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Dark Septate Endophytic Fungi and Thiocyanate Induced Gold Accumulation of *Brassica juncea* and *Amaranthus spinosus* Grown on Gold Mine Tailings

(Kulat Endofit Berseptum Gelap dan Tiosianat Mengaruh Pengumpulan Emas oleh *Amaranthus spinosus* dan *Brassica juncea* yang Ditanam di Sisa Lombong Emas)

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ABSTRACT

Plants can absorb metals, including gold, making them potential as phytomining agents. This study investigated the ability of *Brassica juncea* and *Amaranthus spinosus* inoculated with Dark Septate Endophyte (DSE) fungi and treated with ammonium thiocyanate to accumulate gold (Au) from gold mine tailings. The plants were inoculated with DSE fungi (S14 and S51), referred to as D1 and D2, respectively, and a control group without inoculation (D0). They were grown in four media: Soil (T0), Tailing (T1), Tailing + ammonium thiocyanate 0.62 g/kg (T2), and Tailing + ammonium thiocyanate 1.24 g/kg (T3). Results indicated successful DSE colonisation across treatments, improving root and shoot dry weight, plant height, chlorophyll and carotene contents, and gold uptake. Thiocyanate enhanced gold absorption but caused plant death at high concentrations. *A. spinosus* transported more gold to shoots, while *B. juncea* accumulated more gold in roots. The highest phytomining potential was observed in *B. juncea* inoculated with DSE S14 (D1) in T2 media. These findings highlight the potential of combining plant species, DSE fungi, and chelating agents to optimise phytomining in gold-contaminated sites. DSE fungi not only enhanced gold uptake but also mitigated stress caused by tailings, offering an eco-friendly strategy for metal recovery. Future research should explore scalability and long-term impacts to strengthen phytomining as a sustainable alternative in gold mining reclamation efforts.

Keywords: Amaranth; ammonium thiocyanate; DSE fungus; gold phytomining; phytoremediation

ABSTRAK

Tumbuhan mempunyai keupayaan menyerap logam, termasuk emas, menjadikannya agen fitopelombongan yang berpotensi. Penyelidikan ini mengkaji kemampuan *Brassica juncea* dan *Amaranthus spinosus* yang diinokulasi dengan kulat Endofit Berseptum Gelap (DSE) dan dirawat dengan ammonium tiosianat untuk mengumpulkan emas (Au) daripada sisa lombong emas. Tumbuhan diinokulasi dengan kulat DSE (S14 dan S51) yang dirujuk sebagai D1 dan D2 serta kumpulan kawalan tanpa inokulasi (D0). Tumbuhan ditanam di atas empat media: Tanah (T0), Sisa lombong (T1), Sisa lombong + amonium tiosianat 0.62 g/kg (T2) dan Sisa lombong + amonium tiosianat 1.24 g/kg (T3). Hasil menunjukkan kejayaan kolonisasi DSE dalam semua rawatan, meningkatkan berat kering akar dan pucuk, tinggi tumbuhan, kandungan klorofil dan karotena serta serapan emas. Tiosianat meningkatkan serapan emas tetapi menyebabkan kematian tumbuhan pada kepekatan tinggi. *A. spinosus* mengangkut lebih banyak emas ke bahagian pucuk, manakala *B. juncea* mengumpul lebih banyak emas di akar. Potensi fitopelombongan tertinggi diperoleh oleh *B. juncea* yang diinokulasi dengan DSE S14 (D1) dalam media T2. Penemuan ini menunjukkan potensi gabungan spesies tumbuhan, kulat DSE dan agen pengkelat untuk mengoptimumkan fitopelombongan di kawasan tercemar emas. Kulat DSE bukan sahaja meningkatkan serapan emas tetapi juga mengurangkan tekanan akibat sisa lombong, serta menawarkan strategi mesra alam untuk pemulihan logam. Penyelidikan lanjut harus meneliti kebolehskalaan dan kesan jangka panjang untuk mengukuhkan fitopelombongan sebagai alternatif mampan dalam usaha pemulihan kawasan perlombongan emas.

Kata kunci: Amaranth; amonium tiosianat; fitopelombongan emas; fitoremediasi; kulat DSE

INTRODUCTION

Gold phytomining, which utilises plants to absorb and accumulate valuable metals, presents an innovative approach to recover gold from mine tailings (Krisnayanti et al. 2016). In this study, we explore the potential of two plant species, *Brassica juncea* and *Amaranthus spinosus*, enhanced by the symbiotic colonisation of Dark Septate Endophytic (DSE) fungi and the addition of thiocyanate, a compound known to facilitate gold solubility. This unique combination aims to maximise gold uptake while supporting plant growth under the toxic conditions commonly found in mine tailings. Plants naturally absorb essential elements, including heavy metals, influenced by environmental factors such as growth media composition and exposure to contaminants (Alengebawy et al. 2021). When exposed to tailings rich in heavy metals, plants can serve as bioaccumulators, yet often face toxicity stress that inhibits growth (Emamverdian et al. 2015). DSE fungi, known for their ability to colonise roots and increase tolerance to harsh conditions, can play a crucial role in enhancing both plant resilience and metal uptake efficiency in phytomining applications (Bi et al. 2024). Additionally, thiocyanate enhances gold bioavailability, potentially increasing gold accumulation within plant tissues (Dinh, Dobo & Kovacs 2022), which could make the plant species we used in this study viable candidates for phytomining.

Phytomining is suitable for application on land containing low levels of metal ore, such as tailings. However, along with the presence of heavy metals, its toxicity will interfere with plant growth and development (Wilson-Corral, Anderson & Rodriguez-Lopez 2012). Therefore, efforts are needed to ensure that plants are able to continue to grow with accumulated metals in adequate amounts. Several studies have shown that gold metal can enter and interact with plant cells. This interaction can cause positive or negative responses to plant growth, depending on the concentrations of the gold metal (Ferrari et al. 2021). Although the presence of gold metal at low concentrations does not cause toxicity, higher concentrations of gold metal can cause toxicity, thus affecting plant growth negatively and can produce changes at the physiological, biochemical and molecular levels (Jain et al. 2016; Siddiqi & Husen 2016). Low concentrations of gold metal can activate the function of growth hormone, while higher concentrations have a negative effect on plant growth and biomass production (Siddiqi & Husen 2016). This may occur because the adsorption of gold metal onto the surface of root cell walls causes pore size to decrease and inhibits water transport capacity, thereby reducing plant growth (Ferrari et al. 2021). This is supported by research which concludes that plant growth is reduced under water deficit and plants also show damage and degradation of cell walls and membranes (Saha et al. 2022).

Along with the negative response of plants to high metal concentrations in tailings media, controlling stress conditions is very important to optimise growth and performance of metal accumulation in plants. Utilisation of several microbes, such as DSE fungus, has been recognised as one alternative to support plant growth under sub-optimum conditions. DSE is able to colonise plant roots intra- and intercellularly, forming microsclerotia structures and increasing plant growth without causing disease symptoms (Malicka, Magurno & Piotrowska-Seget 2022), and even can support the plants to survive in extreme conditions or stressful environments such as drought and protects plants from free radicals (Li et al. 2011). Melanin production by DSE also provides antioxidant power and plays a role in fungal survival in stressed environments (Berthelot et al. 2020). Inside the DSE misselia, free oxygen formed by melanin structure provides advantages for plant survival and growth in extreme or stressful environments (Potisek et al. 2021).

DSE is found in many plants, including non-mycorrhizal plant species (Jumpponen & Trappe 1998). Previous research showed that Chinese cabbage (*Brassica campestris* L.) seedlings inoculated with DSE had a dry weight about four times heavier than those not inoculated (Narisawa, Tokumasu & Hashiba 1998). DSE inoculation into the root system of Brassica seedlings can increase plant height, stem diameter, number of roots, and biomass (Xiao et al. 2017). In addition, DSE inoculation caused higher metal accumulation in the canopy (Diene, Sakagami & Narisawa 2014). Other research has proven that the Amaranthaceae family can be associated with DSE fungi (Qin et al. 2017). Previous studies have examined the potential of various plants and fungi for heavy metal accumulation. However, research specifically focused on the phytomining potential of *B. juncea* and *A. spinosus* in gold tailings, especially using DSE fungi combined with thiocyanate, is still limited. This study addresses this gap by exploring the synergistic effects of DSE fungi colonisation and thiocyanate addition, aiming to enhance both tolerance and gold accumulation in these species. The unique combination tested here provides insights into an innovative approach for phytomining in environments with complex metal toxicity, thus contributing to sustainable metal recovery solutions. Therefore, this experiment aimed to analyse morphological and physiological responses, as well as the ability of *B. juncea* and *A. spinosus* inoculated with DSE fungi and the addition of thiocyanate to accumulate gold from gold mine tailings media.

MATERIALS AND METHODS

DSE INOCULUM PREPARATION

The selected DSE strains used in this study were *Cladophialophora nyingchiensis* S51 and *Exophiala pisciphila* S14, obtained from the fungal collection of the Research Centre for Applied Microbiology, National Research and Innovation Agency, Indonesia. Both DSE strains have the ability to produce antipathogenic metabolite compounds. In particular, *C. nyingchiensis* S51 can stimulate the growth of oil palm seedlings and suppress *Ganoderma boninense* attacks (Purba et al. 2024). However, the use of these DSE strains as agents in gold

myco-phytomining has not been explored, and no related reports are available. Previous studies with different DSE strains have demonstrated their potential to stimulate the phytoremediation of heavy metals (Marfuah et al. 2024) and synthetic dyes such as Congo red (Melati et al. 2021). Therefore, *C. nyingchiensis* S51 and *E. pisciphila* S14 also hold potential for stimulating gold phytomining in gold mine tailings, as endophytic fungi within the DSE group are known for their ability to adapt to various abiotic stresses and enhance the resilience of their host plants to these challenges.

The isolate was cultured in malt extract (ME) liquid media to produce sufficient propagules for application to the planting medium. The culturing process was conducted in a 1000 mL Erlenmeyer flask containing ME media, incubated at room temperature on a shaker at 130 rpm for 14 days. After incubation, the culture was filtered to separate fungal biomass and rinsed with sterile water to ensure purity. The DSE suspension was prepared at a concentration of 10% (w/v) by homogenising the fungal biomass, primarily consisting of hyphae, in sterile water as a DSE inoculum stock solution. This concentration was standardised to ensure uniformity across treatments, with the biomass adjusted to approximately follow established protocols (Yuliani et al. 2020).

DSE INOCULATION OF PLANT SEEDS

DSE inoculation was carried out by immersing *B. juncea* and *A. spinosus* seeds in DSE inoculum. The seeds were sterilised with 1% NaOCl for 1 min, followed by soaking in 70% alcohol for 30 s and rinsing with sterile water 3 times. After the sterilisation process, every 150 plant seeds were put into a 2 mL microtube and inoculated with 1.5 mL of DSE isolates mentioned earlier (Sucipto, Munif & Tondok 2015).

PLANTING PROCESS

The planting process started by preparing 144 of 100 g containers for the initial growing medium, consisting of a mixture of soil and compost (1:1 v/v), which was maintained for 2 weeks. Seedlings were then transferred to 144 larger plastic containers (6 kg) for 3 weeks of growth. The growing medium was divided into four treatments: 100% gold tailings (T1), 100% gold tailings + 0.62 g/kg ammonium thiocyanate (T2), 100% gold tailings + 1.24 g/kg ammonium thiocyanate (T3), and control without gold tailings (T0). Ammonium thiocyanate was mixed evenly in the tailings to ensure homogeneity. The media was lightly moistened to prevent leaching and ensure consistent exposure in each treatment.

DSE INOCULATION ON PLANTS

DSE inoculation was given again when the plants were in the growing medium (3 weeks old). The inoculant of 0.5 mL of isolates S14 (*E. phiscipila*) and S51 (*C. nyingchiensis*) was added using a micropipette to the growing medium in a closed room. This dual inoculation approach was expected to enhance plant resilience to stress and improve nutrient uptake by maintaining high DSE colonisation levels throughout different growth stages. After 7 days of inoculation, the plants were treated with different media (T0, T1, T2, and T3).

DSE COLONISATION OBSERVATIONS

Observations on fungal colonisation on roots were conducted according to Phillips and Hayman (1970) with slight modifications. Plant roots were cleaned with distilled water and cut 1 cm. Next, the roots were heated with 3% KOH at 80 °C for 60 min, soaked using 5% H_2O_2 for 30 min, followed by soaking the roots in 1% HCl for 10 min. Then, the roots were stained by soaking the roots in a fuchsin acid solution consisting of 0.1% fuchsin, 1% glycerol and 10% acetic acid for 30 min at 90 °C and fixed with 50% glycerol solution. Next, the roots were observed with a microscope and documented with Optilab 2.0 (Myconos). The percentage of colonisation was determined by preparing 30 root segments (with a length of 2 cm), and the number of colonised root segments was counted under the microscope (Marfuah et al. 2024). Each treatment was repeated three times. The percentage of DSE fungal colonisation on the roots was calculated using the formula: (Number of colonised roots/total observed roots) × 100%.

MORPHOLOGICAL ANALYSIS

Morphological analysis was carried out when the plants entered the germination stage (2 weeks old), after being transferred to the first planting medium (4 weeks old) and 3 weeks after treatment (7 weeks old). Morphological parameters measured were the number of leaves and the dry weight of shoots and roots.

CHLOROPHYLL AND CAROTENE ANALYSIS

Chlorophyll content in the leaves was determined after the plants experienced tailings waste treatments. Leaf samples with a mass of 0.1 g were ground with a mortar, and 10 mL of 80% acetone was added. Samples were placed in 15 mL Falcon tubes and centrifuged at 3000 rpm for 10 min at 4 °C. The absorbance value of the supernatant was measured with a Genesys 20 Thermospectronic Spectrophotometer (Thermo, USA) at wavelengths (λ) 470, 647 and 663 nm. Calculation of chlorophyll and carotene is based on the formula (Lichtenthaler 1987):

$$\text{Chl-a} = 12.25\ A_{663} - 2.79\ A_{647} \qquad \text{Chl-t} = 7.15\ A_{663} + 18.71\ A_{647}$$

$$\text{Chl-b} = 21.50\ A_{646} - 5.10\ A_{663} \qquad \text{Car} = (1000\ A470 - 1.82\ \text{Chla} - 85.02\ \text{Chlb})/198$$

ANALYSIS OF Au CONTENT

Selected samples based on the highest and lowest growth data in each treatment were analysed for their gold content. The Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) instrument was used to analyse the accumulation of gold (Au) content in plant shoots and roots. Then, the obtained Au metal concentration value was used as a basis for calculating the value of translocation factor (TF) (Mattina et al. 2003), bioconcentration factor (BCF) (Zhuang et al. 2007), and phytomining capacity (PhyC) (Kos, Grčman & Leštan 2003).

DATA ANALYSIS

All the data were calculated using Microsoft Excel 365, and ANOVA analysis was carried out using RStudio 4.2.2 followed by the Duncan Multiple Range Test (DMRT) to determine the difference of means among the treatments with $\alpha = 5\%$.

RESULTS AND DISCUSSION

DSE STRUCTURE IN *Brassica juncea* AND *Amaranthus spinosus* ROOTS

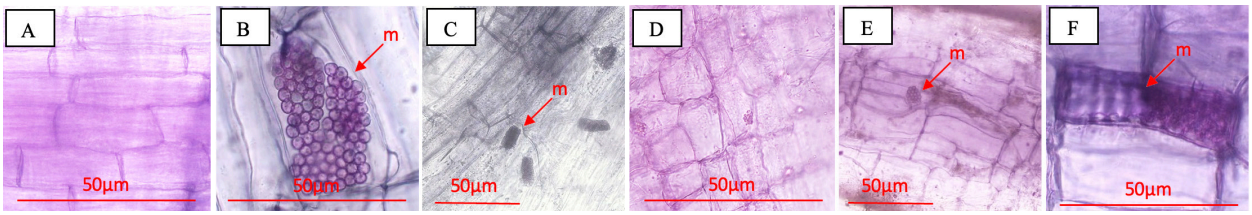
After *B. juncea* and *A. spinosus* plants were inoculated with DSE isolates (S14 and S51), fungal colonisation was characterised by melanin structures on the roots, which were visible through staining with fuchsin acid (Figure 1). DSE-typical microsclerotia were found on the roots of inoculated plants, proving that DSE could colonise the roots of *B. juncea* and *A. spinosus*. In contrast, the roots of control plants (without inoculation) did not show typical DSE structures. DSEs are identified by the presence of dark septate hyphae with conidia or sterile hyphae containing melanin pigment (Zhan et al. 2011). The melanin pigment in DSE fungal hyphae enables these fungi to survive under stressful environmental conditions (Li et al. 2011). DSE colonise plant roots both intra- and intercellularly, forming microsclerotia structures and promoting plant growth without inducing disease symptoms (Narisawa, Tokumasu & Hashiba 1998).

As shown in Figure 2, the highest DSE colonisation was found on T1 media with the maximum colonisation rate of 85%, and *A. spinosus* had the highest colonisation rate among the species, while maximum colonisation was only 72% for *B. juncea*. The treatment with DSE isolate of S14 showed better colonisation ability than S51, especially in T3 media for *A. spinosus* and T0 of *B. juncea* (Figure 1). In this study, DSE inoculation positively affected plant morphology and physiology. Furthermore, the potential of DSE inoculation in enhancing gold accumulation by *B. juncea* and *A. spinosus* grown in gold mine tailings media has been demonstrated.

EFFECT OF DSE INOCULATION ON THE GROWTH OF *B. juncea* AND *A. spinosus*

DSE inoculation increased the growth ability of *B. juncea* and *A. spinosus* compared to plants without inoculation, even though some parameters were not significantly different (Table 1). Results showed that *A. spinosus* had a biomass that was 53.56% higher than *B. juncea* based on the average of all the media. This was supported by the conditions of *A. spinosus* inoculated by the DSE S14 fungus, which had the highest dry weight of shoots and roots. However, the use of tailings media had an impact on reducing plant growth rates. Moreover, increasing the concentration of ammonium thiocyanate also reduced the growth of the *A. spinosus* and *B. juncea* even more. By comparing to soil media (control), tailing treatment (T1) significantly reduced the average plant biomass of both species by 39.21%, followed by tailing+ammonium thiocyanate of 0.62 g/kg (T2) by 67.63%, and tailing+ammonium thiocyanate of 1.24 g/kg (T3) up to 70.28%.

The dry weight of the plant canopy, on average was 21.91% heavier in plants inoculated with DSE S14 and 8.11% higher for the plants inoculated by DSE S51 compared to control plants. Root dry weight was 47.22% heavier in plants inoculated with DSE S14 and 22.84% in plants inoculated with DSE S51 compared to control plants. In this experiment, the tailings media was added with 0.5 kg of compost, and this organic fertiliser became



m = microsclerotia

FIGURE 1. DSE colonisation on the roots of *A. spinosus* and *B. juncea*. (A) *A. spinosus* without DSE inoculation, colonisation of (B) DSE S51 and (C) DSE S14 in *A. spinosus*. (D) *B. juncea* without DSE inoculation, colonisation of (E) DSE S51 and (F) DSE S14 in *B. juncea*

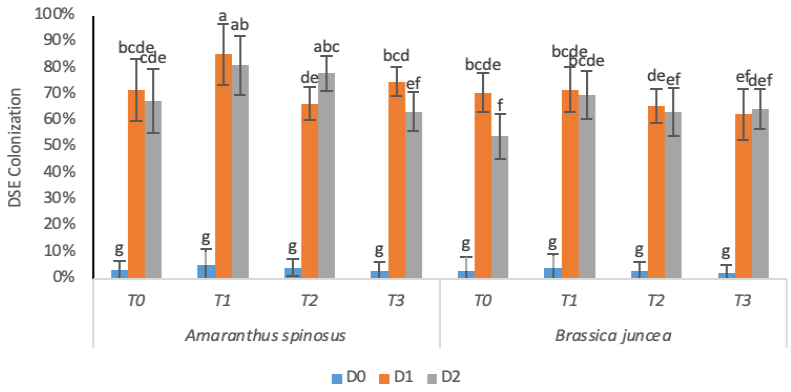


FIGURE 2. DSE colonisation percentage in A. spinosus and B. juncea plants under different media. D0: without DSE; D1: with DSE S14; D2: with DSE S51 inoculation; T0: control media (without tailing); T1: media with tailing; T2: tailing + 0.62 g/Kg thiocyanate; T3: tailing+1.24 g/Kg thiocyanate.

the only source of organic material and macronutrients for the plants. Meanwhile, each plant that was inoculated with DSE S14 and S51 showed a better response compared to plants that were not inoculated with DSE. In this regard, the best values for the number of leaves were possessed by *B. juncea* and *A. spinosus* inoculated with DSE S14, followed by DSE S51. The average number of leaves was 34.79% greater in plants inoculated with DSE S14 and 22.79% greater in plants inoculated with DSE S51 compared to control plants, respectively (Table 1).

The decrease in plant growth in the tailing treatment may have been related to the high concentration of heavy metals such as Pb, Cd, Ag, and Hg contained therein (Hamim et al. 2018). This can happen because heavy metals can inhibit the absorption of water and nutrients, the rate of photosynthesis and enzyme activity (Giannakoula, Therios & Chatzissavvidis 2021; Singh et al. 2018). In addition, because tailings have limited macronutrients that plants need, such as N, P, and K, plant growth and development are increasingly hampered (Setyaningsih, Wulandari & Hamim 2018).

Tailings, which resulted from gold mining, contain several heavy metals and other essential elements as well as non-essential elements (Hamim et al. 2018). The presence of heavy metals in certain amounts can affect the growth and development of a plant (Usman, Al-Ghouti & Abu-Dieyeh 2019). With these limitations, DSE inoculation can help the growth and development of plants affected by metal stress (Diene, Sakagami & Narisawa 2014). Previous experiments showed that DSE fungus inoculation provided a positive response to increase plant growth and biomass production (Marfuah et al. 2024). DSE inoculation significantly improved root growth and nutrient uptake, and facilitated plant growth and survival under heavy metal stress (Hou et al. 2020). DSE inoculation also increases shoot biomass, the number of leaves (He et al. 2021) and leaf area (Santos et al. 2021) under stress, which were the result of increased N and P levels (Li et al. 2023).

CHLOROPHYLL AND CAROTENOID CONTENTS

Chlorophyll and carotenoid contents were measured to determine the physiological response of plants inoculated with DSE fungi and grown on gold mine tailings media (Figure 3). The results showed that chlorophyll content was inconsistent in both plants, regardless of DSEs inoculation, tailings treatment and chelators addition. The chlorophyll content of *A. spinosus* was consistently 19.41% higher than that of *B. juncea*, probably due to species-specific differences in their photosynthetic capacity and tolerance to stress conditions. Previous studies suggest that *A. spinosus* has a more efficient photosynthetic apparatus and a higher antioxidant capacity compared to *B. juncea*, enabling it to maintain better chlorophyll and carotenoid levels under adverse conditions (dos Santos et al. 2017).

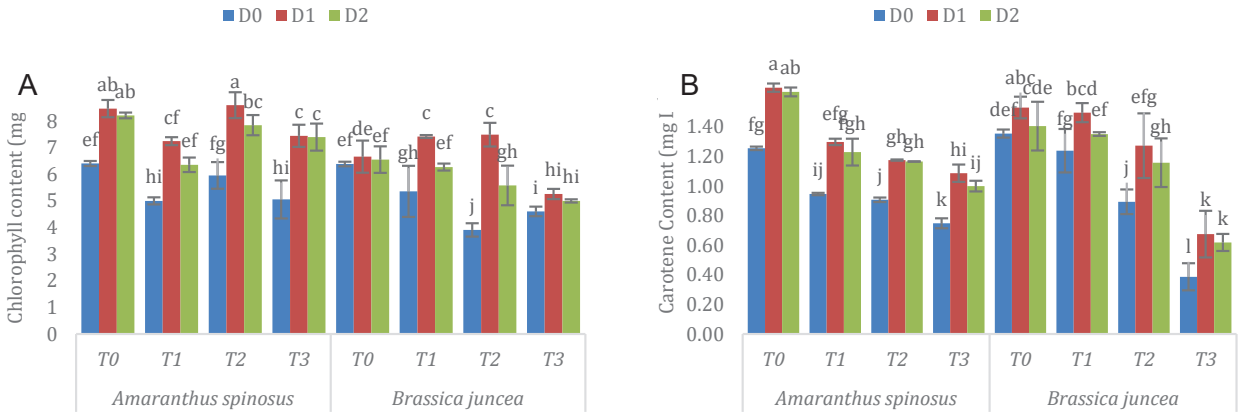
In general, inoculation with the DSE isolate S14 increased the chlorophyll content (Figure 3(A)), indicating the positive role of DSE in enhancing plant stress tolerance. Among the media treatments, soil provided the highest chlorophyll content, which declined with the use of tailings and further decreased in tailings media with increasing levels of ammonium thiocyanate. The lowest total chlorophyll content (3.91 mg/L) was observed in *B. juncea* without DSE inoculation grown on tailings + ammonium thiocyanate (0.62 g/kg), highlighting the species' lower tolerance to stress conditions and reduced ability to maintain photosynthetic pigments under such treatments. In contrast, the highest chlorophyll content (8.59 mg/L) was recorded in *A. spinosus* inoculated with DSE S14 and grown on tailings + ammonium thiocyanate (0.62 g/kg), further supporting the notion that *A. spinosus* is more resilient and better adapted to maintain its physiological processes in challenging environments.

The higher chlorophyll content of the plants inoculated by DSE was in line with the growth improvement of both species, especially with DSE S14 inoculations (Table 1). The impact of DSE inoculation on host plant growth under abiotic stress conditions is generally positive, although it

TABLE 1. Growth response of *A. spinosus* and *B. juncea* on different treatments

DSE strain	Media	SDW (g)		RDW (g)		NL	
		AS	BJ	AS	BJ	AS	BJ
D0	T0	22.15^{a}	10.54^{b}	16.33^{b}	6.97^{bc}	201.25^{ab}	12.39^{a}
	T1	6.45^{de}	5.89^{d}	11.15^{c}	2.57^{de}	186.37^{bc}	9.27^{a}
	T2	12.62^{c}	6.62^{cd}	16.33^{b}	5.57^{c}	176.20^{bc}	9.78^{a}
	T3	5.80^{e}	5.67^{d}	6.98^{d}	1.67^{e}	112.74^{c}	9.21^{a}
D1	T0	23.01^{a}	13.50^{a}	28.20^{a}	9.25^{a}	279.43^{a}	11.91^{a}
	T1	9.32^{d}	6.96^{cd}	17.71^{b}	3.85^{d}	232.87^{ab}	10.19^{a}
	T2	18.04^{b}	7.41^{c}	17.92^{b}	7.79^{ab}	225.82a^{b}	12.80^{a}
	T3	8.86^{de}	6.21^{cd}	13.04^{c}	1.73^{e}	185.65^{bc}	11.26^{a}
D2	T0	22.49^{a}	11.22^{b}	17.68^{b}	8.07^{ab}	217.23^{ab}	10.79^{a}
	T1	6.82^{de}	6.63^{cd}	17.33^{b}	3.14^{de}	213.86^{ab}	9.77^{a}
	T2	15.90^{b}	7.14^{cd}	17.40^{b}	5.69^{c}	218.89^{ab}	11.08^{a}
	T3	6.07^{e}	5.87^{d}	11.99^{c}	1.70^{e}	192.73^{b}	11.59^{a}

AS = *A. spinosus,* BJ = *B. juncea*, SDW = shoot dry weight (g), RDW = root dry weight (g), NL = number of leaves. D0 = without DSE, D1 = with DSE S14 inoculation, D2 = with DSE S51 inoculation. Mean values sharing a similar letter in the column are considered not significant at $p < 0.05$ of the DMRT test



Means represented by the same letter do not show significant difference based on the DMRT test. D0 = without DSE, D1 = with DSE S14 inoculation, D2 = with DSE S51 inoculation

FIGURE 3. (A) Chlorophyll and (B) carotene contents of *A. spinosus* and *B. juncea* in response to DSE inoculation and tailings treatment after 7 weeks of planting and after 5 days of application of ammonium thiocyanate

is varied, depending on the specific DSE species involved (Li et al. 2023). The outcomes of interactions between DSEs and plants were influenced by the particular DSE species present (Li et al. 2023). Similarly, in this study, DSE S14 (*E. pisciphila*) and DSE S51 (*C. nyingchiensis*) demonstrated different effects, with DSE S14 outperforming DSE S51 in enhancing plant growth and chlorophyll content. As a super-metal accumulator, *E. pisciphila* exhibits an exceptional tolerance to toxic metals, which is a crucial trait for remediation of heavy metal-contaminated soils through plant-microorganism bioaugmentation strategies (Cao et al. 2019; Lacercat-Didier et al. 2016). The remarkable tolerance of *E. pisciphila* to high metal concentrations in extreme mining environments is likely because of its specialised detoxification mechanisms for both essential and non-essential metals (Cao et al. 2019). In contrast, limited information is available on the capability of DSE species within the genus Cladophialophora, particularly *C. nyingchiensis*, in enhancing plant adaptation and growth under heavy metal stress.

The carotene content was decreased along with the use of tailings media and ammonium thiocyanate

(Figure 3(B)). The 1.24 g/kg ammonium thiocyanate added to the tailings media caused a significant reduction of carotene up to 95.93% compared to plants grown on the soil media. On average, *A. spinosus* had a carotene content of 5.29% higher than *B. juncea*. Meanwhile, DSE S14 inoculation caused the increase of carotene content by 31.93% compared to plants without DSE inoculation. The treatment with 1.24 g/kg thiocyanate (T3) caused the reduction of carotene up to 71.45% in *B. juncea* plants, without DSE inoculation. Meanwhile, the smallest decrease of carotene content (2.26%) occurred in *B. juncea* plants inoculated with DSE S14 on tailings media without chelators.

Heavy metal content such as lead (Pb) in gold mine tailings might cause cell damage and decrease photosynthetic pigments, such as chlorophyll and carotene, due to an increase in free radicals and reactive oxygen species (ROS) (Andriya et al. 2019; Giannakoula, Therios & Chatzissavvidis 2021). Reduced pigments reflect that tailings had induced plant stress, which disrupts chlorophyll synthesis through inhibition of the enzymes protochlorophyllide reductase and a-aminolaevulinic acid (ALA) dehydratase (Hamim et al. 2019). Inoculation of DSE fungi can reduce these negative impacts by increasing chlorophyll and carotene contents and improve photosynthesis and plant growth, especially under heavy metal stress (dos Santos et al. 2017; Hou et al. 2020).

GOLD (Au) ACCUMULATION, BIOCONCENTRATION FACTOR (BCF), TRANSPORT FACTOR (TF), AND PHYTOMINING CAPACITY

Gold (Au) was selected as the metal for assessing the phytomining potential of *B. juncea* and *A. spinosus*. This study represents the first documentation of DSE fungi (DSE S14 and DSE S51) inoculation against *B. juncea* and *A. spinosus* cultivated in gold mine tailings media with the incorporation of two levels of ammonium thiocyanate. *B. juncea* exhibited an average total gold content 78.85% higher than that of *A. spinosus*. The inoculation of DSE fungi influenced the accumulation of Au in both shoots and roots of the plants grown on gold mine tailings (Table 2). When DSE S14 and S51 fungi were inoculated, both plant species demonstrated an increase in Au accumulation in both roots and shoots. Among the DSE strains, S51 exhibited the most favourable response, demonstrating the highest gold absorption in plants. Specifically, DSE S51 inoculation increased metal accumulation in plants by 46.92% compared to non-inoculated plants, while S14 only increased it by 30.50% (Table 2). Additionally, the use of tailings media supplemented with 1.24 g/kg ammonium thiocyanate yielded the best response, facilitating gold absorption into plant tissues. *B. juncea* effectively extracted Au with a 687.78% higher concentration in its roots compared to *A. spinosus*. *B. juncea* inoculated with S51 and grown in tailings media without a chelator exhibited the highest gold content in its roots, which is 717.95 µg/kg. In the canopy, *A. spinosus* displayed a higher Au concentration than *B. juncea* by 72.99%. However, *B. juncea* inoculated with S14 and cultivated on tailings supplemented with 1.24 g/kg ammonium thiocyanate exhibited the highest gold content in its canopy at 486.49 µg/kg (Table 2). No Au content was detected in the two plants grown in soil media, indicating that Au extraction was influenced by Au content in the media (tailings), DSE inoculation, and Au solubility levels.

B. juncea and *Amaranthu*s sp. are known for their potential to rapidly accumulate metals within a short lifespan (Rahman et al. 2013). Studies indicate that *B. juncea* primarily accumulates metals in the roots, while *A. spinosus* tends to accumulate more in the shoots (Małecka et al. 2019; Napaldet & Buot 2020). Inoculation with DSE fungi enhances metal absorption in plants (Marfuah et al. 2024) and specifically increases gold uptake by stimulating plant enzymes (Domka, Rozpądek & Turnau 2019; Oladipo et al. 2018). DSE also improves the availability of N, P, and K, which correlates with higher gold absorption (Vergara et al. 2017). Ammonium thiocyanate dissolves gold in the medium, with an optimal concentration of 1.24 g/kg (Pisco et al. 2017; Wilson-Corral, Anderson & Rodriguez-Lopez 2012). However, at this level, *B. juncea* and *A. spinosus* only tolerate it for five days, showing signs of wilting and drying, likely due to desiccation from high metal solubility (Narasimha et al. 2003) and poisoning from excessive metal uptake (Emamverdian et al. 2015). Despite these challenges, chelators, including ammonium thiocyanate, accelerate gold absorption in plants (de la Rosa et al. 2009).

Furthermore, the results of total gold analysis on plants can be processed to get the value of phytomining capacity per plant (PhyC), which is not only determined by Au concentration in the plant tissues but also influenced by the dry weight of the plants. The results showed that the value of phytomining capacity in *A. spinosus* plants was greater by 37.89% compared to *B. juncea*. The use of DSE S14 fungi was the best to increase the value of phytomining capacity, which was 78.35% higher than control plants (without DSE fungi). As for the media treatment, T2 gives the highest average phytomining capacity value in plants, which was 4.32 µg/plant. The largest phytomining capacity value was obtained by *A. spinosus* plants inoculated with DSE S14 fungi in T2 media, which was 7.49 µg/plant, while the smallest phytomining capacity value was obtained by *A. spinosus* plants without DSE fungi inoculation in tailing media without chelator, which was only 0.13 µg/plant (Table 2).

Ammonium thiocyanate had an important role in dissolving metals from tailing media, including Au, which induced Au accumulation dramatically, especially in the shoots (Table 2 & Figure 4(A)). It appeared that the phytomining capacity per plant of *A. spinosus* was greater than *B. juncea*, and the use of chelator was only effective for the concentration of 0.62 g/kg of ammonium thiocyanate. The application of thiocyanate up to 1.24 g/kg significantly

TABLE 2. Gold Analysis of *A. spinosus* and *B. juncea* grown on different media. Mean values sharing a similar letter for each column are considered not significant at $p < 0.05$

DSE strain	Media	AuR		AuS (µg/kg)		AuC		PhyC (µg/plant)	
		AS	BJ	AS	BJ	AS	BJ	AS	BJ
D0	T0	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{g}	0.00^{f}
	T1	9.76^{f}	0.00^{f}	2.37^{f}	0.00^{g}	6.85^{e}	0.00^{g}	0.13^{g}	0.00^{f}
	T2	58.06^{bc}	557.66^{bc}	213.70^{d}	42.88^{ef}	121.36^{c}	273.88^{c}	3.38^{d}	3.42^{b}
	T3	119.07^{a}	536.67^{bc}	343.41^{b}	224.50^{de}	222.66^{a}	301.92^{c}	2.82^{d}	2.13^{d}
D1	T0	0.00^{f}	0.00g	0.00^{f}	0.00^{g}	0.00^{e}	0.00^{j}	0.00^{g}	0.00^{f}
	T1	19.93^{def}	73.95^{f}	178.57^{d}	51.29^{def}	74.19^{d}	59.56^{f}	2.00^{e}	0.63^{e}
	T2	41.73^{bcd}	253.40^{e}	375.23^{b}	75.01^{d}	214.93^{a}	165.35^{e}	7.49^{a}	2.46^{cd}
	T3	48.62^{bc}	495.22^{c}	440.48^{a}	490.11^{a}	203.83^{a}	491.47^{a}	4.44^{c}	4.16^{a}
D2	T0	0.00^{f}	0.00^{f}	0.00^{f}	0.00^{g}	0.00^{e}	0.00^{g}	0.00^{g}	0.00^{f}
	T1	34.61^{cde}	645.42^{a}	129.25^{e}	30.90^{f}	58.30^{d}	221.29^{d}	1.36^{f}	2.12^{d}
	T2	66.22^{b}	592.95^{ab}	263.39^{c}	58.03^{hi}	159.95^{b}	297.68^{c}	5.30^{b}	3.87^{ab}
	T3	52.75^{bc}	395.60^{d}	369.52^{b}	365.99^{b}	158.92^{b}	372.70^{b}	2.87^{d}	2.81^{c}

AS = *Amaranthus spinosus,* BJ = *Brassica juncea,* D0 = without DSE, D1 = with DSE S14 inoculation, D2 = with DSE S51 inoculation. AuR = Au content in root, AuS = Au content in shoot, AuC = Au concentration, PhyC = Phytomining capacity per plant

increased the concentration of gold accumulated in plants, but the plants underwent growth inhibition, and the dry weight decreased, which consequently had lower phytomining capacity (Figure 4(B)).

Ammonium thiocyanate is a chemical chelating agent that can increase the solubility of metal elements in the soil, such as Fe, Zn, Cd, Cu, Ni, Pb, and Al, including Au (Azizitorghabeh et al. 2021; Ebbs et al. 2010), so that they can be easily absorbed by plants. The high accumulation of heavy metals in plant tissue due to a process known as 'chelate-induced hyperaccumulation' will cause toxic effects on plants (Kopittke et al. 2010). In addition, ammonium thiocyanate also caused inhibition of plant growth and development because of disturbances in nutrient balance, damage to photosynthetic pigments, changes in amino acid composition and inhibition of antioxidant enzyme activity (Yu & Zhang, 2013). Feng et al. (2023) found that thiocyanate causes disruption of carbon and nitrogen metabolism in rice, thereby causing decreased growth. Yang et al. (2021) also observed that thiocyanate application can cause chloroplast dysfunction in plants, which resulted in the decrease of photosynthesis.

Based on the multivariate analysis, all treatments, including plant species, the application of DSE and media treatments, had a significant effect on Au concentrations and phytomining capacity of the plants (Table 3). Furthermore, the analysis also indicated that tailing and thiocyanate treatments induced Au accumulation in both species, although the increase was greater in *B. juncea* than in *A. spinosus* (Figure 4(A) & 4(B)). The analysis also showed that DSE significantly improves the absorption of Au in *B. juncea* but not in *A. spinosus*. With regard to gold phytomining ability, *A. spinosus* plants had a higher ability than *B. juncea* (Figure 4(B) & 4(D)) because *A. spinosus* had a higher dry weight compared to *B. juncea* (Table 3).

A different response was shown by *A. spinosus*, which experienced an increase in Au absorption and phytomining capacity at a thiocyanate concentration of 0.62 g/kg only and decreased again with higher levels of thiocyanate (Figure 4). The synergistic application of DSE fungi and thiocyanate increased the phytomining potential through increased Au uptake and maintained plant growth (Figure 4). Thiocyanate as a chemical lixiviant improves metals' availability, including Au, which became more available and easier to be absorbed by plants (Azizitorghabeh et al. 2021), while DSE might improve plant ability under metal stress through detoxification mechanisms (Cao et al. 2019).

The analysis of Au content in both media and plants provides essential data for calculating the Bioconcentration Factor (BCF) and Translocation Factor (TF). These metrics are critical for determining the mechanisms of metal remediation in plants. The BCF indicates a plant's capacity to accumulate metals like Au from the media (Table 4), while the TF, derived from the ratio of Au content in shoots to roots, highlights the plant's phytoextraction ability (Figure 4). In this study, *B. juncea* demonstrated a 78.85% higher BCF value compared to *A. spinosus*, indicating a stronger accumulation potential. DSE inoculation, particularly with strains S51 and S14, significantly enhanced the BCF values, increasing gold accumulation by 36.92% and 30.50%, respectively, compared to uninoculated plants.

Despite these enhancements, plants grown in tailings media supplemented with ammonium thiocyanate in the T2 and T3 treatments were categorised as low accumulators (BCF 0.01–0.1) under Wei, Zhou and Mathews (2008) criteria. The TF values showed distinct remediation mechanisms: *A. spinosus* (TF > 1) exhibited phytoextraction, while *B. juncea* (TF < 1) aligned with phytostabilisation. These findings suggest practical applications for targeted remediation strategies. For example, *A. spinosus* could be utilised in environments requiring the removal of metals from contaminated soils, while *B. juncea* may stabilise metals within the soil, reducing their mobility and environmental risks. These insights provide a basis for selecting appropriate plant species tailored to specific phytoremediation objectives.

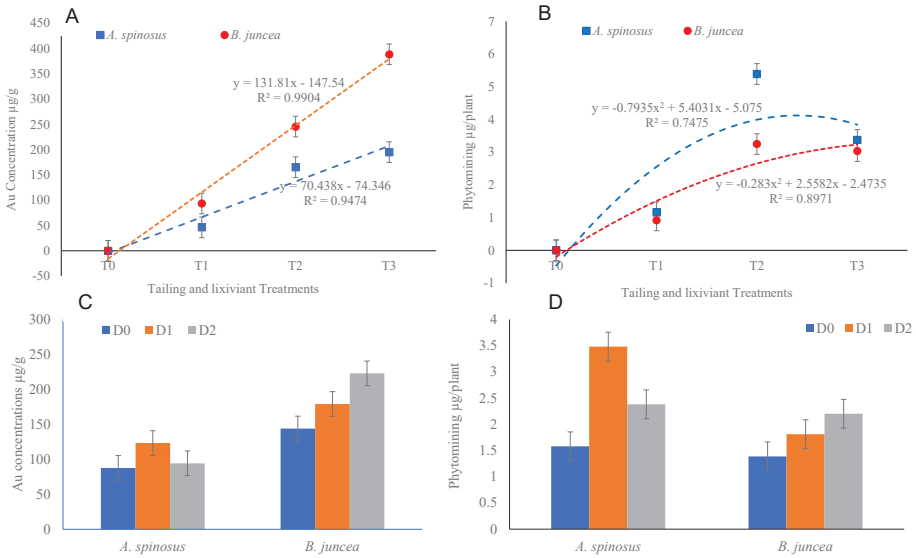
The Translocation Factor (TF) serves as an indicator to assess the mobility of metals from roots to shoots (Mahar et al. 2016). *A. spinosus* exhibits a higher TF value compared to *B. juncea*. This discrepancy arises because *A. spinosus* accumulates more gold in the shoots than in the roots, while *B. juncea* tends to accumulate more gold in the roots than in the shoots. Numerous metals ascend through the xylem to the upper parts of the plant, concentrating in leaves and stems (Pasricha et al. 2021). The highest TF value was observed in *A. spinosus* plants, specifically those inoculated with DSE S14 and grown on tailings + ammonium thiocyanate at 1.24 g/kg media, which was 9.09 (Figure 5). However, high concentrations of ammonium thiocyanate (1.24 g/kg) caused rapid wilting due to phytotoxicity, which affects water uptake, oxidative stress or ion imbalance.

In contrast, the lowest TF value was recorded in *B. juncea* plants inoculated with DSE S51 on media T2, which was 0.04. Encouragingly, DSE inoculation had a

TABLE 3. Multivariate test of between-subject effect of Au concentration and phytomining capacity of *A. spinosus* and *B. juncea*

Source	Dependent variable	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	Au concentration	887773.156[a]	23	38598.833	65.727	<.001
	Fitomining	189.393[b]	23	8.234	58.353	<.001
Intercept	Au concentration	966082.953	1	966082.953	1645.074	<.001
	Fitomining	219.912	1	219.912	1558.395	<.001
Plant species	Au concentration	77259.006	1	77259.006	131.559	<.001
	Fitomining	5.579	1	5.579	39.535	<.001
DSE	Au concentration	16709.632	2	8354.816	14.227	<.001
	Fitomining	11.358	2	5.679	40.245	<.001
Media	Au concentration	622252.874	3	207417.625	353.197	<.001
	Fitomining	140.07	3	46.69	330.868	<.001
Species * DSE	Au concentration	14048.883	2	7024.441	11.961	<.001
	Fitomining	5.881	2	2.941	20.839	<.001
Species * Media	Au concentration	61120.136	3	20373.379	34.692	<.001
	Fitomining	8.692	3	2.897	20.532	<.001
DSE * Media	Au concentration	42570.608	6	7095.101	12.082	<.001
	Fitomining	7.572	6	1.262	8.943	<.001
Species * DSE * Media	Au concentration	53812.019	6	8968.67	15.272	<.001
	Fitomining	10.24	6	1.707	12.094	<.001
Error	Au concentration	14094.194	24	587.258		
	Fitomining	3.387	24	0.141		
Total	Au concentration	1867950.304	48			
	Fitomining	412.692	48			
Corrected total	Au concentration	901867.35	47			
	Fitomining	192.78	47			

(a) R^2 = .984 (Adjusted R^2 = .969), (b) R^2 = .982 (Adjusted R^2 = .966)

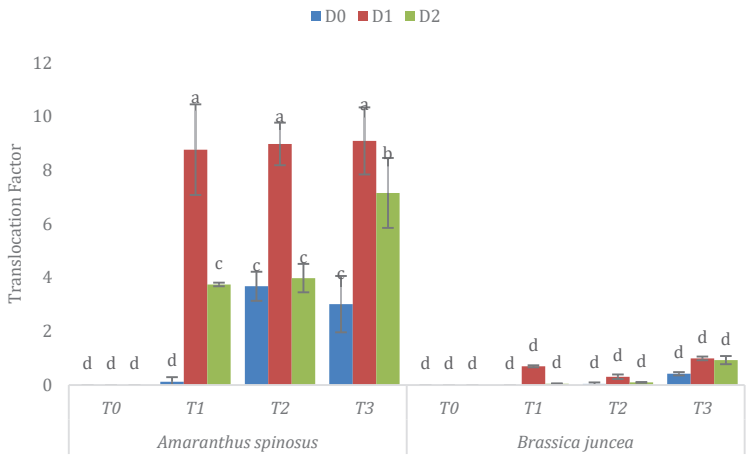


D0 = without DSE, D1 = with DSE S14 inoculation, D2 = with DSE S51 inoculation

FIGURE 4. Au concentration in relation with (A) tailing and thiosyanate treatments, (B) DSE treatments, and phytomining capacity with (C) different tailing and thiocyanate treatments and (D) different DSE treatments of *A. spinosus* and *B. juncea* after 3 weeks planting and after 5 days ammonium thiocyanate application

TABLE 4. Bioconcentration factor of gold (Au) of *A. spinosus* and *B. juncea*. The value followed by a similar letter in all treatments within each species is not significantly different at $p < 0.05$ of the DMRT test

DSE strain	*A. spinosus*				*B. juncea*			
	T0	T1	T2	T3	T0	T1	T2	T3
D0	0.0000^{e}	0.0004^{e}	0.0062^{c}	0.0114^{a}	0.0000^{g}	0.0000^{g}	0.0141^{c}	0.0155^{c}
D1	0.0000^{e}	0.0038^{d}	0.0110^{a}	0.0105^{a}	0.0000^{g}	0.0031^{f}	0.0085^{e}	0.0253^{a}
D2	0.0000^{e}	0.0030^{d}	0.0082^{b}	0.0082^{b}	0.0000^{g}	0.0114^{d}	0.0153^{c}	0.0192^{b}



D0 = without DSE, D1 = with DSE S14 inoculation, D2 = with DSE S51 inoculation

FIGURE 5. Translocation factor (TF) of *A. spinosus* and *B. juncea* treated by DSE inoculation and tailings treatment after 3 weeks planting and after 5 days ammonium thiocyanate application

positive impact on increasing the translocation factor value in both plants cultivated on similar media (Figure 4). For instance, *A. spinosus* plants inoculated with DSE S14 and grown on T3 media demonstrated a TF value increase of up to 201.78%, compared to non-inoculated plants. This suggests that plants inoculated with DSE exhibit an enhanced ability for gold mobilisation from roots to shoots (Li et al. 2011).

CONCLUSIONS

Growth parameters of *A. spinosus* and *B. juncea*, such as leaf number, shoot, and root dry weight, decreased significantly due to the use of gold mine tailings for phytomining. This decrease was inversely proportional to the concentration of ammonium thiocyanate as a chelating agent. Although gold tailings reduced chlorophyll and carotene content, ammonium thiocyanate increased gold uptake in both plants. DSE inoculation significantly reduced the negative effects of gold tailings and ammonium thiocyanate, increasing the capacity of the plants to extract gold in shoots and roots. The best combination for phytomining was found in DSE-inoculated *A. spinosus* S14 with tailings and 0.62 g/kg ammonium thiocyanate. Further research is recommended to understand the mechanism of DSE in reducing tailings-induced stress and chelators at the molecular and physiological levels. Testing alternative chelators, optimising the concentration of ammonium thiocyanate, and analysing the long-term effects of DSE on various environmental conditions and plant species could improve the applicability of this method.

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