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The Effect of the Philippine *Chromolaena odorata (L.)* Ethanol Leaf Extracts on the Germination of *Lycopersicum esculentum*

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Abstract: *Chromolaena odorata (L.)* King and Robinson is a member of the Asteraceae family. Research shows that its diverse range of secondary metabolites contributes to its allelopathic properties but the phytotoxic potential of *C. odorata* from the Philippines has not yet been studied. Ethanol extracts of *C. odorata* leaves were tested on seed germination and seedling growth of *Lycopersicum esculentum* (tomato) in 7 and 14 days observation periods. The lowest concentration level of 0.5% ethanol leaf extracts significantly inhibited germination and seedling growth of *L. esculentum* in both observation periods. Column chromato graphic elution of ethanol extract yielded 11 fractions, each of which was subjected to bioassay. Only fractions 4 and 5 inhibited seed germination of *L. esculentum* at 25% concentration. Phytochemical screening of fractions 4 and 5 are positive for the presence of alkaloids and steroids which may have caused the inhibition of seed germination and seedling growth of *L. esculentum*.

Key words: Allelopathic, *Chromolaena odoratal*, germination, phytotoxi, seedling growth, significantly inhibited

INTRODUCTION

Chromolaen a odorata, a troublesome weed of arable fields, roadsides and plantation crops (Eze and Gill, 1992) is reported to be highly allelopathic compared with some other weeds that are among the world's worst (Ambika and Poornima, 2003). This is caused by allelochemicals that are produced by plants as end products by products and metabolites (Gill, 1992). Allelochemicals include alkaloids, terpenoids, flavonoids, steroids, tannins, phenolic and other compounds which are known to produce toxic effects on some plants (Ilori *et al*., 2010). All parts of *C. odorata* contain allelochemicals but its leaves have been reported having the highest amount of these chemicals (Gill, 1992; Ambika and Jayachandra, 2001; Ambica, 1980). This also agrees with the recent findings which reported that the leaves of *C. odorata* has highest inhibitory effect on germination and on linear growth of paddy and barnyard grass, followed by stems and roots as the lowest (Suwal *et al*., 2010).

There were several researches conducted to explore the potential benefits of *Chromolaena* bioactive compounds. One of these researches showed the effects of some chemical components of *C. odorata* extracts to Coleoptera pests of stored products (Owusu, 2000). Another study revealed the allelopathic activities of *C. odorata* crude methanol extract on the seed germination and seedling growth of *L. esculentum* (Eniola and Fawusi, 1989). There was also a report that it has a protective ability against oxidative damage due to the alkaloids and flavonoids that are isolated from the leaf extracts (Phan *et al*., 2001). In addition, one study established the allelopathic effect of the aqueous extract of *C. odorata* on the growth of *Vigna unguiculata* (cowpea) (Gill, 1992). However, the thorough review of literature indicates that the phytotoxic potential of the Philippine *C. odorata* ethanol leaf extracts on the germination and seedling growth of *L. esculentum* has not yet been examined or investigated.

Hence, this study was conducted to investigate the effect of the Philippine *C. odorata* ethanol leaf extracts on seed germination and seedling growth of *L. esculentum*. Specifically, this study aims to determine which concentration of *C. odorata* ethanol leaf extract has a significant inhibitory effect on the germination of *L. esculentum* and on seedling growth, determine which fractions of *C. odorata* ethanol leaf extracts have significant inhibitory effect on the germination of *L. esculentum* and identify the phytochemicals present in the fractions that inhibit germination of *L. esculentums*.

The result of this study can be used as baseline information in identifying further the bioactive components of *C. odorata* that inhibit germination which

can make it a good herbicide. Additionally, these bioactive components of *C. odorata* may alter mitotic activity in plant and animal cells, thus, it can be a good anti-mitotic or anti-cancer agent.

MATERIALS AND METHODS

Collection of plant material: About 10 kg of young and healthy *C. odorata* were collected from a hilly area in Barangay Bomba, Pagadian city, Western Mindanao, Philippines. These leaves were cleaned and rid of unwanted materials, cut into small pieces and air-dried for 5 days. Then, the dried leaves were pulverized using an electric blender and kept in sealed polyethylene bags.

The Marimar variety (a hybrid tolerant to heat) of tomato (*Lycopersicum esculentum*) seeds of Eastwest Seed Company, Philippines was used as bioassay plant.

Extraction of bioactive components from *C. odorata* leaves: The ethanol extraction followed the method employed by Vital and Rivera (2009) and by Akinmoladun *et al.* (2007). First, the mixture was filtered using clean and dry cheesecloth and re-filtered using Whatman No. 1 filter study. Then, the ethanol was removed from the filtrate through a rotary evaporator (Buchi R110, Japan) under reduced pressure at 40°C temperature and kept in the refrigerator at 4°C until use. The 0.05, 0.5, 2.5 and 5% concentrations of ethanol leaf extracts were used in the conduct of the experiment.

Bioassay of *C. odorata* ethanol leaf extracts: The experimental unit consisted of Petri plates (with a sterile Whatman No. 1 filter study placed at the bottom for root anchorage) which contained 10 mL each of the treatments, namely distilled water (negative control), 0.05, 0.5 and 2.5% ethanol extracts and 5.0% ethanol extract (positive control). *L. esculentum* seeds were allowed to germinate in each of the 30 Petri plates in a room environment with 25-28°C temperature range under 12 h light and 12 h dark photoperiod and with a relative humidity of 70-80%. Photoperiod was established by covering the experimental chamber fully with a black cloth.

Germination of *L. esculentum* seeds treated with *C. odorata* ethanol leaf extracts: On germination 5 mL distilled water was added every 24 h throughout the 7 and 14 days observation periods to prevent drying-up. On the 7th day, the number of seeds that germinated was counted. Similarly, the number of germinated seeds was counted on the 14th day of the second set-up. This was done to determine, if seeds would germinate after a longer period of exposure to the treatments.

Growth of *L. esculentum* seedlings treated with *C. odorata* ethanol leaf extracts: On growth of the seedlings, the adding of 5 mL distilled water was still made every 24 h to prevent drying-up. The growth rate of each seedling was taken by measuring its shoot length daily for 7 days. On the 7th day, seedlings were harvested to measure the shoot length (from the base of the shoot to the uppermost part of the terminal bud of the seedling) and the root length (from the base of the shoot to the root tip) using a ruler.

Measuring the individual seedling shoot length daily in the other identical set-up was also made on the 7th day, treatments were applied on appropriate Petri plates and shoot length was measured continuously for another 7 days. On the 14th day, the seedlings were harvested and their roots and shoot lengths were measured, accordingly. Increase of root and shoot length was obtained by subtracting the root and shoot lengths on day 7 from the length on day 14 as shown below:

- Root length increase = RL on day 14-RL on day 7
- Shoot length increase = SL on day 14-RL on day 7

Fractionation of *C. odorata* ethanol leaf extracts and bioassay of each fraction: About 100 g of *C. odorata* ethanol leaf extract was dissolved in 200 mL analytical grade hexane (Concord, China) and subjected to gravity column chromatographic separations by gradient elution using hexane and ethyl acetate (Aldrich, USA) as the mobile phase (increasing polarity) and silica gel as column packing. The column was eluted following step-wise gradient. Fractions were obtained in the process and were air-dried until they solidified. The individual fraction weight was taken using an analytical balance. The fractions were separately diluted with distilled water to obtain the 25 and 50% concentrations. Then, each dilution was subjected to bioassay. The percentage germination was computed using the following formula:

$$\text{Germination (\%)} \frac{\text{No. of germinated seeds}}{\text{Total number of seeds sowed}} \times 100$$

Statistical analysis: All experiments were conducted in five replicates. The data were subjected to analysis of variance and Tukey's HSD post hoc test was used to determine differences among treatments. The data gathered were analyzed using SPSS Version 17.

Phytochemical screening: The fractions which inhibited germination were brought to the Chemistry Department of the MSU-Iligan Institute of Technology, Iligan city for phytochemical screening. This was done to determine the

presence of alkaloids, flavonoids, saponins, tannins, anthraquinone, steroids and cyanogenic glycosides using standard experimental procedures described by Falodun *et al*. (2008).

RESULTS AND DISCUSSION

Germination of *L. esculentum* seeds: Table 1 shows that the highest mean number of germinated seeds was to those treated with distilled water, the negative control, having 8.8 and 9.0 for 7 and 14 days observation periods, respectively. Those treated with 0.05% ethanol extract have the mean number of 4.2 (7-days period) and 5.4 (14 days periods) while there was no seed germination at all for those treated with 0.5, 2.5 and 5.0% ethanol extracts in both observation periods.

ANOVA results show that there was a significant difference in the mean number of germinated seeds for those treated with varying ethanol extract concentrations in both 7 days ($p<0.05$; $F = 72.967$; $df = 5$) and 14 day ($p<0.05$; $F = 63.038$; $df = 5$) observation periods. The mean numbers (9.04 and 12.06 in 7 day and 14 day periods, respectively) of germinated seeds treated with distilled water (negative control) are higher compared to those treated with 0.05% *C. odorata* ethanol leaf extract. But the latter is significantly higher than those treated with 0.5, 2.5 and 5.0% ethanol extracts at $p<0.05$. These results indicate that there was germination when seeds were treated with 0.05% ethanol extract and there was no germination to those treated with 0.5, 2.5 and 5.0% ethanol extracts.

Growth of *L. esculentum* seedlings

Root and shoot lengths in 7-day period: Table 2 shows that both the mean of root and shoot lengths of

Table 1: Mean (± SD) number of germinated seeds of *L. esculentum* treated with *C. odorata* ethanol leaf extracts in 7 and 14 days observation period

Treatments	Mean number at 7 days	Mean number at 14 days
Distilled water (negative control)	8.8±1.79^a	9.0±0.71^a
0.05% extract	4.2±1.52^a	5.4±0.89^a
0.5% extract	0^b	0^b
2.5% extract	0^b	0^b
5.0% extract (positive control)	0^b	0^b

Table 2: Mean (±SD) number of root and shoot lengths of *L. esculentum* 7 days after being treated with *C. odorata* ethanol leaf extracts on day 1

Treatments	Mean root length (mm)	Mean shoot length (mm)
Distilled water (negative control)	39.82±13.73^a	36.34±20.15^a
0.05% extract	13.60±13.14^a	14.60±14.00^a
0.5% extract	0.00^b	0.00^b
2.5% extract	0.00^b	0.00^b
5.0% extract (positive control)	0.00^b	0.00^b

Mean values having the same superscripts are not significantly different ($p>0.05$)

L. esculentum treated on day 1 of the 7 days observation period with distilled water (negative controls) and those treated with 0.05% ethanol extract increased. However, the seeds treated with 0.5 and 2.5% ethanol extracts have no root length at all after 7 days, similar to those treated with 5.0% ethanol extracts (positive control). Seedlings treated with distilled water have the highest mean of root and shoot lengths (39.82 and 36.34 mm) while those treated with 0.05% ethanol extract have 13.60 mm and 14.60 mm root and shoot lengths, respectively. No growth was observed to those treated with 0.5, 2.5 and 5% extracts. ANOVA results show that there is a significant difference in the root ($p<0.05$; $F = 70.19$; $df = 3$) and shoot ($p<0.05$; $F = 63.560$; $df = 3$) lengths of *L. esculentum* treated with ethanol extract (0.05, 0.5, 2.5 and 5.0%) in the 7-day observation period.

Root and shoot lengths increase in 14 days period: Table 3 shows that the seedlings treated with distilled water (negative control) on day 7 of the 14-day observation period have the highest mean of root and shoot lengths (9.04 and 12.06 mm). The mean of root and shoot lengths (6.70 and 7.92 mm) of seedlings treated with 0.05% ethanol extract also increased while no root and shoot length increase at all for seedlings treated with the higher concentrations (0.5, 2.5%) of ethanol extracts as well as those treated with 5.0% ethanol extract (positive control).

ANOVA results show that there is a significant difference in the root length increase ($p<0.05$; $F = 81.64$; $df = 3$) and the shoot length increase ($p< 0.05$; $F = 67.40$; $df = 3$) of *L. esculentum* seedlings treated with varying concentrations of ethanol extracts (0.05, 0.5, 2.5 and 5.0%) in the 14 day observation period.

These results suggest that *C. odorata* ethanol leaf extracts have a significant effect on seed germination and seedling growth of *L. esculentum* at a low concentration (0.5%) of *C. odorata* ethanol leaf extract.

Germination of *L. esculentum* seeds treated with 11 fractions obtained from *C. odorata* ethanol leaf extracts: Table 4 shows that fractions 1, 2, 3, 6, 7, 8 and 11 have

Table 3: Mean (±SD) number of the root and shoot length increase of *L. esculentum* after being treated with *C. odorata* leaf extracts on day 7 of the 14 days observation period (length increase = length on day 14 length on day 7)

Treatments	Root length	Shoot length
Distilled water (negative control)	9.04±3.08^a	12.06±5.77^a
0.05% ethanol crude extract	6.70±1.85^a	7.92±3.15^a
0.5% extract	0.00^b	0.00^b
2.5% extract	0.00^b	0.00^b
5.0% extract (positive control)	0.00^b	0.00^b

Mean values having the same superscripts are not significantly different ($p>0.05$)

Table 4: Mean (±SD) number of germinated seeds treated with different concentrations of the 11 fractions obtained from *C. odorata* ethanol leaf extracts

Fractions	T1	T2	T3	G (%)
F1	3.80±1.09	3.20±1.30	3.3 ±1.94	68.00[b]
F 2	5.00	4.40±0.89	4.10±1.66	89.60[b]
F 3	5.00	4.80±0.44	3.77±1.78	86.40[b]
F 4	5.00	0.00	0.00	37.60[a]
F 5	4.80±0.44	0.00	0.00	39.20[a]
F 6	5.00±0.0	2.60±1.14	1.30±1.94	57.60[a]
F 7	4.20±1.30	4.60±0.54	0.80±1.75	58.40[a]
F 8	4.00±0.70	2.80±1.92	1.00±1.76	53.60[a]
F 9	5.00±0.0	0.20±0.44	0.30±0.48	42.40[a]
F 10	3.80±1.09	4.20±1.30	0.00	47.20[a]
F 11	4.00±0.70	4.60±0.54	2.45±2.01	74.40[b]

Mean values having the same superscripts are not significantly different (p>0.05). F = Fractions; T1 = distilled water; T2 = 25% fraction; T3 = 50% fraction; T4 = 0.025% colchicine; G% indicates percent germination

Table 5: Phytochemical screening of fractions 4 and 5

F	Alkaloids	Flavonoids	Saponins	Tannins	Anthra Quinone	Steroids	Cyanogenic glycosides
4	+	-	-	-	-	+	-
5	+	-	-	-	-	+	-

+ = Present; - = Absent

higher percentage of germination (above 50%) while fractions 4, 5, 9 and 10 have lower percentage of germination (below 50%). The *L. esculentum* treated with 25 and 50% concentrations of fraction 1, 2, 3, 6, 7, 8, 9, 11 and 25% concentration of fraction 10 have germinated but there was no germination to those seeds treated with both 25 and 50% concentrations of fractions 4 and 5.

ANOVA results show that there was no significant difference in the number of seeds germinated for the different treatments of fractions 1 (p = 0.833), 2 (p = 0.567) 3 (p = 0.424) and 11 (p = 0.093) while there was a significant difference for the different treatments of fractions 4 (p = 0.00), 5 (p = 0.00), 6 (p = 0.002), 7 (p = 0.00), 8 (p = 0.00), 9 (p = 0.00) and 10 (p = 0.00) at $p < 0.05$. However, it was only with fractions 4 and 5 that no seed germination occurred in both 25 and 50% concentrations.

Phytochemical screening: Since, only fractions 4 and 5 inhibited germination of *L. esculentum* seeds treated with both 25 and 50% concentrations, only fractions 4 and 5 were subjected to phytochemical screening. Table 5 shows that the phytochemicals present in fractions 4 and 5 are only alkaloids and steroids. Flavonoids, saponins, tannins, anthraquinone and cyanogenic glycosides are absent in these fractions.

The least effective concentration was 0.5% *C. odorata* ethanol leaf extract and the degree of inhibition elevated as the treatment concentration was increased. This finding agrees with a number of studies dealing with inhibition effects of plant extracts on seed germination (Teerarak *et al*., 2010).

The highest inhibitory effect has been attributed to bioactive chemical components soluble in ethanol (Vital and Rivera, 2009; Tabudlo, 1996; Supnet and Reotutar, 2008; Pisutthanan *et al*., 2005; Owolabila *et al*., 2010; Ngozi *et al*., 2009). In this study, the root length being more affected by ethanol extracts than shoot length is in close agreement with studies which reported that extracts of allelopathic plants have more evident effects on root growth than on shoot growth (Ambika and Jayachandra, 2001). This is expected because it is the roots that are first to absorb allelochemicals from the environment (Turk *et al*., 2002).

The phytochemical screening carried out in this study was specific only to the fractions that inhibited germination (fractions 4 and 5) because several phytochemical screenings have already been carried out for the *C. odorata* leaf extracts (Vital and Rivera, 2009; Tabudlo, 1996; Supnet and Reotutar, 2008; Pisutthanan *et al*., 2005; Owolabila *et al*., 2010; Ngozi *et al*., 2009).

CONCLUSION

This study shows that ethanol extracts have an effective inhibitory effect on seed germination and seedling growth of *L. esculentum.* Fractions 4 and 5 of *C. odorata* ethanol crude extracts have significant inhibitory effect on the seed germination of *L. esculentum* and this could be due to the phytochemicals such as alkaloids and steroids identified from these fractions.

RECOMMENDATIONS

It is recommended that further study should be made to determine the effect of fractions 4 and 5 on chromosome morphology. The chemical structure of the components found in these fractions should also be isolated and elucidated. In addition, more studies should

be done to further identify the bioactive components of *C. odorata* that inhibit germination, making it a promising source of herbicide and to determine, if its bioactive components may alter mitotic activity in plant and animal cells making it a potential anti-mitotic or anti-cancer agent.

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