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Table: 5, Figs: 14

Phytotoxicity of *Ageratum conyzoides* L. ethyl acetate extract on *Amaranthus spinosus* L., *Cyperus rotundus* L. and *Axonopus compressus* L.

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ABSTRACT

We evaluated the herbicidal activity of fractions recovered from the ethyl acetate leaf extract of *Ageratum conyzoides* L. on *Amaranthus spinosus* L., *Cyperus rotundus* L. and *Axonopus compressus* L. growth. The leaves of *A. conyzoides* were defatted with *n*-hexane and then extracted with ethyl acetate. Two subfractions A and B, were recovered after fractionation of the ethyl acetate extract by column chromatography. In pot assays, the ethyl acetate extract of 2-subfractions A and B were applied to *A. spinosus*, *C. rotundus* and *A. compressus* at concentrations of 2, 4, 6, 8 and 10 % and the herbicide (2,4-D at 0.686 kg a.i. ha^{-1}) and distilled water applied as positive and negative controls, respectively. The subfractions showed phytotoxic activity 7-days after the start of the pot assay. Subfraction A at concentrations of 4 % or higher completely suppressed the growth of *A. spinosus* and that of *A. compressus* at 10 % concentration. Thus, subfraction B was less phytotoxic to weeds than subfraction A. The *C. rotundus* weed was less sensitive to both subfractions. GC-MS indicated the presence of caryophyllene (6.40 %), 2-propenoic acid, 3-(2-hydroxyphenyl)-, (E)- (15.35%), precosene II (23.33 %) and phytol (4.33 %) in subfraction A; and caryophyllene (9.61 %), 2-propenoic acid, 3-(2- hydroxyphenyl)-, (E)- (20.82 %), precocene I (3.27 %) and precocene II (29.78 %) in subfraction B. Our results showed that subfraction A had promising herbicidal effects on *A. spinosus* and *A. compressus*, while, subfraction B was herbicidal to *A. spinosus*.

Keywords: *Ageratum conyzoides*, *Axonopus compressus*, bioherbicide, *Cyperus rotundus*, ethylacetate, GCMS, Pot culture, precocene II, subfraction, weeds.

INTRODUCTION

Ageratum conyzoides, popularly known as billygoat, is an annual aromatic plant (Figure 1). It is an invasive weed in tropical and subtropical regions of the world. In Indonesia, it is commonly found in crop fields, yards, roadsides and water edges (11). The *A. conyzoides* produces allelochemicals which exert strong allelopathic effects on crops. The aqueous extracts of its leaf, root, flower and stem were strongly herbicidal to germination and growth of *Parthenium hysterophorus* L. (3) and reduced the germination of two weeds (*Phalaris minor* and *Poa annua*) in wheat (2). Its volatiles are allelopathic to crops like cucumber (*Cucumis sativus* L.), wheat (*Triticum aestivum* L.) and tomato (*Solanum lycopersicum* L.) (14). We showed that *A. conyzoides* had the strongest bioherbicidal potential among *Acacia mangium* Willd., *Pinus merkusii* Jungh.et de Vriese, *Terminalia catappa* L., *Jatropha curcas* L., *Tectona grandis* L.f., *Imperata cylindrica* L., *Cyperus rotundus* L., *Chromolaena odorata* L. and *Axonopus compressus* (Swartz), of 10-plant species tested on *A. spinosus* (6,9). The methanol extract of *A. conyzoides* at 20 %

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A B

Figure 1. *Ageratum conyzoides* L. (A). Single Plant and (B). Population

A B

Figure 2. *Amaranthus spinosus* L. (A). Single Plant and (B). Population

A B

Figure 3. *Cyperus rotundus* L. (A). Single Plant and (B). Population

A B

Figure 4. *Axonopus compressus* L. (A). Single Plant and (B). Population

concentration completely suppressed the *A. spinosus* growth, 7 days after application. This effect was similar to that observed for 2,4-dichlorophenoxy-acetic acid herbicide applied at 0.686 kg a.i.ha^{-1} (8). Its main constituents identified by GC-MS in the methanol extract 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 %)(7).

It is necessary to use certain solvents according to the properties of the desired compound, because difference in solvent polarity results in different amounts and types of allelochemical compounds obtained (12). Our findings also revealed that the among all the extracts of *A. conyzoides* leaves obtained by the maceration method and fractionated with different polarity solvents, the ethyl acetate extract of *A. conyzoides* fraction had the strongest post-emergence herbicidal effects on *A. spinosus* (8). The ethyl acetate extract at 20 % concentration completely controlled *A. spinosus* growth 21 days after application and its effects were similar to herbicide (2,4-dichlorophenoxy-acetic acid). Main constituents identified by GC-MS in ethyl acetate extract were precocene II (16.63 %), neophytadiene (14.94 %), α-methyl linolenate (14.13 %) and phytol (8.24 %) (8). This study aimed to test the herbicidal effects of ethyl acetate leaf extracts of *A. conyzoides* on growth of *Amaranthus spinosus* L., *Cyperus rotundus* L. and *Axonopus compressus* L. *A. spinosus* is one from 18 most serious weeds in the world (24) (Figure 2). *C. rotundus* is one the most perennial weed and is ranked as one of world's worst weed (13) (Figure 3). *C. rotundus* in maize crop reduced yield about 32.4 to 42.3 % (22). *A. compressus* has a creeping stem, it has roots at nodes and reduces crop yields. It is used as cover crop under oil palm plantations (20) (Figure 4).

MATERIALS AND METHODS

The pot culture study was conducted in screen house from August 2021 to July 2022 in the Laboratory of Biology, Organic Chemistry and Weed Science, Syiah Kuala University (USK), Province of Aceh-Indonesia (95°22'34, 49°T longitude, 5°34'3, 44°U latitudes) altitude: 3 m above sea level, annual rainfall: 1.700.5 mm, max temp: 34.40 °C and minimum temp : 22.90 °C.

The experimental treatments consisted of 2-factors (i). Ethyl Acetate Sub fractions: 2 (Subfraction A and B) and (ii). Its Concentrations: 6 (2,4,6,8,10 %), negative control (distilled water) and positive control (2,4-D at 0.686 kg a.i. ha-^{1}). The treatments were replicated thrice in completely randomized design.

Pot culture

Soil was collected up to 20 cm depth from Maheng Village, Aceh Besar (latitude: 5.415°, longitude: 95.451°, Altitude: 1.1 m, annual rainfall: 1.821.2 mm). The soil was dried for 7-days, sieved to remove the plant remains. In each plastic pot (16 cm dia, 13 cm depth) 1 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 June 11, 2022. Seven days after sowing, thinning was done to keep one healthy plant per pot. The purple nutsedge (*Cyperus rotundus*) tubers with single buds were used as planting material in sand and manure 1:1 ratio. Tubers of the same size were collected and germinated in germination box covered with water-absorbing cloth. After sprouting, the tubers were planted in pots @ 1 tuber per pot. The *Axonopus compressus* stolons were cut into segments, put into the water and

sprouted plantlets were planted in pots @ 1 plantlet per pot. In *A. compressus* sprouted stolon segments were used as planting material. Seven days after sowing, thinning was done in both test grasses to keep one healthy plant per pot. After 14 days of sowing (June 26, 2022), the plants were foliar sprayed (4 ml per pot) either with water or plant extract and 2,4-D as per treatments. The pots were irrigated twice daily with 200 ml tap water. Growth parameters of test weeds were recorded as indicated in Table given below.

Growth Parameters of Test weeds

Weed spp	Growth Parameters recorded	DAA
Amaranthus spinosus L.	Dry shoot and root weight, root length and leaf area	7,14,21
Cyperus rotundus L.	Leaf length, numbers of tillers, fresh and dry weight	7,14,21
Axonopus compressus L.	Leaf numbers, stem length, fresh and dry weight	7,14,21

DAA: Days after application

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

Table 1. Rating system used to assess weed control.

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
	20	Poor to deficient weed control, slight crop discolouration, stunting. or stand loss,
	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 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 : 10

Plant extract, source of *A. spinosus* seeds, tubers of *C. rotundus* and stolons of *A. compressus*

A. conyzoides leaves were collected before flowering from Indrapuri district, Aceh Besar (Latitude: 5.436°, Longitude: 95.518°, Altitude: 7.8 m, annual rain fall :1.821.2 mm. The tubers of *C. rotundus* and the stolon of *A. compressus* were obtained from our Experimental Farm, Faculty of Agriculture, USK. The *A. conyzoides* leaves were dried for 2- weeks at room temperature and grinded. The grinded leaves (25 kg) were left for 1 h in 4 L ammonia. Then, they were sequentially extracted 6-times with *n*-hexane and 7-times with ethyl acetate. Each extraction was done with 20 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). This process provided its 250 g mass from ethyl acetate extract. The *A. spinosus* seeds were collected from Meunasah Gle, Sigli, Pidie (Latitude: 5.2317°N, Longitude: 95.5745°E, Altitude: 81 m).

Fractionation

The ethyl acetate extract was fractionated by column chromatography. Cotton was put into the bottom column and sand was heated and sieved through 12 mesh sieve. Then 250 g silica gel was added after soaking in *n*-hexane for 24 h. Sand was added on top of silica gel and finally the extract (50 g). Then, the column was eluted with *n*-hexane: ethyl acetate ratio of 8:2, v/v by keeping no air bubbles in the static phase. Then, the column faucet was slowly opened so that the eluent flowed (15 drops per min). Fractions (100 mL of each) were collected in glass bottles. The column was further eluted with mobile phases as of *n*-hexane: ethyl acetate (8:2; 7:3; 6:4; 5:5; 4:6; 3:7 and 2:8, v/v). The fractions were pooled according to their thin layer chromatography patterns (12). Two subfractions A and B were obtained, these were dissolved in distilled water to prepare concentrations of 2, 4, 6, 8 and 10 %.

GC-MS analysis

GC-MS conditions were as under: Temperature was set at 40°C and held for 72.5 min. 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. 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.

Data analysis

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

RESULTS AND DISCUSSION

WEED CONTROL

(i). *A. spinosus* : The effects of *A. conyzoides* ethyl acetate extract at various subfraction on control of *A. spinosus*, *C. rotundus* and *A. compressus* weeds at 7 DAA can be seen in Figure 5 and at 21 DAA in Figure 6. The ethyl acetate *A. conyzoides* subfraction A extract killed the *A. spinosus* at concentrations of 4 % or higher, which was similar to synthetic herbicide (2,4-D) at 7 DAA (Figure 5). Field observations showed that the application of ethyl acetate extract of *A. conyzoides* subfraction A at 4 % and 6 % concentrations at 1 DAA caused mild wilting, leaf discolouration, leaf curling, drying and petiole shrinking in weeds, which were included in the category of severe effects based on Frans and Talbert (1977) (10) died at 4 DAA. These extracts at 8 % concentration lead to yellowing and drying of leaves and stem and killed plants at 3 DAA. Application of 10 % concentration resulted in death of *A. spinosus* at 1 DAA with drying and yellowing in all parts, stems shrinking and leaves fall off (100 % control), however, 2,4-D at 1 DAA controlled 85.47 % *A. spinosus.* Our previous finding also showed that application of the ethyl acetate *A. conyzoides* extracts subfraction A at 10 % concentration killed 100 % weeds at 1 DAA. In this study, we found that

application of the ethyl acetate *A. conyzoides* extracts subfraction A at 4 % concentration killed 100 % *A. spinosus* weed at 4 DAA.

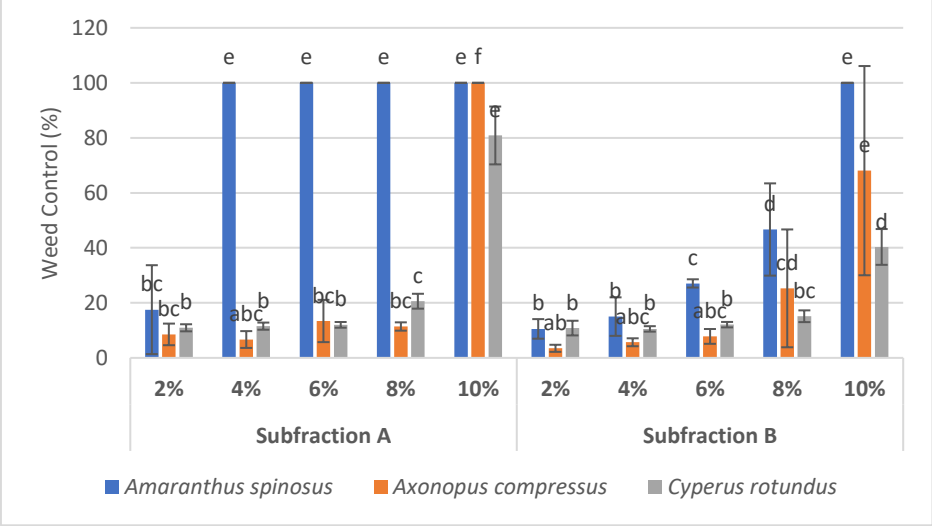

Figure 5. Effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on control of *A. spinosus*, *C. rotundus* and *A. compressus* weeds at 7 DAA.

The application of ethyl acetate extract of *A. conyzoides* subfraction B on *A. spinosus* at 1 DAA at 2, 4, 6 and 8 % concentrations induced symptoms viz., leaves were withered, curled and yellow spots appeared. The 10 % applied concentration on spiny amaranth caused the leaves to fall and dry with pale stems and shrinking. At 7 DAA, the growth was completely ceased similar to impact caused by herbicide 2,4-D.

(ii). *C. rotundus* : The application of ethyl acetate extract of *A. conyzoides* subfraction A at concentrations of 2, 4, 6 and 8 % caused the initial symptoms on *C. rotundus* at 1 DAA, in the leaf colour and turning brown. There were yellow spots on leaves. Concentration of 10 % at 1 DAA exhibited more visible symptoms such as brownish discolouration of leaves and yellow spots appeared and slight wilting of leaves. It was observed that 1 DAA caused 77.33 % inhibition of *C. rotundus* plants growth over control, while 80.87 % inhibition at 7 DAA. However at 14 DAA and 21 DAA, *C. rotundus* had recovered (65.53 % and 42.80 % respectively) (Figure 5). It was noticed that the leaves that were green in colour but still had slightly yellowish colour showing the traces of applied ethyl acetate extract of billygoat weed subfraction. However, these symptoms were not visible in newly growing leaves.

(iii). *A. compressus* : Field observations showed that the application of ethyl acetate extract of *A. conyzoides* subfraction A at 2 and 4 % concentrations on *A. compressus* at 1 DAA did not display any symptoms, but 6 and 8 % concentrations caused yellow spots on the leaves. At 10 % concentration, the symptoms were yellowish brown spots on the leaves and they withered and died at 5 DAA, with all the leaves drying out and growth

completely ceased at 7 DAA (Figure 5). On 7 DAA at 2, 4, 6 and 8 % concentration, the visible symptoms began to decrease, but leaves were still slightly yellowish and many plants started to grow new shoots. On 21 DAA, at concentrations of 2, 4, 6 and 8 % the plants had recovered (Figure 6). The leaves were fresh green, but there was still light yellow colour on tips of the leaves. At 10 % concentration, the weeds plants were killed.

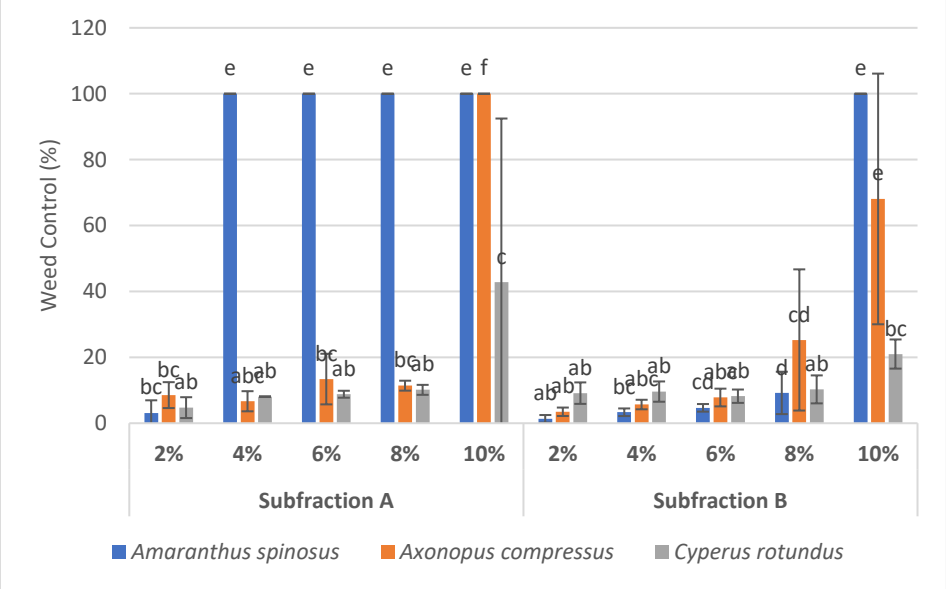

Figure 6. Effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on control of *A. spinosus*, *C. rotundus* and *A. compressus* weeds at 21 DAA.

SEEDLINGS GROWTH

(i). *A. spinosus* : The ethyl acetate *A. conyzoides* extracts strongly varied in their effects on leaf area, root length (Figure 7), shoot and root dry weight (Figure 8) of *A. spinosus* and these alterations depended on both the extract fraction and concentrations applied. *A. spinosus* exposed to the ethyl acetate *A. conyzoides* extract subfraction A starting at concentration 4 % or higher and subfraction B concentration of 10 % at 21 DAA killed weed plants. This was because allelochemicals 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 hinder hormonal activity so that cell division and elongation in the shoot areas, root dry weight, leaf area and root length of *A. spinosus* was inhibited (7). Figure 7 also showed that the application of ethyl acetate *A. conyzoides* extracts subfraction A concentration of 2 % increases root length of *A. spinosus*. 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 (17,23,25,26).

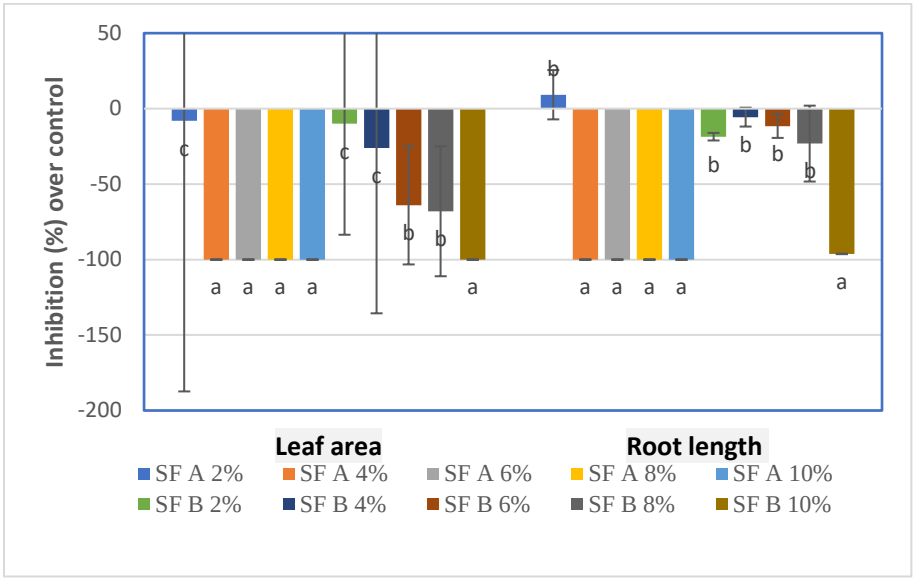

Figure 7. Inhibitory effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on leaf area and root length of *A. spinosus* at 21 DAA.

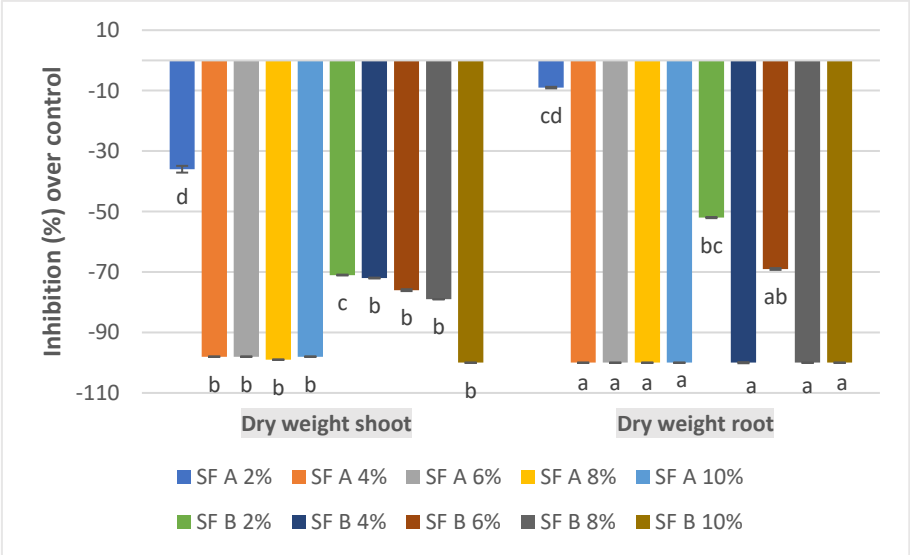

Figure 8. Inhibitory effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on shoot and root dry weight of *A. spinosus* at 21 DAA.

(ii). *C. Rotundus* : The *A. Conyzoides* Ethyl Acetate extracts differed in their effects on leaf length, numbers of tillers (Figure 9), fresh and dry weight (Figure 10) of *C. rotundus*. These extracts were inhibitory and stimulatory to length of leaf, numbers of tillers, fresh and dry

weight of *C. rotundus*. Various responses occurred because of higher concentrations and selective properties of allelochemical effects on target plants (1).

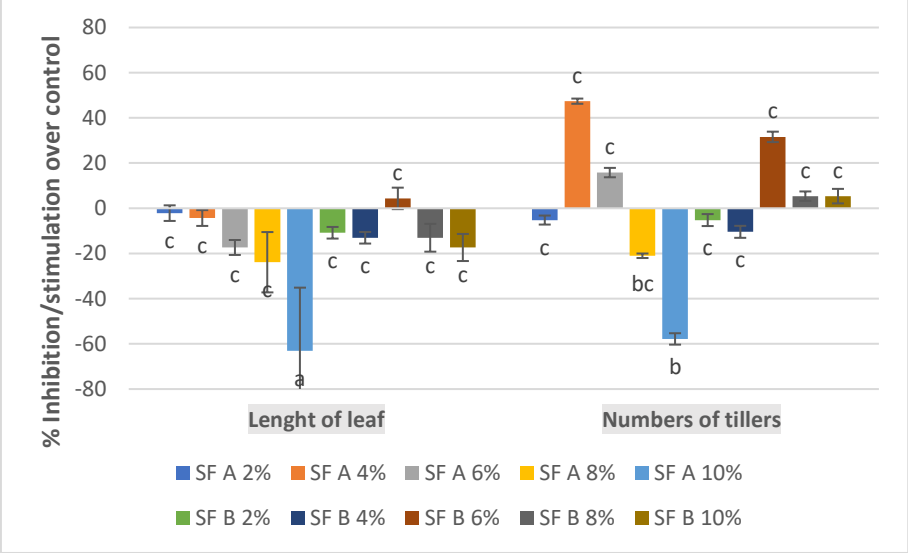

Figure 9. Effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on leaf length and numbers of tillers of *C. rotundus* at 21 DAA.

The plants exposed to ethyl acetate *A. conyzoides* extract subfraction A concentration 10 % were the most inhibited with reduced length of leaf, numbers of tillers, fresh and dry weight when compared with the *C. rotundus* treated with distilled water at 21 DAA (Figure 9 and 10). The growth of *C. rotundus* plants treated with ethyl acetate *A. conyzoides* subfraction A at 10 % concentration was inhibited. There were plant growth inhibitions (63.01 % inhibition of leaf length, 57.82 % inhibition in numbers of tillers, 82.32 % inhibition in fresh weight, 82.37 % in dry weight of *C. rotundus*, respectively) over control. Figure 9 and 10 also showed the stimulatory effects on *C. rotundus* plants growth treated with ethyl acetate *A. conyzoides* subfraction A. The concentration of 4 % increased the numbers of tillers (47.39 % stimulation) and it was similar in plants exposed to subfraction A concentration 6 % (15.80 % stimulation), subfraction B concentration at 6 %, 8 %, 10 % (caused 31.60 %, 5.37 %, 5.37 % stimulation, respectively) over control. However, there were decrease in dry weight generated by ethyl acetate *A. conyzoides* subfraction A and subfraction B extract on length of leaf, numbers of tillers, leaf area, fresh and dry weight of *C. rotundus*, this confirmed that the *C. rotundus* plants growth was restricted. This was perhaps due to allelochemical compounds contained in subfraction A and B can inhibit hormonal activity, so that cell division and elongation in the shoot and root areas were inhibited. Allelochemical at high concentration inhibits the formation of nucleic acid, proteins and adenosine triphosphate (ATP). If ATP was reduced, cell metabolism will also decrease (19).

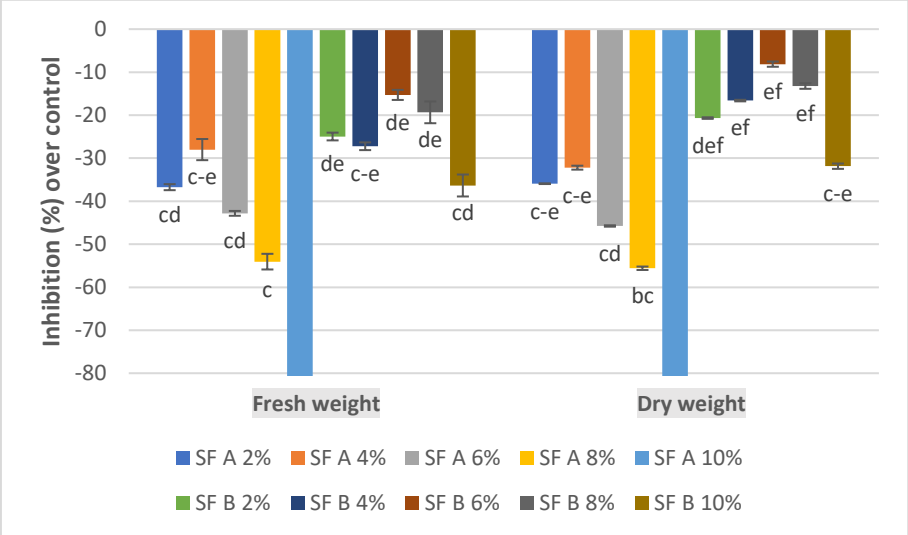

Figure 10. Effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on fresh and dry weight of *C. rotundus* at 21 DAA.

(iii). *A. compressus*: The ethyl acetate *A.conyzoides* subfraction A extract concentration of 10 % provided complete control of *A. compressus* (100 %) mortality (Figure 11 and 12). These figures also showed that there were inhibitory and stimulatory effects of applied ethyl

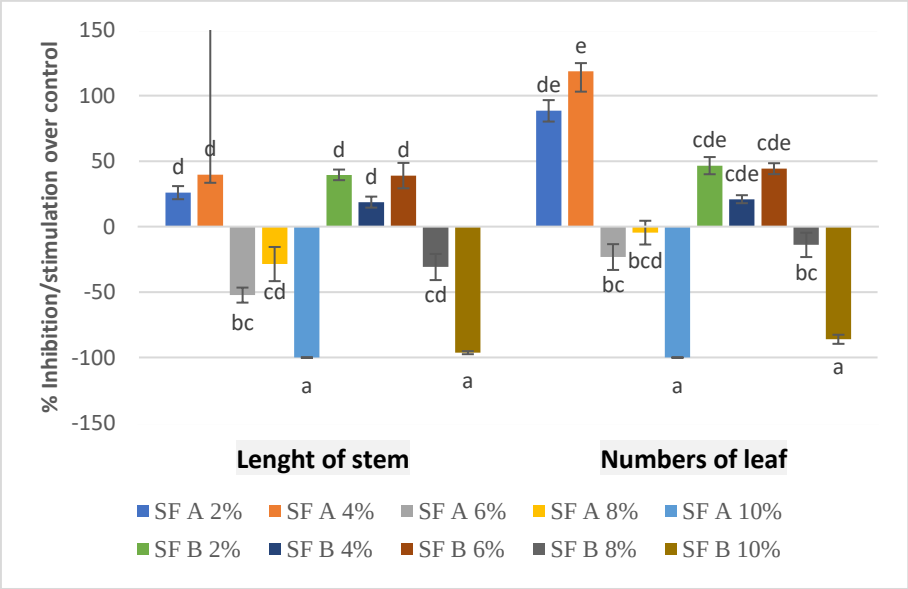

Figure 11. Effects of ethyl acetate extracts of *A. conyzoides* subfractions A and B concentrations on stem length and leaf numbers of *A. compressus* at 21 DAA.

acetate *A. conyzoides* extract subfraction at different concentrations. Stem length, numbers of leaf, fresh and dry weight of *A. compressus* exposed to the ethyl acetate *A. conyzoides* extract subfraction A concentration of 10 % were similar to *A. compressus* plants exposed to the ethyl acetate *A. conyzoides* extract subfraction B concentration of 10 % and 2,4-D. Allelochemicals can act simultaneously on several processes, producing varied responses for each one, depending on the concentration of compound (18,19).

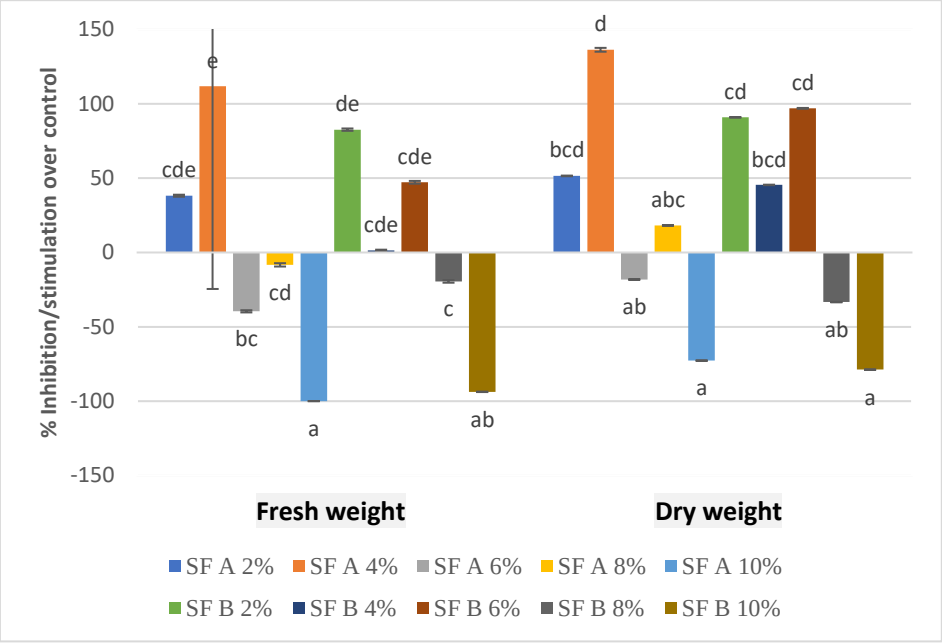

Figure 12. Effects of ethyl acetate extracts of *A. cnyzoides* subfractions A and B concentrations on fresh and dry weight of *A. compressus* at 21 DAA.

From the three types of test weeds, we observed the variable inhibition for the applied *A. conyzoides* ethyl acetate extract at various subfractions and concentrations. The application of ethyl acetate extract of *A. conyzoides* subfraction A to *A. spinosus* weeds, caused the death of *A. spinosus* starting at concentrations of 4 %, 6 %, 8 % and 10 %, but application of ethyl acetate extract of *A. conyzoides* subfraction B caused the death of *A. spinosus* at a concentration of 10 % against *A. spinosus* weeds at 7 DAA, equivalent to the application of synthetic herbicides (2,4-D) (Figure 5).

The application of ethyl acetate extract subfraction A against *C. rotundus* based on the results of observations at 1, 7, 14 and 21 DAA, the highest control was at 10 % concentration, which was at 1 and 7 DAA at 77.33 % and 80.87 % respectively and it was included in the category of severe influence (10). However, at 14 and 21 DAA there was decrease of 65.53 % and 42.80 % only and was included in the category of moderate influence(10). So due to the application of ethyl acetate extract of *A. conyzoides* weed

subfraction A on *C. rotundus*, it showed recovery process every week. For the application of subfraction B ethyl acetate extract to *C. rotundus* weeds also showed symptoms at 1 day after application. The best concentration was found 10 %, with 42.20 % control included in category of moderate effect. While other concentrations were included in the mild category. At 7 DAA observations, all treatments showed a visible decrease in the leaves recovery.

The application of subfraction A ethyl acetate extract against *A. compressus*, the maximum control (67.33 %) of *A. compressus* was found at 10 % concentration at 1 DAA, it was moderate category effect, while at 7 DAA, the control was 100 % which was in the severe effect category (10). Thus the ethyl acetate application extract of *A. conyzoides* weed subfraction A at 10 % concentration absolutely killed *A. compressus* at 7 DAA. The application of subfraction B ethyl acetate extract to *A. compressus* weeds also provided weed control, its effect could be seen from the percentage of weed control at 1 DAA, which was at 10 % concentration and was the best concentration to control 53.67 % weeds (moderate category) (10). While the other concentration treatments had mild effects. At 7 DAA, the 10 % concentration increased the weed control to 83.20 % i.e. severe effect. While at 14 and 21 DAA the 10 % concentration showed a decrease of 77.67 % and 74.73 %, which indicated that the weeds were recovering.

Based on the description above, it can be concluded that the application of ethyl acetate extract was very efffective to control *A. spinosus*. This is because *Amaranthus* sp has a high sensitivity to allelochemicals. The occurrence of variation in the inhibition of various weeds was owing to the differences in the physiology and morphology of weed types. There are 3-important roles (herbicides, environment and application methods) that affects the selectivity of herbicide (16). The roles of plants consist of morphological differences, absorption and translocation, physiological differences, differences in biophysics, biochemical reactions, growth rate and plant age. The role of herbicides consists of molecular shape, herbicide concentration, formulation and mode of action. The environment can also specify all the factors that affects the selectivity of herbicides (light, wind, temperature, wind, soil type). Furthermore, the method of application and the time of application are very essential in determining the degree of success in controlling the weed.

GC-MS Analyses of the Extracts

Major constituents identified in the ethyl acetate *A. conyzoides* extracts subfraction A are presented in Table 4 and all constituents in Table 3. The GC-MS analysis indicated that ethyl acetate *A. conozoides* extract subfraction A contained mainly acetamide (5.70 %), caryophyllene (6.40 %) (sesquiterpene), 2-propenoic acid, 3-(2-hydroxyphenyl)-,(E)- (15.35 %) (fatty acid), precosene II (23.33 %)(phenolic), n-hexadecanoic acid (6.62 %)(fatty acid), hexadecanoic acid, ethyl ester (5.17 %)(fatty acid), phytol (4.33 %)(sesquiterpene) and 9,12,15-octadecatrienoic acid (6.70 %) (Table 2). These compounds contributed 73.60 % 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* and *A. compressus* either acting alone or exerting a synergistic effect. In our last finding, we found that ethyl acetate *A. conyzoides* subfraction was composed of tetradecanoic acid, ethyl ester (10.26 %), precocene II (9.39 %), octadecanal (8.23 %), 9,12,15-octatadecatrienic, methyl

Table 3. All constituents in the Ethyl acetate *A. conyzoides* extract subfraction A

No.	Retention time (min)	Compound	Compound Content (%)
1	4.16	Acetamide	5.70
2	4.324	Cyclopentane, 1-ethyl-3-methyl-,trans-	0.30
3	6.327	Styrene	0.27
4	6.551	2-Butenoic acid, 2-methyl-	0.53
5	11.680	1,2,3-Propanetriol, 1-acetate	0.56
6	13.864	Heptanediamide, N,N'-di-benzoyloxy-	0.51
7	15.996	1,2,3-Propanetriol, 1-acetate	0.53
8	18.435	Benzamide	0.36
9	19.108	Phenol, 2-methoxy-3-(2-propenyl)-	0.46
10	20.169	4-Hydroxy-2-methoxybenaldehyde	0.48
11	20.812	Caryophyllene	6.40
12	21.200	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	1.35
13	21.805	Precocene I	2.14
14	22.404	cis-ß-Farnesene	0.52
15	22.955	7-epi-cis-sesquisabinene hydrate	0.25
16	23.332	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6- methylene-, [S-(R*,S*)]-	2.40
17	24.812	Caryophyllene oxide	1.51
18	25.172	Demethoxyencecalinol	0.44
19	25.870	6-tert-Butyl-8-(methoxymethyl)-2,2- dimethyl-4H-1,3-benzodioxine	0.49
20	26.472	Precosene II	2.33
21	27.652	Spiro-1-(cyclohex-2-ene)-2'-(5'- oxabicyclo[2.1.0]pentane), 1',4',2,6,6- pentamethyl	0.24
22	27.839	Estafiatin	0.64
23	28.465	Methyl benzoate, imine	0.27
24	29.271	E-7-Octadecene	0.44
25	30.240	Neophytadiene	0.85
26	30.849	Spiro[4-methyl-5-oxo-10,13-dioxatricyclo[7.3.1.0(4,9)]tridecane]-2,2'- oxirane	0.27
27	31.965	Hexadecanoic acid, methyl ester	1.67
28	32.737	n-Hexadecanoic acid	6.62
29	33.291	Hexadecanoic acid, ethyl ester	5.17
30	35.284	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.36
31	35.502	Phytol	4.33
32	36.032	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	6.70
33	36.495	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	0.41
34	36.692	Hexadecanamide	2.15
35	36.927	Ethyl 14-methyl-hexadecanoate	0.37
36	37.376	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]-	2.78
37	39.787	Methyl 2-hydroxy-octadeca-9,12,15- trienoate	1.56
38	46.753	2,2,4-Trimethyl-3-(3,8,12,16-tetramethylheptadeca-3,7,11,15-tetraenyl)-cyclohexanol	0.34
39	52.415	Stigmasterol	0.68
40	53.170	W-18	0.37
41	54.374	W-18	0.25

Table 4. Main constituents in the ethyl acetate *A. conyzoides* extract subfraction A and subfraction B

No.	Retention Time (min)	Compound	Compound Content (%)	
			SF A	SF B
1	4.16	Acetamide	5.70	-
2	20.81	Caryophyllene	6.40	9.61
3	21.20	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	15.35	20.82
4	21.812	Precocene I	-	3.27
5	23.33	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	-	3.20
6	26.47	Precosene II	23.33	29.78
7	32.74	n-Hexadecanoic acid	6.62	4.72
8	33.29	Hexadecanoic acid, ethyl ester	5.17	4.78
9	35.50	Phytol	4.33	-
10	36.03	9,12,15-Octadecatrienoic acid	6.70	-
		Total area	73.60	76.18

SF : Subfraction

ester (7.32 %), 10-heneicosene (c,t)(5.19 %) and neophytadiene (5.09 %)(7). 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-spectrum antifungal agent, with allatocidal and insect-growth regulator activities (15). Its Table 4, main constituents in the ethyl acetate *A. conyzoides* extract subfraction A and subfraction B phytotoxic effect was reported on radish, mungbean, tomato and ryegrass seedlings (14). The compositions of ethyl acetate subfraction A extracts also included the presence of caryophyllene (6.40 %). The *Senecio salignus* extract adversely affected the photosynthesis in *Physalis ixocarpa* and *Echinochloa crus-galli* due to 2-sesquiterpenes: B-caryophyllene and caryophyllene oxide (21). The compositions of ethyl acetate subfraction A extracts also contained phytol (4.33 %), which interacts and damages the structure of phospholipid bilayer of cell membrane (5). In the case of the ethyl acetate of *A. conyzoides* subfraction B, major constituents identified in the extracts were (Table 4), caryophyllene (9.61 %), 2-Propenoic acid, 3-(2- hydroxyphenyl) (20.82 %)(fatty acid), Precocene I (3.27 %) (phenolic), Cyclohexene, 3-(1,5- dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-(3.20 %), Precocene II (29.78 %)(phenolic), n-Hexadecanoic acid (4.72 %)(fatty acid) and hexadecanoic acid, ethyl ester (4.78 %)(fatty acid) with 76.18 % of the total composition. In our last finding, we have found out 1-octadecyne (38.57 %), phytol (11.24 %), di-tert-utylphosphine-d (5.17 %) and 1-hexadecine (4.08 %).

All constituents identified in the ethyl acetate *A. conyzoides* extracts sub-fraction AB were shown in Table 5.

Table 5. All constituents in the Ethyl acetate *A. conyzoides* extract subfraction B

No.	Retention time (min)	Compound	Compound content (%)
1.	4.024	Acetamide	0.56
2.	6.456	2-Butenoic acid, 2-methyl-	0.87
3.	1.677	1,2,3-Propanetriol, 1-acetate	1.09
4.	1.779	Cyclopropanecarboxamide, N-benzoyloxy-	0.55
5.	15.993	1,2,3-Propanetriol, 1-acetate	0.82
6.	19.108	Eugenol	0.63
7.	20.163	Benzaldehyde, 3-(chloroacetoxy)-4-methoxy-	0.56
8.	20.819	Caryophyllene	9.61
9.	21.190	2-Propenoic acid, 3-(2-hydroxyphenyl)-, (E)-	20.82
10.	21.812	Precocene I	3.27
11.	22.407	cis-ß-Farnesene	0.72
12.	23.332	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-	3.20
13.	24.809	Caryophyllene oxide	1.80
14.	25.172	Demethoxyencecalinol	0.46
15.	25.870	2,4,5,5,8a-Pentamethyl-6,7,8,8a-tetrahydro5H - chromene	0.48
16.	26.465	Precocene II	29.78
17.	27.842	3,5a,9-Trimethyl-3a,5,5a,9b-tetrahydro3H,4H-naphtho [1,2-b]furan-2,8-dione	0.50
18.	29.271	1-Hexadecanol, 2-methyl-	0.41
19.	30.243	7-Hydroxy-6,9a-dimethyl-3-methylenedeca-hydro-azuleno[4,5-b]furan-2,9-dione	0.43
20.	31.964	Hexadecanoic acid, methyl ester	1.76
21.	32.679	n-Hexadecanoic acid	4.72
22.	33.284	Hexadecanoic acid, ethyl ester	4.78
23.	35.281	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	1.53
24.	35.498	Phytol	2.97
25.	35.981	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	1.54
26.	36.491	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	0.81
27.	36.665	Hexadecanamide	1.41
28.	37.379	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, acetate, [R-[R*,R*-(E)]]-	2.19
29.	39.352	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11- dodecamethyl	0.49
30.	39.773	Methyl 2-hydroxy-octadeca-9,12,15- trienoate	0.84
31.	40.508	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	0.40

Chromatogram of ethyl acetate *A. conyzoides* extract subfraction A and B identified by GC-MS were shown in Figure 13 and 14 respectively.

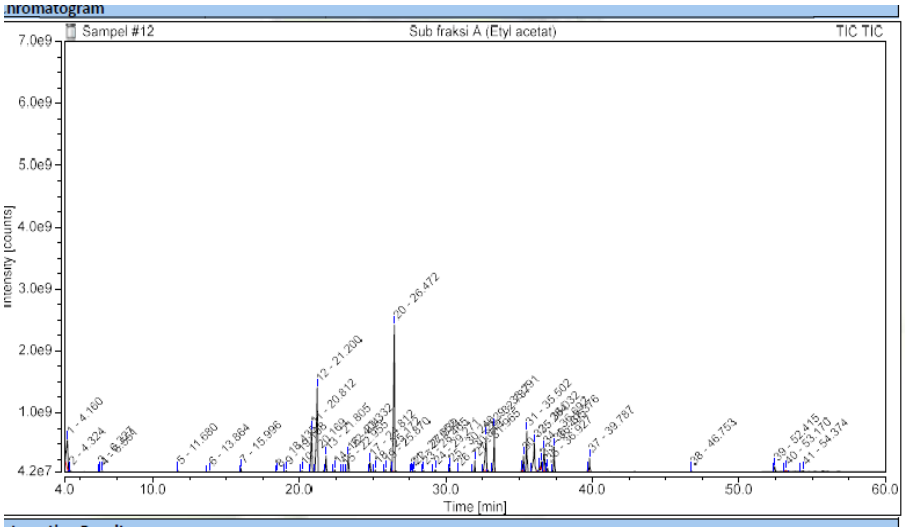

Figure 13. Chromatogram the ethyl acetate *A. conyzoides* extract subfraction A identified by GC-MS

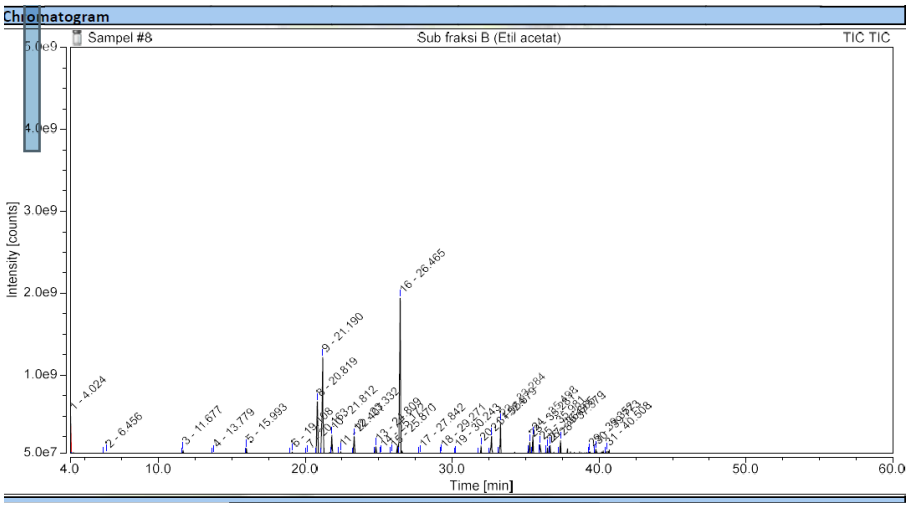

Figure 14. Chromatogram the ethyl acetate *A. conyzoides* extract subfraction A identified by GC-MS

CONCLUSIONS

Analysis of weed control, plant growth (leaf area, dry shoot and root weight and root length) indicated that the ethyl acetate extract of *A. conyzoides* subfraction A had the

strongest post-emergence herbicidal effects on *A. spinosus.* At seven days after application, the ethyl acetate *A. conyzoides* extract subfraction A at concentration of 4 % or higher killed 100 % *A. spinosus* weed plants similar to synthetic herbicide 2,4-D. Likewise, also at seven days after application, the ethyl acetate extract of *A. conyzoides* subfraction B at concentration 10 % also killed 100 % *A. spinosus* plants. However, the *A. conyzoides* extracts differed in their effects on growth of *C. rotundus* [weed control (%), length of leaf, numbers of tillers, fresh and dry weight] and *A. compressus* [weed control (%), numbers of leaf, length of stem, wet and dry weight]. Subfraction A showed the promising control of *A. spinosus* and *A. compressus* and the subfraction B the control of *A. spinosus.* It was concluded that subfraction B was less phytotoxic to test weeds than subfraction A. The *C. rotundus* was less sensitive to both subfractions A and B.

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DECLARATION

We declare that all authors of this Ms. have made substantial contributions. We did not exclude any author who substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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