Allelopathic Screening of Several Weed Species as Potential Bioherbicides

G Erida¹², N Saidi³, Hasanuddin² and Syafuddin²

¹Doctoral Program in Agricultural Sciences, Syiah Kuala University, Darussalam, Banda Aceh 23111, Indonesia;
²Department of Agrotechnology, Faculty of Agriculture, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia
³Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Syiah Kuala, Darussalam, Banda Aceh 23111, Indonesia

E-mail: ginaerida@gmail.com

Abstract Many plants that are considered to be weeds have the potential to exhibit allelopathy; they might therefore be used as a herbicides to control other weeds. The aim of this study was to identify the best potential bioherbicide against spiny Amaranthus spinosus growth at various concentrations from among five weed species: Imperata cylindrica, Cyperus rotundus, Chromolaena odorata, Ageratum conyzoides, and Axonopus compressus. The study followed a non-factorial, completely randomized design, examining 17 treatments with three replicates. Extracts of the leaves, tuber, and/or rhizome of the five sample plants were obtained using maceration and were applied to the indicator weed A. spinosus at concentrations of 10%, 20% and 30%. A synthetic herbicide (2,4-dichlorophenoxyacetic acid at 0.686 kg/ha) was used as a positive control and distilled water as a negative control. The application of allelopathic extracts of the weeds at various concentrations significantly affected the height growth rate, the percentage of weed control, leaf area, root length, and shoot and root dry weight growth of A. spinosus. The greatest inhibition on A. spinosus growth at 7 days after application was observed with a 20% concentration of A. conyzoides, followed by 20% concentrations of C. rotundus, C. odorata.

1. Introduction

Weeds growing in crop fields compete with the planted crops for light, moisture, and other essential nutrients [1]. In addition, some weeds interfere with the crop plants by producing allelochemicals, which inhibit the growth and development of the crops [2-4], and some act as hosts for pests and diseases [5]. As a result, weeds can be serious pests that can damage crops, reducing the quality and yield and increasing the cost of production.

Weeds are generally controlled either through conventional methods or by using herbicides. Although chemical herbicides can be effective for controlling weeds, they are associated with risks such as a negative impact on soil [6], the development of resistance to herbicides [7], harm to non-targeted organisms, the disturbance of the ecology as a whole, and remnant chemical residues in the environment [8]. An increased awareness of risks involved in the use of herbicides has resulted in greater emphasis on searching for alternative methods of weed control that are safe for the environment. One such approach is allelopathy, which is recognized as a natural method of weed control [3,9-12].

Allelopathy can be defined as the effect of one plant on another plant through the release of chemical compounds into the environment [13]. Allelochemicals can be advantageous, stimulating the target organism, or they may harm the other plant [13,14]. A great variety of natural compounds are known to cause allelopathy, with secondary metabolites constituting the most important group of allelochemicals [13,15]. Known allelochemicals include simple water-soluble organic acids and unsaturated lactones,
long-chain fatty acids and polyacetylenes, naphthoquinone, anthraquinones and complex quinones, simple phenols, benzoic acid and derivatives, cinnamic acid and derivatives, flavonoids, tannins, terpenoids, steroids, amino acids and polypeptides, alkaloids and cyanohydrins, sulfides and glucosides, purines and nucleotides, coumarins, thiocyanates, lactones, and acetogenins [16]. It has been reported that allelochemicals are present in almost all plant tissues, stems, roots, leaves, and fruits. Allelochemicals are released from the plant residues via various processes such as root exudation, leaching, decomposition, and volatilization [13,15].

Perennial weeds have allelopathic potential that can severely affect crop survival and productivity. Such weeds were used in the present study. Cogon grass (Imperata cylindrica) extracts have inhibitory effects on the seed germination and seedling growth of butterfly pea (Centrosema pubescens) [17]. In addition, extracts of ≥10% of concentrations of I. cylindrica significantly reduced the early seedling growth of the weed Parthenium hysterophorus [18]. A previous investigation showed that 15% concentrations of purple nutsedge (Cyperus rotundus) extracts reduced the germination percentage of finger millet (Eleusine coracana Gaertn.) by 48% and 60% [19]. This extract has also been shown to inhibit the germination and growth of several broadleaf weed species [20] and of maize [21]. Conversely, blanket grass (Axonopus compressus) may increase the yield of oil palm crops when used as a soil cover, when compared to beans [22]. Siam weed (Cromolaena odorata) leaf and root extracts have been shown to inhibit the germination and growth of 10 herbaceous plants, with the leaf extracts providing the strongest inhibition [23]; at 15% concentration, they inhibit the germination and growth of C. pubescens seedlings [17], the germination of chickpea (Cicer arietinum) seeds in the laboratory [24], and the germination of mung beans (Vigna radiata) and the shrub Mimosa invisa [25]. Intercropping annual billygoat weed (Ageratum conyzoides) in citrus orchards may effectively suppress weeds and control insect pests and diseases [26,27,28]. It has the potential to be used as a natural herbicide for weed control in paddy fields [29]; however, leaf debris of A. conyzoides deleteriously affect the easy growth of rice by releasing water-soluble phenolic acids into the soil [12].

The essential aspect of a bioassay test for identifying allelopathy is to detect allelopathic action in the selected species [30,31]. The most common target species are plants with fast germination, uniformity, and sensitivity. For this reason, spiny amaranth (Amaranthus spinosus) was selected as the target plant for the present study because it is known to exhibit rapid germination, uniformity, and sensitivity [32], and it can behave as an active competitor [33].

The aim of this study was to identify the best potential bioherbicide against the growth of A. spinosus from among five weed species, described above, that are known to have an allelopathic influence on other plants, by investigating extracts at various concentrations.

2. Materials and methods

2.1. Design and setting

This study followed a non-factorial, completely randomized design consisting of 17 treatments with three replicates per treatment and two plants per treatment. The negative control was distilled water, and the positive control was a conventional herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), at 0.686 kg/ha. The treatments comprised various concentrations (10%, 20%, and 30%) of extracts from all parts of five plants: I. cylindrica (rhizome), C. rotundus (tuber), C. odorata (leaves), A. conyzoides (leaves), and A. compressus. The indicator weed A. spinosus was grown from seeds.

The study was conducted from May to October 2015 at the Science of Weed Laboratory, Faculty of Agriculture, the Laboratory of Biology (Herbarium) and the Laboratory of Chemistry, Faculty of Mathematics and Natural Sciences, and the Experimental Farm, Faculty of Agriculture, Syiah Kuala University, Indonesia (Unsyiah).

2.2. Sample preparation of plant extracts and the indicator plant

Samples of I. cylindrica, C. rotundus, and C. odorata were obtained from Meunasah Jambe, Sigli, Pidie, A. conyzoides from Indrapuri, Aceh Besar, and A. compressus from the gardens of the Faculty of
Agriculture, Unsyiah, Banda Aceh. The \textit{A. spinosus} samples were obtained from Meunasah Gle, Sigli,Pidie. The collected samples were identified by a botanist at the Faculty of Mathematics and Natural Sciences Herbarium, Biology, Unsyiah.

Optimally grown leaves of \textit{A. conyzoides} and \textit{C. odorata}, tubers of the same color and shape of \textit{C. rotundus}, rhizomes of \textit{I. cylindrica}, and all parts of \textit{A. compressus} were dried for 2 weeks at room temperature, cut into pieces, and then crushed with a mortar and pestle. Approximately 1 kg (dry weight) of material was obtained from each plant species. The mashed material was transferred into a can and was extracted using methanol for 24 h by maceration. The resulting filtrate was then evaporated using a rotary evaporator (R-210/R-215, Buchi Corp.) at a temperature of 40°C to yield a concentrated extract.

2.3. Phytochemical testing
Phytochemical testing for alkaloids, steroids, terpenoids, saponins, and flavonoids was performed in the organic chemistry laboratory of the Natural Materials Division at Unsyiah. For the alkaloid test, 0.5 g samples extracted from the source plant were basified using ammonia and were then added to 1 mL of chloroform and crushed. The filtrate was added to 10 mL 5% hydrochloric acid, shaken vigorously, and kept still until the hydrochloric acid and chloroform formed two separate layers. The hydrochloric acid layer was divided among three tubes, each of which was used for testing the presence of alkaloids by adding Mayer’s, Dragendorff’s, and Wagner’s reagents. The presence of alkaloids was also tested using thin-layer chromatography, in which a small amount of concentrated ammonia was added to a fresh 0.5 g sample of the plant extract. This was stirred until mixed, left for an hour, and the same amount of dichloromethane was added and left for 30 min until separation occurred. The dichloromethane fraction was speckled on a chromatography plate and eluted using ethyl acetate and hexane (8:2) and inserted into the chamber. After removing it from the chamber, it was inserted into an acid cabinet and evenly sprayed with Dragendorff’s reagent and then heated on a hotplate until the color changed to brown.

Terpenoid and saponin tests were performed to identify steroids. In these tests, 0.5-g samples from the source plant were finely crushed and extracted with hot methanol. The filtrate obtained was concentrated to obtain the methanol extract, which was extracted again with dichloromethane. The dichloromethane layer was tested for steroids and terpenoids using the Liebermann–Burchard reagent. The insoluble residue in dichloromethane layer was vigorously shaken. The presence of terpenoids and steroids was also tested using thin-layer chromatography. A 0.5-g sample was diluted with 1 mL of ethyl acetate and then speckled on a thin-layer chromatography plate and eluted using the ethyl acetate eluent system described above for approximately a minute or until the boundary mark was reached. The plate was then left to dry, sprayed with vanillin sulfate and heated on a hotplate until a color change was noted, which indicated steroids, terpenoids, or if a stable form was noted after approximately 30 min, which indicated the presence of saponins. This was then hydrolyzed with up to 4 mL of 2 N hydrochloric acid and filtered and tested for triterpene and steroid saponins using the Liebermann–Burchard reagent.

For the flavonoid test, 0.5 g leaf samples were extracted using methanol and then concentrated; the concentrated sample was then extracted again using n-hexane. The residue was extracted with 10 mL of 80% ethanol and added to 0.5 mg magnesium metal and 0.5 M hydrochloric acid. A pink or purple color indicated the presence of a flavonoid. The presence of phenolic compounds was tested by treatment with FCl\textsubscript{3} solution [34].

2.4. Preparation of growth media and planting spiny amaranth (\textit{A. spinosus})
The topsoil layer was obtained from Lampakuk Village, Aceh Besar, taken to a depth of 20 cm. The soil was dried for 7 days, separated from the plant remains, sieved, and put into 1 kg plastic pots. Selected \textit{A. spinosus} seeds were soaked in water for 2 h and grown in the pots (with up to five seeds per pot). At 7 days after planting, selective pruning reduced the number of plants to one per pot. The selected plants were those that exhibited good growth and showed uniformity. The plants were watered (200 mL/pot of water purified by reverse osmosis) twice a day at regular intervals. Any water that flowed out through the pot holes was returned to the pot.
2.5. Application of plant extracts

Plant extracts were applied to each experimental treatment apart from the control. Water was calibrated before the application to determine the amount of solution required (8 mL for each plant). The liquid extract was separately sprayed on all parts of the plant for each treatment and replicated.

2.6. Measures

Percentage control of *A. spinosus* observed at 7, 14, and 21 days after the application (DAA) of each extract were recorded. The level of inhibition was assessed by five observers using a 0–100 rating system of weed control (Table 1).

| Rating (%) | Description of main categories | Detailed description |
|------------|---------------------------------|----------------------|
| 0          | No effect                       | No weed control      |
|            |                                 | No crop reduction or injury |
|            |                                 | Very poor weed control |
| 10         | Slight effect                   | Slight crop discoloration or stunting |
|            |                                 | Poor weed control     |
| 20         | Slight effect                   | Some crop discoloration, stunting, or stand loss |
|            |                                 | Poor to deficient weed control |
| 30         | Moderate effect                 | Crop injury more pronounced, but not sustained |
| 40         | Moderate effect                 | Deficient weed control |
| 50         | Severe effect                   | Moderate injury, crop usually recovers |
| 60         | Severe effect                   | Deficient to moderate weed control |
| 70         | Severe effect                   | Weed control somewhat less than satisfactory |
|            |                                 | Heavy crop injury and stand loss |
| 80         | Severe effect                   | Satisfactory to good weed control |
| 90         | Severe effect                   | Crop nearly destroyed, with only a few surviving plants |
| 100        | Complete effect                 | Very good to excellent weed control |
|            |                                 | Only occasional crop plants left alive |
|            |                                 | Complete weed destruction |
|            |                                 | Complete crop destruction |

Table 1. The rating system used to assess weed control

Weed height rate was measured with a ruler from the bottom of the stem to the top of the plant. Measurements were conducted at 0–7, 7–14, and 14–21 DAA. The formula used to find the height growth rate was as follows:

\[
HGR = \frac{H_2-H_1}{T_2-T_1}
\]  

(1)

Where HGR is the height growth rate; H1 and H2 are the starting and ending heights, respectively; and T1 and T2 are the start and end times.

At 21 DAA, the leaf area was measured using a leaf area meter, and the root length was measured by pulling the plant from the pot and measuring the roots using a ruler. Dry weights of shoots and roots were measured by placing them in an oven for 48 h at 60°C and then weighing them using an analytical scale.
2.7. Data analysis
All the observed parameters were analyzed using analysis of variance. If an effect of treatment was observed, post hoc testing was performed using Duncan’s new multiple range test at a 5% probability level. The analyses were performed using the SPSS version 16 (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Phytochemical testing
The results of the phytochemical tests are shown in Table 2.

Table 2. Results of phytochemical testing of plant extracts

| Plant Species | Alkaloid | Terpenoid | Saponin | Steroid | Flavonoid | Phenolic |
|---------------|----------|-----------|---------|---------|-----------|---------|
| I. cylindrica | −        | +         | −       | +       | +         | +       |
| C. rotundus   | −        | +         | −       | −       | +         | +       |
| C. odorata    | −        | +         | −       | +       | +         | +       |
| A. conyzoides | −        | +         | −       | +       | −         | +       |
| A. compressus | −        | +         | −       | +       | +         | +       |

+ Present; − Not present.

All five plant extracts contained terpenoids and phenolic compounds. All except C. rotundus contained steroids, and all except A. conyzoides contained flavonoids. None of the extracts contained saponins. A previous study found that I. cylindrica contained saponins, tannins, arundoin, femenol, isoarborneol, cylindrin, stigmasterol, simiareno, campesterol, β-sitosterol, scopeletin, scopolin, p-hydroxybenzaldehyde, catechol, chlorogenic acid, oxalate acid, d-malic acid, citric acid, potassium, and 5-hydroxytryptamine and its leaves contained polyphenolic compounds [35]. C. rotundus has been found to contain cyperene, flavonoids, sitosterol, and ascorbic acid [36], whereas C. odorata has been found in various studies to contain palmitic acid, flavonoids, alkaloids/terpenoids, tannin/polyphenols, triterpenoids, steroids, and saponin [37]; and tannins, phenols, alkaloids, coumarin, and flavonoids [38]. Leaves of A. conyzoides contain alkaloids, flavonoids, tannins, saponins, cardiac glycosides, anthraquinones, and terpenoids along with minerals, vitamins, and other compounds of known pharmacological activity [39]. It has been reported that A. conyzoides contains mono- and sesquiterpenes, chromene, chromone, benzoazurin, and coumarin as well as flavonoids, triterpenes, and steroids [40]. In addition, another researcher found that A. conyzoides L contained ageratochromene and its derivatives, monoterpenes, and sesquiterpenes as major volatile components as well as flavones under adverse conditions [26,27].

3.2. Height growth rate of A. spinosus
The results of analysis of variance applied to the plant extracts at various concentrations showed a highly significant effect on the height growth rates at 0–7, 7–14, and 14–21 DAA. Mean values for height growth rates of A. spinosus are presented in Table 3.

Table 3 shows that 20% of A. conyzoides extract was the most effective for inhibiting the height growth rate of A. spinosus at 7 DAA. This concentration may kill 100% of A. spinosus as a target plant. The inhibition of height growth rate can be prevented using allelochemical compounds contained in the extracts, which can inhibit the hormonal activity facilitating cell division and elongation in the apical shoot and root areas. Results of phytochemical test showed that A. conyzoides contains allelochemical compounds such as phenols, terpenoids, and steroids (Table 2). In a previous study, it has been reported that allelochemical compounds inhibit growth by inhibiting auxin activity during cell division and elongation [41].
I. artemisiifolia essential oils) and N. may disturb cell division in the meristem.

Gibberellin, which promotes cell division, cell enlargement, and stem elongation; permeability process and osmotic inhibition of Mg^2+ and ATPase activity; in addition, they inhibited non-absorption and disturbed the biosynthesis process and osmotic inhibition [44]. Allelochemicals especially phenols, can reduce cell membrane permeability [45]. Cell elongation may be inhibited by phenolic compounds that affect the hormone gibberellin, which promotes cell division, cell enlargement, and stem elongation; gibberellin inhibition may disturb cell division in the meristematic tissue, thus inhibiting elongation of stem internodes [46].

Secondary metabolite compounds from the terpenoid group may act as plant growth inhibitors [47]. Neophytadiene, a diterpene terpenoid contained in Nepeta ranjensis (a plant from the Balkans used for essential oils) and catnip (Nepeta cataria), inhibits growth in the shoots of ragweed (Ambrosia artemisiifolia) [48]. Steroids have the potential for allelopathy and are able to inhibit the germination of the grass Echinochloa crus-galli and the legume Senna obtusifolia [49].

### Table 3. Height growth rate of A. spinosus following treatment with plant extracts

| Treatment          | Average height growth rate (cm) |
|--------------------|--------------------------------|
|                    | 0–7 DAA | 7–14 DAA | 14–21 DAA |
| 2,4 D              | 2.34    | def      | 0.00      | a         | 0.00      | a         |
| Distilled water    | 2.66    | def      | 2.77      | de        | 3.09      | ef        |
| 10%                | 2.16    | c-f      | 2.73      | cd        | 3.18      | f         |
| *I. cymindrica*    | 2.79    | f        | 3.38      | def       | 3.19      | f         |
| 30%                | 2.90    | ef       | 3.42      | ef        | 3.04      | ef        |
| 10%                | 2.23    | c-f      | 3.01      | d-f       | 2.67      | c-f       |
| *C. rotundus*      | 2.62    | c-e      | 3.04      | d-f       | 2.68      | c-f       |
| 30%                | 1.83    | cd       | 2.87      | d-f       | 2.33      | cd        |
| 10%                | 2.59    | d-f      | 3.24      | d-f       | 2.79      | d-f       |
| *C. odorata*       | 2.65    | d-f      | 3.10      | d-f       | 2.79      | d-f       |
| 30%                | 2.21    | c        | 2.24      | c         | 2.13      | c         |
| 10%                | 1.91    | cd       | 2.91      | d-f       | 2.40      | c-e       |
| *A. conyzoides*    | 20%     | 0.00     | 0.00      | a         | 0.00      | a         |
| 30%                | 1.81    | b        | 1.45      | b         | 1.35      | b         |
| 10%                | 2.99    | ef       | 3.47      | f         | 3.18      | f         |
| *A. compressus*    | 20%     | 2.95     | 3.26      | d-f       | 2.91      | d-f       |
| 30%                | 3.00    | d-f      | 3.13      | d-f       | 2.73      | d-f       |

Numbers followed by the same letter in the same column were not significantly different at the 5% probability level (Duncan’s new multiple range test). Data were transformed by $\sqrt{x + 0.5}$. DAA: Days after application; 2,4 D: 2,4-Dichlorophenoxyacetic acid.

The inhibition of height growth rate also occurs through the activity of phenolic compounds, which inhibit mitosis in the embryo that results in the inhibition of cell division at the growing point, affecting the height growth rate [13,42]. Phenolic compounds destroyed the spindle threads during metaphase, reducing the mitosis index by producing excessive prophasic condensation and metaphasic chromosomes. This ultimately caused metaphase accumulation and a significant increase in cell percentage through chromosome deviations. This primarily resulted in a change in microtubular axis formation, which subsequently resulted in the formation of some spindle rods and asymmetric convergence from the chromosomes. When cell proliferation is inhibited, cell propagation in plant organs may also be inhibited; growth may therefore decrease or stop, and the cell numbers and their sizes may not increase [43].

Phenolic compounds reduced the synthesis of total carbohydrate, protein, and nucleic acids (both DNA and RNA) owing to the distribution of the phosphorylation channel or inhibition of the activation of Mg^2+ and ATPase activity; in addition, they inhibited non-absorption and disturbed the biosynthesis process and osmotic inhibition [44]. Allelochemicals especially phenols, can reduce cell membrane permeability [45]. Cell elongation may be inhibited by phenolic compounds that affect the hormone gibberellin, which promotes cell division, cell enlargement, and stem elongation; gibberellin inhibition may disturb cell division in the meristematic tissue, thus inhibiting elongation of stem internodes [46].

Secondary metabolite compounds from the terpenoid group may act as plant growth inhibitors [47]. Neophytadiene, a diterpene terpenoid contained in Nepeta ranjensis (a plant from the Balkans used for essential oils) and catnip (Nepeta cataria), inhibits growth in the shoots of ragweed (Ambrosia artemisiifolia) [48]. Steroids have the potential for allelopathy and are able to inhibit the germination of the grass Echinochloa crus-galli and the legume Senna obtusifolia [49].
3.3. Percentage growth control of A. spinosus

The results of analysis of variance applied to the plant extracts at various concentrations showed a highly significant effect on the percentage control of A. spinosus at 7, 14, and 21 DAA. Mean values for percentage of control of A. spinosus are presented in Table 4.

Table 4. Mean percentage growth control of A. spinosus by the plant extracts

| Treatment       | The Mean percentage control of A. spinosus (%) |
|-----------------|-----------------------------------------------|
|                 | 7 DAA | 14 DAA | 21 DAA |
| 2,4 D           | j     | a      | a      |
| Distilled water | 0.00  | 0.00   | 0.00   |
| 10% I. cylindrica| 16.00 | 45.67  | 58.00  |
| 20% I. cylindrica| 24.17 | 47.17  | 62.50  |
| 30% I. cylindrica| 34.50 | 52.17  | 65.50  |
| 10% C. rotundus | 50.17 | 40.83  | 40.83  |
| 20% C. rotundus | 85.83 | 67.67  | 67.67  |
| 30% C. rotundus | 91.50 | 76.67  | 76.67  |
| 10% C. odorata  | 50.17 | 40.83  | 40.83  |
| 20% C. odorata  | 78.00 | 74.33  | 74.33  |
| 30% C. odorata  | 93.83 | 82.33  | 82.33  |
| 10% A. conyzoides| 58.17 | 56.67  | 56.67  |
| 20% A. conyzoides| 78.00 | 74.33  | 74.33  |
| 30% A. conyzoides| 93.83 | 82.33  | 82.33  |
| 10% A. conyzoides| 100.00| 100.00 | 100.00 |
| 20% A. compressus| 13.33 | 41.67  | 41.67  |
| 30% A. compressus| 49.67 | 51.67  | 51.67  |

Numbers followed by the same letter in the same column were not significantly different at the 5% probability level (Duncan’s new multiple range test). Data were transformed by arcsin. DAA: Days after application; 2,4 D: 2,4-Dichlorophenoxyacetic acid.

Table 4 shows results for the assessed percentage growth control of A. spinosus. Notably, the 20% concentration of A. conyzoides extract had the highest control percentage of 100% at 7 DAA. These scores were categorized as a complete effect [50]. A previous study showed that a 20% extract of A. conyzoides had a greater inhibitory effect than that of Cleome viscosa on the growth of Sesamum indicum. L [51].

The field observations recorded that following the application of the 20% extract of A. conyzoides, the leaves of A. spinosus had the appearance being burnt and rolling down on the first day. This was followed by a partial loss of leaves on the second day. On the third day, all the leaves fell off and the shoots died, even though the stem remained upright. By the fifth day, all A. spinosus plants had died.

Table 4 also shows that at 7 DAA, the application of the C. odorata extract had a greater effect on A. spinosus than the application of the I. cylindrica extract. This is in accordance with the findings in a previous study on the germination and seedling growth of Centrocema pubescens [17].

Various responses occur due to higher concentrations and the selective nature of the effects of allelochemicals on target plants [10,32,52,53]. Allelochemicals stimulated or inhibited plant growth depending on their concentration [54,55].

At DAA 7, 30% C. odorata, C. rotundus, and A. conyzoides extracts did not result in the death of A. spinosus, although many remnants of extracts were observed on the leaves, which gave the leaves a burnt and rolling down appearance. The percentage control of A. spinosus following the application of 30% C. odorata and 30% C. rotundus decreased over the longer observation time period. This is in accordance with the previous findings that reported that the active ingredients of bioherbicides are readily biodegradable [13]. In the present study, the percentage control of A. spinosus following the application
of *I. cylindrica* extract increased over time. This is in contrast to the other researcher who reported that bioherbicide qualities of *I. cylindrica* were unstable [6]. The concentration of the extract affects the control level and mortality rates of the indicator plant. However, if the concentration is not sufficient to cause death, then natural activities of life still continue [56]. Following the application of *I. cylindrica* and *A. compressus* extracts, the stem of *A. spinosus* appeared abnormally pale compared to its original color 7 DAA. It has been reported that the application of extracts of *I. cylindrica* and *C. rotundus* at concentrations of 30%, 45%, and 60% reduced the growth of *A. spinosus* [57]. In the present study, the application of a synthetic herbicide (2.4 D at a dose of 0.686 kg/ha) caused the death of *A. spinosus* by 14 DAA. Thus, the application of 20% *A. conyzoides* extract resulted in faster death of *A. spinosus* than the application of the synthetic herbicide.

At 7 DAA, the second highest inhibition of *A. spinosus* was observed with the application of 30% *A. conyzoides* extract followed by 30% *C. odorata* extract; this result was not significantly different from that observed with the application of the *C. rotundus* extract. Furthermore, a high level of inhibition of *A. spinosus* was also seen following the application of *I. cylindrica* and *A. compressus* extracts.

### 3.4. Leaf area, root length, and shoot and root dry weights of *A. spinosus*

The results of analysis of variance applied to the plant extracts at various concentrations showed a highly significant effect of these extracts on the leaf area, root length, and dry weight of shoots and roots. Mean values for the aforementioned parameters of *A. spinosus* are presented in Table 5.

**Table 5.** Mean leaf area, root length, and shoot and root dry weights of *A. Spinous* following the application of the plant extracts.

| Treatment        | Leaf Area (cm²) | Root length (cm) | Dry weight of shoot (g) | Dry weight of root (g) |
|------------------|-----------------|------------------|-------------------------|------------------------|
| 2.4 D            | 0.00 a          | 0.00 a           | 0.58 a                  | 0.00 a                 |
| Distilled water  | 37.17 bc        | 33.17 bc         | 8.47 c                  | 2.30 e                 |
| 10% *I. cylindrica* | 31.50 bc      | 23.00 ab         | 31.50 b                 | 0.85 bc                |
| 20% *I. cylindrica* | 36.60 bc      | 25.08 bc         | 36.60 b                 | 0.59 b                 |
| 30% *I. cylindrica* | 36.95 bc      | 30.50 bc         | 36.95 b                 | 0.95 b                 |
| 10% *C. rotundus*  | 32.37 bc        | 31.42 bc         | 32.37 B                 | 0.62 b                 |
| 20% *C. rotundus*  | 38.12 bc        | 32.17 bc         | 38.12 B                 | 0.85 bc                |
| 30% *C. rotundus*  | 30.45 bc        | 35.50 c          | 30.45 B                 | 0.67 b                 |
| 10% *C. odorata*   | 39.88 bc        | 31.75 bc         | 39.88 B                 | 0.98 b                 |
| 20% *A. conyzoides* | 41.40 bc      | 26.67 bc         | 41.40 b                 | 0.65 b                 |
| 30% *A. conyzoides* | 31.33 bc      | 22.17 bc         | 31.33 b                 | 0.92 bc                |
| 10% *A. compressus* | 67.98 c        | 27.33 bc         | 67.98 b                 | 0.98 b                 |
| 20% *A. compressus* | 0.00 a         | 0.00 a           | 0.00 a                  | 0.00 a                 |
| 30% *A. compressus* | 53.43 cd       | 37.92 c          | 53.43 c                 | 1.63 c-e               |
| 30% *A. compressus* | 41.28 bc       | 31.58 bc         | 41.28 b                 | 0.75 b                 |

Numbers followed by the same letter in the same column were not significantly different at the 5% probability level (Duncan’s new multiple range test). Data were transformed by $\sqrt{x} + 0.5$. 2,4 D: 2,4-Dichlorophenoxyacetic acid.

Table 5 shows the leaf areas, root lengths, and dry weights of the shoots and roots of *A. spinosus* after the application of the various extracts. The results for the 20% *A. conyzoides* extract were not significantly different from those of the synthetic herbicide (the positive control). By 7 DAA, *A. spinosus* plants were completely destroyed, so further measurements were not possible. We believe this was because the allelopathic compounds in the *A. conyzoides* extract are phenolic compounds, terpenoids, and steroids that inhibit metabolic processes such as cell division, especially in the leaf. In a prior study,
it has been reported that a Senecio salignus extract affected photosynthesis in Physalis ixocarpa and E. crus-galli and identified two sesquiterpenes responsible for this: β-caryophyllene and caryophyllene oxide. A transient fluorescent chlorophyll indicated that 100 μg/mL β-caryophyllene had a major effect on photosynthesis in P. ixocarpa plant in vivo by inhibiting photosystem II by transforming the active centers to “heat sinks” or the formation of silent reaction centers unable to reduce QA. β-caryophyllene also induces chlorosis on treated leaves [58].

Gallic acid compounds contained in A. conyzoides can inhibit the growth of other plants through denaturing proteins. The reduction in macromolecular components resulted in the inhibition of protein synthesis, which also resulted in the inhibition of protoplasm synthesis [59]. This subsequently inhibited the process of cell division and cell elongation, causing narrowing of A. spinosus leaves. Other study reported that the residue obtained from an aqueous acetone shoot extract of A. conyzoides inhibited the germination and the growth of roots and shoots of Lactuca sativa, Digitaria sanguinalis, and Amaranthus caudatus [60]. Another one reported that the sesquiterpenes β-caryophyllene and caryophyllene oxide inhibited the dry biomass of P. ixocarpa plants, with β-caryophyllene having the greater effect [58]. β-Caryophyllene has been shown to inhibit root elongation in P. ixocarpa and E. crus-galli seedlings [13]. The morphological and physiological effect of phenolic acids on susceptible plants include reductions in leaf expansion, leaf production, net carbon assimilation rate, and stomatal conductance as well as decreased leaf water potential due to the reduced osmotic potential and turgor pressure and lower nutrient contents in the roots and shoots.

A plant’s dry weight reflects the accumulation of organic compounds synthesized by the plant from water and carbon dioxide. If the synthesis process is interrupted, this will result in a decrease in the dry weight [61].

4. Conclusion

The phytochemical testing showed that all five of the tested allelopathic plant extracts, I. cylindrical, C. rotundus, C. odorata, A. conyzoides, and A. compressus, contained terpenoids and phenolic compounds; all but C. rotundus contained steroids, and all but A. conyzoides contained flavonoids. None contained saponin. All of the plant extracts at the different concentrations affected the control of the target plant, A. spinosus, resulting in changes in the height growth rate, leaf area, root length, and shoot and root dry weights. The 20% A. conyzoides extract showed the greatest inhibition of the growth of A. spinosus at 7 DAA, resulting in 100% control; this was followed by 20% C. rotundus (86% control), C. odorata (78%), I. cylindrical (24%), and A. compressus (13%).

Acknowledgements

The authors would like to thank the Directorate of Research and Community Service, Directorate General of Research and Development Strengthening Ministry of Research, Technology and Higher Education for research funding

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