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# The presence of Nickel Sulphate in soil influences the synthesis of flavonoids and phenols in garlic (*Allium sativum* L.), changing its allelopathic potential

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Producing Allelochemicals and secondary metabolites in plants is influenced by heavy metals. The impact of Nickel Sulphate (NiSO4.6 H2O) stress on producing flavonoids and phenols, and also garlic's (Allium sativum L.) allelopathic potential on weeds(*Echinocloa crus-gali* and *Amaranthus retroflexus)* are discussed in this article. For 8 weeks, garlic plants were cultivated in sterile soil polluted with 100 mg/kg Nickel Sulphate. Control plants were cultivated on sterile soil. The plants were then collected, and the bulbs and leaves were dried at 50 ° C before being crushed into powder in a mortar. In sterile petri plates coated with a No. 1 filter paper of wahtman, five ml of garlic powder solutions (0.75 %, 1.5 %, and 2.25 % V/V) were put. Within each petri dish, 25 A.retroflexus seeds and 20 E.crus-gali seeds were put on filter paper and cultured for one week (under the temperature of 25±2 C° in ambient light). E. crus-gali and A. retroflexus seedling growth (root and shoot length) and the percentage of germination were measured. When garlic bulbs were compared to control plants, the content of total flavonoids and phenols increased significantly. In addition, raising the concentration of the solution in the medium enhanced the inhibitory impact of the garlic solution.

**Keywords: Allelopathic potential, phenol, Flavonoid, *Amaranthus retroflexus, Allium sativum , Echinocloa crus-gali*.**

## INTRODUCTION

Weeds decrease crop growth and production by excreting allelochemicals (Yousaf, Zahoor et al. 2013, Gholamin and Khayatnezhad 2020, Bi, Dan et al. 2021), therefore altering ecosystem balance (Wille, Thiele et al. 2013, Jia, Khayatnezhad et al. 2020, Ma, Ji et al. 2021). Though synthetic herbicides have been effectively employed to control weeds in most crop areas throughout the world, constant use of these chemicals may enhance the resistance of weed to these herbicides (Bich and Kato-Noguchi 2012, Huang, Wang et al. 2021, Rodríguez 2021). On the contrary, allelopathic compounds have been shown to be helpful against resistant weeds (Li, Mu et al. 2021, Radmanesh 2021).

Nickel compounds are well-known heavy metals that have an impact on plant growth and development (Seregin and Kozhevnikova 2006). Yet, nickel sulphate in soil has been found to increase the production of secondary metabolites like phenols and flavonoids in garlic (Khayatnezhad and Gholamin 2020, Guo, She et al. 2021, Hewitt 2021, Peng, Khayatnezhad et al. 2021).

Garlic (*Allium sativum* L.) is a common plant across the Mediterranean, including Iran. Allicin, the most significant component in garlic, converts to allinase enzyme when the plant organs are broken or disturbed (Ankri and Mirelman 1999, Ma, Khayatnezhad et al. 2021).

This activity might be beneficial in reducing weeds in farms by using allelopathic approaches. Plants' allelopathic potential rises in response to adverse environmental circumstances such as light and temperature stressors, drought, malnutrition, and soil metal pollution, allowing them to compete and thrive in the ecosystem (Si, Gao et al. 2020, Sun, Lin et al. 2021, Yin, Khayatnezhad et al. 2021).

## MATERIALS AND METHODS

### Preparing soil and planting

The soil utilized in this study was sterilized at 121 C°. For each treatment, four 25 kg soil mesh plastic boxes (30 x 50cm) were randomly assigned. Garlic bulbs were placed in a surface and for 10 minutes sterilized in 5% sodium hypochlorite, before being planted in boxes filled with sterile soil (control) and Nickel-Sulphate-contaminated soil (treatment) at Tarbiat Modares University greenhouse, and for eight weeks exposed to ambient light, humidity and with an average temperature ranging 7-18 C°. Garlic bulbs and Leaves were then dried in a 50 C° oven for 24 hours before being cut and crushed into a powder. To make the aqueous stock extract solutions, 7.5 g of the powder was then soaked in 100 ml of DDW and shaken for 48 hours. DDW was used to create aqueous solutions (0.75 %, 1.5 %, and 2.25 % w/v) from the stock solution, which was then filtered twice using filter papers (whatman no 1). Target seeds were placed on a surface and sterilized in sodium hypochlorite aqueous solution of 5% for 15 minutes before being rinsed 3 times with DDW. 20 E. crus-gali seeds and 20 A. retroflexus seeds were put in triplicate in petri dishes (10 diameter) coated with Whatman no 1 paperEach treatment petri plate received 5 ml of each aqueous extraction. Control plates received sterile DDW. After 7 days, the percentage of seed germination and seedlings growth were measured.

### Total flavonoid measutment

Acidic ethanol with 1% HCl was used for homogenizing 0.1 g of frozen garlic leaves and bulbs, which were centrifuged at 12000×g for 15 minutes and the supernatant was heated for 10 minutes at 80 C°. Sample absorbance was measured at 270, 300, and 330 nm after cooling. Flavnoid content was measured using an extraction coefficient of 33,000 cm-1. M-1 (Gholamin and Khayatnezhad 2020, Kabir, Arefin et al. 2021).

### Total phenol measurement

3 ml of methanol was used to combine and homogenize the samples for phenol extraction. This extraction was combined with 0.1 N Folin-Ciocaltive reagent in a volume of 0.5 ml. when 5 minutes passed, 1 ml 0f 20% $Na_2CO_3$ was inserted into the mix and left at room temperature for 10 minutes. The supernatant absorbance was determined at 730 nm. The results were given in mg of GA / 100 g of extracted materials (Gholamin and Khayatnezhad 2021, Huma, Lin et al. 2021).

### Statistical analysis

The SPSS 16.0 software program was used for analyzing the data, using one-way variance analysis. Duncan's tests were used to perform mean separations. Differences with a p-value of 0.05 were deemed significant.

## RESULTS AND DISCUSSION

Plant bulbs cultivated in soil polluted with Nickel Sulphate had substantially higher flavonoid content than control plants ($P \geq 0.05$)., but not in leaves that showed no change ($P \geq 0.05$) (Fig.1). ww

When plants were cultivated in Nickel Sulphate treated soil, the total phenol content of the leaves was considerably lower, but the total phenol content of the bulbs was higher. ($P \geq 0.05$) (Fig. 1).

### The impact of garlic bulb and leaves aqueous extraction on seed germination and seedling growth in E. crus gali and A. retroflexus

The least germination of the seeds of *E. crus gali* happened in 2.25% aqueous extractions of garlic plants leaf and bulb that was grown in soil polluted with Nickel Sulphate whereas the least germination of *A. retroflexus* occurred in 1.5% and 2.25% (Fig. 2) of solutions. The bulb and leaf extracts' germination percentage varied (Figs 2 and 3). The shoot length of A. retroflexus and E. crus-gali was decreased by an aqueous extraction of garlic leaves cultivated in Nickel Sulphate (Table 1). The least inhibition was observed with 0.75 % and 1.5 % leaf extract, and the highest inhibition was observed with 2.25 % solution. The highest doses of leaf and bulb extracts of garlic plants cultivated in Nickel Sulphate inhibited root development of E.crus-gali and A. retroflexus (P 3 0.05).

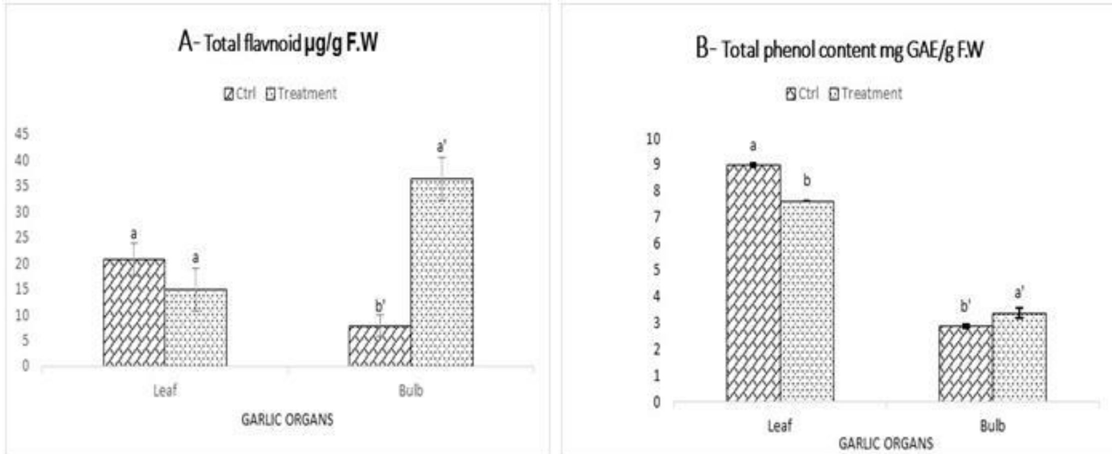


**Figure1 : Effect of Nickel Sulphate ($NiSO_4.6H_2O$) on (A) total Flavnoid and (B) Phenol content of garlic leaf and bulb. Means sharing the same letter in a column do not differ significantly at the 5% according to the Duncan test.**

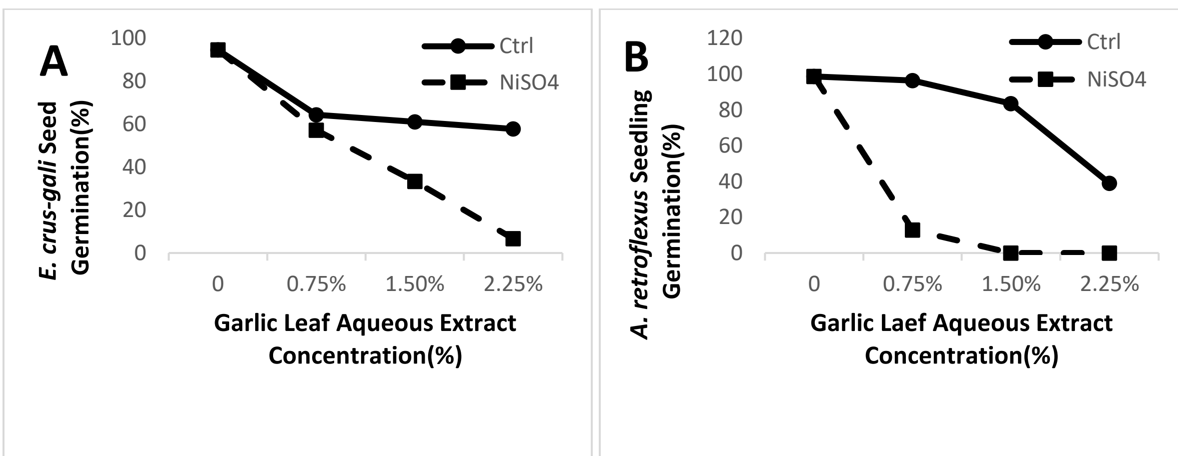


**Figure 2: Percent change in germination of (A) *E. crus-gali* and (B) *A. retroflexus* seeds under leaf extraction of garlics which grown in control and Nickel Sulphate treatment soil.**

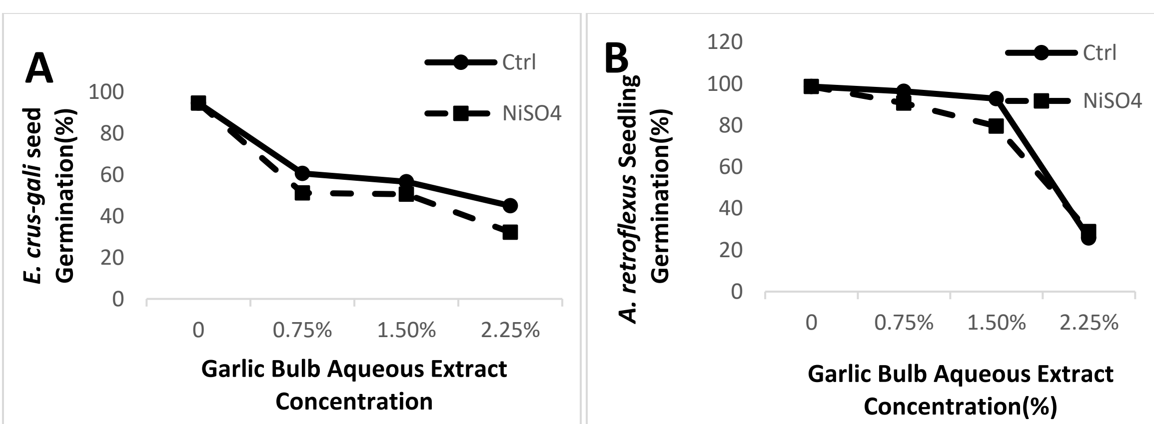


**Figure 3: Percent change in germination of (A) *E. crus-gali* and (B) *A. retroflexus* seeds under bulb extraction of garlics which grown in control and Nickel Sulphate treatment soil.**

**Table 1: Decreased percentage of shoot and root lengths of *E. crus-gali* and *A.retroflexus* seedlings under leaf extraction of garlic grown in sterile and nickel sulphate treatment soil (compare to control).**

| Aqueous extract | Shoot Lenght | | Root Lenght | |
|---|---|---|---|---|
| | *E. crus-gali* | *A.retroflexus* | *E. crus-gali* | *A.retroflexus* |
| **Sterile (0.75%)** | 19.57%[a*] | 15.75%[a] | 22.45%[a] | 28.46%[a] |
| **Sterile (1.5%)** | 39.46%[b] | 24.65%[b] | 70.33%[b] | 61.53%[b] |
| **Sterile (2.25%)** | 60.85%[c] | 70.34%[c] | 83.05%[bc] | 87.69%[cd] |
| **$NiSO_4$ (0.75%)** | 62.46%[c] | 100%[d] | 76.27%[c] | 80%[c] |
| **$NiSO_4$ (1.5%)** | 93.02%[d] | 100%[d] | 94.49%[d] | 100%[d] |
| **$NiSO_4$ (2.25%)** | 95.71%[d] | 100%[d] | 95.76%[d] | 100%[d] |

* Means sharing the same letter in a column do not differ significantly at the 5%, according to the Duncan's test.

**Table 2: Decreased percentage of shoot and root lengths of *E. crus-gali* and *A.retroflexus* seedlings under bulb extraction of garlic grown in sterile and nickel sulphate treatment soil (compare to control).**

| Aqueous extract | Shoot Lenght | | Root Lenght | |
|---|---|---|---|---|
| | *E. crus-gali* | *A.retroflexus* | *E. crus-gali* | *A.retroflexus* |
| **Sterile (0.75%)** | 18.91%[a*] | 22.60%[a] | 56.92%[a] | 28.46%[a] |
| **Sterile (1.5%)** | 56.75%[b] | 47.94%[b] | 72.30%[c] | 61.53%[b] |
| **Sterile (2.25%)** | 87.56%[abc] | 79.45%[c] | 90%[d] | 87.69%[cd] |
| **$NiSO_4$ (0.75%)** | 75.67%[c] | 29.45%[a] | 30.76%[b] | 80%[c] |
| **$NiSO_4$ (1.5%)** | 91.08%[de] | 43.15%[b] | 72.30%[c] | 100%[d] |
| **$NiSO_4$ (2.25%)** | 96.48%[e] | 100%[d] | 92.30%[d] | 100%[d] |

* Means sharing the same letter in a column do not differ significantly at the 5%, according to the Duncan's test.

For example, the shortest shoot length was determined in 2.25 % of garlic bulb aqueous extract where no root or shoot development of A.retroflexus occurred (Tables 1 and 2).

According to the findings, garlic leaves and bulbs' aqueous extraction reduces the seed germination and seedling growth of *E. crus-gali* and *A. retroflexus*, while the inhibition is increased when treated with Nickel Sulphate. Increased inhibition is expected to be associated with increasing concentrations of leaf and bulb extracts (Tables 1 and 2 and Figs 2 and 3) to the extent that the germination percentage of the root and shoot growth of the target weed seeds decreased to zero.

According to Kong, Hu et al. (2002), the allelopathic capacity of plants enhanced under severe and stressful conditions, and allelopathy and stress were positively connected. Furthermore, it has been demonstrated that some secondary metabolites, like flavonoids and phenols, are generated under both natural and stressed circumstances (Gholamin and Khayatnezhad 2020, Karasakal, Khayatnezhad et al. 2020, Xu, Ouyang et al. 2021).

Allelopathic effects of phenolic chemicals impacts the seed germination, plant development, and growth negatively (Khayatnezhad and Gholamin 2020, Cheng, Hong et al. 2021, Tao, Cui et al. 2021, Wang, Shang et al. 2021, Zheng, Zhao et al. 2021, Zhu, Liu et al. 2021). Furthermore, flavonoids and phenols play significant functions in plant interactions, such as signaling pathways involved and regulating allelopathic potentials (Zhu, Saadati et al. 2021).

Djurdjevic, Dinic et al. (2004) investigated the allelopathic potential of *Allium ursinum* on Amaranth, Wheat and lettuce seed and seedling through determining leaf's, bulb's and root's phenolic acid and total phenols. He discovered that aqueous extracts of bulbs had greater inhibitory effects on seedling growth and seed germination of target plants than leaves. He also demonstrated that the phenolic chemicals produced by A. ursinum in soil totally prevented target plant seed germination and seedling growth (Cheng, Hong et al. 2021, Wang, Shang et al. 2021, Zheng, Zhao et al. 2021, Zhu, Liu et al. 2021).

Inducing plant inner system is another reason of increasing allelopatic activity of garlic's aqueous extraction under Nickel Sulphate stress that takes place for protecting themselves against the stress of heavy metal (Ren and Khayatnezhad 2021) notably in leaves rather than bulbs. Plant species may use these chemicals to defend themselves.

Hou, Li et al. (2021), (Huang, Wang et al. 2021) hypothesized that garlic's allelopathic chemicals against microorganisms are connected to allicin compounds that can impede germinating and developing invasive microorganisms.

## CONCLUSION

Increasing garlic bulb's flavonoid and phenol content under the stresses of Nickel Sulphate suggest that phenols and flavnoids metabolism increases garlic's allelopathic potentials in connection with sulphur compounds metabolism, particularly allicin that is the garlic's main secondary metabolite. It is recommended to repeat the experiment in the following years to achieve more reliable results.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## ACKNOWLEGEMENT

This paper was from my own master thesis.

## AUTHOR CONTRIBUTIONS

Sina Mohammadzadeh conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version.



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