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Comparative Allelopathic Effect of Imperata cylindrica and Chromolaena odorata on Germination and Seedling Growth of *Centrosema pubescens*

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Abstract. The effect of aqueous extract of leaf, stem and root of *Imperata cylindrica* and *Chromolaena odorata* at concentrations of 0, 5, 10 and 15% on germination and seedling growth of *Centrosema pubescens* were investigated. The experimental design used was completely randomized design with three replications. Results of the study showed that plant species, plant parts and their concentration had a significant effect ($P < 0.05$) on parameters measured. Aqueous extracts of both plants at all concentration levels inhibited germination percentage, germination rate, shoot and root growth of *Centrosema pubescen* and these effects were directly proportional to the concentration of extract. *Chromolaena odorata* extract was more inhibitory than *Imperata cylindrica* extract and the leaf extracts showed the most inhibitory effect.. Root growth of *Centrosema pubescens* was more sensitive to allelopathic effects of both test plants than on shoot growth. These results suggested that potential allelopathic substances produced by both plant species may hinder the success of introduction of *C. pubescens* into grassland invaded by the two weeds. Further studies need to be conducted to investigate their allelopathic behavior under field conditions against their associated species and mechanism of inhibition involved.

Index Terms: *Centrosena pubescens*, *Chromolaena odorata*, *Imperata cylindrica*, aqueous extract, phytotoxicity

I. INTRODUCTION

Grazing based livestock husbandry plays an important role in the development of livestock in the tropics. Since the last four decades, forage supply from grassland area in Indonesia has been continuously decreasing, mainly due to conversion of grassland to crop cultivation and invasion of noxious exotic plants. Invasion of world's grassland by shrub plants is among the dominant changes in the earth's vegetation during the last two centuries (Polley *et al*., 2003).

In Indonesia, there are some exotic plants that have invaded grassland areas and causing ecosystem degradation. One of them is *Chromolaena odorata* (L.) King and Robinson (*C. odorata*) (C. odorata). This weed is a perennial scrambling shrub of neotropical origin forming a tangled bushes up to 3 m that can form dense thicket, thereby reducing grazing area and forage production.

In the pasture area owned by Faculty of Animal Science Hasanuddin University in Enrekang regency, Indonesia, the weed have covered more than 50% grassland area thus severely reducing carrying capacity of grassland. Lack of forage because of reducing carrying capacity generally occur during the dry season and during the season, many cattle grazing on the grassland are abort or die because of starvation or consuming the weed.

Besides invaded by *C. odorata*, there are large areas of grassland in Indonesia infested by *Imperata cylindrica* (*I. cylindrica*). The weed is considered to be one of the ten most troublesome and problematic weedy species in the world (Holm, 1969). In Indonesia, there are 10 million of total grassland area dominated by *I. cylindrica* (Tjitrosodirdjo, 1993). Although it can be used as animal feed, the low quality making this grass only suitable used as feed for four weeks of regrowth (Chadhokar, 1977).

The improvement of natural grassland invaded by the two weed species can be done through introduction of suitable improved species species by replacing existing species, or introduction of improved legume species by oversowing improved species into natural grassland. Replacing existing species with improved ones by full cultivation needs high cost. Therefore, in natural grassland, oversowing seems to be the most realistic.

Oversowing legume species like *Centrosema pubescens* (C. pubescens) into existing grassland may pose problems, because the two weeds have been known to have allelopathic effects on seeds of many crops. A review on the literatures indicates that study on allelopathic effects of *I. cylindrica* and *C. odorata* on seed of *C. pubescens* has not been done yet. Therefore, this study was undertaken to determine the effect of aqueous extracts of the two weed species on seed germination and seedling growth of *C. pubescens*.

II. MATERIALS AND METHOD

A.Collection and extraction of plant materials

The study was conducted under laboratory conditions from November until December 2014. *I. cylindrica* and *C. odorata* plants were randomly uprooted and collected at their flowering stage from campus of Hasanuddin University, Makassar, Indonesia (5^{o}08'23"S, 119^{o}25'19" E). The seeds of *C. pubescens* were collected from the plant growing naturally in campus of Hasanuddin University. Before using, the seeds were steeped in water to determine their viability; those that floated were not used.

B.Preparation of extracts

Plant parts, i.e. leaf, rhizome and root of fresh mature *I. cylindrica* and leaf, stem and root of *C. odorata* plants were cut into 1 – 2 cm pieces, put in paper bags and oven dried at 70^{o} C for 48 hours and then ground. Aqueous extract were prepared by extracting 15 g of ground plant materials with 100 ml distilled water in the blender for five minutes. After allowing to stand for several minutes, the blended materials were filtered through double-layered cheesecloth. The filtrate was considered to be of 15% concentration.The filtrate then was diluted with distilled water to make extracts of 5 and 10% concentrations. Distilled water was used as control.

C.Germination and bioassay studies

Forty seeds of *C. pubescens* were placed on filter paper in petri dish, then 10 ml of each extract concentration from *I. cylindrica* and *C. odorata* were added to each petri dish. Three replications for each extract and concentration of each plant were used. Petri dishes were incubated at 27 - 32^{o} C for one week. A final reading was taken for percent of germination and length of shoot and root on the seventh day after incubation. The length of shoot and root were measured using ruler by taking ten seedling per treatment at random. However, when germination percentage was less than 10%, all seedlings were used as sample. Percentage of seed germination and growth reduction of shoot and root were calculated as follows:

$$\text{Percents of seed germination} = \frac{\text{No.of seed germinated}}{\text{Total number f seeds}}$$

$$\text{Growth reduscton} = C - T/C \times 100\%$$

T – length of treatment organ

C – length of control organ

D.Statistical Analysis

The experiment was conducted using the Completely Randomized Design (CRD) with three replications. Data obtained were subjected to analysis of variance using SPSS software version 16 and means separation was made using least square difference method at 0.05 probability level.

III. RESULTS AND DISCUSSION

The results of the effects of aqueous extract of leaf, stem and root of *I. cylindrica* and *C. odorata* on germination and seedling growth are presented in Table 1.

Table 1. Effects of aqueous extract of *I. cylindrica* and *C. odorata* on seed germination (% of the control) and seedling growth of *C. pubescens*

Plants	Plant parts	Concen-tration (%)	Germina-tion (%)	Shoot		Root	
				Length (cm)	Reduction (%)	Length (cm)	Reduction (%)
Control		0	100.0a	2.30a	0.00a	2.82a	0.00a
I.cylindri-	Leaf	5	57.4b	1.75b	23.9a	0.98b	65.2b

ca.		10	51.1b	1.42bc	38.3ab	0.72bc	74.5bc
		15	25.5b	0.96c	58.3b	0.12c	95.7c
	Rhizome	5	95.8a	1.83a	20.4a	1.43b	49.3a
		10	83.0a	1.71ab	25.7ab	0.72c	74.5b
		15	76.6a	1.23b	46.5b	0.61c	78.4b
	Root	5	95.8a	1.93a	16.1a	1.95b	30.9b
		10	89.4a	1.79ab	22.2ab	1.67bc	40.8b
		15	83.0a	1.45b	40.0b	1.31c	53.5b
C.odorata.	Leaf	5	56.2b	0.98b	57.4b	0.92b	67.4b
		10	35.2bc	0.72b	68.7bc	0.60bc	78.7bc
		15	0.00c	0.00c	100.0c	0.00b	100.0c
	Stem	5	98.1a	. 1.60b	30.4b	1.51b	46.5b
		10	49.0b	0.51bc	77.8c	0.42b	81.1c
		15	24.5b	0.36c	84.8c	0.38b	86.5c
	Root	5	98.3a	1.82ab	20.9a	1.79b	36.5b
		10	61.3ab	1.76b	30.4bc	1.48bc	47.5bc
		15	49.0b	1.05c	54.3c	1.01c	61.0c

Means within a column followed by the same superscript letter are not significantly different at 0.05 probability letter.

Aqueous extract of plant parts of *I. cylindrica* and *C. odorata* exhibited allelopathic effect on germination, shoot and root lengths of *C. pubescens*. The allelopathic effect varied among plant species, plant parts and extract concentration levels. Maximum allelopahic effect was recorded in *C. odorata* extract with 15% concentration that completely inhibited seed germination and subsequently reduced the seedling growth of tested plant (Table 1).

The phytotoxic effects of *I. cylindrica* and *C. odorata* on seed germination and seedling growth had been widely reported. In *I. cylindrica*, it had been revealed by Koger and Bryson (2004), Samad *et al*, (2011) and in *C. odorata* by Suwal *et al*., (2010), Devi and Dutta (2012) and Sahid and Yusoff (2014). This indicates that all plant parts of the two weeds species contained allelopathic substances that affected the seeds of *C. pubescens.*

The phytotoxic effect of *C. odorata* in this study was contrary to findings of Ilori *et al*. (2010) and Ambika and Poornima (2004) who reported that *C. odorata* extract stimulated the seedling growth of *Celosia argentea* and soybean, respectively. This observation might be due to high concentration of allelochemical present in the current study. This was in agreement with Einheling *et al*. (1982) that allelochemicals have to be present above threshold concentration for impact.

In the present study, phytotoxic effect of extracts was found to be higher in *C. odorata* than in *I. cylindrica.* Irrespective of plant parts and their concentration, germination percentage was lower in *C. odorata* treated seeds (52.6%) than in *I. cylindrica* treated seeds (70.4%). Reduction of shoot and root length also also were higher .in *C. odorata* treated seeds (58.6 and 67.7%, respectively) than in *I. cylindrica* treated seeds (32. and 64%, respectively) (Table 1). The phytotoxic effect of *C. odorata* was also reported to be higher than *Parthenium hysterophorus* on (Masum *et al*. 2012), but was lower than *Cyperus esculentus* and *Panicum maximum* extracts (Usuah *et al*., 2013). This indicates that aqueous extracts of *C. odorata* exerted higher degrees of inhibition than *I. cylindrica.* Varied responses occur because of higher concentration and the selectivity of allelochemicals on the target plants (Inderjit and Duke, 2003).

From the present work it was observed that although all plant part extracts s had strong allelopathic effect on seed germination and seedling growth of *C.pubescens,* but highest inhibition was found in the leaf leaf extract. Irrespective of plant species and concentration, germination percentage was lower in C. odorata leaf treated seeds (30.5%) compared to other plant parts (63.4%). The germination percentage of *I cylindrica* leaf treated seeds was also lower (44.7) than other plant parts (89.5%) (Table 1). The higher inhibitory effect of aqueous extracts of *I. cylindrica* leaf compared to other plant parts extracts on germination had been reported (Anjun *et al*. (2005) and Samad *et al*. (2005). The same results also had been reported in *C. odorata* (Eze and Gill, 1992),

Devi and Dutta (2012), Suwal *et al*. (2010), Gill *et al*. (2015). The highest inhibiting effect of leaf extracts of the two weed species could be due to the high accumulation of allelochemicals in the top meristems of the plants (Gella *et al*., 2013, Gill et al., 2015). and the high quantity of photochemical in the leaf (Kanchan and Jayachandra, 1980). The phytotoxic activity of *I. cylindrica* leaf extract associated with the presence of p-coumaric and o-coumaric, gentistic, benzoic, p-hydroxybenzoic acids, vanillin and p-hydroxybenzaldehyde in the leaf (Eussen and Niemann, 1981), in addition to caffeic, ferulic, chlorogenic, and syringic acids (Hussain Abidi, 1991). In *C. odorata*, phenolics, alakaloids and amino acids were the main allelochemicals (Ambika and Jayachandra, 1984).
Results of the present study also showed that phytotoxicity of the two plant extracts were concentration dependent because there were increment in inhibition with increasing concentration of extract. This results are in agree with Fariba *et al*. (2007) who stated that allelochemicals stimulated or inhibited plant growth depending on their concentration.
Both plant extracts showed inhibitory effect on shoot and root length but exerted more inhibitory effects on the root than on the shoot of tested species (Table 1). The more sensitive of root over shoot of maize as affected by *C. odorata* extract had also reported by Masum *et al*. (2012) These also were in line with Shahrokhi *et al*. (2012) that aqueous extract of *Amaranthus retroflexus* had the greatest inhibitory effect on root length of wheat seedling. The present results were in agreement with those of Angiras *et al*. (1988) that radicle growth in maize was more inhibited by aqueous extracts of *Echinochloa crus-galli* and *Cyperus rotundus* than shoot growth. This is because root is the first organ that absorbs the allelochemicals from the environment. Besides, root tissue has greater permeability compared to shoot tissue (Nishida, 2005).

IV.CONCLUSION

The present study clearly indicates that r both *Imperata cylindrica* and *Chromolaena odorata* extracts, especially in their leaf have inhibitory effects on seed germination and seedling growth of *Centrosema pubescens*. Further studies are required to evaluate the inhibitory effects of both plants under field conditions. It is recommended than both weeds, especially should be removed from the field before *Centrosema pubescens* seed introduced into the *I. cylindrica* and *C. odorata* infested area.

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