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Table: 2, Figs: 5

# Herbicidal activity of *n*-hexane billygoat weed (*Ageratum conyzoides* L.) extracts on *Amaranthus spinosus* L.

**G. Erida*, N. Saidi[1], Rosmaiti[2], Rahmaddiansyah[3], E. Nurahmi and Jumini**
Department of Agrotechnology, Faculty of Agriculture,
Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
E. Mail: ginaerida@gmail.com, ginaerida@unsyiah.ac.id

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## ABSTRACT

We tested *Ageratum conyzoides* L. *n*-hexane leaf extract fractions for their ability to inhibit the growth of *Amaranthus spinosus* L. The column chromatography was used to separate the *n*-hexane extract into its three subfractions, A, B and C. In pot culture, these subfractions were applied to *A. spinosus* at 2 %, 4 %, 6 %, 8 % and 10 % concentrations. The herbicide (2,4-D at 0.686 kg a.i. $ha^{-1}$) and distilled water were positive and negative controls, respectively. The subfractions showed phytotoxic activity 7-days after application. All subfraction had promising herbicidal effects on *A. spinosus* i.e. suppressed its growth. Seven day after aplication (DAA) subfraction A supressed growth at 8 % concentrations or higher, subfraction B suppressed at 2 % concentration or higher and subfraction C at 4 % concentration or higher. The subfraction B, had the fastest effect on *A. conyzoides* at 2 % concentration followed by subfraction C at 4 % and subfraction A at 8 %. GC-MS detected the presence of compounds in **Subfraction A** : 2h-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl (13.85 %), caryophyllene oxide (12.69 %), caryophyllene (12.20 %), precocene I (9.15 %), phytol (3.57 %), squalene (3.04 %). **Subfraction B**: 2h-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl (25.60 %), caryophyllene (11.13 %), caryophyllene oxide (6.19 %), precocene I (5.93 %), squalen (5.77 %) and **subfraction C:** 2h-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl (36.48 %), precocene I (2.27 %), caryophyllene (2.20 %), caryophyllene oxide (2.14 %).

**Keywords**: *Ageratum conyzoides*, *Amaranthus spinosus*, billygoat weed, column chromotograplhy, GCMS, herbicidal, *n*-hexane, pot culture, precocene II, subfraction.

## INTRODUCTION

Weeds cause yield losses upto 34 %, greater than the losses caused by other agricultural pests (24). Herbicides are used to control weeds but their repeated use causes : negative impacts on soil (23), development of weeds resistance to herbicide (4,12), to pollutes the environment and dangerous human health (2). Hence, the herbicidal potential of plant substances (allelochemicals) is being investigated (7,8).

*Ageratum conyzoides* L. (family Asteraceae) is a tropical plant, found in some regions of Africa, Asia and South America. In Indonesia, it is commonly found in crop fields, yards, roadsides and water edges (19). It is popularly known as billygoat- weed, is an annual aromatic plant (Figure 1) and has a large variety of secondary metabolites with biological activities (14). The *A. conyzoides* produces allelochemicals, which exert strong allelopathic effects on crops. It is an aggressive weed in crops and cause severe yield losses by interfering in growth and development of crops (5). Aqueous extracts of *A. conyzoides*

*Correspondence author, [1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas syiah Kuala, Banda Aceh 23111, Indonesia, [2]Department of Agrotechnology, Faculty of Agriculture, Universitas Samudra., Langsa 24354, Indonesia, [3]Department of Agribusiness, Faculty of Agriculture, Universitas syiah Kuala, Banda Aceh 23111, Indonesia.

leaf, root, flower and stem posses strong herbicidal potential against germination and growth of *Parthenium hysterophorus* L. (25). The aqueous extract of *A. conyzoides* flower, root, stem and leaf significantly reduces the seeds germination and seedling growth of mungbean [*Vigna radiata* L. (31)]. The growth of rice was severely inhibited in *A. conyzoides* leaf debris amended soils (6). We showed that *A. conyzoides* had the strong bioherbicidal potential among the *Imperata cylindrica* L., *Cyperus rotundus* L., *Chromolaena odorata* L., *Axonopus compressus* (Swartz), *Acacia mangium* Willd., *Pinus merkusii* Jungh.et de Vriese, *Terminalia catappa* L., *Jatropha curcas* L. and *Tectona grandis* L.f. on *Amaranthus spinosus* L. (15,17). *A. conyzoides* methanol extract at 20 % concentration completely suppressed the *A. spinosus* growth (Figure 2), 7 days after application. This effect was similar to that observed for 2,4-D herbicide applied at 0.686 kg a.i. $ha^{-1}$ (17).

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

It is necessary to use certain solvents according to the properties of desired compound, because solvent of different polarity results in obtaining different amounts and types of allelochemical compounds (20). Our findings also revealed that among all the extracts of *A. conyzoides* leaves obtained by the maceration method and fractionated with different polarity solvents, the *n*-hexane extract of *A. conyzoides* fraction had the post-emergence herbicidal effects on *A. spinosus* (16). The *n*-hexane extract at 20 % concentration controlled *A. spinosus* growth (74.28 %) at 21 days after application (16). Main compounds identified by GC-Ms in *n*-hexane extract were: 2h-1-benzopyran, 6,7-

dimethoxy-2,2-dimethyl (59.22 %), caryophyllene (25.47 %), 2.6.10.14.18.22-Tetracosahexaene (5 %) and 1,8-cineole (3.9 %). Based on this research, we studied the potencial of herbicides at the subfraction level of *n*-hexane extract on the growth of *A. spinosus* weed. *A. spinosus* was used as a target plant because of its fast uniform germination, sensitivity to allelochemicals and active competitor with crops (1,32) and one of 18 most serious weeds in the world (38).

## MATERIALS AND METHODS

The research was carried out at Syiah Kuala University (USK), Province of Aceh, Indonesia, from August 2021 to July 2022 in the Laboratory of Biology, Organic Chemistry and Weed Science. Lab bioassays were done from (18 June 2022 to 20 July 2022. Pot studies in screen house were conducted 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: 1700.5 mm, max temp: 34.40 °C and minimum temp: 22.90 °C.

### Experimental

The experimental treatments consisted of 3 Factors: (i). *A. conyzoides n*-hexane extracts subfractions: 3 (A,B,C), (ii). Concentrations 5 (2, 4, 6, 8, 10 %) and controls 2 (Positive Control: 2,4-D, Negative Control: Distilled Water). The treatments were replicated thrice in completely randomized design (CRD). The seeds of *A. spinosus* were gathered in Meunasah Gle, Sigli and Pidie. Dr. Saida Rasnovi Botanist, identified the plants.

### *A. spinosus* plant extracts

The leaves of *A. conyzoides* were gathered in Aceh Besar's Indrapuri district. These leaves were air dried for two weeks at room temperature and then grinded. The 25 kg grinded leaves were placed in 4 L ammonia for 1.0 h. They were then extracted 6-times using *n*-hexane. Each extraction took three days and used 20 L of solvent. The organic fractions recovered after extraction with each solvent were filtered, mixed and dried in a rotary evaporator (20). We obtained 250 g of *n*-hexane extract.

### Fractionation

The *n*-hexane extract was fractionated by column chromatography. Cotton was put into the bottom column and sand was heated and sieved using 12 mesh sieve. Then, 250 g silica gel was added after soaking for about 24 h with *n*-hexane. Sand was added on top of the silica gel and finally the extract (50 g). Then, the column was eluted with *n*-hexane: ethyl acetate at a ratio of 9:1, 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 accommodated in glass bottles. The column was further eluted with mobile phases as of *n*-hexane:ethyl acetate (9:1; 8:2; 7:3; 6:4; 5:5; 3:7 and 2:8, v/v). Those subfractions were suspended in distilled water to prepare concentrations of 2, 4, 6, 8 and 10 %. According to their thin layer chromatography patterns, pools of fractions were created (20). The three obtained subfractions are A, B, and C. The subfractions were prepared into concentrations of 2, 4, 6, 8 and 10 % by suspending them in distilled water.

**Pot culture**

We collected soil up to 20 cm depth from the Lampakuk Village, Aceh Besar. The soil was dried for 7- days and then sieved to remove plant residue etc. One kg soil was placed in each plastic pot (16 cm dia and 13 cm depth). On June 11, 2022, 5-unsterilized of *A. spinosus* seeds were immersed in water for 2.0 h and thereafter sown in each pot at 2 cm depth. When the seedlings were 14 days old, the plants were foliar sprayed (4 ml per pot) with either water or plant extract as per treatments. Two hundred ml tap water was used to irrigate the pots twice a day. Weed control (%) was determined at 7, 14, and 21 DAA (Days After Application). Whereas, dry shoot and root weight, root length and leaf area, were measured at 21 (DAA). Using a 0-100 rating scale, the *A. spinosus* weed control (%) was evaluated based on 5 observations (Table 1). After oven drying for 48 h at 60 °C or until reaching a constant dry weight, the dry weight of shoots and roots were recorded. The root length was measured, after washing with tap water.

Table 1. Rating system used to assess weed control.

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

Source:(18)

**GC-MS analysis**

The analysis used single quadrupole GC-MS systems contain one mass filtering quadrupole that require chromatographic temperature ramps and spectral scan rate to be matched in order to maximize the number of compounds that can be routinely identified for metabolite profiling. The following GC-MS conditions were used a temperature of 40 °C was maintained for 72.5 min. The split ratio was 99:8, the total flow was 58.8 ml/m, the column flow was 0.55 ml/m, and the cleaning flow was 3.0 ml/m. Based on retention periods

and comparisons of mass spectra with those from the Willey-NIST library, compounds were identified. They were measured in terms of relative areas.

**Statistical analysis**

At a 5 % probability level, Duncan's new multiple range test and analysis of variance (F test) were applied to all data. The SPSS version 16 programme (SPSS Inc., Chicago, IL) was used to conduct the analysis.

## RESULTS AND DISCUSSION

### WEED CONTROL

At various concentrations, each subfraction of the *n*-hexane extract of *A. conyzoides* caused 100 % death of *A. spinosus* plants. The effects of *A. conyzoides n*- hexane extract at various subfraction on *A. spinosus* at 7 and 21 DAA are shown in Figure 3. The *n*-hexane *A. conyzoides* subfraction A extract caused the death of *A. spinosus* at 8 % concentrations or higher, which was similar to synthetic herbicide (2,4-D) at 7 DAA (Figure 3).

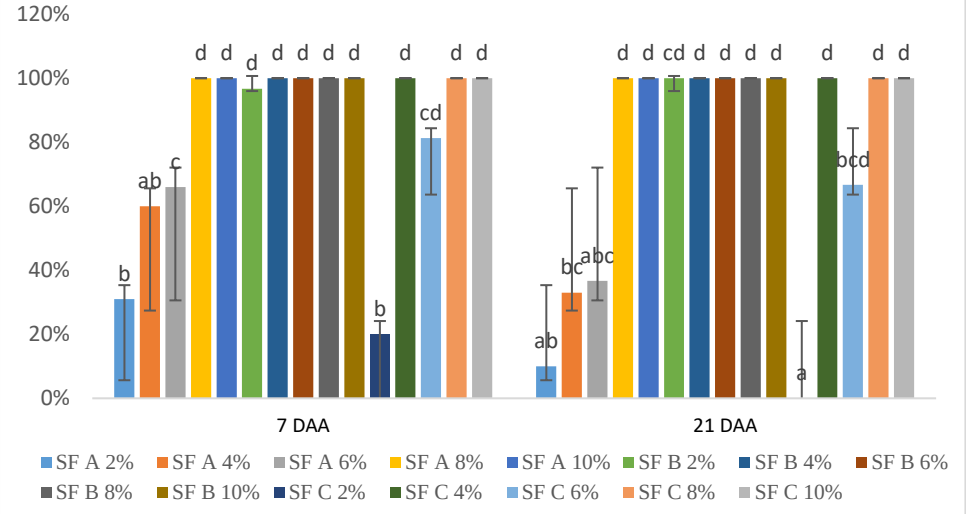


Figure 3. Effects of *n*-hexane extracts of *A. conyzoides* at different subfraction (SF) at 7 DAA and 21 DAA on weed conrol ( %) of *A. spinosus.*

**Subfraction A :** Field observation for weed control were done on test plants based on Frans and Talbert (Table 1). These showed that the application of *n*-hexane extract of *A. conyzoides* subfraction A with concentration of 2 % exhibited symptoms like the edges of the leaves curled and turned white, and some dry leaves tips curled up. After few days, the weeds revived and some new leaves grew. The application of 4 % concentration showed some dry leaves and some withered leaves and also some new leaves were growing. At 6 % concentration, besides some dry leaves one green leaf was left with dry edges. In conclusion, at 6 % concentration, some leaves died but many of them grew again. At concentration of

8 % and 10 %, all leaves withered and weeds completely died. The phytotoxic symptoms were different between extract and synthetic herbicide (2,4-D). The symptos of 2,4-D not only caused leaf chlorosis, but also stem fell and turn into brown like burning.

**Subfraction B:** The application of *n*-hexane extract of *A. conyzoides* subfraction B on *A. spinosus* at 2 % concentrations showed moderate damage, withered stems and shoots, leaves curl and dry at the ends. The following day, the weeds died, marked by dry and stems withered and turned white. At 7 DAA, the weeds had completely dried up but few leaves emerged from the new shoots. The application of 4 %-10 % extract showed severe damage; the leaves dried and curled and wilted. The whole weeds died, leaves dried and whole and stems withered and turned white.

**Subfraction C :** The application of *n*-hexane extract of *A. conyzoides* subfraction C on *A. spinosus* at 2 % concentrations showed the leaves were wilting. But after 3 DAA, the new leaves start growing again, and no more reaction from *A. conyzoides* subfraction C extract. Concentration at 4 % showed the leaves completely wilted, but the weeds were still green, but died at 4 DAA. At concentration of 6 %, 8 %, and 10 %, plants died quickly (2 DAA).

Due to the allelochemicals influences on the target plant's increased concentration and selectivity (1), many reactions took place. The *A. spinosus* weed control (%) increased with the increasing *A. spinosus* concentration (Figure 3). At 7 DAA, 4 % concentration caused 100 % inhibition and 81.33 % inhibition at 6 % concentration, the control was rather irregular with respect to tested concentration. This bias was most likely caused by unanticipated changes in environmental variables (light, $CO_2$ temperature, soil moisture, relative humidity, rain, or wind) that occurred during or after extract spraying (21). Environmental variables may directly affect the efficiency of plant extracts applied post-emergence by changing pathways for penetration and translocation or indirectly by changes in the physiological stage of the weed (11).

## PLANT GROWTH

**Leaf area :** Regarding the leaf area (Figure 4) at 21 DAA, distilled water-treated *A. spinosus* plants had their largest leaf area (275.97 $mm^2$). Because they died at 7 DAA after application, *A. spinosus* plants treated with *n*-hexane at *A. conyzoides* subfraction A concentrations of 8-10 %, subfraction B concentration 2-10 %, and subfraction C concentration 4-10 % were not present after 21 DAA (all 100 % inhibition). This was due to the allelochemicals present in the extract of *A. conyzoides*, which impeded the hormonal function. The physiological functions of weeds (transpiration, photosynthesis and respiration), are impaired by allelochemicals absorption. This blocks the hormone action, which in turn prevents cell proliferation and elongation in the leaf regions (26).

**Shoot Dry Weight, Root Dry Weight and Root Length** : The effects of *n*-hexane *A. conyzoides* extracts on the shoot and root dry weight and root length of *A. spinosus* varied significantly. These variations depended on both the extract and the concentrations used. *A. spinosus* death was caused by exposure to *n*-hexane *A. conyzoides* subfraction A extract concentrations of 8 % or higher, subfraction B concentrations of 2 % or higher and subfraction C concentrations of 4 % or higher (Figure 5). The reductions in dry weight caused by the *n*-hexane extracts on the roots and shoots provide proof that the *A. spinosus* plants' ability to produce organic matter

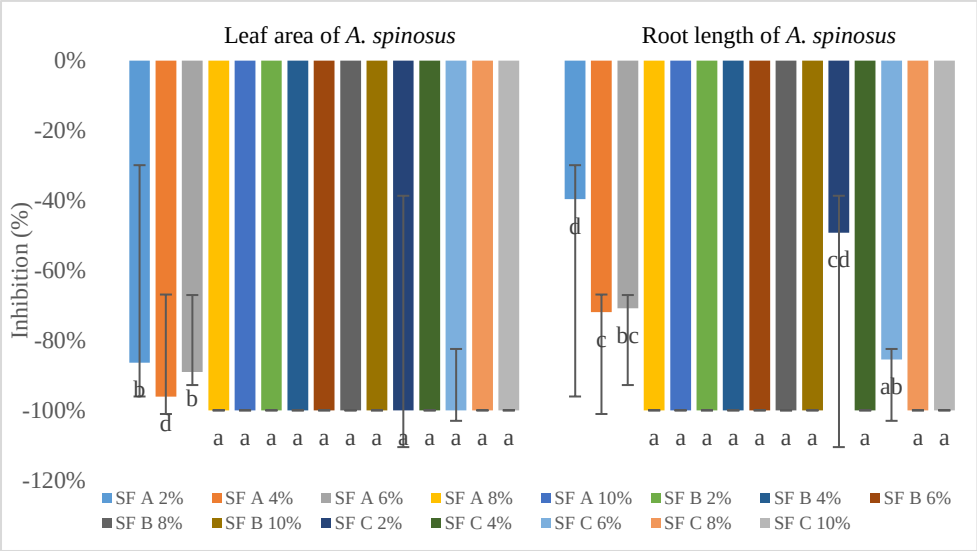


Figure 4. Inhibitory effects of *n*-hexane extracts of *A. conyzoides* at different subfraction (SF) at 21 DAA on Leaf area and root length of *A. spinosus.*

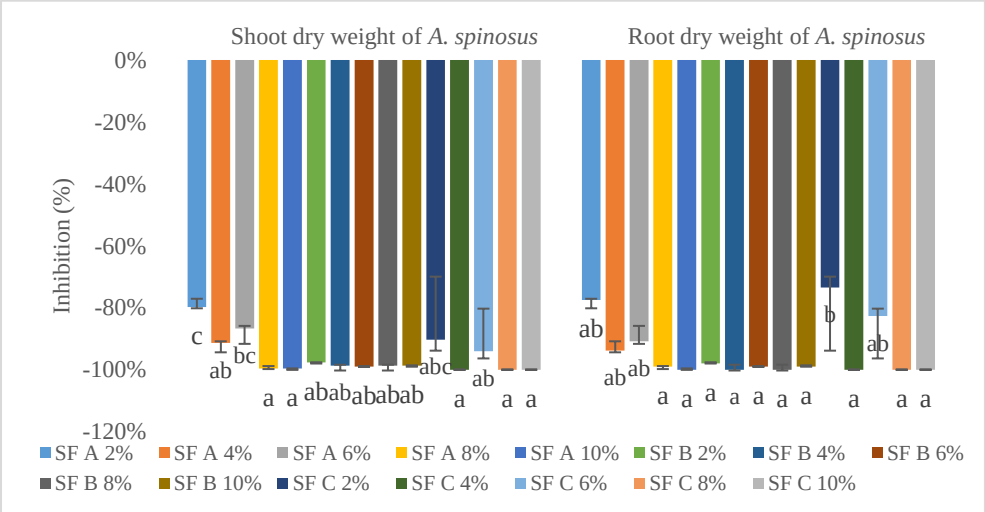


Figure 5. Effects of *n*-hexane extracts of *A. conyzoides* at different subfraction (SF) at 21 DAA on shoot dry weight and root dry weight of *A. spinosus*

was constrained. High amounts of allelochemical substances can prevent the synthesis of proteins, nucleic acids and adenosine triphosphate (ATP), which would slow down the cell metabolism (34).

## GC-MS ANALYSIS

Main constituents identified in the *n*-hexane *A. conyzoides* extracts subfraction A, B and C are presented in Table 2.

Tabel 2. Main constituents identified in *A. conyzoides n*-hexane subfractions A, B, C extract by GC-MS

| No. | RT | Compound name | Compound content (%) | | |
|---|---|---|---|---|---|
| | | | SF A | SF B | SF C |
| 1 | 20.74 | Caryophyllene | 12.20 | | |
| 2 | 21.76 | Precocene I | 9.15 | | |
| 3 | 22.50 | cis-ß-Farnesene | 4.88 | | |
| 4 | 23.45 | (3R,3aR,7R,8aS)-3,8,8-Trimethyl-6 methyleneoctahydro-1H 3a,7- methanoazulene | 8.89 | | |
| 5 | 24.84 | Caryophyllene oxide | 12.69 | | |
| 6 | 26.40 | 2H-1-Benzopyran, 6,7-dimethoxy-2,2- dimethyl | 13.85 | | |
| 7 | 35.58 | Phytol | 3.57 | | |
| 8 | 46.84 | Squalene | 3.04 | | |
| 9 | 20.88 | Caryophyllene | | 11.13 | |
| 10 | 21.86 | Precocene I | | 5.93 | |
| 11 | 23.39 | Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)] | | 5.68 | |
| 12 | 24.87 | Caryophyllene oxide | | 6.19 | |
| 13 | 26.49 | 2H-1-Benzophyran, 6,7-dimethoxy-2,2-dimethyl | | 25.60 | |
| 14 | 29.81 | 6-(1'-hydroxyethyl)-7-methoxy-2,2-dimethylchromene | | 5.83 | |
| 15 | 46.81 | Squalene | | 5.77 | |
| 16 | 18,39 | 2H-1-Benzopyran, 6,7-dimethoxy-2,2- dimethyl | | | 36.48 |
| 17 | 20,84 | Caryophyllene | | | 2.20 |
| 18 | 21,83 | Precocene I | | | 2.27 |
| 19 | 24,84 | Caryophyllene oxide | | | 2.14 |
| 20 | 36,22 | Hexadecanamide | | | 2.33 |
| 21 | 39,61 | Methyl 2-hydroxy-octadeca-9,12,15-trienoate | | | 5.10 |
| Total area | | | 68.27 | 66.13 | 50.52 |

RT: Retention Time, SF: Subfraction,

**Subfraction A:** The GC-MS analysis indicated that *A. conyzoides n*-hexane extract subfraction A contained mainly 2h-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl (precocene II) (13.85 %), caryophyllene oxide (12.69 %), caryophyllene (12.20 %), (3R,3aR,7R,8aS)-3,8,8-trimethyl-6 methyleneoctahydro-1H 3a,7- methanoazulene (8.89 %), precocene I (9.15 %), phytol (3.57 %) and squalene (3.04 %). These compounds constitute 68.27 % of the total composition identifiedd by GC-MS and were likely involved in phytotoxicity effects of *n*-hexane subfraction A extract on *A. spinosus* either acting alone or exerting synergistic effects. In our previous findings, the main compounds in *A. conyzoides n*-hexane fraction were : 2h-1-benzopyran, 6,7-dimethoxy-2,2- dimethyl (59.22 %) (precocene II) (phenolic), caryophyllene (25.47 %) (sesquiterpene), 2,6,10,14,18,22-tetracosahexaene (squalene) (5 %) and 1,8-cineole (3.90 %) (16).

**(i). Phenolics :** 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* (9). It is

a wide-spectrum antifungal agent, with allatocidal and insect-growth regulator activities (30). Its phytotoxic effects have been reported on radish, mungbean, tomato and ryegrass seedlings (29)

**(ii). Sesquiterpenes :** Two sesquiterpenes (β-caryophyllene and caryophyllene oxide) isolated from the *n*-hexane extract of *Senecio salignus* DC., inhibited ATP synthesis (36). β-caryophyllene can inhibit the growth of *Brassica campestris* L. and *Raphanus sativus* L. seedlings (39). β-caryophyllene is a component of the essential oil of *Artemisia lavandulaefolia* which suppresses the growth of *Achyranthes japonica* (Miq.) seedlings (27). Extracts of chickpea (*Cicer arietinum* L.) and black daisy seeds showed that the main compounds consisting of eugenol, β caryophyllene and caryophyllene oxide caused a marked reduction in the length of roots and shoots of *Vicia sativa*, *Sinapis arvensis* L. and *Vicia narbonensis* L. (3). The squalene (2,6,10,14,18,22-tetracosahexaene) belonged to triterpenes (antioxidants).

**(iii). Monoterpenes :** 1,8-cineole was another major compound of the *n*-hexane extract of subfraction A of *A. conyzoides*. The phytotoxicity of some eucalyptus is caused by the presence of various compounds in their essential oils, one of which is 1,8-cineole. As one of the main components found in essential oils, 1,8-cineole has bioherbicidal activity and has been tested on other weeds (35,37). 1,8-cineole inhibited the germination and seedling growth of silverleaf nightshade (*Solanum elaeagnifolium* Cav.) (40), affects cell proliferation and DNA synthesis in plant meristems (33) inhibits root growth and DNA synthesis in the root apical meristem of *B. campestris* (28). It is one of the main components of eucalyptus essential oil and has been used as a pre-emergence herbicide (13). *Eucalyptus globulus* Labill essential oil could be used to control *Echinochloa cruss-galli* (L.) Beauv. due its post-emergence herbicidal activity (22).

The compositions of *n*-hexane subfraction A extracts also included the presence of caryophyllene (12,20). The *Senecio salignus* extract adversely affected the photosynthesis in *Physalis ixocarpa* Brot ex. Hornem and *E. crus-galli* due to 2-sesquiterpenes: B-caryophyllene and caryophyllene oxide (36). The compositions of *n*-hexane subfraction A extracts also contained phytol (3.57 %) a compound that had been reported as a bioherbicide, which interacts and damages the structure of phospholipid bilayer of cell membrane (10).

**Subfraction B:** *n*-hexane extract of *A. conyzoides* subfraction B contained mainly : 2h-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl (25.60 %), caryophyllene (11.13 %), caryophyllene oxide (6.19 %), precocene I (5.93 %), 6-(1'-hydroxyethyl)-7-methoxy-2,2-dimethylchromene (5.83 %), squalen (5.77 %) and cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene- (5.68 %) with a total composition of 66.13 %.

**Subfraction C:** The *n*-hexane extracts of *A. conyzoides* extract subfraction C contained mainly: 2h-1-benzophyran, 6,7-dimethoxy-2,2-dimethyl (precocene II) (36.48 %), methyl 2-hydroxy-octadeca-9,12,15-trienoate (5.10 %), hexadecanamide (2.33 %), precocene I (2.27 %), caryophyllene (2.20 %), caryophyllene oxide (2.14 %) in subfraction C showing the total composition by 50.52 %.

## CONCLUSIONS

Analysis of leaf area, weed control (%), shoot and root dry weights and root length indicated that different subfractions of *A. conyzoides n*-hexane extract had strong post-emergence herbicidal activity on *A. spinosus*. The strongest phytotoxic effects were from *n*-hexane extracts of *A. conyzoides* subfraction B, which caused complete death only at 2 % concentration or higher at 7 DAA. The *n*-hexane extract subfraction A applied at 8-10 % concentration and subfraction C concentration 4-10 % generated phytotoxicity similar to 2,4-D. Hence, *n*-hexane extract of *A. conyzoides* subfraction B provided promising control of *A. spinosus* followed by subfraction C and subfraction A. However, *A. conyzoides* effects 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.

## 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.

## DECLARATION

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

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## PUBLISHER NOTE