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THE INFLUENCE OF TWO TYPES OF EXTRACTS OBTAINED FROM *TAGETES ERECTA* FLOWERS ON RADISH SEEDS

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Abstract

This research paper aimed to evaluate the influence of two types of extracts from the flowers of Tagetes erecta on radish seeds. The extracts were obtained in two organic solvents, ethanol 70% and propylene glycol - PG-50%. Studies were carried out on the phenolic profile, dry matter and antioxidant activity by the DPPH method. The results highlighted that the extract in ethanol had a high concentration only in the total content of flavonoids (6,574 RE mg/mL) compared to the extract in PG (5,111 RE mg/mL) and slightly higher content in polyphenols was found in the case of the PG extract variant. The extract in ethanol 70% registered a higher redox potential (EC50-0.65µl/ml extract). The monitoring of the effect of the extracts was carried out by applying the radish seed germination bioassay. Thereby, the extracts in ethanol showed moderately phytotoxic activity at 0.50% concentration (Gi<80%) and strongly phytotoxic at 1.50% (Gi<50%), and in the case of the extract in PG, they showed moderately phytotoxic activity at 0.50% concentration and a stimulatory effect at 1.50% concentration (Gi>100%).

Key words*: germination bioassay, Tagetes erecta, biostimulants.*

INTRODUCTION

Recently, the use of products based on biologically active substances in the agricultural and horticultural industry (Rodino et al., 2015), to the detriment of conventional fertilizers and pesticides, has seen a significant increase due to the growing awareness of the population regarding their benefits on plants and the environment (Seufert et al., 2012). Some plants, due to the nature of their composition, show resistance to the attacks of insects and non-beneficial microorganisms. This resistance manifests itself through different processes, including the modification of the biology of pests, a process also called antibiosis (Koch et al., 2016; Fabrick et al., 2020). Besides the plants whose active substances have been exploited to develop products for plant protection, we can mention neem oil, *Chrysanthemum cinerariaefolium*, *Derris elliptica*, *Nicotiana*, and *Ryania* (Kandar, 2015). *Tagetes* species show increasing interest from researchers due to the rich composition of phytochemical compounds with antioxidant properties. *Tagetes erecta* L. is an annual or perennial plant, mostly herbaceous, which is part of the sunflower family (*Asteraceae*), known as an ornamental plant, which presents itself in the form of yellow or intense orange flowers, originally from Mexico (Lokesh et al., 2015). The studies carried out (Buse Dragomir & Nicolae, 2013; Xu et al., 2011) indicate that the *Tagetes erecta* species exhibits nematicidal, fungicidal, repellent and insecticidal activity, and the roots were used in agriculture for the nematicidal activity that persists for a long period.

According to a study carried out by Devika and Justin (2014), regarding the identification of

phytochemical compounds by the GC-MS analysis method, on two types of *Tagetes erecta* methanolic extracts (flowers and leaves), it was highlighted that in the *Tagetes erecta* flower extract, were identified 31 phytochemical compounds, and 19 phytochemical compounds were recorded in the extract obtained from the leaves. In leaves of *Tagetes erecta*, were found phenolic compounds with strong antioxidant activities such as caffeic acid, p-coumaric acid and quercetin (Mir et al., 2023). Quercetin (Grajek et al., 2015) shows efficacy against a wide spectrum of bacteria, being effective on both Gram-positive and Gram-negative bacteria. It acts (Wang et al., 2018) by destroying the membrane walls and at the same time inhibits their development. On the other hand, *Tagetes erecta* flowers are rich in flavonoid compounds, including patulitrin, which has a strong antimicrobial activity, especially on pathogens (Mekvimol et al., 2020). In recent years, in agricultural management, more and more products based on natural substances are used for the protection of plants, through the method of cultivation by rotation, this also has applicability in the field of horticulture (Santos et. al., 2015). Thus, this study aims to evaluate the phytostimulant or phytotoxic potential (allelopathic activity of some phytochemical compounds), of two types of extracts obtained from *Tagetes erecta* flowers, in different organic solvents (PG 50% and ethanol 70%), by maceration at room temperature, by applying the germination biotest on radish seeds.

According to Kulbat (2016), some phenolic compounds, such as gallic acid and p-coumaric acid, can exhibit an allelopathic effect on plants. The seed germination bioassay is used, in general, to determine the presence of some phytotoxic or phytostimulant compounds present in the composition of an extract, but also to test its salinity level (Zucconi et al., 1985).

At the same time, were carried out studies to quantify the total compounds of polyphenols and flavonoids, to determine the dry substance from the extracts, as well as the antioxidant activity by the DPPH method.

MATERIALS AND METHODS

Plant material and method of obtaining two extract variants

The dried and ground flowers of the organic culture (*Tagetes erecta*) originating from Bulgaria, were purchased from the trade. To prepare the extracts, the *Tagetes erecta* flowers were washed with purified water and dried in an incubator at a temperature of approximately 40ºC. Were prepared two variants of the extract from marigold flowers (*Tagetes erecta*), in propylene glycol (PG) 50% (DOW Europe Germany, supplier: Medchim) and in ethanol 70% (absolute ethyl alcohol, CHIMREACTIV, Romania) with a ratio of extraction 1:20 (w/v) (Perisoara et al., 2023). The extracts were obtained by maceration in the dark, at room temperature, for 21 days and occasional stirring. After filtering, two extracts of *Tagetes erecta* flowers with fluid consistency of reddish-brown color were obtained (in ethanol 70% and PG 50%). Extracts were stored in the refrigerator (4°C) until future studies and analyses.

Quantitative phytochemical screening

Determination of Phenol Content (TPC)

The method used to quantify the total polyphenol content (TPC) was after the one reported by Sidhu and Saxena (2017). The principle of the method consists of the ability of polyphenols to reduce the Folin-Ciocalteu reagent in the alkaline environment to blue-green carbon oxides with maximum absorption at 765 nm. Thus, 10 µl and respectively 5 µl of the sample were taken, over which was added separately, an amount of methanol required for a total volume of 0.2 mL, followed by the addition of 2.5 mL of Folin-Ciocalteu reagent (diluted with distilled water in a ratio of 1:10 v/v). The mixture was left to incubate for 10 min., at room temperature and protected from light, after which 2 mL of Na_2CO_3 reagent (20%) was added. After incubation at room temperature for 120 min, the absorbance values were recorded at the wavelength of 765 nm. Absorbance was read using a PerkinElmer Lambda 25 UV-VIS spectrophotometer. The concentration of polyphenols was calculated from the calibration curve recorded with caffeic

acid in the concentration range of 1-11 µg/mL (R^2 = 0.9974). For the method described, two determinations were performed for each sample and reported as means. The results were expressed in caffeic acid equivalents (CAE) mg/mL test sample extract. All the reagents used (caffeic acid, Folin-Ciocalteu reagent, Na_2CO_3 20% and methanol), were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Determination of Flavonoid Content (TFC)

The colorimetric method based on the property of flavones to react with aluminium chloride in a potassium acetate medium was used to determine the flavone content. Following the reaction, a stable, yellow-chelated complex is formed, whose spectrum shows an absorption maximum in the range of 370-450 nm. The method used to identify TFC was that reported by Cha-Chi et al. (2002), with minor modifications. Rutin was used as a reference reagent to obtain the calibration curve. Thus, amounts of 10 µl and respectively 5 µl of the sample were taken, over which were added 1.5 mL of methanol, 0.1 mL of aluminium chloride (10%), 0.1 mL of 1M potassium acetate and a quantity of distilled water necessary for a total mixture volume of 2.9 mL. After a gentle stirring, the resulting mixture was left to stand for 30 min in the dark, after which the absorbance was read at 415 nm. The absorbance was read using the PerkinElmer Lambda 25 UV-VIS spectrophotometer. The concentration of flavones from the extracts was determined by extrapolation from the calibration curve with rutin, having a dose range of 5-50 µg/mL (R^2 = 0.9996). The samples were processed in duplicate and the results were expressed in rutin equivalents (RE) mg/1 mL sample-extract test. The reagents used rutin aluminium chloride and potassium acetate, were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Evaluation of antiradical capacity

Blank sample: DPPH standard (0.04%) diluted in methanol (2,2-Diphenyl-1-picrylhydrazil) (Sigma-Aldrich Chemie GmbH, Germany) was used. From the obtained standard solution, a sample of 1 mL of the solution was taken, which was mixed with 0.5 mL of methanol (Honeywell, France), then 0.5 mL of distilled water was added, and it was left to rest for 20 min., at room temperature and protected from light. After incubation, the absorbance values at 520 nm were recorded. The extract samples taken in the work were subjected to 1:10 dilution with distilled water. From the diluted extract samples, the following volumes were taken: 1.5 µl, 2 µl, 2.5 µl, 3 µl, 6 µl, 9 µl, 12 µl (in the case of the Fenugreek extract conditioned in PG 50%) and 3 µl, 6 µl, 9 µl, 12 µl, 15 µl (in the case of Fenugreek extract in 70% ethanol) and mixed separately with 500 µl of methanol, 1,000 µl of DPPH reagent (0.04%), over which was added an amount of water required for a total sample volume of 2,000 µl. The samples were left to incubate at room temperature, protected from light, for 20 min. After incubation, the absorbance values were recorded at 520 nm. The absorbance was read using the PerkinElmer Lambda 25 UV-VIS spectrometer equipped with a sample thermostatting system. The whole experiment was worked in triplicate and the results were reported as means. The results are presented as % inhibition as a function of the Log of the applied dose, thus calculating the effective concentration, EC_{50}.

Determination of dry matter

To determine the dry substance from the extracts taken in the study, the gravimetric method SR 7487 - Determination of extractable substances with solvents was used, undergoing slight modifications. This consists of evaluating the amount of product remaining after removing volatile compounds after applying a heat treatment (105°C). Used heat-resistant weighing ampoules, which were dried in an oven at 105°C and cooled in a desiccator at 25°C. This drying-cooling process is carried out in three cycles, the ampoule being weighed at the end of each cycle. At the end, the three weighings are averaged and reported as the final mass (m1) of the weighing vial. An amount of 20 mL of each extract sample is taken and added to the thermostatic water bath, at 95°C, to remove the solvent. Process followed by one hour of drying the two samples in the oven at 105°C. Finally, the samples are cooled in a desiccator for 30 min and weighed, noting the mass m2. The dry matter is determined according to the formula:

(1) [(m2 – m1)] / V x 1000 x 1000 mg/l,

- m1 - mass of the empty capsule, in grams,
- m2 - mass of capsule with residue, in grams,
- V - the volume of the sample (ml).

Seed Germination Bioassay

The purpose of the study was to evaluate the behaviour of *Tagetes erecta* flower extracts in ethanol 70% and propylene glycol 50% on radish seeds. The method used in the radish seed germination study was modified and adapted according to the procedure developed by researchers Miteluț and Popa (2011), Ghayal et al. (2018) and Cristea et al. (2024). The two extracts were studied at concentrations of 0.10%, 0.50%, 1.00% and 1.50%, dilutions were made with distilled water, and the results were reported to the control sample (distilled water); the same dilutions were made for the solvents too (prolylene glycol 50% and ethanol 70%). Before the start of the study, Petri dishes - Corning Petri dishes, with diameter 100 mm and 200 mm, were disinfected with 70% isopropyl alcohol (Contec IPA) and allowed to dry for 24 h. The filter paper - IDL GMBH, blue type, with a diameter of 90 mm, was sterilized in a hood, equipped with a UV lamp for 30 min. on each side. Radish seeds were washed with purified water and dried in an oven at approximately 30°C. After the end of the sterilization process, filter papers were placed in the Petri dishes, on top of which 10 radish seeds of similar size were added and 5 mL of the sample obtained after the dilutions were pipetted. In the case of the control sample, the method is similar, replacing the 5 mL of the sample with 5 mL of distilled water. The plates were deposited in the incubator for 5 days, at a controlled temperature (25°C ± 1°C). The experiment was done in duplicate for all samples. In the end, the germinated seeds were counted - G and the length of the roots - L were measured. The obtained results were processed to determine the germination percentage (GP), the relative seed germination index - RSG, the relative root growth index- RRG and the germination index - Gi, using the following calculation formulas (Yuan et al., 2018):

(2) GP - $\frac{\text{Number of germinated seeds}}{\text{Total number of seeds}}$ / x 100%;

(3) RSG - $\frac{\text{Number of germinated seeds in the sample}}{\text{Number of germinated seeds in the control}}$ x 100%;

(4) RRG - $\frac{\text{Mean root length of germinated seeds in the sample}}{\text{Mean root length of germinated seeds in the control}}$ x 100%;

(5) $Gi = {}^{G}/_{G0} \times {}^{L}/_{L0} \times 100$, where,

- G represents the number of germinated seeds on the sample substrate,
- G_0 represents the number of germinated seeds in the control,
- L represents the mean length of plant roots per substrate sample,
- L_0 represents the mean length of the plant roots on the control substrate.

From the specialized data (Emino et al., 2004; Ravindran et al., 2017), the Gi value may indicate a phytostimulant or phytotoxic effect of the extracts on the studied seeds. The germination index values are determined by interpreting and processing the data obtained regarding the relative root growth index and the relative seed germination index. Obtaining a Gi value lower than 50% reveals a strong phytotoxic activity of the extract, a Gi value as close as possible to 0% highlights extreme phytotoxicity of the extract, a Gi value between 50-80% highlights moderate phytotoxic activity, a Gi value between 80-100% reveals an extract with non-phytotoxic activity on the plant, and a Gi value over 100% highlights a product with phytostimulating properties. The study ended when more than 65% of the seeds in the control sample had germinated and/or developed roots at least 20 mm long (EPA, 1996; Alamri et al., 2018).

Statistical analysis

The germination, TPC and TFC studies were performed in duplicate and the antioxidant activity study was performed in triplicate, and the results being reported as mean, standard deviation (±) and relative standard deviation (RSD) were calculated. The statistical analysis was performed using Microsoft Excel 2019. The results were interpreted using the t-test: Paired Two Sample For Means to compare the phytostimulation activity or phytotoxicity of the studied extracts. The *p-value*<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSIONS

Quantitative phytochemical screening

In the analysis to determine the content of polyphenols, the Folin-Ciocalteu method was used, and the results were expressed in caffeic acid equivalents (CAE). According to the results obtained (Table 1) regarding the total polyphenol content (TPC) of the two variants of *Tagetes erecta* flower extract, it is highlighted that the extract in PG 50% recorded a slightly higher amount of polyphenols (4,815 CAE mg/ mL), compared to the extract variant in 70% ethanol (4,549 CAE mg/mL). We can say that a better yield regarding the content of polyphenolic compounds was obtained in the case of the PG extract variant. However, some studies reveal the fact that the yield regarding the amount of phytochemical compounds obtained in an extract depends to a large extent on the amount of water used in the extractive mixture. However, in a study carried out by Gong et al. (2011), regarding the chemical composition of some extracts in ethanol (at different concentrations) and water, from *Tagetes erecta*, it was found that the highest concentration of polyphenols was obtained in the case of the extract variant in ethyl alcohol/water (7:3 v/v), 62.33 ± 1.81 GAE/g, and the lowest concentration was obtained in the case of the aqueous extract version, 8.50 ± 0.10 GAE/g. Also, Burlec et al. (2022), following their study on the extracts obtained from *Rudbeckia hirta* and *Tagetes erecta* in different solvents (water, ethanol and ethyl acetate), reported a higher concentration of polyphenols in the case of ethanolic extracts, in both plant species studied. While the extracts in ethyl acetate registered the lowest concentration level in polyphenols, in both types of plants. According to the studies carried out by the researcher Yilmaz et al. (2006), solvent mixtures composed of water and polar alcohols (ethanol, methanol) are more effective regarding the extraction of a higher amount of phytochemical compounds with antioxidant activity, compared to the use of a single solvent. Polyphenols in general exhibit different degrees of polarity, and the selection of a solvent with a polarity as close as possible to what is desired to be extracted, is the key element in ensuring an efficient extraction (Kaczorová et al., 2021). At the same time, polar solvents are effective in extracting a wide range of polyphenols, due to their ability to interact with functional polar groups (Rivas-García et al., 2024).

The content of flavonoids (TFC) was determined by the spectrophotometric method, being in the range of 5,111 RE mg/mL – 6,574 RE mg/mL. The highest value was recorded in the case of the ethanolic extract. Kushwaha et al. (2020), reported a higher concentration of both polyphenols and flavonoids in the methanolic extracts of *Tagetes patula* (of different varieties), compared to the aqueous extracts. Another reference study is the one carried out by Siddhu and Saxena (2017), on *Tagetes erecta* extracts obtained in chloroform, ethyl acetate and methanol, where the highest concentration in flavonoids was observed in the case of the extract variant in methanol (13.43 ± 0.43 mg RE/g extract), and the lowest concentration in flavonoids (7.057 ± 0.66 mg RE/g extract) was seen in the case of the extract variant in chloroform.

Flavonoid compounds are characterized by the property of chelating both metallic copper ions (Cu_{2+}) and iron ions (Fe_{2+}), which contributes to their antioxidant activity. Also, with the help of hydroxyl groups or carbon groups present in the mixture form stable complexes with metal ions (Burlec et al., 2021). As a result of our study, it was observed a higher concentration of flavonoids, compared to the amount of polyphenols, a fact observed in both variants of Marigold (*Tagetes erecta*) extracts studied. Contrary to the results obtained in our study, the specialized literature (Norziah et al., 2015), reveals the fact that polar solvents (methanol and ethanol) can extract phenolic compounds with large molecular size (polyphenols), and aqueous extraction systems better extract flavonoids (phenolic compounds with small molecular size). An observation regarding the differences in the content of polyphenols and flavonoids in the two extracts obtained from *Tagetes erecta* flowers could be given by the polarity of the solvent system used, but also by the variety of phytochemical compounds and the complexity of the composition of the plant (Kowalczyk et al., 2013; Rivas-García et al., 2024).

Table 1. The results of TPC, TFC and antioxidant activity

Sample	TPC (CAE) mg/mL	TFC (RE) mg/mL
Tagetes erecta flower extract in PG 50%	4,815	5,111
Tagetes erecta flower extract in ethanol 70%	4,549	6,574

Evaluation of antiradical capacity

The antioxidant capacity of the two variants of *Tagetes erecta* flower extract was determined by the DPPH spectrophotometric method. The method is characterized by the discolouration of the stable free radical DPPH, by the delocalization of an electronic charge, which manifests itself on the entire surface of a molecule and the measurement of the absorbance at the wavelength of (λ) 520 nm. This is directly proportional to reduced free radicals in the mixture (solution). Antiradical activity is defined by the number of antioxidant substances useful to reduce the initial DPPH concentration by half (50%) and is defined as the effective concentration (EC 50). The results are presented in Table 2, as % of inhibition as a function of the Log of the applied dose, thus also calculating the effective concentration (EC 50). According to the obtained results, the ethanolic extract recorded the most pronounced antioxidant response (EC 50-0.65 µl/mL extract). Ciobanu and his colleagues (2021), highlighted in their study of three variants of propolis, that the most pronounced antiradical activity was obtained in the case of the extract variant with the highest concentration of phenolic compounds present in the mixture. According to the data from the specialized literature (Selma et al., 2014), the extracts that present in their composition a higher concentration of phenolic compounds (polyphenols or flavonoids) will present a higher antioxidant activity. This is also confirmed in our study, where the extract from *Tagetes erecta* flowers in ethanol had a higher concentration of flavonoids, compared to the extract obtained in propylene glycol.

Xu et al. (2015), conducting a study on the antioxidant activity of some *Tagetes erecta* flower extracts obtained at different temperatures (subcritical water extraction SWE), found that the extracts that had in their composition a higher concentration of phenolic compounds showed higher antiradical activity. There are studies in the specialized literature (Leopoldini et al., 2004) where it was highlighted that gallic acid is one of the most active phenolic compounds regarding the transfer of hydrogen atoms and electrons. At the same time Rice-Evans et al. (1996), support these studies by demonstrating the effect of the chemical structure of phenolic compounds on antiradical activity.

Table 2. Antioxidant activity (DPPH) of *Tagetes erecta* flower extracts

Sample	DPPH		
	The regression equation	The regression coefficient	EC 50 µl/ml extract
Tagetes erecta flower extract in PG 50%	y = 74.02x - 10.774	R^2= 0.9755	0.75
Tagetes erecta flower extract in ethanol 70%	y = 74.479x - 10.286	R^2= 0.9828	0.65

Determination of dry matter

Table 3 highlights the results obtained regarding the determination of the dry substance (residue) from the two variants of *Tagetes erecta* extract, where the highest amount of dry matter was obtained in the case of the extract obtained in propylene glycol (25,920 mg/L). If we were to correlate with the results previously obtained, the *Tagetes erecta* flower extract in PG 50%, had a slightly higher amount of TPC, compared to the studied ethanolic extract. But a point that must be taken into account would be the nature of the solvent in which the extraction was done. Propylene glycol has a higher molecular weight, compared to the ethyl alcohol used in the extraction process of the second variant of the extract. A study reported by Perisoara et al. (2023), regarding the determination of the dry matter of three extracts from *Foenum-graecum* seeds in various solvents (ethanol 40%, propylene glycol 50% and ethanol 70%), showed that the extract richest in phenolic compounds (ethanol 40%) has the largest amount of dry matter, followed by the extract in propylene glycol 50% and the one in ethanol 70%. These results are directly proportional to the amount of phenolic compounds, both flavonoids and polyphenols, found in each of the three variants.

Table 3. Results regarding the final residue obtained from the two variants of *Tagetes erecta* extract

Sample	m1 (g)	m2 (g)	The result obtained (mg)	Sample volume (mL)	The final result (mg/L)
Tagetes erecta flower extract in PG 50%	24.5854	25.1038	5,184	20.00	25,920
Tagetes erecta flower extract in ethanol 70%	21.3445	21.7371	3,926	20.00	19,630

Seed Germination Bioassay

The seeds can absorb various substances from the treated environment, be they nutrients or substances with an allelopathic effect, and this is best reflected in the way the roots develop and grow (Ravindran et al., 2017; Baca et al., 1990; Oncel et al., 2000; Araujo & Montero, 2005). The effect on some plants, whether stimulating or phytotoxic, is indicated by the value of the germination index (Gi). This is determined by quantifying the values obtained in the case of the germination index (RSG) and the relative growth of the roots (RRG) (Ravindran et al., 2017). In the current study, the effect of the two variants of *Tagetes erecta* extract (ethanol 70% and PG 50%) at different concentrations on radish seeds was compared, with the solvents used in the extraction process. This was done to reveal the potential stimulating or phytotoxic effect of the obtained extracts.

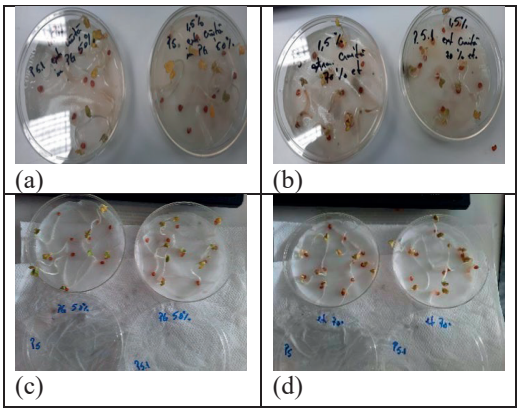

Figure 1. Seedling growth germination bioassay of radish seeds: (a) 1.50% *Tagetes erecta* in PG 50%; (b) 1.50% *Tagetes erecta* in ethanol 70%; (c) 1.50% ethanol 70%; (d) 1.50% propylene glycol 50%

Interpretation of the germination percentage (GP%) was achieved by reporting the number of germinated seeds from the sample/control to the total number of seeds tested (Luo Y. et al., 2017). The germination percentage (GP%), obtained after treating the radish seeds with the two variants of *Tagetes erecta* extract, but also with its solvents, at concentrations of 0.10%, 0.50%, 1.00% and 1.50%, fell between 90-100%. As can be seen from Table 4, the lowest values were observed in the case of *Tagetes erecta* extract in 70% ethanol, at concentrations of 0.10%, and 0.50%. However, in the case of treating radish seeds with the PG 50% solvent, a lower percentage (90%) was observed in the case of the positive control (distilled water). If we were to compare the results obtained in the case of *Tagetes erecta* extract in 70% ethanol with the solvent, it can be observed that in the case of the solvent, the seeds had a germination percentage of 100% at all concentrations studied. Regarding the extract of *Tagetes erecta* in PG 50%, it obtained a germination percentage of 100% at all concentrations compared to the tested solvent. The latter registered GP values (%) below 100% at concentrations of 1.00% and 1.50% (GP - 95%).

Table 4. Germination bioassay results - germination percentage (GP)

Sample	% sample/control				
	0.10	0.50	1.00	1.50	Martor
Tagetes erecta flower extract in PG 50%	100% ± 5.50 RSD = 57.97	100% ± 0 .00 RSD = 0.00	100% ± 0.00 RSD=0 .00	100% ± 0.00 RSD = 0.00	100% ± 0.00 RSD = 0.00
Tagetes erecta flower extract in ethanol 70%	90% ± 1.40 RSD = 15.71	90% ± 0.00 RSD = 0.00	100% ± 0.00 RSD = 0.00	95% ± 0.00 RSD = 0.00	95% ± 0.71 RSD = 7,44
Ethanol 70%	100% ± 0.00 RSD = 0.00	100% ± 0.00 RSD= 0.00	100% ± 0.00 RSD =0.00	100% ± 0.00 RSD = 0.00	100% ± 0.00 RSD = 0.00
Propylene glycol 50%	100% ± 0.00 RSD = 0.00	100% ± 0,70 RSD= 7.44	95% ± 0.70 RSD = 7.44	95% ± 0.00 RSD = 0.00	90% ± 0.00 RSD = 0.00

RSG values (%) were obtained by reporting the number of germinated seeds from the extract sample to the number of germinated seeds from the control sample (Cristea et al., 2024). In the case of the results obtained for the relative germination index - RSG, they were: 90-100% for *Tagetes erecta* extract in PG 50%, similar results being observed in the solvent. However, at different concentrations. In the extract, we can observe RSG values of 100% at concentrations of 1.00% and 1.50%. In the case of the solvent, the RSG values decrease directly in proportion to the increase in the dose of the

test sample. For the 70% ethanolic extract, the results were between 94.73-105.26%. The highest value was recorded at the concentration of 1.00% extract (statistically insignificant compared to the solvent *(p>0.05)*). The solvent recorded values of 100% at all tested doses. Following the results obtained, we can see that the relative germination index is improved compared to both the solvent and the positive control (distilled water), in both extract variants, being closely related to the concentration of phenolic compounds found in each extract. This was also observed by Perisoara et al. (2022), where they highlighted, following the germination study of *Tagetes erecta* extract in 40% ethanol on radish and cucumber seeds, the fact that the extract had a positive, statistically significant influence on radish and cucumber seeds, compared to the positive control and the solvent, at all tested concentrations.

Table 5. Germination biotest results - relative germination index (RSG)

Sample	% sample/control				
	0.10	0.50	1.00	1.50	Martor
Tagetes erecta flower extract in PG 50%	95%	90%	100%	100 %	100 ±0.00
Tagetes erecta flower extract in ethanol 70%	94.73 %	94.7%	105.20 %	94.73%	95± 0.71
Ethanol 70%	100%	100%	100%	100%	100± 0.00
Propylene glycol 50%	100%	95%	95%	90%	90± 0.00

The data on the relative growth index of the roots were obtained by reporting the average of the roots developed in the tested extract sample to the average of the length of the roots in the control sample. The values obtained regarding the RRG% index fell within 33.74-103.23%. The highest values were recorded in the case of the ethanolic extract of *Tagetes erecta* (103.70%), at the concentration of 0.10%, followed by the extract in PG (103.23%), at the highest concentration tested (1.50%). After treating the radish seeds with the two extract variants, the RRG index was improved (Table 6), compared to both the control and the solvent. Thus, the *Tagetes erecta* extract in PG compared to the solvent (PG), recorded higher values at all tested doses. This is due to the beneficial effect shown by the phenolic compounds present in the composition of the extract. Regarding the ethanolic extract, compared to the solvent (ethanol 70%), the RRG values were improved only at the concentrations of 0.10% and 1.00%. This could be explained by the presence of a higher concentration of flavonoids in the composition of the extract, showing an allelopathic effect on the plant at the concentrations of 0.50% and 1.50%. Following the germination study conducted by Buse Dragomir & Nicolae (2013), of the extract obtained from the roots of *Tagetes erecta* on the seeds of *Brassica oleracea* (var. *capitata* and var. *botrytis*), tested at different concentrations (2.00%, 5.00%, 10.00 and 20.00%) it was found that the length of the roots decreased significantly compared to the control, directly proportional to the increase in the dose of the tested extract.

Table 6. The results of the germination bioassay - the relative root growth index a (RRG)

Sample	% sample/control				
	0.10	0.50	1.00	1.50	Martor
Tagetes erecta flower extract in PG 50%	*86.3% (6.95 cm ± 0.33 RSD = 4.88)	86.0% (6.92 cm ± 1.46 RSD = 21.13)	92.10% (7.4 cm ± 0.43 RSD =5.91)	103.2% (8.30 ± 1.57 RSD = 18.98)	**8.0 cm ± 2.41 RSD = 29.97
Tagetes erecta flower extract in ethanol 70%	103.7% (4.9 cm ± 4.05 RSD =82.83)	67.5% (3.19 cm ± 2.71 RSD = 85.11)	94.49% (4.46 cm ± 3,11 RSD = 69,83)	51.64% (2.44 cm ± 0.42 RSD = 17,38)	4.72 cm ± 5.22 RSD = 7.44
Ethanol 70%	88.56% (5.77 cm ± 2.71 RSD = 8.08)	82.9% (5.40 cm ± 1.32 RSD = 24.46)	*77.9% (5.08 cm ± 0.86 RSD = 16.98)	56.63% (3.69 cm ± 2.85 RSD = 0.42 77.41)	6.51 cm ± 0.84 RSD = 12,91
Propylene glycol 50%	74.13% (6.35 cm ± .48 RSD = 7.57)	72.7% (6.23 cm ± 3.59 RSD = 57.65	33.74% (2.89 cm ± 2.04 RSD = 70.71	62.46% (5.35 cm ± 0.93 RSD = 17.44	8.56 cm ± 1.01 RSD = 11.85

*statistically significant compared to control (p>0.05).
**Average root length of germinated seeds expressed in cm

Gi values (%) were obtained by combining the results obtained in the case of RSG and RRG (Sobarzo-Bernal et al., 2021). In Italian legislation, the germination index is included in the list of quality assurance regulations regarding the commercialization of composts (Cesaro et al., 2015). According to the results highlighted in Table 7, the values of Gi (%), fell between 32.05-103.23%. Thus, it can be observed that the extract of *Tagetes erecta* in PG 5% showed a phytostimulant effect (Gi - 103.23%) at the maximum tested concentration (1.50%). At the same time, it has a moderate phytotoxic effect at a concentration of 0.50%

(Gi - 77.47%), registering a Gi value (%) below 80%. Compared to the solvent, Gi values (%) were significantly improved at all doses studied. In the case of *Tagetes erecta* extract in ethanol 70%, a non-phytotoxic effect was observed at 0.10 and 1.00% concentrations, recording values of Gi (%) close to 100% (98.24 and 99.47%, respectively). The ethanolic extract showed a strong phytotoxic effect at 1.50% concentration (Gi - 48.92%). Such an effect was also found in the case of the PG 50% solvent, at a concentration of 1% (Gi - 32.05%), recording a Gi value (%) below 50%. The study reported by Mavi (2014) regarding the treatment of aged seeds of eggplant (*Solanum melongena* L.) with two types of extract obtained from the flowers of *Tagetes erecta* and *Tagetes patula*, to improve the germination rate and the way the seedlings develop, it was observed that the percentage of germination was statistically improved compared to the control and the Hydropriming method, for both types of extract (GP - 82 and 80%, respectively). From the obtained data, a close connection can be observed between the composition of phenolic compounds, the antioxidant activity and the results obtained in terms of the germination study of extracts from radish seeds. The allelopathic effect can be given by the high concentration of phenolic compounds and most of them are reported as phytotoxic compounds (El-Gawad et al., 2015; Elshamy et al., 2019). According to the data in the literature (Li et al., 2010; Anwar et al., 2021), the phytotoxic effect manifests itself through different processes, namely, by the inhibition of respiration, the absorption capacity of nutrients, but at the same time, it can affect the process and activities of the enzyme-linked receptors.

The ethanolic extract of *Tagetes erecta*, presented a higher concentration of flavonoids, and this was found in the manifestation of a more pronounced antioxidant activity, but also a strong phytotoxic effect. Similar results were observed by Santos et al. (2015) studying the phytotoxic activity of two *Tagetes* species (*Tagetes erecta* and *Tagetes patula*), reporting a higher antioxidant activity at the maximum tested concentration (4,000 $\mu g \cdot mL^{-1}$). The ability to interfere with DPPH radicals, but also the power to reduce Fe_2 ions can be related to the high content of phenolic compounds (flavonoids) (Siriamornpun et al., 2012). Some studies (Huckelhoven & Kogel 2003) have shown that certain phytochemical compounds with antioxidant activity can intervene in the process of plant development and germination. Instead, the extract of *Tagetes erecta* in PG 50%, having a more balanced content of phenolic compounds, led to the manifestation of a phytostimulating effect on the radish seeds. For both extracts, these effects were observed at the highest concentrations tested.

Table 7. Germination biotest results - Germination index Gi

Sample	% sample			
	0.10	**0.50**	**1.00**	**1.50**
Tagetes erecta flower extract in PG 50%	82.06	77.47	92.10	103.23
Tagetes erecta flower extract in ethanol 70%	98.24	63.95	99.47	48.92
Ethanol 70%	88.56	82.96	77.97	56.63
Propylene glycol 50%	74.13	69.10	32.05	56.21

*semnificativ statistitic comparativ cu martor (*p>0.05)*.
**Average root length of germinated seeds expressed in cm.

CONCLUSIONS

Two variants of *Tagetes erecta* flower extract were obtained using different extraction solvents that are part of the category of concentrated alcohols (ethanol 70% and propylene glycol 50%). These extracts were obtained by maceration, without the intervention of any heat treatment. The main objective regarding the use of these types of solvents and the method of obtaining the extracts was to extract compounds in the highest possible quantity, with potential phytostimulant and antimicrobial (fungicide) effect and not to damage potential extractable compounds. This could be proven by the high content of phenolic compounds (polyphenols and flavonoids) obtained in the two extract variants. At the same time, the work offers valuable information regarding the obtaining in an economical and environmentally friendly way of future natural products based on biologically active substances, intended for the protection and phytostimulation of plants.

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