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Table: 2, Figs : 5

Herbicidal effects of *n*-hexane, ethyl acetate and methanol extracts of billygoat weed (*Ageratum conyzoides* L.) leaveson *Amaranthus spinosus* L. growth

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ABSTRACT

The herbicidal activity of *Ageratum conyzoides* L. extracts was evaluated on the weed *Amaranthus spinosus* L. The leaves of *A. conyzoides* were sequentially extracted with *n*-hexane, ethyl acetate and methanol. In pot culture, these extracts were applied to *A. spinosus* plants at concentrations of 5 % - 20 %. Positive and negative controls were 2,4-D (2,4-dichlorophenoxy-acetic acid) applied at 0.686 kg a.i.ha^{-1} and distilled water. The *A. conyzoides* extracts had variable effects on leaf area, and shoot and root dry weight and weed control (%) of *A. spinosus*. The ethyl acetate extract was most inhibitory than *n*-hexane extract, while the methanol extract had no effect. Twenty one days after application, the applied ethyl acetate extract at 20 % concentration completely controlled the *A. spinosus* similar to 2,4-D. Main constituents identified by GC-MS in ethyl acetate extract were: 1,8-cineole (3.90 %), caryophyllene (25.47 %), precocene II (59.22 %), and 2,6,10,14,18,22-tetracosahexaene (5 %). In *n*-hexane extract were: precocene II (16.63 %), neophytadiene (14.94 %), phytol (8.24 %), and α-Methyl linolenate (14.13 %) and in the methanol extract were: 1,8-cineole (3.78 %), precocene II (9.16 %), neophytadiene (20.6 %), phytol (14.12 %) and 9,12,15-octadecatrienoic acid methyl ester (9.36 %). The ethyl acetate extract proved promising to control the weed *A. spinosus*.

Keywords: *Ageratum conyzoides*, *Amaranthus spinosus*, billygoat weed, ethyl acetate, extract, growth, herbicidal, neophytadiene, precocene II.

INTRODUCTION

Weeds had negative impacts on crops, these exceeds the losses caused by insect pests and diseases (16). Weeds are controlled using synthetic herbicides, resulting in environmental pollution and development of resistance in weeds (1,6,15). An alternative to classical chemical weed control is the use of botanical herbicides, consisting of extracts prepared from plant parts (2,21). Aqueous extracts from shoots of Indonesian perennial plants (*Acacia mangium* Willd, *Pinus merkusii* Jungh. et de Vriese, *Tectona grandis* L.F., *Terminalia catappa* L. and *Jatropha curcas* L.) and weed species (*Imperata cylindrica* L., *Ageratum conyzoides* L., *Cyperus rotundus* L., *Chromolaena odorata* L., and *Axonopus compressus* (Swartz) Beauv) were screened for phytotoxicity against *Amaranthus spinosus* L., (Amaranthaceae), a hard-to-control weed in orchards. The extract of billy goat weed (*A. conyzoides*, Asteraceae) applied at a concentration of 20 % completely suppressed the *A. spinosus* growth 7 days after its application (9,10). *A. conyzoides* (Figure 1) is an annual invasive weed native from tropical America that has now naturalized worldwide, invasive

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Figure 1. *Ageratum conyzoides* L.: (A) whole aerial part and (B) leaves and flowers.

weed native from tropical America that has now naturalized worldwide, particularly in Southeast Asia including India, China, Japan, Indonesia and Korea (17,19). In Indonesia this weed is commonly found in crop fields, yards, roadsides and water edges (11). In crop fields, *A. conyzoides* decreased the growth and yield of crops, as it produces allelochemicals harmful to crop plants. The phenolics present in its leaf extract and residues negatively interfered with growth and development of wheat crop (25). Phenolics released as root exudates and *A. conyzoides* residues suppressed the growth of rice (*Oryza sativa* L.) (3). The volatile oil and the aqueous extract of *A. conyzoides* were allelopathic to many crops including radish, mungbean and ryegrass (18). This study aimed to test the herbicidal activity of *n*-hexane, ethyl acetate and methanol leaf extracts of *A. conyzoides* on growth of weed *A. spinosus* in pot assays. A GC-MS analysis of the extracts was done to identify the allelochemicals involved.

MATERIALS AND METHODS

The study was conducted from April to July 2019 in the Laboratory of Biology, Chemistry and Weed Science, Syiah Kuala University (USK), Aceh Province, 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 latitude), altitude: 3 m, Annual rainfall : 2718 mm, max temp : 33.66 °C and minimum temp : 24.16 °C.

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. The *A. conyzoides* leaves were dried for 2-weeks at room temperature and grinded. The grounded leaves (15 kg) were left for 1 h in 1 L of ammonia. Then, they were sequentially extracted 6-times with *n*-hexane, 7-times with ethyl acetate and 3-times with methanol. Each extraction was done with 5 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 (12). The dry residues of the n-hexane, ethyl acetate and methanol extracts were suspended in distilled water to prepare concentrations of 5, 10, 15 and 20 %.

Experimental Design

The experimental treatments consisted of 3 factors : (i). Extracts 3 (n-haxane, ethylacelate and methanol), (ii). extract concentration 4 (5,10,15,20 %) and control 2 (Positive control : 2,4-D and Negative control : Distilled water). The treatments were replicated 4-times in non-factorial completely randomized design.

Pot culture

Soil was collected upto 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* were soaked in water for 2 h and 5 seeds were sown per pot at 2 cm depth on May 15, 2019. Seven days after sowing, thinning was done to keep one healthy plant per pot. After 21 days of sowing, 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.

Table 1. Rating system used to assess weed control

Effects	Rating	Effects Descriptiom
No effect	0	No weed control No crop reduction or injury
Slight	10	Very poor weed control Slight crop discoloration or stunting Poor weed control
	20	Some crop discoloration. 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

Source: 9

Growth parameters of *A. spinosus* (leaf area, dry shoot and root weight) weed control (%), were recorded 7, 14, 21 and 28 days after foliar spray. The leaf area was measured using leaf area meter model GA-5 (Tokyo Photo Electric CO, LTD). 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 for 48 h or until achieving constant dry weight.

GC-MS analysis

Each extract was subjected to GC-MS (Shimadzu GC-MS QP2010S spectrometer 70 eV). Injector temperature was 280 °C, split mode, and sampling time was 1 minute. Column temperature program was: first the temperature was set at 40 °C for 5 min, then it was increased for 10 min to reach 270 °C (23 °C /min), and finally it was held at 270 °C for 60 min. The detector temperature was 280 °C. Carrier gas was He, pressure was 10.9 Kpa, total flow was 58.8 ml/m, column flow was 0.55 ml/m, cleaning flow was 3.0 ml/m and split ratio was 99:8. Analyses were performed in a Rtx-5MS column (30.00 m, 0.25 mm diameter, 0.25 µm). Detector had an El (Electron Impact) ionizing type of 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.

Statistical analysis of Data

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

RESULTS AND DISCUSSION

Leaf area

The *A. conyzoides* extracts strongly affected the leaf area of *A. spinosus* and these effects depended on both the applied extract and their concentrations (Figure 2).

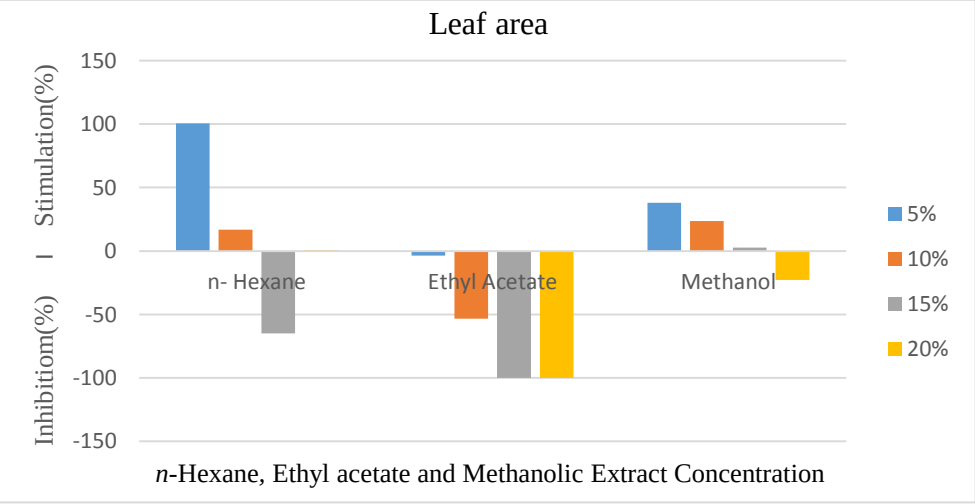

Figure 2. Effects of applied *n*-hexane, ethyl acetate and methanol extracts of *A. conyzoides* on leaf area of *A. spinosus* at 21 DAA

(i). *n*-Hexane Extract: The 5 and 10 % extracts stimulated the leaf area (100 % and 16.7 % respectively), while the 15 % extract inhibited the leaves growth (65 %). The leaf area was not affected < 20 % concentration over control

(ii). Methanol Extract: Its 5 %, 10 % and 15 % concentrations at 21 DAA stimulated the leaf area (38.10 %, 23.60 %, and 2.60 %). While the 20 % extract inhibited the leaf growth by 22.70 %.

(iii). Ethyl Acetate Extract: All the concentrations of ethyl acetate extract inhibited the leaf growth of *A. spinosus*. The 5 and 10 % extracts were less toxic and caused only 3.50 % and 53.40 % reduction in the leaf expansion, respectively. However, the higher concentrations (15 % and 20 %) completely suppressed the leaf area, starting from 14 day after application (DAA).

Allelochemicals absorption impairs the weed physiological processes (transpiration, photosynthesis and respiration), which decreases the production of photosynthates (22). One day after the application of ethyl acetate and the *n*-hexane extracts of *A. conyzoides*, the leaf size was reduced and the leaf expansion was delayed in *A. spinosus*. Our findings indicated that aerial parts of *A. conyzoides* were rich in phytotoxic compounds of low and moderate polarity, which are sequentially extracted by *n*-hexane and ethyl acetate, respectively. But these were not detected in the methanolic extract.

Weed control

The 20 % ethyl acetate extract inhibited the growth of *A. spinosus* and provided 90 % control one week after application. A complete suppression comparable to the herbicide 2,4-D treatment was observed after 21 DAA (Figure 3). One day after the application of the ethyl acetate at 20 % concentration, caused slight leaf and stem chlorosis (38.45 %). In the next few days, the aerial parts of *A. spinosus* become burned and with rolling of leaves and leading to weed death. These phytotoxic symptoms differed from 2,4-D which not only

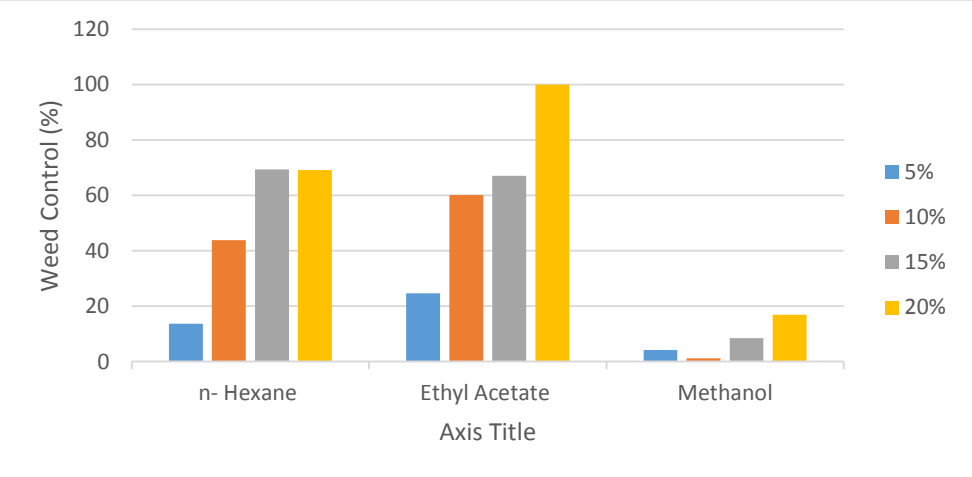

Figure 3. Effects of *n*-hexane, ethyl acetate and methanol extracts of *A. conyzoides* at 21 DAA on weed control (%) of *A. spinosus.*

caused leaf chlorosis but also killed stem. The *n*-hexane extract, controlled *A. spinosus*, when applied at higher concentrations. However control was inconsistent, with the concentration tested such as 40 % inhibition at 10 % and 20 % concentrations, and 90 % inhibition at 15 % concentration. .This bias was likely due to the unexpected variations in environmental factors such as light, CO_2, temperature, soil moisture, relative humidity, rainfall, or wind, acting during or after extract spraying (13). Environmental factors can impact the effectiveness of plant extracts in post-emergence application, directly by altering the penetration and translocation mechanisms or indirectly through modifications in the weed physiological stage (5). The methanol extract was completely inactive showing the idea that it contained low levels or did not contain phytotoxic compounds. The interactions of the chemical compounds can be additive, synergistic and antagonistic (16)

Shoot and root dry weights

The inhibitory and stimulatory influences of extracts on shoot dry weights were recorded at 21 DAA (Figure 4).

Shoot dry weight: Application of *n*-hexane extract on *A. spinosus* plants tested at 5 and 10 % concentrations stimulated the shoot dry weights by 20.0 % and 32.80 % respectively, over control. All concentrations of methanol extracts were stimulatory. However, the ethyl acetate extracts of 5, 10, 15 and 20 % concentrations inhibited the shoot dry weight by 36.80 %, 84.79 %, 84.37 % and 76.07 %, respectively.

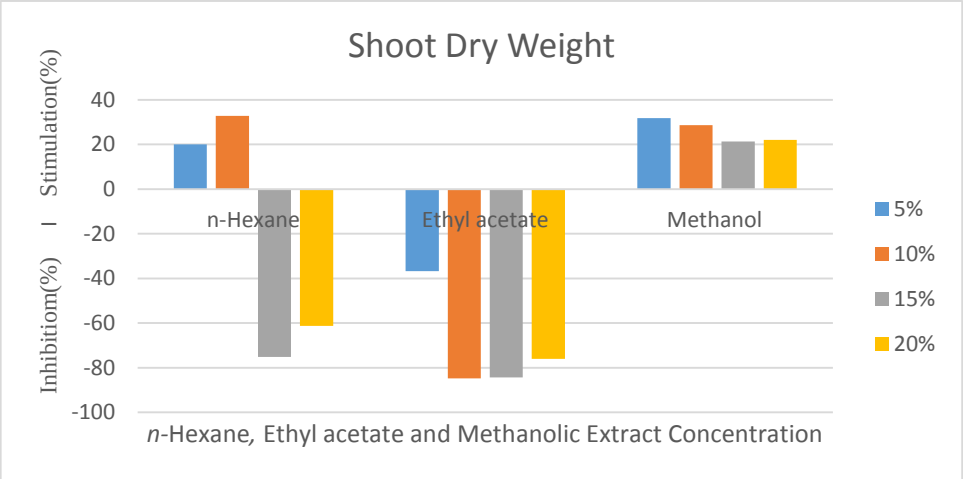

Figure 4. Effects of *n*-hexane, ethyl acetate and methanol extracts of *A. conyzoides* at 21 DAA on shoot dry weight of *A. spinosus*

Root dry weight: The *n*-hexane extracts were inhibitory at 15 and 20 % concentrations and decreased root dry weight by 75.10 % and 61.30 % at 21 DAA (Figure 5). The *A. spinosus* plants treated with 20 % ethyl acetate extract caused inhibiton of 79.59 % over control (distilled water). The decrease in dry weight caused by the *n*-hexane and ethyl acetate extracts on roots and shoots confirmed that these extracts restricted biomass production in *A. spinosus* plants. Allelochemical can be beneficial or harmful to target organisms (20,24).

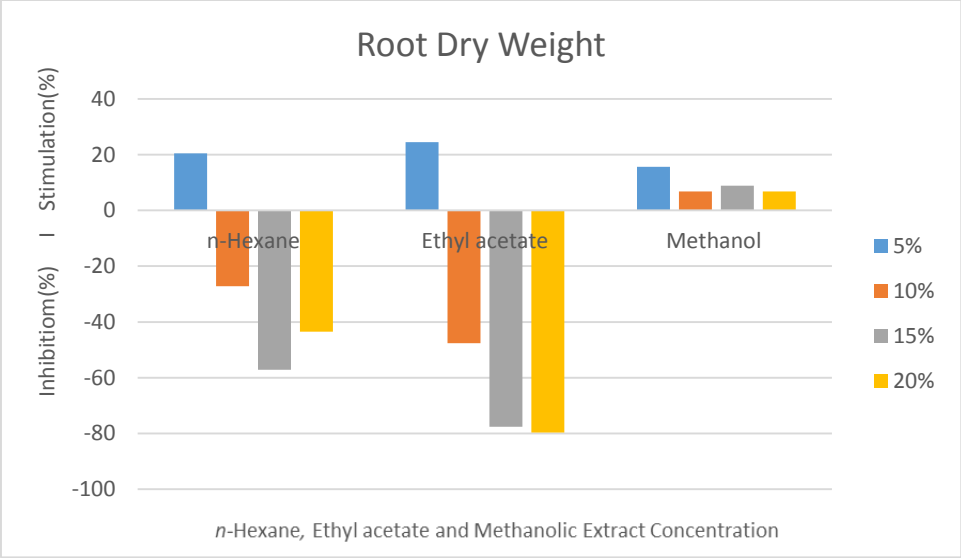

Figure 5. Effects of *n*-hexane, ethyl acetate and methanol extracts of *A. conyzoides* at 21 DAA on root dry weight of *A. spinosus*

GC-MS analyses of extracts

Major constituents identified in the extracts of *A. conyzoides* are presented in Table 2. The GC-MS analysis indicated that the *n*-hexane extract contained mainly precocene II (59.22 %), followed by caryophyllene (25.47 %), 1,8-cineole (3.90 %) and 2,6,10,14,18,22-tetracosahexaene (5 %). These compounds accounted for 93.59 % of the total composition analysed by GC-MS and likely were involved in the moderate phytotoxicity observed for the *n*-hexane on *A. spinosus*, either acting alone or exerting a synergic joint action. 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* (4). It is a wide-

Table 2. GC-MS analyses of the *n*-hexane, ethyl acetate and methanol extracts recovered from the aerial parts of *Ageratum conyzoides*.

No.	Retention time (min)	Compound name	Compound content (%)		
			Hexane extract	Ethyl acetate extract	Methanol extract
1	7.688	1,8-cineole	3.90	-	3.78
2	19.637	caryophyllene	25.47	-	-
3	25.903	Precocene II	59.22	16.63	9.16
4	29.798	Neophytadiene	-	14.94	20.6
5	30.747	Phytol	-	8.24	14.12
6	35.157	α-Methyl linolenate	-	14.13	-
7	35.352	9,12,15-octadecatrienoic acid methyl ester	-	-	9.36
8	47.152	2,6,10,14,18,22-tetracosahexaene	5.00	-	-
		Total area	**93.59**	**53.94**	**57.02**

spectrum antifungal agent, with allatocidal and insect-growth regulator activities (19). Its phytotoxic effect was reported on radish (*Raphanus raphanistrum* L.), mungbean (*Vigna radiata* L.), tomato (*Solanum lycopersicum* L.) and ryegrass seedlings (18). Caryophyllene and 1,8-cineole are oxygenated sesquiterpenes constituting essential oils of several aromatic plants. For example, 1,8-cineole is main constituent of the *Eucalyptus* oils and other plant essential oils with potential as pre-emergence herbicides (26,27) and it was used previously as pre-emergence herbicide. β-caryophyllene showed strong inhibitory effects on dry biomass of *Physalis Ixocarpa* Brot. ex Hornem and root elongation in seedlings of *P. ixocarpa* and *Echinochloa crus-galli* L. (23). In the ethyl acetate and methanol extracts, their main constituents were 53.94 % and 57.02 % elucidated in their GC-MS compositions. They contained precocene II although at lower levels (16.63 % and 9.16 %, respectively) than the *n*-hexane extract. The diterpenoids neophytadiene (14.94 %) and phytol (14.94 %) were also the constituent of these extract. Neophytadiene isolated from *Nepeta cataria* L. species inhibited shoot growth of ragweed (*Ambrosia artemisiifolia* L.) (7). The methanol extract also had 1,8-cineol (3.78 %). The α-Methyl linolenate (14.13 %) and 9,12,15-octadecatrienoic acid methyl ester (9.36 %) are fatty acid esters that were unique in the ethyl acetate and the methanolic extracts, respectively. They can aid in the penetration of hydrophilic constituents through the leaf cuticular layers (14). The shared composition observed for the ethyl acetate and methanol extracts suggested that non-volatile constituents also participated in the phytotoxic activity of the ethyl acetate extract. This is also supported by the phytotoxic activity of aqueous extracts from *A. conyzoides* parts which contained non-volatile phenolic compounds (i.e. kaempferol, quercetin and their glycosides; single phenolics such as gallic, protocatechuic, benzoic and sinapic acids; tannins), coumarins and non-volatile terpenes (8), that were probably also extracted along with the ethyl acetate.

CONCLUSIONS

Analysis of leaf area, dry shoot and root weights and weed control (%) indicated that the ethyl acetate extract of *A. conyzoides* had the strongest post-emergence herbicidal effects on *A. spinosus*. While *n*-hexane and methanol extracts had moderate herbicidal effects and no herbicidal effects, respectively. The ethyl acetate extract at 20 % concentration required 21 days to cause phytotoxicity similar to 2,4-D herbicide. Hence, it may be developed as promising herbicide against *A. spinosus*. However, its effect could not be explained by its volatile constituents detected by GC-MS.

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CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

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PUBLISHER NOTE