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# ALLELOPATHIC POTENTIAL OF *Codiaeum variegatum* L. Rumph. Ex A. Juss. ON GERMINATION, EARLY GROWTH AND BIOCHEMICAL CHARACTERISTICS OF *Lolium perenne* L.

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**ABSTRACT.** In this study, the allelopathic potential of aqueous extracts of *Codiaeum variegatum* L. Rumph. Ex A. Juss. (40%, 20%, 10%, 5%, 2.5%, and 1.25%) on germination, early growth, and physiological activity of the selected plant recipient *Lolium perenne* L. was investigated. The obtained results confirmed the existence of allelopathic potential, which differed in its possibilities of adverse effects on the morphological and physiological characteristics of the recipient plant. The monitoring of the germination characteristics of the recipient plant showed that the tested extracts exhibited a concentration-dependent effect, i.e., stronger concentrations had a stronger inhibitory effect. A weaker negative effect on the growth of the selected species was observed, and it was shown that the root length of the seedling was usually under the stronger inhibitory effect of higher concentrations of extracts, in contrast to the length of the shoot, where all the applied extracts had a significant stimulatory effects. Moreover, allelochemicals of *C. variegatum* were shown to interfere with the synthesis of phenolic compounds and antioxidant capacity of *L. perenne* seedlings, the values of which decreased with increasing concentration of test extracts.

**Keywords:** allelopathy, croton, English ryegrass, total phenolics, total flavonoids, antioxidative activity

## INTRODUCTION

*Codiaeum variegatum* L. Rumph. Ex A. Juss. (croton) is a popular ornamental plant that belongs to the family Euphorbiaceae. It is native to the Molucca Islands of Indonesia and the tropical rainforests of the Philippines, Papua New Guinea, and Australia. It was first introduced to many tropical and warm regions where it grows easily, and later to Europe and the United States (NJOYA *et al*., 2021). *C. variegatum* is an evergreen shrub that usually reaches a height of 0.5 to 2 m (NASIB *et al*., 2008). It is a very attractive garden and potted species with decorative value.

Phytochemical analysis revealed that extracts of *C. variegatum* have the highest content of alkaloids and phenolics whose composition has been identified and quantitatively studied (HASSAN *et al*., 2014; SAFFOON *et al*., 2014; MOHAMED *et al*., 2019; ABO-ZEID *et al*., 2019). The most abundant alkaloids are glaucine, oxoglaucine, and hemiarginine (HASSAN *et al*., 2014;

NJOYA *et al*., 2021). The following phenolic acids were present in the leaf extracts: chlorogenic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic acid, trans- and cis-p-coumaric acid, trans- and cis-ferulic acid (NAIDU, 1988; NJOYA *et al*., 2021). Compared to other phenolic acids, ellagic acid is dominant (SAFFOON *et al*., 2014). The extracts also contain a variety of flavonoids, including epicatechin, rutin hydrate, vitexin, isovitexin, apigenin, orientin, and vicenin-2, as identified in studies by HASSAN *et al*. (2014) and ABO-ZEID *et al*. (2019). Terpenoids are also present in significant quantities, comprising 20-30% of the extract. The most abundant diter penoids are α-amyrin, β-sitosterol, ent-trachyloban-3-one, and ent-18-hydroxy-trachyloban, as reported by HASSAN *et al*. (2014). In addition, the specific phytochemical composition con tributes to the healing properties of this species (JHA *et al*., 2016).

Under horticultural conditions, it has been observed that *C. variegatum* may have a potential allelopathic effect on other ornamental herbaceous plants (MUSHTAQ and SIDDIQUI, 2017). Allelopathy is a biochemical phenomenon with ecological implications, whereby a donor plant produces chemical compounds that can have either harmful or beneficial effects on a recipient plant. These compounds are released into the environment, and their mechanisms of action are visible in the first stages of the growth and development of recipient plants. The effects may include inhibition of germination, reduction of seedling growth, changes in cell membrane permeability, enzyme activities, cell division and elongation, respiration, and photosynthesis. These primary effects can lead to secondary signs of allelopathic activity, as noted in studies by SCAVO *et al*. (2018) and SCAVO and MAUROMICALE (2021).

*Lolium perenne* L. (English ryegrass) is a perennial grass that can reach a height between 10 and 90 cm (CASTLER and DUNCAN, 2003). It is one of the most important forage species that is a high-quality feed for livestock due to its nutritional value and it is a common park grassland species (QAWASMEH *et al*., 2012). It is native to Europe, Asia, and North Africa, but has also been able to adapt to conditions in several temperate regions in the Americas, Australia, and New Zealand (QAWASMEH *et al*., 2012).

The aim of this study was to determine the allelopathic potential of *C. variegatum* under experimental conditions, building on our observations of *C. variegatum* potential during pot cultivation. Considering the favorable germination dynamics and the park landscape concept where these plants are commonly found in gardens, *L. perenne* was chosen as the recipient plant for testing. The study began with the assumption that the extract of *C. variegatum* leaves would inhibit the germination of *L. perenne* seeds and the synthesis and biological activity of secondary metabolites belonging to the group of phenolic compounds.

## MATERIALS AND METHODS

### *Experimental design*

The leaves of potted *C. variegatum* cultivated at the Institute of Biology and Ecology, Faculty of Science in Kragujevac were used. Seeds of *L. perenne* were obtained from commercial sources ("Bioproduct" Čačak). Since there are insufficient data on the allelopathic potential of *C. variegatum*, in the first part of the experiments, the seeds of *L. perenne* were treated with different concentrations of aqueous *C. variegatum* extracts, and then the physiological response of the seedlings of the selected plant was observed. The seeds are exposed to different concentrations of aqueous *C. variegatum* extracts to determine which concentrations of the extracts would achieve the greatest allelopathic potential. By following this procedure selected concentrations were: 40%, 20%, 10%, 5%, 2.5%, and 1.25%. In the next phase, the influence of selected concentrations on germination characteristics and early growth of *L. perenne* seedlings was investigated. The second group of experiments included the study

of the influence of selected concentrations of extracts on the amount of total phenolic compounds, the amount of flavonoids, and total antioxidant activity.

### *Preparation of aqueous extracts - infusums of C. variegatum*

Water extracts - infusums were made from fresh leaves of potted *C. variegatum* by measuring 40 g of grinded fresh plant material and adding 100 mL of distilled water. The mixture, with stirring, was left for 48 h. After that, the mixture was filtered through Whatman No. 1 filter paper. From the obtained 40% extract, dilutions were made with distilled water in order to obtain extracts in concentrations of 20%, 10%, 5%, 2.5%, and 1.25%.

### *Procedure of experiment*

Seeds were surface sterilized with a 0.1% sodium hypochlorite solution (NaClO) to remove epiphytic microflora and washed several times with distilled water (pH 7). Thirty seeds were placed in Petri dishes containing Whatman filter paper No 1 and treated with 5 ml of aqueous *C. variegatum* leaf extract at concentrations of 40%, 20%, 10%, 5%, 2.5%, and 1.25%. Untreated seeds served as a control sample. The treated seeds were incubated together with the untreated ones in an air chamber (temperature 23 ± 2°C, photoperiod 16/8 h, humidity 60%) for three weeks. The experiment was performed three times in three replicates.

### *Germination parameters*

Seeds were considered germinated when a seedling at least 2 mm long was formed. Germinated seeds were recorded every day until the total number of germinated seeds was constant. All parameters were calculated according to ESPANANY *et al*. (2016) using the following equations:

*Germination percentage*

$$\mathrm{GP} = \frac{\text{Total seeds germination}}{\text{Total number of planted seeds}} \times 100$$

*Mean time of germination*

$$\mathrm{MTG} = \frac{\sum n_i \times t_i}{\sum n_i}$$

$n_i$ = number of newly germinated seeds in the time *i*
$t_i$ = time from the start of experiment to the observation (in days)

*Rate of germination*

$$\mathrm{RG} = \sum \frac{\mathrm{G}}{\mathrm{t}}$$

G = seed germination at 1-day intervals (the percentage)
t = total germination period (in days).

*Germination uniformity*

$$U = \frac{\mathrm{GP}}{\mathrm{MTG}}$$

### *Morphological traits and vigor index*

Measurements were made three times in three series. Root and shoot lengths were measured with a digital caliper. Measurements of fresh weight were made using an analytical balance. The evaluation of the vigor test to predict seed quality was determined using the following formulas (KHARB *et al*., 1994):

$$\text{Seedling Length Vigor Index (SLVI)} = (\text{Mean shoot length} + \text{Mean root length}) \times \text{FGP}$$

$$\text{Seedling Weight Vigor Index (SWVI)} = \text{Mean seedling weight} \times \text{FGP}$$

FGP = Final germination (%).

### *Preparation of methanolic infusums from plants*

A methanolic infusum was prepared from the aerial part of the plants by weighing 20 mg of dry plant material and adding 20 mL of methanol. The mixture was stirred for 48 hours and then filtered through Whatman filter paper No 1. The resulting infusum was used immediately after preparation to determine total phenolic compounds, total flavonoids, and total antioxidant activity.

### *Determination of total phenols in plant infusums*

The total phenolic content in the plant infusums was determined using the spectrophotometric method (SINGLETON *et al*., 1999). Methanolic infusum mg/ml, Folin-Ciocalteu reagent and $NaHCO_3$ were used to prepare the samples for analysis. 0.5 mL of methanolic infusum, 2.5 mL of Folin-Ciocalteu reagent and 2 mL of $NaHCO_3$ were used to prepare the samples for analysis. Samples were incubated at a temperature of 45˚C for 15 min, then absorbance was measured at a wavelength of $\lambda_{max}$ = 765 nm. A calibration curve was constructed based on different concentrations of gallic acid (from 0.02 to 0.1 mg/mL), which was used as a standard. The amount of total phenolic compounds was expressed in gallic acid equivalents (mg GAE/g dry weight).

### *Determination of total flavonoids in plant infusums*

The content of flavonoids in methanolic plant infusums was determined by the spectrophotometric method (QUETTIER-DELEU *et al*., 2000). Sample preparation was performed by mixing the 1 mL of infusum with 1 mL of $AlCl_3$ followed by incubation at room temperature for 1 hour. Absorbance was measured using a spectrophotometer at a wavelength of $\lambda_{max}$ = 415 nm. A calibration curve was constructed based on different concentrations of rutin (from 0.02 to 0.1 mg/mL), which was used as a standardand the calibration curve was constructed. The total flavonoid content was expressed as rutin equivalent (mg RUE/g dry weight).

### *Evaluation of antioxidant activity*

The ability of the herbal infusums to neutralize DPPH radicals was investigated using the spectrophotometric method (TAKAO *et al*., 1994; STANKOVIĆ, 2011). For each sample, a series of double dilutions were prepared in the concentration range from 500 to 0.97 μg/ml. Samples were prepared by mixing 1 mL of diluted infusum and 1 mL of DPPH solution, followed by incubation at room temperature for 30 min. Absorbance was measured using a

spectrophotometer at a wavelength of $\lambda_{max}$ = 517 nm. The percentage of inhibition was calculated using the following equation:

$$\% \text{ inhibition} = \left( \frac{\text{A of control} - \text{A of sample}}{\text{A of control}} \right) \times 100$$

### *Statistical analysis*

Results obtained using the SPSS statistical data processing software package (SPSS for Windows, version 21) are reported as the mean of nine replicates ± standard error (SE). The collected data were analyzed using ANOVA and LSD post-hoc test with a significance threshold of $p \leq 0.05$.

## RESULTS AND DISCUSSION

### *Germination parameters*

The germination characteristics of *L. perenne* were studied in response to different concentrations of *C. variegatum* extracts, with germination percentage (GP), mean germination time (MTG), germination rate (RG), and germination uniformity (U) measured and presented in Table 1. The highest GP was observed in the treatment with 1.25% extract (GP = 95%), followed by the control treatment (GP = 94.45%). As the concentration of the extracts increased, the percentage of total germinated seeds decreased, and a complete inhibition effect was observed at 40% concentration, where no germination occurred during the experimental period. This suggests that the allelochemicals in the extract acted at the very first stage of germination, inhibiting imbibition and preventing complete germination. The highest and lowest GP values were associated with the highest and lowest RG (RG = 68.29 at 1.25% treatment; RG = 21.09 at 20% treatment). Similar patterns were observed for germination uniformity, with the highest values recorded in the control and 1.25% extract treatments (U = 20.48), and the lowest value recorded in the 20% extract treatment (U = 5.95). MTG was shortest in the 2.50% extract treatment (MTG = 4.52).

Table 1. Effect of different concentrations of *C. variegatum* extracts on germination percentage (GP), mean germination duration (MTG), germination rate (RG), and germination uniformity (U) of *L. perenne*;

| Treatment | GP | MTG | RG | U |
|---|---|---|---|---|
| **Control** | 94.45 ± 0.65 | 4.64 ± 0.08 | 66.89 ± 1.84 | 20.48 ± 0.19 |
| **1.25%** | 95.00 ± 1.61 | 4.72 ± 0.21 | 68.29 ± 3.98 | 20.36 ± 1.20 |
| **2.50%** | 89.45 ± 2.24 | 4.52 ± 0.08 | 65.13 ± 3.28 | 20.02 ± 0.79 |
| **5%** | 88.89 ± 1.92 | 5.14 ± 0.02 | 60.42 ± 2.47 | 17.29 ± 0.33 * |
| **10%** | 82.23 ± 0.64 * | 5.75 ± 0.14* | 52.37 ± 0.67* | 14.35 ± 0.20* |
| **20%** | 38.90 ± 8.34* | 7.21 ± 0.68* | 21.09 ± 5.82* | 5.95 ± 1.80* |
| **40%** | / | / | / | / |

The results are presented as the mean of the measurement ± standard error. An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) compared to control values.

Secondary metabolites, including allelochemicals, can have various effects on plant seeds, ranging from beneficial to inhibitory (RADWAN *et al*., 2019). The inhibitory effects of allelochemicals on seed germination can be attributed to their synergistic impact on various

physiological processes in the recipient plant (ISLAM *et al*., 2018). IRSHAD and CHEEMA (2004) report that allelochemicals can significantly affect seed germination and plant productivity by blocking cell division and hydrolysis of reserve nutrients.

The allelopathic potential of the *C. variegatum* varied, according to an analysis of the germination characteristics of *L. perenne*. Differences in allelopathic potential were expected given the different concentrations of the extracts. The higher concentrations of the extracts had a stronger negative effect on seed germination. The obtained results are in agreement with those of previous studies, which confirmed that lower concentrations of allelochemicals have a weaker allelopathic effect, while higher concentrations show a stronger allelopathic effect (NORSWORTHY, 2003; DHIMA *et al*., 2009; SINGH *et al*., 2013; RAVLIĆ *et al*., 2014). The stronger inhibitory effect is primarily brought on by allelochemicals acting directly without transformation or degradation (ABBAS *et al*., 2014). Additionally, the authors note that other researchers have observed a dependence of allelopathic potential on the application rate, with a stronger inhibitory effect at higher allelochemical concentrations. This has been noted by Qasem (1995), El-Khatib et al. (2004), and Yarnia (2010).

### *Growth characteristics*

The presented results on the effects of different treatments on the early growth of the recipient plant are summarized in Table 2. The treatment with 2.50% extract resulted in the most significant increase in root length (6.20 cm), followed by the 1.25% extract treatment (6.19 cm), both showing significant differences compared to the control. Conversely, all other treatments had an inhibitory effect on root length compared to the control. In terms of shoot length, all treatments had a stimulatory effect, with values ranging from 7.88 to 9.64 cm. The treatment with 20% extract had the highest shoot length values, showing a statistically significant difference compared to the control. The treatment with 5% and 2.5% extract had the most observable impact on fresh mass.

SISODIA and SIDDIQUI (2010) indicated that the different phytotoxic effect of *C. variegatum* extracts on the growth of selected seedlings was due to different chemical constituents in these plants. The lesser negative effect on growth and greater effect on seed germination is the result of different mechanisms of action of allelochemicals (IMAN *et al*., 2006). The results obtained were similar to those of previous studies confirming that *C. variegatum* extracts can stimulate shoot elongation of selected plants (SISODIA and SIDDIQUI, 2010). The reduction in root length and stimulation of shoot elongation indicates that the adsorbed allelochemicals are distributed in an organ-specific manner. It is noteworthy that reduced root length in response to allelopathic compounds is a common phenomenon observed in many plant species, including the recipient plant used in this study. This is thought to be a result of higher absorption and accumulation of allelochemicals which can lead to toxicity and inhibition of root growth, due to direct contact with the filter paper (CORREIA *et al*., 2005).

### *Vigor index*

Analysis of the SLVI indicated that treatments with 1.25% extract (1384.08) and 2.50% extract (1312.48) were most favorable for promoting germination and early developmental stages of *L. perenne*. For the SWVI analysis, a stimulating effect was observed for all applied treatments (except for the treatment with 20% extract).

It is well known that the Vigor test evaluation is more crucial than the standard germination test for predicting seed quality because it assesses not only the viability of the seed but also its capacity to grow plants under unfavorable environmental conditions (ARUMUGAM *et al*., 2008; BOJOVIĆ *et al*., 2018). Concentrations up to 5% show slight deviations from the control sample, with the plant tending to respond better to external influences when treated with

extract than with the control sample, while concentrations above 5% show an inhibitory effect that increases up to 40% and completely inhibits germination of the test species.

### *Total phenolic and flavonoid contents*

Tab. 3 shows the results for total phenolics obtained in above-ground plant parts. The concentrations of total phenolics ranged from 6.75 to 9.83 mg GAE /g dry weight. The highest amount of phenolic compounds was measured in the control treatment and followed by the values obtained in the treatment with 2.50% extract (9.57 mg GAE/g dry weight). The lowest amount of these metabolites was measured in the treatment with 20% extract with a statistically significant difference compared to the control.

The amount of flavonoids in methanolic infusums of aerial plant parts under the influence of different extracts of *C. variegatum* is expressed as the rutin equivalent and shown in Tab. 3. The obtained values for the amount of flavonoids in the shoots ranged from 11.22 to 15.51 mg RUE/g dry weight. Similar to phenols, the highest values were recorded in the treatment with 2.50% extract. They were followed by the values measured in the control treatment (14.18 mg RUE/g dry weight), while the lowest values were measured at 20% extract.

Table 2. Effect of different concentrations of *C. variegatum* extracts on shoot and root length (cm), fresh weight (g), seedling length growth index (SLVI), and seedling weight growth index (SWVI) of *L. perenne*;

| Treatment | Root | Shoot | Fresh weigth | SLVI | SWVI |
|---|---|---|---|---|---|
| **Control** | 5.89 ± 0.11 | 7.88 ± 0.17 | 0.010 ± 0.00 | 1300.87 ± 22.92 | 0.944 ± 0.04 |
| **1.25%** | 6.19 ± 0.21 | 8.37 ± 0.22 | 0.012 ± 0.00 | 1384.08 ± 44.13 | 1.107 ± 0.13 |
| **2.50%** | 6.20 ± 0.20 | 8.46 ± 0.19 | 0.015 ± 0.00* | 1312.48 ± 55.18 | 1.297 ± 0.07* |
| **5%** | 5.67 ± 0.66 | 8.69 ± 0.18 | 0.016 ± 0.00* | 1278.07 ± 87.11 | 1.426 ± 0.04* |
| **10%** | 5.46 ± 0.46 | 8.67 ± 0.27 | 0.012 ± 0.00 | 1162.20 ± 54.63 | 1.003 ± 0.08 |
| **20%** | 3.75 ± 0.08* | 9.64 ± 0.80* | 0.013 ± 0.00 | 327.13 ± 20.46* | 0.482 ± 0.09* |

The results are presented as mean of measurement ± standard error. An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) compared to control values.

Table 3. Effect of different concentrations of *C. variegatum* infusums on total phenolic (mg GAE /g dry weight) and flavonoid contents (mg RUE/g dry weight) of *L. perenne*;

| Treatment | Total phenolic content | Flavonoid content |
|---|---|---|
| **Control** | 9.83 ± 0.37 | 14.18 ± 0.20 |
| **1.25%** | 8.40 ± 1.04* | 13.79 ± 1.89 |
| **2.50%** | 9.57 ± 0.10 | 15.51 ± 0.29 |
| **5%** | 8.71 ± 0.13* | 13.45 ± 1.13 |
| **10%** | 8.27 ± 0.14 | 13.41 ± 1.07 |
| **20%** | 6.75 ± 0.05* | 11.22 ± 0.62 |

The results are presented as the mean of the measurement ± standard error. An asterisk (*) indicates a statistically significant difference ($p \leq 0.05$) compared to control values.

The recipient plant's phenolic and flavonoid content are both impacted by the extracts of *C. variegatum* with the effects on the amount of total flavonoids almost identical to those for the content of total phenols.. The differences in the content of total phenolics and total flavonoids in the selected species could be due to the specific response of the species to the increased concentration of *C. variegatum* extracts, indicating different adaptation mechanisms

for tolerance to different concentrations of allelochemicals. The amount of phenolic compounds depends on their function in the plant itself, i.e., their role in protection against various biotic and abiotic factors to which the plant is exposed during its growth and development (ALONSO-AMELOT *et al*., 2004; LATTANZIO *et al*., 2006). The role of flavonoids in the process of biochemical communication of plants with the environment is equally important (TREUTTER, 2005). Accordingly, differences in the quantitative characteristics of phenolic compounds and flavonoids may be due to their unequal distribution and accumulation in plants, which is closely related to the direct influence of the biochemical composition of *C. variegatum* (SAFFOON *et al*., 2014).

### *Antioxidant activity*

The values obtained for DPPH inhibition varied according to the treatment applied (Tab. 4). The initial infusum dilutions clearly showed the highest percentage of inhibition for the 2.50% treatment, followed by the 10% treatment, while the other treatments showed lower values compared to the control. This is evident when considering the values obtained for percentage DPPH inhibition. The percentage of DPPH inhibition, however, decreased in comparison to the values obtained for the control sample as the initial dilution of the plant infusum increased.

Phenolic acids are reported to be the most abundant phenolic allelochemicals in *C. variegatum*, with ellagic acid playing a dominant role (SAFFOON *et al*., 2014; NJOYA *et al*., 2021). Therefore, the presence of phenolic compounds in *C. variegatum* could be responsible for the inhibitory effect of the used extracts on the antioxidant activity of *L. perenne*. The obtained results are in line with earlier research that demonstrated a direct relationship between the allelochemicals of *C. variegatum* and a reduction in antioxidant capacity (BOONMEE and KATO-NOGUCHI, 2019).

## CONCLUSION

The study has provided evidence that *C. variegatum* possesses allelopathic properties that can significantly inhibit the germination and early growth of *L. perenne*. Our findings suggest that the allelopathic effect of this species is directly correlated with the concentration of the extract solution, with increasing concentration leading to a proportional increase in inhibition of germination and growth. Notably, our results also indicate that a 40% solution of *C. variegatum* leaf extracts can completely inhibit germination of *L. perenne*. Additionally, this study has demonstrated that the allelopathic effect of *C. variegatum* on recipient seedlings is related to the biochemical composition of the extracts used, which can disturb the synthesis of secondary metabolites and antioxidant activity. The obtained results are significant both scientifically and practically because allelopathic influence is a complex process that is difficult to distinguish from plant competition for nutrients in the field, compared to laboratory conditions.

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Table 4. Effect of different concentrations of *C. variegatum* extracts on total antioxidant activity of *L. perenn*e;

| **Infusum concentration (µg/ml)** | **Control** | **Treatment** | | | | |
|---|---|---|---|---|---|---|
| | | **1.25%** | **2.5%** | **5%** | **10%** | **20%** |
| **500** | 40.92 ± 3.90 | 39.41 ± 7.36 | 42.49 ± 3.66 | 39.62 ± 1.84 | 41.39 ± 4.95 | 29.44 ± 2.43 |
| **250** | 25.48 ± 2.73 | 23.70 ± 3.07 | 23.63 ± 1.98 | 24.80 ± 2.42 | 23.77 ± 2.36 | 21.24 ± 2.16 |
| **125** | 21.31 ± 2.59 | 20.56 ± 3.04 | 19.06 ± 1.01 | 18.44 ± 1.48 | 17.90 ± 2.15 | 17.08 ± 1.31 |
| **62.5** | 18.17 ± 1.73 | 17.14 ± 1.50 | 16.73 ± 1.78 | 16.87 ± 1.97 | 16.87 ± 1.69 | 16.26 ± 1.38 |
| **31.25** | 17.21 ± 1.82 | 16.26 ± 1.48 | 16.12 ± 1.66 | 15.92 ± 1.66 | 16.12 ± 1.49 | 15.98 ± 1.43 |
| **15.62** | 16.87 ± 1.69 | 15.92 ± 1.43 | 15.78 ± 1.67 | 15.57 ± 1.67 | 15.92 ± 1.49 | 15.71 ± 1.49 |
| **7.81** | 16.39 ± 1.68 | 15.64 ± 1.41 | 15.57 ± 1.67 | 15.37 ± 1.67 | 15.64 ± 1.54 | 15.44 ± 1.43 |
| **3.9** | 16.12 ± 1.70 | 15.37 ± 1.39 | 15.30 ± 1.68 | 15.16 ± 1.67 | 15.30 ± 1.61 | 15.23 ± 1.43 |
| **1.9** | 15.92 ± 1.70 | 15.10 ± 1.38 | 15.03 ± 1.69 | 14.96 ± 1.67 | 15.03 ± 1.61 | 14.96 ± 1.48 |
| **0.97** | 14.89 ± 2.12 | 14.55 ± 1.36 | 14.82 ± 1.69 | 14.75 ± 1.67 | 14.82 ± 1.61 | 14.62 ± 1.60 |

The results are presented as the mean of the measurement ± standard error.

## References:


[1] ABBAS, T., TANVEER, A., KHALIQ, A., SAFDAR, M.E., NADEEM, M.A. (2014): Allelopathic effects of aquatic weeds on germination and seedling growth of wheat. *Herbologia* **14** (2): 12–25.

[2] ALONSO-AMELOT, E.M., OLIVEROS, A., CALCAGNO-PISARELLI, P.M. (2004): Phenolics and condensed tannins in relation to altitude in neotropical *Pteridium* spp. A field stady in the Venezuelan Andes. *Biochemical Systematics and Ecology* **32**: 969–981. doi: 10.1016/j.bse.2004.03.005.

[3] ARUMUGAM, M., RAJANNA, M. P., GOWDA, R. (2008): Seed dormancy and seedling vigor as influenced by planting time environment and date of harvest in rice (*Oryza sativa* L.). *Caspian Journal of Environmental Sciences* **6:** 1–9.

[4] BOJOVIĆ, B., JAKOVLJEVIĆ, D., ĆURCIĆ, S., STANKOVIĆ, M. (2018): Phytotoxic potential of common nettle (*Urtica dioica* L.) on germination and early growth of cereals an vegetables. *Allelopathy Journal* **43** (2): 175–186.

[5] BOONMEE, S., KATO-NOGUCHI, H. (2019): Comparative study on allelopathic Potential of medicinal plants against the growth of Cress and Barnyard grass. *Environmental Control in Biology* **57** (3): 75–81.

[6] CASTLER, M.D., DUNCAN, R.R. (eds) (2003): Turfgrass biology, genetics, and breeding. *John Wiley & Sons*. Hoboken, NJ, USA.

[7] CORREIA, N.M., CENTURION, M.A.P.C., ALVES, P.L.C.A. (2005): Influence of *Sorghum* aqueous extracts on soybean germination and seedling development. *Ciencia Rural* **35** (3): 498–503.

[8] DHIMA, K.V., VASILAKOGLOU, I.B., GATSIS, TH.,D., PANOU-PHILOTHEOU, E., ELEFTHEROHORINOS, I.G. (2009): Effects of aromatic plants incorporated as green manure on weed and maize development. *Field Crops Research* **110**: 235–241.

[9] EL-KHATIB, A.A., HEGAZY, A.K., GALAL, H.K. (2004): Allelopathy in the rhizosphere and amended soil of *Chenopodium murale* L. *Weed Biology and Management* **4**: 35–42.

[10] ESPANANY, A., FALLAH, S., TADAYYON, A. (2016): Seed priming improves seed germination and reduces oxidative stress in black cumin (*Nigella sativa*) in presence of cadmium. *Industrial Crops and Products* **79**: 195–204.

[11] HASSAN, E.M., HASSAN, R.A., EL-TOUMY, S.A., MOHAMED, S.M., OMER, E.A. (2014): Phenolic metabolites and antioxidant activity of *Codiaeum variegatum* CV. spirale. *Journal of Pharmacy Research* **8** (5): 619–623.

[12] IMAN, A., WAHAB, Z., RASTAN, S.O.S., HALIM, M.R.A. (2006): Allelopathic effect of sweet corn and vegetable soybean extracts at two growth stages on germination and seedling growth of corn and soybean varieties. *Journal of Agronomy* **5**: 62–68.

[13] IRSHAD, A., CHEEMA, Z.A. (2004): Influence of some plant water extracts on the germination and seedling growth of barnyard grass (*Echinochola crus-galli* (L.) beauv). *Biological Sciences-PJSIR* **47** (3): 222–226.

[14] ISLAM, A.K.M., YEASMIN, S., QASEM, J.R.S., JURAIMI, A.S., ANWAR, M.P. (2018): Allelopathy of medicinal plants: current status and future prospects in weed management. *Agricultural Sciences* **9** (12): 1569–1588.

[15] JHA, U., SINGH, A., OSWAL, R.J. (2016): *In-vitro* antilithiatic effect of ethanolic extract of *Codiaeum variegatum* (L.) Blume. *Asian Journal of Pharmaceutical Technology and Innovation* **4** (17): 95–102.

[16] KHARB, R.P.S., LATHER, B.P.S., DESWAL, D.P. (1994): Prediction of fieldemergence through heritability and genetic advance of vigour parameters. *Seed Science and Technology* **22**: 461–466.

[17] LATTANZIO, V., LATTANZIO, V.M.T., CARDINALI, A. (2006): Role of phenolics in the resistance mechanisms of plants againstfungal pathogens and insects. *Phytochemistry: Advances in Research* **661** (2): 23–67.

[18] MOHAMED, N.E., EL-MASRY, R.A., AWAD, A.E., BADR, H.A. (2019): Chemical composition and antibacterial activity of *Codiaeum variegatum* leaves. *Zagazig Journal of Agricultural Research* **46** (4): 1133–1140.

[19] ABO-ZEID, M.A.M, FARGHALY, A.A., HASSAN, E.M., ABDEL-SAMIE, N.S. (2019). Phenolic compounds of *Codiaeum variegatum* spirale lessened cytotoxic and genotoxic effects of mitomycin C in mice somatic and germ cells. *Cytology and Genetics* **53**: 494–501.

[20] MUSHTAQ, W., SIDDIQUI, M.B. (2017): Allelopathy studies in weed science in India–A review. *International Journal of Natural Research Ecology and Management* **2** (6): 99–103.

[21] NAIDU, G.P. (1988): Antifungal activity in *Codiaeum variegatum* leaf extract. *Current Science* **57** (9): 502-504.

[22] NASIB, A., ALI, K., KHAN, S. (2008): *In vitro* propagation of croton (*Codiaeum variegatum*). *Pakistan journal of botany* **40** (1): 99–104.

[23] NORSWORTHY, J.K. (2003): Allelopathic potential of wild radish (*Raphanus raphanistrum*). *Weed Technology* **17**: 307–313.

[24] NJOYA, E.M., FEWOU, P.M., NIEDERMEYER, T.H. (2021): *Codiaeum variegatum* (L.) Rumph. ex A. Juss. (Euphorbiaceae): An overview of its botanical diversity, traditional uses, phytochemistry, pharmacological effects and perspectives towards developing its plant-based products. *Journal of Ethnopharmacology* **277**: 114244.

[25] QASEM, J.R. (1995): Allelopathic effects of *Amaranthus retroflexus* and *Chenopodium murale* on vegetable crops. *Allelopathy Journal* **2** (1): 49–66.

[26] QAWASMEH, A., OBIED, H.K., RAMAN, A., WHEATLEY, W. (2012): Influence of fungal endophyte infection on phenolic content and antioxidant activity in grasses: interaction between *Lolium perenne* and different strains of *Neotyphodium lolii*. *Journal of agricultural and food chemistry* **60** (13): 3381–3388.

[27] QUETTIER-DELEU, C., GRESSIER, B., VASSEUR, J., DINE, T., BRUNET, C., LUYCKX, M., CAZIN, M., CAZIN, J.C., BAILLEUL, F., TROTIN, F. (2000): Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *Journal of ethnopharmacology* **72** (1-2): 35–42.

[28] RADWAN, A.M., ALGHAMDI, H.A., KENAWY, S.K. (2019): Effect of *Calotropis procera* L. plant extract on seeds germination and the growth of microorganisms. *Annals of Agricultural Sciences* **64** (2): 183–187.

[29] RAVLIĆ, M., BALIČEVIĆ, R., LUCIĆ, I. (2014): Allelopathic effect of parsley (*Petroselinum crispum* Mill.) cogermination, water extracts and residues on hoary cress (*Lepidium draba* (L.) Desv.). *Poljoprivreda* **20** (1): 22–26.

[30] SAFFOON, N., UDDIN, R., SUBHAN, N., HOSSAIN, H., REZA, H.M., ALAM, M.A. (2014): In vitro anti-oxidant activity and HPLC-DAD system based phenolic content analysis of *Codiaeum variegatum* found in Bangladesh. *Advanced pharmaceutical bulletin* **4** (Suppl 2): 533–541.

[31] SCAVO, A., MAUROMICALE, G. (2021): Crop allelopathy for sustainable weed management in agroecosystems: Knowing the present with a view to the future. *Agronomy* **11** (11): 2104.

[32] SCAVO, A., RESTUCCIA, A., MAUROMICALE, G. (2018): Allelopathy: principles and basic aspects for agroecosystem control. *Sustainable Agriculture Reviews 28: Ecology for Agriculture* **28**: 47–101. doi: 10.1007/978-3-319-90309-5_2

[33] SINGH, N.B., KUMAR, S., SINGH, D., YADAV, K. (2013): Allelopathic effects of different phenological stages of *Cassia occidentalis* L. on *Parthenium hysterophorus* L. *Iranian Journal of Plant Physiology* **3** (4): 817–828.

[34] SINGLETON, V.L., ORTHOFER, R., LAMUELA-RAVENTÓS, R.M. (1999): Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology* **299**: 152–178.

[35] SISODIA, S., SIDDIQUI, M.B. (2010): Allelopathic effect by aqueous extracts of different parts of *Croton bonplandianum* Baill. on some crop and weed plants. *Journal of Agricultural Extension and Rural Development* **2** (1): 22–28.

[36] STANKOVIĆ, M.S. (2011): Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac Journal of Science* **33**: 63–72.

[37] TAKAO, T., KITATANI, F., WATANABE, N., YAGI, A., SAKATA, K. (1994): A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Bioscience, Biotechnology, and Biochemistry* **58** (10): 1780–1783.

[38] TREUTTER, D. (2005): Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology* **7** (6): 581–591.

[39] YARNIA, M. (2010): Comparison of field bindweed (*Convolvulus arvensis* L.) and bermuda grass (*Cynodon dactylon* L.) organs residues on yield and yield components of bread wheat (*Triticum aestivum* L.). *Advances in Environmental Biology* **4**(3): 414–421.