-D herbicide which not only generated leaf chlorosis but also stem fall and turned into brown like burning. Figure 2 also showed that the application of ethyl acetate extract subfraction C and D concentrations of 10% did not cause weed mortality (10% inhibition and 0%, respectively) at 21 DAA, had a similar percentage of weed control with distilled water application (0%)). The ethyl acetate subfraction C and D extract concentration 10% at 21 DAA were completely inactive reinforcing the idea that it contained low levels, did not contain phytotoxic compounds or antagonistic effect. This antagonistic is in accordance with the statement by Rice (1984) that the nature of the chemical compounds that interact can be additive, synergistic and antagonistic [8]. Various responses occurred because of higher concentrations and selective properties of allelochemical effect on target plants [20]. Generally, Figure 2 also showed the higher concentration given to *A. spinosus*, the higher the value of *A. spinosus* weed control percentage. However, control was somewhat inconsistent with respect to the concentration tested with inhibition of about 100% at a concentration of 10% and 91.11% at a concentration of 15% at 14 DAA on application subfraction A. This bias was likely due to the unexpected variation of environmental factors such as light, CO_2, temperature, soil moisture, relative humidity, rainfall or wind, acting during or after extract spraying [23]. Environmental factors can impact the effectiveness of plant extracts applied in post-emergence directly by altering penetration and translocation mechanisms or indirectly through modifications in the weed physiological stage [24].

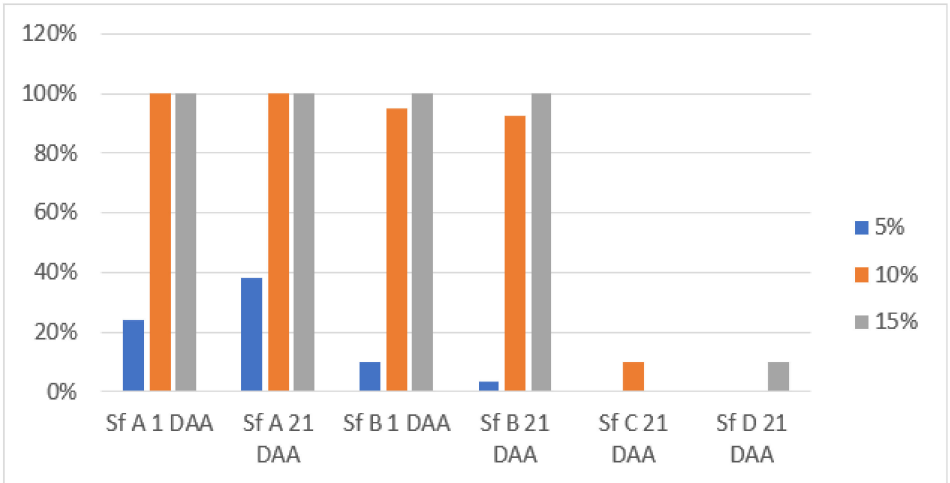

Figure 2. Effects of ethyl acetate extracts of *A. conyzoides* at different subfraction (sf) at 1 DAA and 21 DAA on weed control (%) of *A. spinosus*.

3.2. Shoot Dry Weight, Root Dry Weight and Root Length

The ethyl acetate *A. conyzoides* extracts strongly differed in their effect on shoot dry weight of *A. spinosus* and these differences depended of both the extract and concentrations applied (Figure 3). Shoot dry weight exposed by ethyl acetate *A. conyzoides* subfraction A extract concentration 10% caused mortality of *A. spinosus* with 100% inhibition and

subfraction B concentration 10% with 95% inhibition and concentration 15% with 100% inhibition at 1 DAA. This was because allelochemicals compounds contained in the extract of *A. conyzoides* can inhibit hormonal activity. Allelochemical absorption usually impairs physiological processes such as transpiration, photosynthesis and respiration which in turn lead to inhibit hormonal activity so that cell division and elongation in the shoot areas was inhibited [25]. The shoot dry weight of water-sprayed plants (distilled water) of *A. spinosus* increased with time until it reached 10.51 g at 28 DAA. It follows the same trend in plants exposed to the ethyl acetate extracts subfraction B tested at 5% and subfraction C and D at all concentrations. Although some of the main compounds contained in the ethyl acetate extract of *A. conyzoides* subfraction C and D include secondary metabolites that generally can inhibit plant growth, in this study some increase plant growth. This is in line with that allelochemicals stimulate or inhibit plant growth depending on concentration and target plants or can be additive, synergistic and antagonistic [26–29].

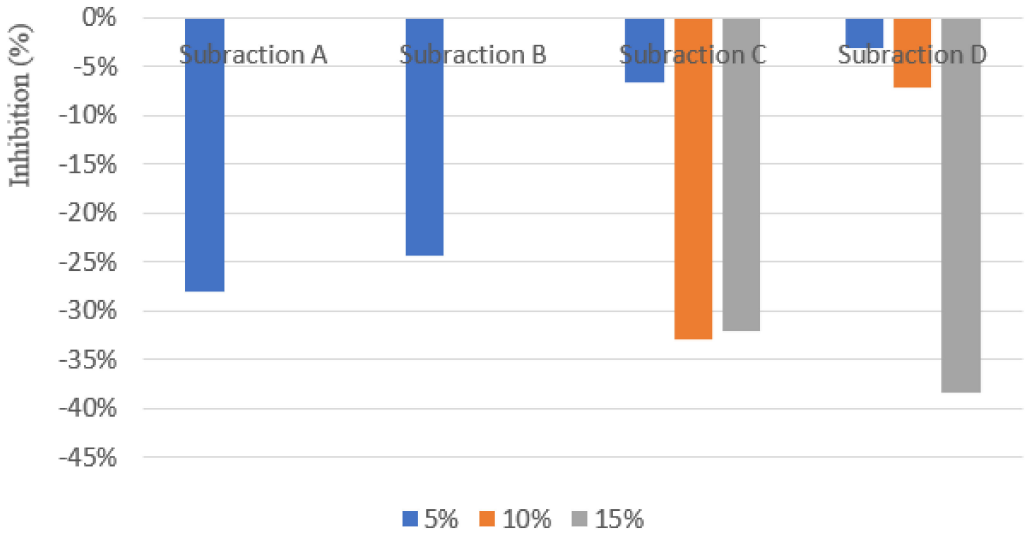

Figure 3. Effect of ethyl acetate *A. conyzoides* extracts at different subfractions on shoot dry weight of *A. spinosus* at 21 DAA.

Figure 3 showed that *A. spinosus* exposed by ethyl acetate extracts subfraction A and B concentration 10% caused the highest inhibition (100% inhibition) and subfraction D concentration 5% had the lowest dry shoot weight (3.10% inhibition) followed by subfraction C concentration 5% (6.52% inhibition) and subfraction D concentration 10% (7.18% inhibition) over control (distilled water) at 21 DAA. At last observation (28 DAA) showed that *A. spinosus* exposed by ethyl acetate extracts subfraction D concentration 10% had the highest dry shoot weight (1.14% stimulation) over control.

In the case of the root dry weights (Figure 4), *A. spinosus* plants treated with ethyl acetate *A. conyzoides* subfraction A and B concentration 10–15% were absent at 21 DAA (both 100% inhibition) because they were die at 1 DAA after application. Figure 4 also showed that the lowest *A. spinosus* plants treated with ethyl acetate *A. conyzoides* subfraction D concentration 5% reached a maximum dry root weight (57.93% stimulation) and concentration 10% reached 28.78% stimulation over control at last observation (28 DAA). It follows the same trend in plants exposed to subfraction D concentration 10% (28.78%), subfraction C concentration 5% (8.11% stimulation) and concentration 15% (11.8% stimulation) over control (distilled water).

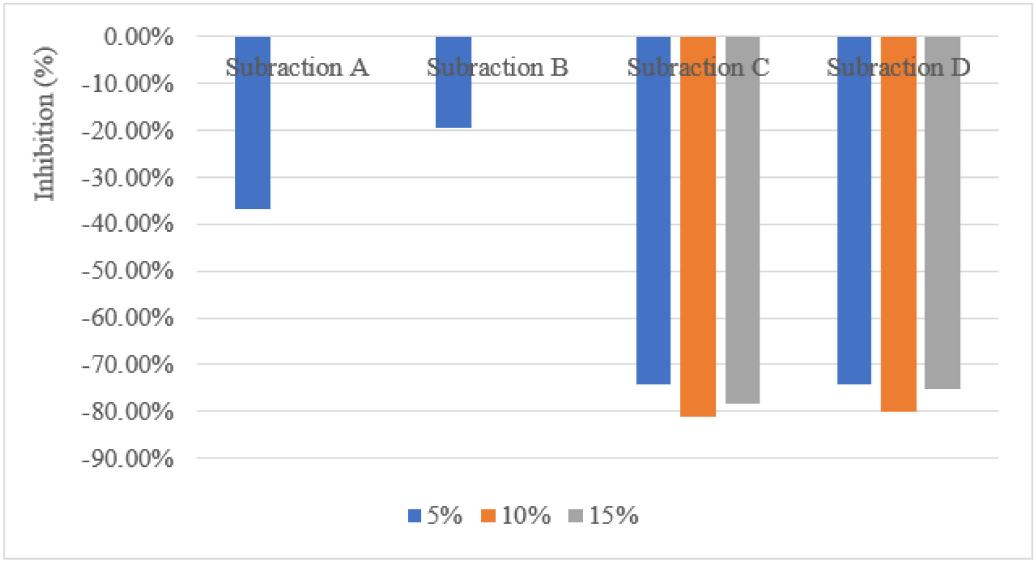

Figure 4. Effect of ethyl acetate *A. conyzoides* extract at different subfractions on root dry weight of *A. spinosus* at 21 DAA.

In the case of root length (Figure 5), there were inhibitory and stimulatory growth plants. Plants exposed to the ethyl acetate *A. conyzoides* extract subfraction A and B concentration 10–15% were absent at 21 DAA because they would die at 1 DAA. *A. spinosus* plants treated with ethyl acetate *A. conyzoides* sub-fraction B concentration 5% reached a maximum root length (10.55% stimulation), and it was similar in plants exposed to subfraction C concentration 5% (9.60% stimulation). There were absences and decreases in dry weight generated by the ethyl acetate *A. conyzoides* subfraction A and subfraction B extract on roots, shoots and root length confirm that the *A. spinosus* plants were restricted in their production of organic matter. This was due to allelochemical compounds contained in subfractions A and B that can inhibit hormonal activity so that cell division and elongation in the shoot and root areas were inhibited. Allelochemical compounds at high concentrations can inhibit the formation of nucleic acids, proteins and adenosine triphosphate (ATP). If ATP was reduced, cell metabolism will also be reduced [8].

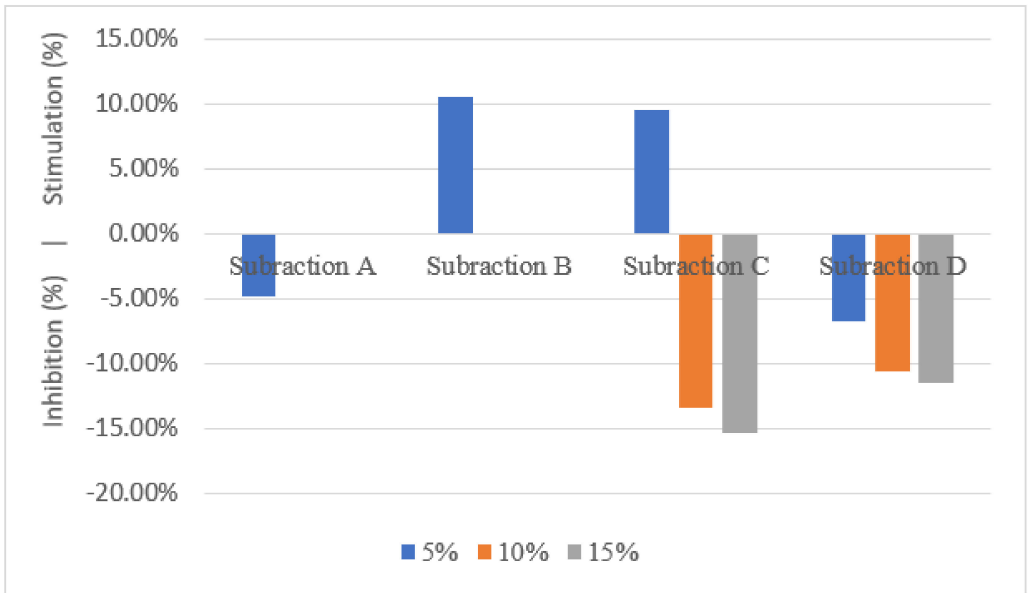

Figure 5. Effect of ethyl acetate *A.* conyzoides extracts at different subfractions on root length of *A. spinosus* at 21 DAA.

3.3. GC-MS Analyses of the Extracts

Major constituents identified in the ethyl acetate *A. conyzoides* extracts subfraction A are presented in Table 2. The GC-MS analysis indicated that ethyl acetate *A. conyzoides* extracts subfraction A contained mainly tetradecanoic acid, ethyl ester (10.26%) followed by precocene II (9.39%) (phenolic), octadecanal (8.23%) (steroid), 9,12,15-octadecatrienoic acid, methyl ester (7.32%) (fatty acids), neophytadiene (5.09%) (terpenoids), 10-heneicosene (c, t) (5.19%), 2-pentadecanone (3.68%), and 2,6,10,15,19,23 hexamethyl- (squalene) (3.63%) (triterpenes). These compounds participated with 52.79% of the total composition integrated by GC-MS and likely were involved in the complete effect of phytotoxicity observed for the ethyl acetate subfraction A extract on *A. spinosus* either acting alone or exerting a synergistic. From our previous finding, we have found ethyl acetate *A. conyzoides* fraction in precocene II (16.63%) and neophytadiene (14.94%). Precocene II, a methoxy derivative of 2,2-dimethylchromene, is usually in high concentrations in the essential oils from the aerial parts of *A. conyzoides* (Chahal et al., 2021). It is a wide- spectrum antifungal agent, with allatocidal and insect-growth regulator activities [11]. Its phytotoxic effect was reported on radish, mungbean, tomato and ryegrass seedlings [16]. The compositions of ethyl acetate subfraction A extracts also shared the presence of the diterpenoids neophytadiene (5.09%). Neophytadiene isolated from *Nepeta* species inhibited shoot growth of ragweed (*Ambrosia artemisiifolia*) shoots [30]. In the case of the ethyl acetate of *A. conyzoides* subfraction B, major constituents identified in the extracts were (Table 2), 1-octadecyne (38.57%) followed by phytol (11.24%), di-tert-butylphosphine-d (5.17%), 1-hexadecine (4.08%) and 1-pentadecene (3.83%) with 62.89% of the total composition. In our previously finding, we have found ethyl acetate *A. conyzoides* fraction in phytol (8.24%). Phytol, which interacts and damages the structure of the phospholipid bilayer of the cell membrane [31]. Extract ethyl acetate of *A. conyzoides* subfraction C contained mainly bannamurpanin (26.01%) followed by octadecanal (12.69%) and allobarbital (8.53%) (Table 2) with a total composition of 47.23%. Bannamurpanin, a compound of the class of flavonoids, is one of a group of phenolic and octadecanal compounds in a group of steroid compounds. The ethyl acetate extracts of *A. conyzoides* extracts subfraction D contained mainly octadecanal (30.52%) followed by bannamurpanin (24.06%), 1,8-cineole (15.75%), trans-dodec-5enal (12.28%) and phytol (8.26%) showing the total composition by 90.87% elucidated in their GC-MS composition. In our previously finding, we have found out 1,8-cineole as much as 3.90% in n-hexane fraction and 3.78% in methanol fraction. 1,8-cineole is oxygenated sesquiterpenes constituting essential oils of several aromatic plants. For example, 1,8-cineole is the main constituent of the *Eucalyptus* oils and other plant essential oils with potential as pre-emergence herbicides [32,33], although it was previously used as preemergence herbicides. Their composition also shared the presence of phytol, a compound that had been reported as a bioherbicide.

Table 2. Major compounds identified in the *A. conyzoides* ethyl acetate subfraction A, B, C, D extract by GC-MS.

NO	Retention Time	Compound Name	Compound Content (%)				Similarity Quality (%)
			Subfraction A	Subfraction B	Subfraction C	Subfraction D	
1	6.12	1,8-cineole				15.75	95
2	24.02	precocene II	9.39				92
3	26.06	allobarbital			8.53		65
4	27.18	1-pentadecene		3.83			95
5	28.12	octadecanal				30.52	89
6	28.23	octadecanal			12.69		89
7	28.29	1-octadecyne		38.57			89
8	28.36	octadecanal	8.23				90
9	28.98	phytol				8.26	89
10	29.08	phytol		11.24			90
11	31.30	1-hexadecine		4.08			96

Table 2. *Cont.*

NO	Retention Time	Compound Name	Compound Content (%)				Similarity Quality (%)
			Subfraction A	Subfraction B	Subfraction C	Subfraction D	
12	33.25	9,12,15-octadecatrienoic, methyl ester	7.32				94
13	35.00	tetradecanoic acid, ethyl ester	10.26				89
14	35.16	10-heneicosene (c,t)	5.19				93
15	35.53	2-pentadecanone	3.68				84
16	36.79	trans-dodec-5enal				12.28	86
17	45.08	2,6,10,15,19,23-hexamethyl-(squalene)	3.63				95
18	53.36	bannamurpanin				24.06	64
19	54.17	bannamurpanin			26.01		54
20	55.29	neophytadiene	5.09				91
21	55.42	di-tert-butylphosphine-d		(5.17%)			75
		Total area	52.79	62.89	47.23	90.87	

4. Conclusions

Analysis of weed control, dry shoot and root weights and root length indicated that the ethyl acetate extract of *A. conyzoides* subfraction A had the strongest post-emergence herbicidal effects on *A. spinosus* (100% inhibition) followed by ethyl acetate *A. conyzoides* subfraction B (95% inhibition), while ethyl acetate *A. conyzoides* subfraction C dan D extracts were had slight effects (13% and 10%, respectively) at concentration 10% and synthetic herbicide (2,4-D at 0.686 kg a.i. ha^{-1}) (23% inhibition) at one day after application. At 21 days after application, the root length of *A. spinosus* exposed to the ethyl acetate subfraction B and C at low concentrations (5%) stimulated the growth of the *A. spinosus* plant. Hence, the ethyl acetate *A. conyzoides* subfraction A and B concentration 10% may be developed as a promising herbicide against *A. spinosus*. However, its effect could not be explained by its volatile constituents detected by GC-MS.

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References

1. Jabran, K.; Mahajan, G.; Sardana, V.; Chauhan, B. Allelopathy for weed control in agricultural systems. *Crop. Prot.* **2015**, *72*, 57–65. [CrossRef]
2. Inderjit; Wardle, D.; Karban, R.; Callaway, R.M. The ecosystem and evolutionary contexts of allelopathy. *Trends Ecol. Evol.* **2011**, *26*, 655–662. [CrossRef]
3. De-Martino, L.; Mancini, E.; de Almeida, R.; De Feo, V. The anti-germinative activity of twenty seven monoterpenes. *Molecules* **2010**, *15*, 6630–6637. [CrossRef]
4. Baeshen, A.A. Morphological and elements constituent effects of allelopathic Activity of some medicinal plants extracts on Zea mays. *Int. J. Curr. Res. Acad. Rev.* **2014**, *2*, 135–143.

5. Annett, R.; Habibi, H.R.; Hontela, A. Impact of glyphosate and glyphosate-based herbicides on the freshwater environment. *J. Appl. Toxicol.* **2014**, *34*, 458–479. [CrossRef]
6. Cahyanti, L.D.; Sumarni, T.; Widaryanto, E. Potential allelopathy of pine leaf (*Pinus* spp.) as bioherbicide on pigweed (*Portulaca oleracea*). *IOSR J. Environ. Sci. Toxicol. Food Technol.* **2013**, *7*, 48–53. [CrossRef]
7. Casimiro, G.S.; Mansur, E.; Pacheco, G.; Garcia, R.; Leal, I.C.R.; Simas, N.K. Allelopathic Activity of Extracts from Different Brazilian Peanut (*Arachis hypogaea* L.) Cultivars on Lettuce (*Lactuca sativa*) and Weed Plants. *Sci. World J.* **2017**, *2017*, 1–7. [CrossRef]
8. Rice, E.L. *Allelopathy*, 2nd ed.; Academic Press: New York, NY, USA, 1984; p. 424.
9. Erida, G.; Saidi, N.; Hasanuddin; Syafruddin. Allelopathic Screening of Several Weed Species as Potential Bioherbicides. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *334*, 012034. [CrossRef]
10. Erida, G.; Saidi, N.; Hasanuddin; Syafruddin. Herbicidal Potential of Methanolic Extracts of *Pinus merkusii* Jungh. et de Vriese, *Acacia Mangium* Willd., *Jatropha Curcas* L., *Tectona Grandis* L.f. and *Terminalia Catappa* L. on *Amaranthus Spinosus* L. *Allelopath. J.* **2020**, *49*, 201–216.
11. Kong, C.; Hu, F.; Xu, X.; Liang, W.; Zhang, C. Allelopathic plants. Ageratum conyzoides L. *Allelopath. J.* **2004**, *14*, 1–12.
12. Kohli, R.K.; Batish, D.R.; Singh, H.P.; Dogra, K.S. Status, invasiveness and environmental threats of three tropical American invasive weeds (*Parthenium hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.) in India. *Biol. Invasions* **2006**, *8*, 1501–1510. [CrossRef]
13. Grainge, M.; Ahmed, S. *Handbook of Plants with Pest-Control Properties*; John Wiley & Sons Limited: Chichester, UK, 1988; 470p.
14. Singh, H.P.; Batish, D.R.; Kohli, R. Allelopathic Interactions and Allelochemicals: New Possibilities for Sustainable Weed Management. *Crit. Rev. Plant Sci.* **2003**, *22*, 239–311. [CrossRef]
15. Batish, D.R.; Kaur, S.; Singh, H.P.; Kohli, R.K. Nature of interference potential of leaf debris of *Ageratum conyzoides*. *Plant Growth Regul.* **2008**, *57*, 137–144. [CrossRef]
16. Kong, C.; Hu, F.; Xu, T.; Lu, Y. Allelopathic Potential and Chemical Constituents of Volatile Oil from *Ageratum conyzoides*. *J. Chem. Ecol.* **1999**, *25*, 2347–2356. [CrossRef]
17. Harborne, J.B. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*; Chapman and Hall: London, UK, 1998.
18. Dayan, F.; Duke, S. Clues in the Search for New Herbicides. In *Allelopathy: A Physiological Process with Ecological Implications*; Reigosa, M., Pedrol, N., Gonzalez, L., Eds.; Springer: Amsterdam, the Netherlands, 2006; pp. 63–83.
19. Tigre, R.; Silva, N.; Santos, M.; Honda, N.; Falcão, E.; Pereira, E. Allelopathic and bioherbicidal potential of Cladonia verticillaris on the germination and growth of Lactuca sativa. *Ecotoxicol. Environ. Saf.* **2012**, *84*, 125–132. [CrossRef] [PubMed]
20. Adler, M.J.; Chase, C.A. Comparison of the Allelopathic Potential of Leguminous Summer Cover Crops: Cowpea, Sunn Hemp, and Velvetbean. *HortScience* **2007**, *42*, 289–293. [CrossRef]
21. Nice, G.; Johnson, B.; Jordan, T. Weed management in pastures: Spiny pigweed. *Purdue Ext.* **2011**, *4*, 1–4.
22. Fransfried, R.E.; Talbert, R.E. Design of Field Experiments and the Measurement and Analysis of Plant Responses. In *Research Methods in Weed Science*; Truelove, B., Ed.; Southern Weed Science Society: AL, USA, 1977; p. 19.
23. Hasan, M.; Ahmad-Hamdani, M.; Rosli, A.; Hamdan, H. Bioherbicides: An Eco-Friendly Tool for Sustainable Weed Management. *Plants* **2021**, *10*, 1212. [CrossRef]
24. Cobb, A.H.; Reade, J.P. *Herbicides and Plant Physiology*; John Wiley & Sons: Hoboken, NJ, USA, 2011.
25. Kaab, S.; Rebey, I.; Hanafi, M.; Hammi, K.; Smaoui, A.; Fauconnier, M.; De Clerck, C.; Jijakli, M.; Ksouri, R. Screening of Tunisian plant extracts for herbicidal activity and formulation of a bioherbicide based on Cynara cardunculus. *S. Afr. J. Bot.* **2019**, *128*, 67–76. [CrossRef]
26. Tesio, F.; Vidotto, F.; Ferrero, A. Allelopathic persistence of *Helianthus tuberosus* L. residues in the soil. *Sci. Hortic.* **2012**, *135*, 98–105. [CrossRef]
27. Vidotto, F.; Tesio, F.; Ferrero, A. Allelopathic effects of *Ambrosia artemisiifolia* L. in the invasive process. *Crop. Prot.* **2013**, *54*, 161–167. [CrossRef]
28. Sikolia, S.F.; Ayuma, E. Allelopathic effects of *Eucalyptus saligna* on germination growth and development of *Vigna unguiculata* L. Walp. *IOSR J. Environ. Sci. Toxicol. Food Technol.* **2018**, *12*, 15–24.
29. Rehman, S.; Shahzad, B.; Bajwa, A.A.; Hussain, S.; Rehman, A.; Alam Cheema, S.; Abbas, T.; Ali, A.; Shah, L.; Adkins, S.; et al. Utilizing the Allelopathic Potential of Brassica Species for Sustainable Crop Production: A Review. *J. Plant Growth Regul.* **2018**, *38*, 343–356. [CrossRef]
30. Dmitrović, S.; Perišić, M.; Stojić, A.; Živković, S.; Boljević, J.; Nestorović Živković, J. Essential oils of two Nepeta species inhibit growth and induce oxidative stress in ragweed (*Ambrosia artemisiifolia* L.) shoots in vitro. *Acta Physiol. Plant.* **2015**, *37*, 1–15. [CrossRef]
31. Chuang, P.-H.; Lee, C.-W.; Chou, J.-Y.; Murugan, M.; Shieh, B.-J.; Chen, H.-M. Anti-fungal activity of crude extracts and essential oil of *Moringa oleifera* Lam. *Bioresour. Technol.* **2007**, *98*, 232–236. [CrossRef]
32. Sotero, V.; Suarez, P.; Vela, J.E.; Sotero, D.G.; Fujii, Y. Allelochemicals of three Amazon plants identified by GC-MS. *Int. J. Eng. Appl. Sci. (IJEAS)* **2016**, *3*, 1–7.
33. Singh, H.P.; Batish, D.R.; Kohli, R.; Saxena, D.B.; Arora, V. Effect of Parthenin—A Sesquiterpene Lactone from Parthenium hysterophorus—On Early Growth and Physiology of Ageratum conyzoides. *J. Chem. Ecol.* **2002**, *28*, 2169–2179. [CrossRef] [PubMed]