Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 19, issue 2, 2009, pp.295-301

STUDY ON THE ALELOPATIC ACTION OF THE WATERY EXTRACT OF ARTEMISIA *ABSINTHIUM* L. UPON THE GERMINATION OF THE CARYOPSES AND OF THE GROWTH OF THE PLANTLETS OF *TRITICUM AESTIVUM* L., *LOLIUM PERENNE* L. AND OF *BROMUS INERMIS* L.

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ABSTRACT. The paper has had as an objective evaluating the phyto-toxic effect, respectively alelopathic of the watery extracts prepared from various organs of the white wormwood plants (*Artemisia absinthium* L.), upon the germination of the wheat caryopses (*Triticum aestivum* L.), ryegrass (*Lolium perenne* L.) and of brome grass (*Bromus inermis* L.), and of the growth of the plantlets resulted from their embryos, in the conditions of their germination on filter paper humidified with such solutions, used in various concentrations (5, 15, 25, 50, 75 şi 100%). After 7 days from germination, there was determined the length of the small roots and that of the small stems of the plantlets. The results obtained denote a strong negative influence exercised by these extracts, both upon the germination of the caryopses and of the growth of the plantlets of the species subject to testing, especially of the solution that was prepared from inflorescence of white wormwood. The alelopathic effect, respectively inhibiting upon the wheat plantlets, of ryegrass and of brome grass are intensified along with the increase of the concentration of the watery extract used as germination and growth substratum. The strongest negative reaction, at the three types of extracts, was manifested by brome grass, and the most resistant species was that of wheat.

Keywords: alelopaty, germination, growth, stimulation, inhibition

INTRODUCTION

The white wormwood (*Artemisia absinthium* L.), is a herbaceous plant, perrennial, common in all regions of our country, in the sunny areas of plain, hill or in the ruderal places, or in the arid and even stony soils. It belongs to the order Sinandrales and is part of the *Asteraceae* family. The wormwood plants contain, as secondary products of metabolism, etheric and flavone oils (artemisina). The total etheric oil has a characteristic smell, a bitter taste, green colour (other times blue or brown), and contains tojonă, cadinen, felandren and azulene (Pârvu, 1991).

From the specialty literature it is known that the wormwood exercises an alelopatic effect upon many species of plants, by the emanations of etheric oils (under the form of vapours), at the level of the leaves. The action of the foliar emanations of white wormwood was studied ever since 1940, by Bode. The starting point of these studies has consisted in observing the fact that, in the vicinity of the wormwood plots there was noticed the producing of a strong inhibition of the growth of the plants of *Foeniculum vulgare*, even at the distance of 1 m from it. In nature, the plants of white wormwood have triggered the stopping of the growth to a series of other vegetal species which were in their vicinity, i.e.: *Levisticum officinale, Carum carvi, Ocimum basilicum, Melissa officinalis, Salvia sclarea, Nepeta cataria*, and others (cf. Chircă şi Fabian, 1973).

The mollecule supposed to be at the origin of the alelopatic properties of the white wormwood is the artemisinin, organic compound with a brute formula: $C_{25}H_{22}O_4$ (fig.1), which – in a pure form – forms crystals under the form of white needles, shiny, silky, having the melting point at the temperature of 120ºC.

Within the experiments made by us, on the alelopaty (Corbu, 2004; 2007; Corbu & Cachiţă, 2006; Corbu, Cachiţă, Şipoş, 2007 a, b; 2008), we have aimed at continuing this research, so that in the present paper we present our results referring to the effect of the watery extracts, prepared from the organs of the white wormwood plants, upon the germination of caryopses and of the growth of the wheat (*Triticum aestivum* L.), ryegrass (*Lolium perenne* L.) and of brome grass (*Bromus inermis* L.) plantlets.

MATERIALS AND METHODS

From *roots*, *leaves* (devoid of petiole) and of *flowers* of white wormwood, organs collected during the *summer time* (in the stage of maturity of the plants), we have prepared the total watery extracts, as follows:

- 250g of vegetal material (leaves or flowers) were cut into small fragments, which were ground in the presence of 500 ml distilled water for 30 minutes; the vegetal material thus obtained was left in standby, to macerate, for 12 hours, at room temperature, respectively $22^o \pm 2^oC$, after which the supernatant was decanted, and the extract was filtered through filter paper and kept, until usage (2-3 days), in the dark, at

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Article received: September 2009; published: November 2009

the temperature of 4ºC; the roots were razored through a plastic razor. The filtered material resulted was considered as being a reference watery extract (the „mother" solution), the concentration obtained being evaluated as of 100%. In the moment of using the extracts, starting from the „mother" solution, there were made dillutions with distilled water, there being obtained the final concentrations of : 5% (V_1), 15% (V_2), 25% (V_3), 50% (V_4), 75% (V_5) and 100% (V_6), constituting 6 experimental variants, which were utilised in the biotestings carried out in the prezent paper.

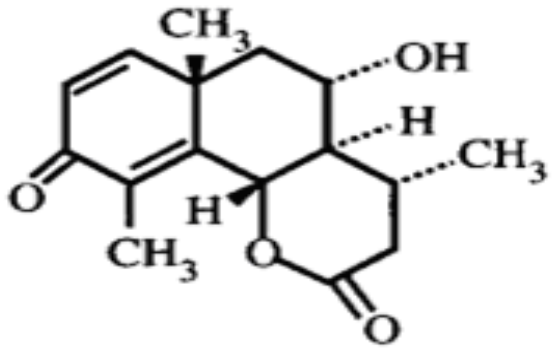

Fig. 1 The structure of artemisinin (according to Cowan, 1999)

For the germination of the seed material there was proceeded to the placing of it in colorless cases, made of plastic material, transparent, on filter paper humidified each with 3ml of extract, this being used as a substratum both in the germination period of the caryopses, and on the duration of the increase of the plantlets resulted from the embryos of the germinated beads. The germination was carried out in the conditions of the laboratory at a temperature of 23-25°C, in indirect light, with specific daily variations; at the proof lot, the germination was produced on a filter paper humidified with distilled water (variant V_0).

At *four days* from placing the seed material to germinate, the germinated seeds were counted, in the case of each type of extract, and at *seven days* there have been caried out biometric measurements regarding *the growth in length* of the small roots and stems, respectively of the entire plantlet. The reactions of the caryopses and the presence in the substratum of germination of the watery extract were compared to the results obtained at the proof lot, caryopses germinated on filter paper as well, humidified with distilled water, biometrical values regarded as being of reference, respectively of 100%. The results of the study were analysed statistically through the analysis of the variance (Săulescu & Săulescu, 1967).

RESULTS AND DISCUSSIONS

The comparative analysis of the effects of the watery extracts prepared from organs of white wormwood plant, regarding the germination of the caryopses of wheat, ryegrass and of brome grass(fig. 2) has revealed specific reactions of the plantlets, according to the *type* and the *concentration* of the extract used, as well as of the *sensitivity* of the receipting species of the alelo-chemical substances. Thus the watery extract prepared from *roots* of wormwood, administrated in low concentrations - of 5% and 15% (variants V_1-V_2) – did not exercised any action , significant from a statistical point of view upon the germination of the wheat, ryegrass and of brome grass caryopses (fig. 2 A). In average concentrations, of 25 - 50% (variants V_3-V_4), and maximum ones, of 75-100% (variants V_5 –V_6), the above-mentioned extract has inhibited the germination of the seed material, at the three vegetal species taken into study, the phyto-toxic effects being maximum, and highly significant from a statistical point of view, at the concentration of total extract, non-diluted (variant V_6).

The watery extract obtained out of *leaves* of white wormwood (fig. 2 B), in concentration of *5%* (variant V_1), has exercised positive effects upon the germination of the wheat and of brome grass caryopses, there being registered increases of 0,67% at wheat and respectively of 0,72% at brome grass, values which, however, as a result of the calculations of analysis of the variance were non-significant from a statistical point of view (fig. 2, variant V_1); on the other hand, the increased concentrations of 75% and 100% (fig. 2, variants V_5 and V_6 of treatment) have manifested strong inhibiting effects, with percentage differences compared to the proof lot comprised between 67,57% and 71,01% and respectively between 84,46% and 100%, values which are considered as being highly significant statistically.

The strongest inhibiting effect upon the germination of the wheat, ryegrass and of brome grass caryopses, has had the extract obtained from the *flowers* of white wormwood. From the data presented in figure 2 C, one can notice the fact that, under the action of the alelopatic compounds existent in the extract of wormwood flowers, there was produced a decrease of the number of caryopses germinated as the concentration of the extract used in experiments increased. Thus the germination was significantly reduced in the presence of the extract prepared from *flowers* of wormwood, starting with the concentration of 25%. At this concentration, at wheat the percentage of germination was of 66.89%, and of 67.15% at ryegrass, and at the concentration of 50% of the extract, the wheat caryopses have sprung in a percentage of 56.86% and of 22.63% at ryegrass.

The strongest inhibition of the germination was registered at brome grass, at a concentration of the extract prepared from flowers of white wormwood of 25% (fig. 2, C, variant V_3), at which the germination percentage was of 59.49%, while at the concentration of 50% of the extract (fig. 2, C, variant V_4), this process was only at 11,68%. The extract of *flowers* of wormwood at the concentration of *75%* has *stopped* totally the germination of the seed material at ryegrass

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and brome grass, while at the wheat caryopses, the springing up of the beans was *inhibited* with 70,43% (fig. 2, C, variant V_5). At the concentration of 100% (fig. 2, C, variant V_6), of the extract obtained from *flowers* of wormwood, at the three vegetal species taken into study, the caryopses no longer germinated.

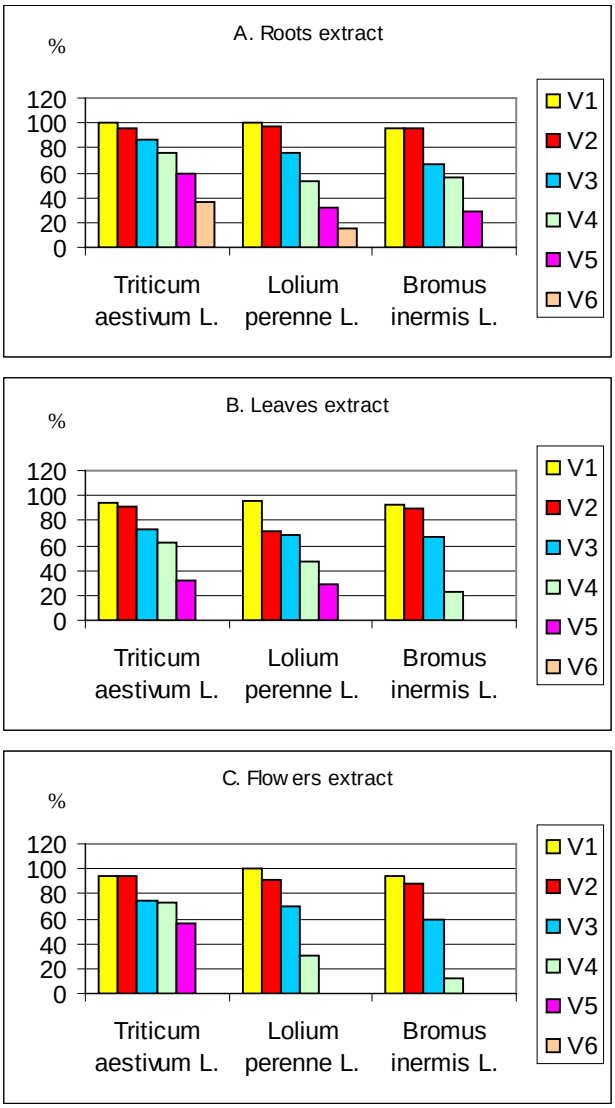

Fig. 2 Expressing in percentage values of the germination of the wheat caryopses *(Triticum aestivum* L.), of reygrass *(Lolium perenne* L.) and of brome grass (*Bromus inermis* L.), which took place of filter paper humidified with watery extract prepared from roots (A), leaves (B), or flowers(C) of white wormwood (*Artemisia absinthium* L.), in the fourth day from placing the beads to germinate, with relation to the values registered at the proof lot, germination carried out on a similar substratum humidified with distilled water (variant V_0), values considered as reference values, respectively of 100% (where: V_1= extract 5%; V_2= extract 15%; V_3= extract 25%; V_4= extract 50%; V_5= extract 75%; V_6= extract 100%).

The growth of the plantlets, the same with the results obtained in the process of germination of the seed material at wheat, ryegrass and at brome grass, has registered differences between the effects of the extracts of wormwood, according to the type of organ from which they were prepared and to the concentration of the solution tested. With the exception of the extracts prepared from *root* and of *leaves* of white wormwood, in concentration of 5% (variant V_1) and of 15% (variant V_2) (fig. 3), which did not exercise a significant effect upon the growth of the plantlets germinated on filter paper humidified with these extracts, all the other concentrations tested, have inhibited – highly significantly from a statistical point of view – the growth in length of the plantlets, with values that have fluctuated between 6,61% and 87, 94% (see also fig. 4).

Comparatively with the extracts prepared from *roots* and of *leaves*, the watery extract obtained from *flowers* of wormwood has exercised strongly inhibiting effects upon the growth of the plantlets taken into study. Like in the case of germination, the highest sensitivity was presented by the plantlets of brome grass to which the growth of the small root was inhibited with 84.29%, and that of the small stem with 41.67%; the average dimensions of the entire plantlet were with 64.76% more reduced in the conditions of the germination of the seeds on a substratum humidified with extract in a concentration of 25% (fig. 3, variant V_3). The extract in a concentration of 50% (fig. 3, variant V_4) has triggered a diminishing of the average heights of the small roots with 92.64%, whereas of the small stems with 70.83%, phenomenon which, at the level of the entire plantlet has represented an inhibiting of the growth of 82.64%. At the superior concentration tested, of 75% of the extract prepared of flowers of white wormwood, the process of growth of the plantlets of ryegrass and of brome grass has ceased, while that of the plantlets of wheat was only strongly inhibited, the difference as to the proof lot being of 93.81% (fig. 3, variant V_5). The extract of the wormwood flowers, in a maximum concentration tested, of 100% (fig. 3 and fig. 4, variant V_6), has blocked the forming and the growth of the plantlets of the three vegetal species analyzed.

Between the proof plantlets (variant V_0) and the ones grown on the substratum moisture with extract of white wormwood flowers, in a concentration of 25% (variant V_3), and of *50%* (variant V_4), there was noticed an obvious morphological difference, manifested both by reduced dimensions and by the curving and *necrozation* of the top of the small root (fig. 4). A particular negative alelopathic reaction has consisted in decreasing the number of secondary small roots formed at the level of the embryonic small roots, as well as a colorization of the coleoptiles, respectively of the small leaves of the plantlets of brome grass and ryegrass.

Our results regarding the germination of the seed material and the growth of the plantlets taken into study, under the action of the watery extract prepared from organs of white wormwood plant, are in accordance greatly with the data previously noticed by the authors Chircă and Fabian (1973), authors who

have studied the effect of the extracts from roots and from the leaves of white wormwood upon the germination and growth of white mustard plantlets (*Sinapis alba* L.), in (*Linum usitatissimum* L), water-cress (*Lepidium draba* L.) and of wheat (*Triticum aestivum* L.) Thus the reactions of these plantlets have differed greatly according to the type of the extract used; respectively the one prepared from leaves has exercised a much stronger *inhibiting* effect than the one made from roots. But also the watery extract obtained from roots has triggered *inhibition*, the dicotyledonous plants presenting, however a more reduced sensitivity to the alelopathic action of the prepared material, comparatively with the *wheat.*

Funke (1943) studying the influence produced by the white wormwood upon the neighboring plants, has noticed that the *inhibiting* action exercised by it has manifested itself at the level of weeds, which are met as a rule on the plots of land where one can find as well the white wormwood; of these, the species of the type *Senecio* have always proved to be the most sensitive to the secretions of the white wormwood leaves, while at the species of *Stelaria* the inhibition of growth was much more reduced (Chircă and Fabian, 1973).

The research made by Plhák (1971), regarding the action of the volatile emanations from the aerial parts of the white wormwood plants, upon other species have proved the selectivity of their effect; thus, at the rye plantlets, he has noticed that the growth of the little roots is more *inhibited* than that of the aerial parts. Our results are in concordance with the ones signaled by Plhák, since the watery extracts – prepared from parts of wormwood plant- have *inhibited stronger*, at the vegetal species with which we have experimented, the growth of the *small roots* than the elongation of the *little stems*.

By the research made by Delabays and Mermillod (2002), the alelopathic properties of the wormwood, already outlined in the laboratory and the glass-house, have been confirmed in the field. The results of the experiments organized in the field, by incorporating into the soil of the dry leaves, picked up from two varieties of wormwood (characterized by a differentiated contents of *artemisinin*) pleads in favor of the role played by *artemisinin* in exercising the *inhibiting* and *phyto-toxic* effects of this compound, particularly upon germination and the weeds growing. The research made by the above-mentioned authors, with varieties of wormwood poor in *artemisinin*, suggest the presence in the leaves of other *phyito-toxic* molecules, with *alelopatic* effects, capable of *inhibiting* germination and the growth of the plants in their immediate vicinity

CONCLUSIONS

The alelopathic potential of the watery extracts prepared from plants of white wormwood differ according to the type of the organ used as vegetal material macerated in water, to the concentration of the finite product and to the vegetal species used as biotest.

The strongest inhibiting effects were exercised by the watery extract prepared out of the white wormwood flowers, which indicates the fact that, compared with the roots and leaves, he flowers contain increased concentrations of aleopathic compounds.

The alelopathic effect, i.e. inhibiting upon the wheat, ryegrass and brome grass plantlets intensifies along with the increasing of the concentration of the watery extract used as a substratum of germination and growth.

The strongest negative reaction, at the three types of extracts, has been with the brome grass, while the most resistant species was that of wheat.

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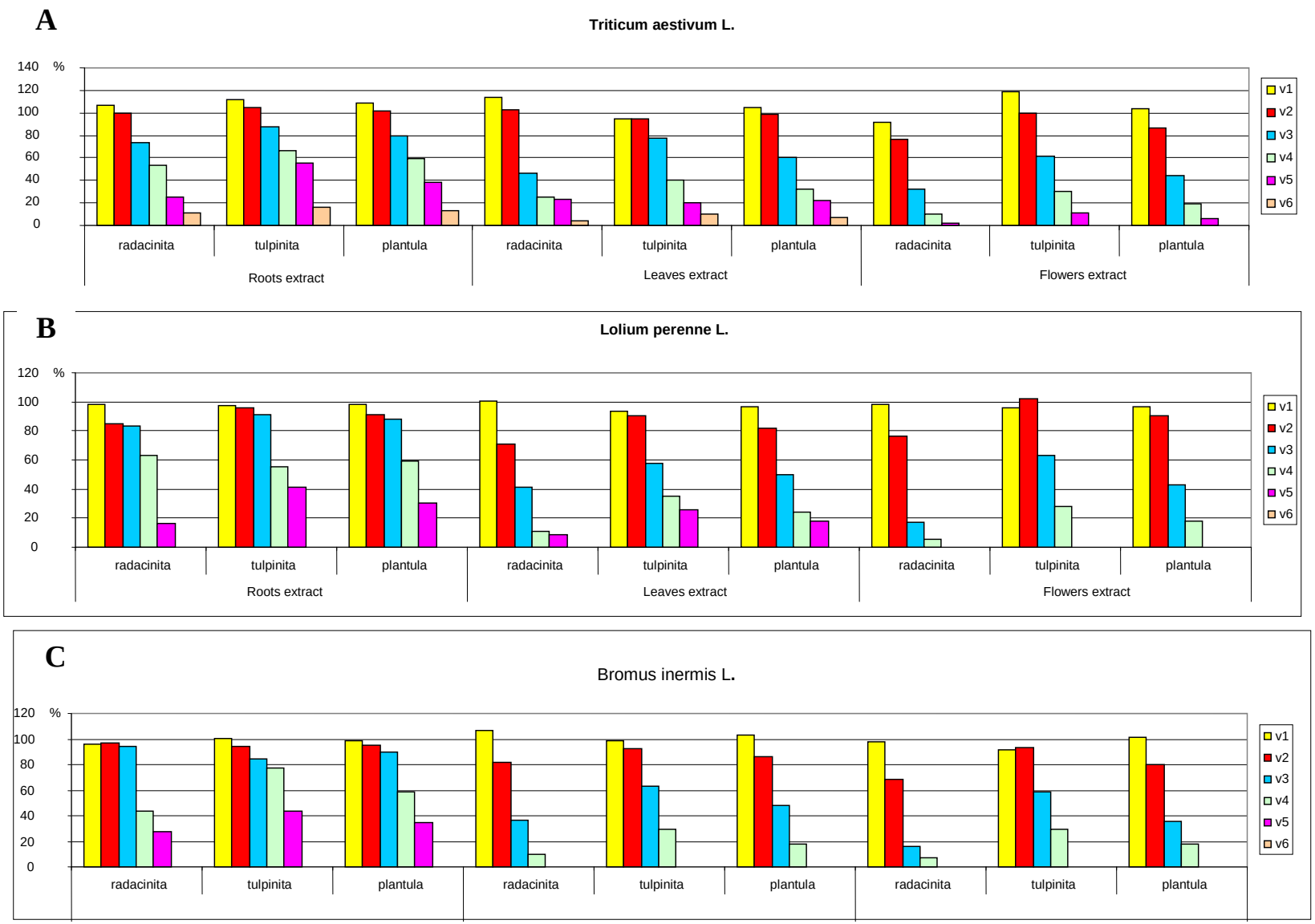

Fig. 3 Expressing in percentage values of the growth of the wheat plantlets (*Triticum aestivum* L.) (A) of reygrass (*Lolium perenne* L.) (B), and of brome grass (*Bromus inermis* L.) (C), at *seven days* from placing the caryopses to germinate on a substratum humidified with watery extract prepared of roots, leaves or flowers of white wormwood (*Artemisia absinthium* L.), in concentration of 5% (V_1), 15% (V_2), 25% (V_3), 50% (V_4), 75% (V_5), 100% (V_6), the percentage calculations being made with relation to the values registered at the proof lot, caryopses germinated on filter paper humidified with distilled water, values considered as reference values, respectively of 100% (V_0)

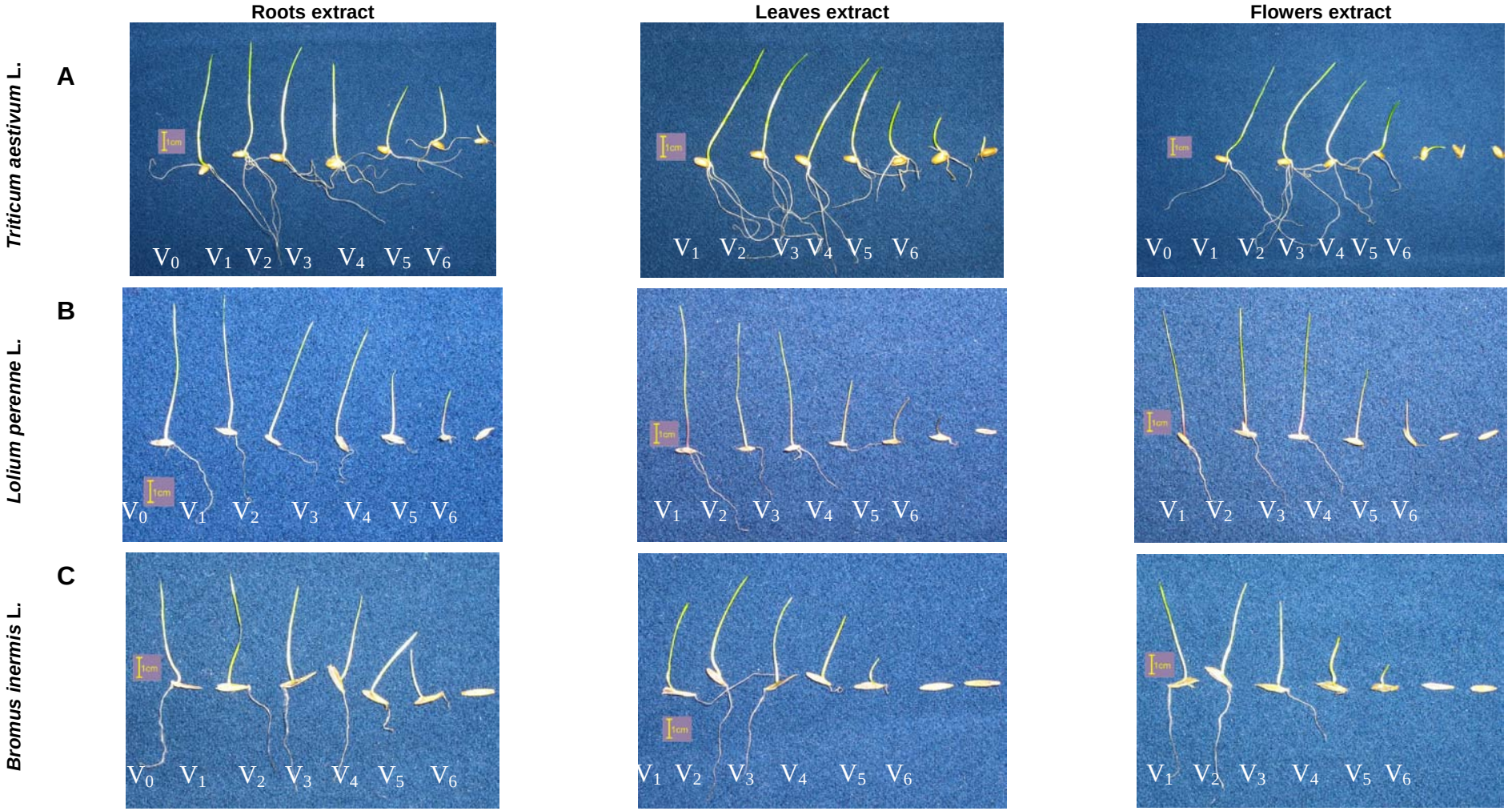

Fig. 4 Comparative aspects regarding the growth of the wheat plantlets (*Triticum aestivum* L.) (A), reygrass (*Lolium perenne* L. (B), and brome grassnearistat (*Bromus inermis* L. (C), under the influence of the watery extract prepared from roots, leaves and flowers of white wormwood (*Artemisia absinthium* L.), used in concentrations of 5% (V_1), 15% (V_2), 25% (V_3), 50% (V_4), 75% (V_5), 100% (V_6), *at seven days* from placing the cariopses to germinate; the proof lot reference - considered - 100% - consisted of the substratum moistured with distilled water (V_0).