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**INHIBITORY EFFECT OF AQUEOUS LEAF EXTRACT OF
BANDOTAN (*Ageratum conyzoides*) AGAINST THE GROWTH OF RED
CHILLI (*Capsicum annuum*)**

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ABSTRACT

Bandotan (*Ageratum conyzoides* L.) is known as a very invasive weed. Wherever the weed was found, it showed inhibitory effects against plant crops and led to significant ecological and economic impacts. However, among the crop plants, bird's eye chilli (*Capsicum frutescens*), instead, showed inhibitory activities against *A. conyzoides*.

In order to determine whether the chilli plants resistant to bandotan allelopathy, bandotan leaf extract was tested against the growth of red chilli (*Capsicum annuum*). By using completely randomized design, 25 red chilli plants were grouped into five consist of 5 plants each. Each plant was grown individually in a poly bag containing mixture of soil and compost in a ratio of 2:1. Group 1 is the chillies given 0% (v/v) bandotan leaf extract as the control. Group 2, 3, 4 and 5 are the plants treated with extract at the concentration of 25%, 50%, 75% and 100% respectively. After treatment for one week the chillies are harvested and all the study parameters namely plant height, fresh and dry weight, and concentration of chlorophyll were assessed. The results showed, plant height of red chillies significantly decreased by the extract of 50% or higher. The fresh and dry weight of red chillies was significantly decreased by treatment of extract above 25%. However, crude water extract of bandotan showed no significant effects on the concentration of chlorophyll a and b as well as the total chlorophyll. Given red chilli that treated with crude leaf extract of bandotan showed the decrease in plant height and weight it can be concluded that *A. conyzoides* containing suppressive allelochemicals against red chili plants. These facts assert *Capsicum annuum* does not resistant to inhibitory activities of the *A. conyzoides* allelopathy.

KEYWORDS: *bandotan*, *billy goat weed*, *allelopathy*, *Ageratum conyzoides*, *red chili*, *Capsicum annuum*.

INTRODUCTION

Bandotan, the Indonesian name for billygoat weed (*Ageratum conyzoides*), is one of the herbaceous plants that are worth mentioning as a cosmopolitan weed due to spread almost all over the world, including Australia, Micronesia, Polynesia, Japan, New Caledonia, Palau, Philippines, Samoa, Tonga, Cambodia, China, Indonesia, Singapore, Taiwan, Thailand, Maldives, Mauritius, India^[1] and Bangladesh^[2]. The invasive nature of the plant is evident because wherever the weed was found, it showed inhibitory effects against the growth of plant crops^[3] and leading to significant ecological and economic impacts.^[4] In the invaded area, *Ageratum conyzoides* not only reduce the productivity but also the diversity of plant species.^[5]

On the crop plants, crude extract of *A. conyzoides* has been reported to be negatively allelopathic against growth, germination percentage, root and shoot growth, and fresh and dry weight of sesame of *Sesamum indicum* seedlings^[6,7], seed germination and seedling vigour of Chickpea (*Cicer arietinum* L.).^[8]

If *A. conyzoides* indeed very invasive then the question is, are there crop plants resistant to this weed? Based on the observations made by chance in a crop land where bird's eye chilli peppers (*Capsicum frutescens* L.) are grown intercropping with eggplant and string bean there was an interesting phenomenon. Under each plant canopy of the chillies there was no *A. conyzoides* could grow, even though outside of the canopy, *conyzoides* is a dominant weed. Such phenomenon led to speculation that the chilli plants, particularly the bird's eye chilli, resistant to inhibitory properties of *A. conyzoides*.

On the other hand, chilli plants itself have been reported to have negative allelopathic properties against other plants. Siddiqui and Zaman^[9], for example, by testing effect of capsicum leachate from *Capsicum annuum* on *Vigna radiata*, suggested that capsicum leachates inhibited the seed germination, root and shoot growths and reduced accumulation of the chlorophyll and porphyrin contents of the plant. Another study showed that *C. annuum* has competitive advantage over *Amaranthus lividus* weeds when both plant planted together.^[10]

Given the phenomenon of inhibition against *A.conyzoides* shown by *C. frutescens*, while the pepper plants which were reported to have allelopathic properties is *C.annuum* then the allelopathic relationship between *A.conyzoides* and *C. annum* still need to be clarified. This study, for that reason, was aimed to investigate allelopathic effects of crude extract of *A.conyzoides* against the growth of red chilli (*C. annum* L.).

MATERIALS AND METHODS

Bandotan Weeds Extract

Plant parts of the bandotan weed (*Ageratum conyzoides*) used in this study were the leaves. Fresh leaves of about 100 g milled into powder and soaked in 100 ml of aquades for 24 hours. After being filtered using Whatman No.1 filter paper, the filtrate collected in erlenmeyer flask and is noted as a stock solution. The stock solution was diluted in accordance with the treatment concentrations designed for the experiment.

Chilli Plant Seedlings

The herb plant designed as the allelochemical recipient in this study is red chilli (*Capsicum annum* L). The chilli seeds used are produced by East West Indonesia Ltd. The seeds were sown on a mixture media in the poly bags consisted of soil and compost in a ratio of 1:2. The compost applied in the study are the product of Trubus Mitra Swadaya Ltd. The chillies were allowed to grow for three weeks before being transferred to test media.

EXPERIMENTAL DESIGN AND TREATMENTS

By using completely randomized design, 25 chilli seedlings were grouped into five consist of 5 plants each. Each chilli seedling was grown individually in a poly bag containing freshly mixture of soil and compost in a ratio of 2:1. Group 1 is the chillies given 0% (v/v) bandotan leaves extract as the control. Group 2, 3, 4 and 5 are the plants treated with bandotan extract at a concentration of 25%, 50%, 75% and 100% respectively. Application of aqueous leaf extract on tested plants is done by watering the growing medium with 20 ml of the extract. After being allowed to grow for one week the whole chilli plants are harvested and all the study parameters were assessed.

Study Parameters

The allelopathic effects of crude extract of bandotan leaves on red chilli plants were based on the parameters assessed one week after extract application. The study parameters are: plant height, dry weight, and concentration of chlorophyll a dan b.

Plant height is the total length of the plant measured (using ruler meter) from soil surface level to highest peak of the crop.

Plant dry weight was determined by measuring plant weight after the samples were dried in an oven at 60°C.

Concentration of chlorophyll a dan b.

The concentration of chlorophyll a and b was determined by following Miazek^[11] protocol as follows. The fresh leaves of red chilli with a weight of 0.1g milled in a mortar and then dissolved in 95% ethanol. After filtration the chlorophyll contained in the filtrate measured spectrophotometrically using a UV-1800 UV-VIS Spectrophotometer from Shimadzu. The concentration of chlorophyll a and chlorophyll b in the ethanolic extract of chilli leaves calculated using equations below.

$$\text{Chla} = 13.36.A664 - 5.19.A648 \quad (1)$$

$$\text{Chlb} = 27.43.A648 - 8.12.A664 \quad (2)$$

In the equation (1) and equation (2), A664 mean absorbance at wavelength 664 nm, while A648 mean absorbance at wavelength 648 nm. By multiplying the absorbance values by $[v/(w \times 1000)]$, where v is the volume of solvent and w is the weight of plant tissue, the concentration of chlorophylls (in mg/g plant tissue) are obtained.

Statistical Analysis

Study results presented as mean±standard error (SE) and One-way analysis of variance (ANOVA) with LSD test was used to determine the significant differences between the means at the 5% level.

RESULTS AND DISCUSSION

Effects of aqueous leaf extract of the bandotan weed on the plant height of red chillies are presented in Table 1. Based on the results of analysis of variance (ANOVA) and the LSD test at $\alpha=5\%$ it is clear that leaf extract of bandotan with a concentration of 50% or more very markedly suppressed the growth of red chillies. Fresh and dry weight of of the whole plant of *C. annuum* after given the crude extract of leaf extract of *A.conyzoides* are presented respectively in Table 2 and Table 3. Based on the results of ANOVA and the LSD test at $\alpha=5\%$, crude extract of bandotan in a concentration of 25% or higher significantly decrease the fresh and dry weight parameters of red chilli plants.

The possible effects of crude extract of bandotan leaves against the chlorophyll content of red chilli plant has also determined and the results are presented in Table 3. Given all the F-values of ANOVA against chlorophyll a (2.519761), chlorophyll b (1.01619) and total chlorophyll (1.994461) are smaller than F-criterion (2.886), it can be affirmed that the content of chlorophyll in the leaves of red chilli is not affected by the aqueous leaf extract of the *A.conyzoides*.

Table 1: Effect of crude leaf extract of bandotan weeds (*A. conyzoides*) on the plant height of red chillies (*Capsicum annuum* L.).

Concentration of Extract	Plant Height (cm)					Mean \pm SE
	1	2	3	4	5	
0%	9	10	12	11	10.5	10.5 \pm 0.98 ^a
25%	10	10	10.5	9	9	9.7 \pm 0.59 ^a
50%	8.5	7	9	8.5	8	8.2 \pm 0.66 ^b
75%	8	8.5	7.5	7.5	9	8.1 \pm 0.57 ^b
100%	9	9	7.5	8.5	8.5	8.5 \pm 0.54 ^b
ANOVA of the data are as follows: F= 9.02439 , P-value =0.000247, Fcrit.= 2.886 LSD test results: The values of Mean \pm SE followed by the same superscript are not significantly different at $\alpha = 5\%$						

Table 2: Effect of crude leaf extract of bandotan weeds (*A. conyzoides*) on the plant fresh weight of red chillies (*Capsicum annuum* L.)

Concentration of Extract	Fresh Weight (g)					Mean \pm SE
	1	2	3	4	5	
0%	34.2	39.8	37.2	28.6	36.5	35.26 \pm 3.70 ^a
25%	25.5	30.9	29.8	30.8	36.5	29.76 \pm 3.44 ^b
50%	22.2	26	24.2	21.1	25	23.84 \pm 1.77 ^c
75%	18.8	16.2	17	18.5	20.3	18.16 \pm 1.41 ^d
100%	20	17.1	19.6	17.4	18	18.42 \pm 1.15 ^d
ANOVA results of the data are as follows: F= 34.45664 , P-value =0.000, Fcrit.= 2.886 LSD test results: The values of Mean \pm SE followed by the same superscript are not significantly different at $\alpha = 5\%$						

Table 3: Effect of crude leaf extract of bandotan weeds (*A. conyzoides*) on the plant dry weight of red chillies (*Capsicum annum L.*).

Concentration of Extract	Fresh Weight (g)					Mean ± SE
	1	2	3	4	5	
0%	17.1	29.1	23.6	20.5	23	22.66 ± 3.87 ^a
25%	15.9	19.9	17.1	20	17.6	18.10 ± 1.58 ^b
50%	12	15.2	12	9.3	9.4	11.58 ± 2.12 ^c
75%	3.9	3.7	8.7	9.7	13.1	7.82 ± 3.52 ^c
100%	11.9	8.1	10	9.3	12.2	10.30 ± 1.52 ^c
ANOVA results of the data are as follows: F=19.56547, P-value =0.000, Fcrit.= 2.886						
LSD test results: The values of Mean ± SE followed by the same superscript are not significantly different at α = 5%						

Table 4: Effect of crude leaf extract of bandotan weeds (*A. conyzoides*) on the chlorophyll content of red chillies (*Capsicum annum L.*)

Concentration of Extract	Concentration of Chlorophyll (mg/g plant tissue)		
	Chl a	Chl b	Total
0%	11.49±0.942	15.25±0.141	26.742±1.801
25%	10.68±0.691	15.432±0.294	26.11±1.272
50%	8.41±0.259	14.312±0.178	22.72±0.724
75%	9.27±0.430	15.242±0.302	24.512±1.405
100%	10.25±0.562	15.328±0.099	25.576±1.032
F	2.519761	1.01619	1.994461
P-value	0.073491	0.422756	0.13421
F-criterion	2.886	2.886	2.886

Based on the data presented in Tables 1, 2, 3 and 4, it can be suggested that the aqueous leaf extract of *A. conyzoides* significantly inhibit the growth of red chilli plants, but exhibit no significant influence on its chlorophyll content. These findings seem a bit odd, because similar allelopathic studies that use the same parameters suggested that if an allelochemical compound inhibited the growth of a plant, it will also decrease the chlorophyll content.^[12] The reasonable explanation to this contradiction is the aqueous extracts of bandotan leaves do not contain a typical-chlorophyll allelochemical, or if any, the concentration is too low to affect chlorophyll metabolism.

Bandotan weeds, indeed, contained numerous bioactive including alkaloids (echinatine, lycopsamine), sterol (brassicasterol, dihydrobrassica sterol, spinasterol), chromene (ageratochromene dimer, precocenei(7-methoxy-2,2'-dimethylchromene), sesquiterpene (B-

caryophyllene, caryophyllene epoxide), flavonoid (kaempferol-3, 7-diglucopiranoside), isoflavone, terpenes (α -pinene, β -pinene, methyleugenol, ocimene, eugenol), and some secondary metabolites such as caffeic acid and fumaric acid.^[13] In a more limited scale study, from leaf extract of *A. conyzoides*, Agbafor et al.^[14] was only able to isolate some classes of bioactive such as alkaloids, flavonoids, tannins, saponins, cardiac glycosides, terpenoids, anthraquinones.

As suggested by Li et al.^[15], among the allelopathic phytochemical, phenolic compounds such as chlorogenic, gallic, p-hydroxybenzoic, protocatechuic, caffeic, and 3,5-dinitrobenzoic acids is the significant determinant. *A. conyzoides* contained phenolic compound such as p-coumaric acid, gallic acid, ferulic acid, p-hydroxybenzoic acid, and anisic acid which can only effectively extracted from plant by using organic solvent, acid or bases. Sultana et al.^[16] for example, was able to isolate phenol by using acetone and n-hexane as the solvent.

Despite the crude leaf extracts of bandotan weeds showed no significant effect on chlorophyll content of red chillies, but the extracts evidently exhibit inhibitory effects on the plant growth. The inhibitory effects of the aqueous leaf extract of bandotan allegedly due to the phytotoxic activities of the plant. Phytochemicals such as by alkaloids, flavonoids, tannin, and terpenoids showed phytotoxic and insecticidal activities.^[17, 18]

Phenomena of growth inhibition shown by *A. conyzoides* under the plant canopy of *Capsicum frutescens*, as referred in the introduction section, most likely not due to the resistance of the chillies to allelochemicals, but because of other factors that still need to be investigated further.

CONCLUSION

Given red chilli treated with aqueous leaf extract of bandotan weed showed the decrease in plant height and weight it can be concluded that the crude extract of *A. conyzoides* containing suppressive allelochemicals against red chilli plants. These facts assert *Capsicum annuum* does not resistant to inhibitory activities allelochemical of *A. conyzoides*.

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