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Growth Stimulating and Fungicidal Properties of Succinic Acid Complexes with Silver, Copper and Boron Ions During Pre-Sowing Treatment of Soybean Seeds

Sana K. Kabdrakhmanova,[1,*] Ainur K. Kabdrakhmanova,[1,*] Esbol Shaimardan,[1, 2] Kydyrmolla Akatan,[3,*] Madiar M. Beisebekov,[2] Bagadat S. Selenova,[1] Roza A. Aubakirova,[3] Aida Maussumbayeva,[4] Sabu Thomas[5,*] and Tulegen M. Seilkhanov[6]

Abstract

The synthesis of novel monoligand metal complexes involving the succinic acid anion is a topic of keen interest when exploring compounds with multifaceted biological properties, such as antimicrobial and antifungal activities. In this context, the exploration of complexes involving copper, silver, and boron ions becomes particularly intriguing. Succinic acid, serving as a natural analog of salicylic acid, exhibits the potential to enhance plant systemic resistance against pathogens when combined with antimicrobial elements. This not only bolsters the mechanisms of defense but also augments production processes, promotes growth, and ultimately leads to higher crop yields. For pre-sowing treatment of soybean seeds, complexes of succinic acid with copper, silver and boron ions were used, synthesized in this work to study the biostimulating and fungicidal action. The resulting complexes were characterized using scanning electron microscopy, X-ray diffraction and infrared spectroscopy. The research delved into the biological efficacy of succinates concerning soybeans with the aim of enhancing germination rates while mitigating the presence of phytopathogens and dwarfism. Fourier-transform infrared (FTIR) analysis provided confirmation of the establishment of novel bonds between metal ions and the oxygen atoms within the carboxyl group of succinic acid. The succinic acid monoligand complexes with copper, silver, and boron ions exhibited distinct surface morphologies with a monoclinic crystal structure, as revealed through scanning electron microscopy analysis. These complexes demonstrated dual effects, both stimulating and fungicidal in nature, significantly enhancing germination rates by 19.7-24.4 times and reducing susceptibility to phytopathogenic organisms like *Fusarium* and *Alternaria tenuis Nees* by 2.75-8.66 times when compared to the control sample. Furthermore, the complexes led to a reduction in dwarfism by 1.44-7.0 times.

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1. Introduction

Currently, plant protection from pathogens is achieved mainly through the application of fungicides. The negative consequences of the application of such chemical preparations are known to include pollution of the environment and the agricultural products themselves with residual chemicals, including heavy metals and nitrates, among other compounds hazardous to human health. A basic tendency in the field of plant protection concerns reduction of the usage of pesticides, and their subsequent replacement with environmentally friendly alternatives.[1] Metal ions accelerate the drug action and efficiency of therapeutic compounds.[2] In addition, biologically active ligands are quite important in coordination compounds. Mixed ligand complexes also play a key role in magneto chemistry, analytical chemistry and photochemistry, among others.[2,3] These complexes provide vital information on enzymes, such as their activation. Furthermore, these complexes offer protection against pathogenic microorganisms.[3] Recently, eco-bioligands with d-elements

[1] *Department of Chemical and Biochemical Engineering, Satbayev University, Almaty, 050000, Kazakhstan.*
[2] *Scientific Center of Composite Materials, Almaty, 050000, Kazakhstan.*
[3] *National Scientific Laboratory, S. Amanzholov East Kazakhstan University, Oskemen, 070020, Kazakhstan.*
[4] *Higher School of Natural Sciences, I. Zhansugurov Zhetysu University, Taldykorgan, 040009, Kazakhstan.*

have been used for various purposes, such as catalysts, medicine, ion-exchange synthesis, porous material production and pesticides.[4-7] Moreover, metal-organic frameworks (MOFs), based on metal carboxylates are interesting, not just for their structural diversity, but also due to their biological activities in DNA binding and chemical nuclease synthesis, as well as their anti-tumor, antibacterial and antifungal properties.[8] Justina *et al.* observed that drugs administered in the form of metallic complexes exhibit modified pharmacological and toxicological properties.[9] For example, platinum and Cu^{2+} complexes drugs have efficient bioactivity against diseases. In this regard, mixed ligand complexes are important in industrial field. Substantial researches in chemistry have been devoted to the synthesis and characterization of biologically active compounds that would be used to curb organisms causing illnesses and death to both plants and animals.

In plants, copper is involved in the synthesis of iron-containing enzymes, activates photosynthesis, carbohydrate metabolism, plant respiration and helps for formation of P and B vitamins.[10,11] Moreover, copper is a vital element, reacts with amino acids,[11,12] carboxylic acids, and nitrogenous bases.[13–15] Investigators have done researching work on DL-alanine, succinic acid (SA) and its derivatives.[1,3,8,9] The valuable property of succinic acid is that four electron donor oxygen atoms give a special ability to bind inorganic fragments and form secondary blocks through carboxylate groups.[16-18] This is due to the fact that ligands of d-element complexes increase antibacterial and fungicidal properties and biological efficiency. Further, allows to use these complexonates as ecological and biological products in order to reduce the usage of fungicides and pesticides. The succinic acid has shown the inhibitory ability in relation to the bacteria Erwinia Carotovora and the fungus Penicillium Casei. Also, important property of SA, its derivatives and copper ions are their bactericidal and fungicidal activity. The copper complex has an increased potent antifungal effect and could be suggested as a potential antifungal agent in the treatment.[19-21] The complex of succinic acid with silver has a cytotoxic effect against gram-positive/gram-negative bacteria and fungi. The activity of silver-based complexes is associated with good stability, water solubility, reduction and oxidation ability.[22] Researchers[23] have done investigation in regard borate-ethylenediamindisuccinate complex (B-EDDYAK) on the biochemical composition of the cultivated medicinal plant Kalanchoe. Boron complex with succinic acid has demonstrated good biological activity against pathogens. Moreover, very promising direction is the usage of complexes of succinic acid and its derivatives as a biostimulant and adaptogen of plants.[24-26] The scientists[27] established that, using of SA as a means of pre-sowing seed treatment contributes to an increase in the number of stems per plant, plant height, pigment and nitrogen content in tissues, leaf surface area, and also increases plant resistance to pathogens. The composition based on Polyvinylpyrrolidone (PVA) and starch in presence of "Maxim" fungicide was developed[28] in order to encapsulate sunflower seeds, which affects positively to seed germination as well as formation and development of sunflower seedlings. It was found that, relatively good increase in sunflower seeds with a starch composition (1 %) is observed in case of "Maxim" fungicide of different concentrations (5 % and 10 %) under controlled conditions: PVA (5 %) (1:4)/ Maxim 10%). As a result, the germination was high, in average of 97.48 %. The data shows that an increase in the concentration of "Maxim" fungicide promotes an increase in germination, thereby decreasing the number of sprouted sunflower seeds. In this regard, can be concluded that, 5 % and 10 % solution of "Maxim" fungicide is acceptable for the destruction of fungal diseases under controlled conditions. In addition, the relevance of this problem is that crop shortages (60%) due to harmful diseases, insufficient macro- and microelements determine the use of promising technologies for pre-sowing seed treatment.[29] Therefore, scientists are looking for new ways to solve problems associated with the treatment of seeds for sowing.

This work is devoted to the preparation of complexes of Cu^{2+}, Ag^{+} and $B^{3+}$with succinic acid, studying of their physical and chemical properties, as well as to test the fungicidal activity of the complexes against soybean pathogens. The complexes with metals and succinic acid have a positive effect on seed germination.

[5] *School of Energy Materials, Mahatma Gandhi University, Kottayam,686560, India.*
[6] *The Laboratory of Engineering Profile of NMR Spectroscopy, Sh. Ualikhanov Kokshetau University, Kokshetau,020000, Kazakhstan.*
**Email:* ainurkabdrahmanova@mail.ru (A. K. Kabdrakhmanova), sanaly33@mail.ru (S. K. Kabdrakhmanova), ahnur.hj@mail.ru (A. Kydyrmolla), sabuthomas@mgu.ac.in (S. Thomas)

2 Materials and methods

2.1 Chemicals and materials

Deionized water (H_2O), silver nitrate ($AgNO_3$), succinic acid ($(CH_2)_2(CO_2H)_2$), monosodium phosphate (NaH_2PO_4), disodium phosphate (Na_2HPO_4), copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium carbonate (Na_2CO_3), and boric acid (H_3BO_3) were obtained from Sigma-Aldrich (Bangalore, India). All reagents were of analytical grade and were used without additional purification.

2.2 Preparation of succinic acid/Me complex

The preparation of the SA/Me complex was conducted following the scheme presented in Fig. 1.

2.2.1 Preparation of a complex of Cu^{2+} and succinic acid

Copper (II) carbonate was obtained through the reaction of Na_2CO_3 (0.023 mol, 2.46±0.1 g) with $CuSO_4 \cdot 5H_2O$ (0.023 mol, 5.8±0.1 g). Copper (II) succinate was then produced via

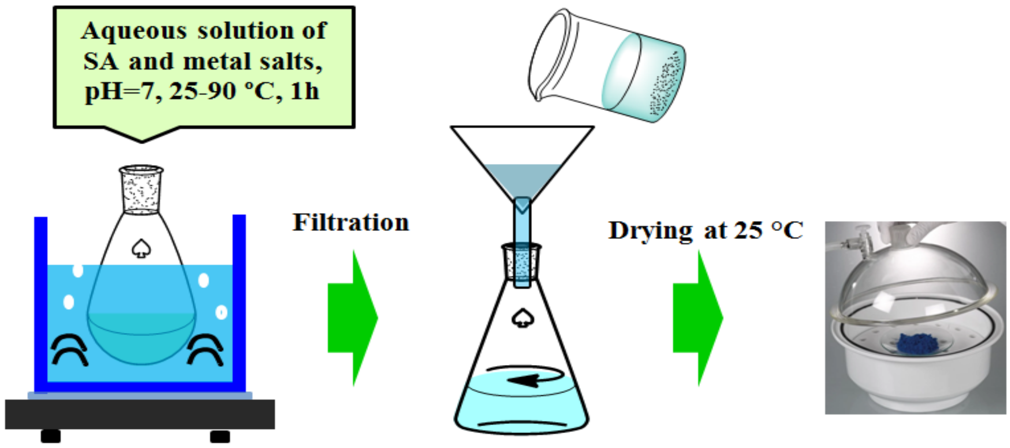


Fig. 1 Preparation Scheme for SA/Me Complex.

a reaction between an aqueous solution of succinic acid (0.023 mol, 2.74±0.1 g) and the aqueous suspension of copper (II) carbonate. After the reaction, the precipitate was dried and crushed into a blue, needle-like powder of succinic acid and copper (SA/Cu) complex, with a yield of 92% (Fig. 2a).

2.2.2 Preparation of a complex of Ag^+ and succinic acid

KOH solution was used to alter the pH of a 20 mL (3.0 mmol/L) aqueous solution of succinic acid to 7.0. In darkroom conditions, 20 mL (6.0 mmol) of Ag (I) nitrate was added to the succinic acid solution. A white precipitate formed immediately. The residue was then collected by filtration and dried in a desiccator with phosphorus pentoxide (P_4O_{10}), while being protected from any light. Afterwards, the dried residue was crushed, resulting in a white powder of succinic acid and silver (SA/Ag) complex, with a yield of 90% (Fig. 2b).

2.2.3 Preparation of a complex of B^{3+} and succinic acid

0.11±0.01g (1 mmol/L) succinic acid was dissolved in 20 mL of deionized water, then 0.168±0.01g (2 mmol/L) of sodium bicarbonate was added to form the sodium salt of succinic acid. Boric acid (2 mmol) was added to the solution and completely dissolved. The mixture was stirred at 90±5 °C for 20 min. 20 mL of ethyl acetate was then added to initiate crystallization. Following 8 days, white crystals were obtained, which were then crushed into a powder of succinic acid and boron (SA/B) complex, with a yield of 88±3% (Fig. 2c).

2.3 SEM analysis

SEM analysis was conducted to ascertain the surface morphology of the complexes and the particle shapes. The surface morphologies of the complexes were examined using a high-resolution JSM-6390 scanning electron microscope (JEOL, Japan). The study was conducted in standard mode. Measurements were carried out in high vacuum mode using a secondary electron detector at an accelerating voltage of 15 kV. Since the complexes are organic compounds, the surface was coated with gold to improve electron conductivity properties, and then it was mounted on an aluminum strip using carbon tape.

2.4 XRD analysis

The crystal structures of complexes were studied via X-ray diffraction on a X´PertPRO diffractometer (Malvern Panalytical Empyrean, Netherlands) using monochromatized copper (CuKα) at a scan speed of 0.05º for 10 s, with a K-Alpha1 wavelength of 1.54187 Å. Measurement in reflection mode, using an aluminium rectangular multi-purpose sample holder (PW1172/01), was performed at a diffraction angle 2θ between 10° and 80°, with the X-ray tube voltage at 40 kV,

(a) SA/Cu **(b) SA/Ag** **(c) SA/B**

Fig. 2 Complex of Me and succinic acid.

current intensity at 30 mA, and a measurement time of each step of 0.5 s. As the sample is a metal-organic compound, these parameters were configured for the XRD study.

2.5 FTIR analysis

FTIR analysis of the complexes were were performed on an Spectrum 400 FT-IR spectrometer (Perkin Elmer, USA), with a resolution of 1 cm^{-1} at a range of 400-4700 cm^{-1}, in accordance with the standard method and using an accessory to measure attenuated total reflection (ATR) and specular-diffuse reflection (SDR), at a temperature of 25±1 °C. The structure of the complexes was confirmed by their respective functional groups present in the IR spectrum.

2.6 Biological efficiency

In order to determine the fungicidal properties and the biostimulating action of the SA/Cu, SA/Ag and SA/B complexes, soybean seeds were treated with them, each at a concentration of 0.1%, using an SMBYC-400 coating machine (Shuoman, China) at a temperature of 20-23±1 °C. Germination was performed in the laboratory and any infections by pathogens were studied using standard methods.[30,31] Incubation was carried out for 2 weeks at room temperature, following which the type of pathogen causing any infection was determined. So as to ascertain the species of mycophilic fungi, samples were removed from any infected samples using a sterile dissecting needle and then placed in a drop of water on a glass slide, with determination of fungi and any other pathogens carried out systematically under an XSZ-146 microscope (Hinotek, China).[32]

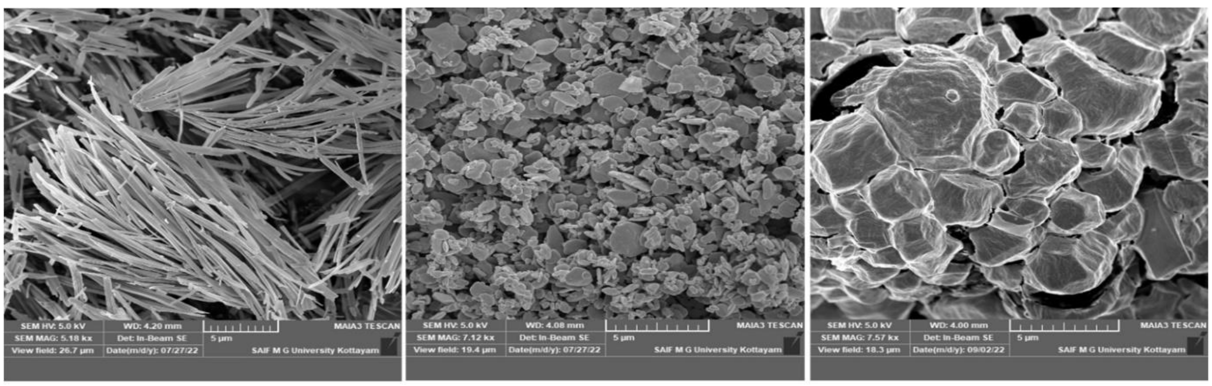
3. Results and Discussion

3.1 SEM analysis

In Fig. 3, the scanning electron microscopy (SEM) analysis illustrates the surface morphology of the acquired complexes. Notably, the SA/Cu complex's surface exhibits a configuration of slender, ribbon-like fibers (Fig. 3a). These fibers are notably clustered together, forming a substantial matrix structure. The observed fibers exhibit lengths in the range of about 20 to 40 μm, accompanied by widths spanning from 200 to 500 nm. Analysis revealed that the spherical grains exhibit dimensions spanning from 200 nm to 2.5 μm. It was discovered that the size of the spherical grains ranges from 200 nm to 2.5 μm. Furthermore, the smaller grains are stacked atop the larger ones, resulting in a coarser appearance. Conversely, the SA/B complex depicted in Fig. 3c is characterized by tightly arranged spherical grains exhibiting diverse shapes and sizes, encompassing a range of 3 to 20 μm. While the granules share a resemblance in shape to those observed in SA/Ag, its surfaces lack smoothness.

3.2 XRD analysis

X-ray phase analysis was carried out to determine the crystal structure of the obtained complexes. Peaks corresponding to 2Θ values of 16.05°, 19.98°, 25.33°, 25.98°, 31.44°, 32.42°, 37.98°, 38.42°, and 42.03° were identified in the diffractogram of succinic acid. Succinic acid features a monoclinic crystal structure with the space group P 1 21/c 1, as illustrated in Fig. 4a. Additionally, the crystal structure of succinic acid aligns well with the ICDD PDF-4/AXIOM database and exhibits strong congruence with the findings from research by Ref. [33]. In Fig. 4b, the 2Θ values characteristic of the SA/Cu complex were 12.66°, 15.72°, 16.81°, 18.68°, 21.73°, 24.89°, 26.52°, 29.8°, 36.88, 40.68°, 43.09°, 46.43°, 52.89°. The SA/Cu complex exhibits a characteristic single-crystal structure, characterized by a monoclinic crystal lattice. In Fig. 4c, the Θ values characteristic of the SA/Ag complex were equal to 12.26°, 18.7°, 21.47°, 24.57°, 29.41°, 30.22°, 36.77°, 38.03°, 39.30°, 40.68°, 42.64°, 48.28°, 49.21°, 57.72°. The SA/Ag complex displays prominent peaks corresponding to the (111), (200), and (220) planes within the face-centered cubic structure system. In Fig. 4d, the 2Θ values characteristic of the SA/B complex were 10.30°, 12.15°, 20.44°, 22.97°,25.95°, 27.91°, 30.56°, 32.16°, 35.16°, 36.54°, 41.26°, 43.22°, 46.66°. Lastly, the SA/B complex demonstrates a typical single-crystal structure.



(a) SA/Cu (b) SA/Ag (c) SA/B

Fig. 3 SEM images of complexes.

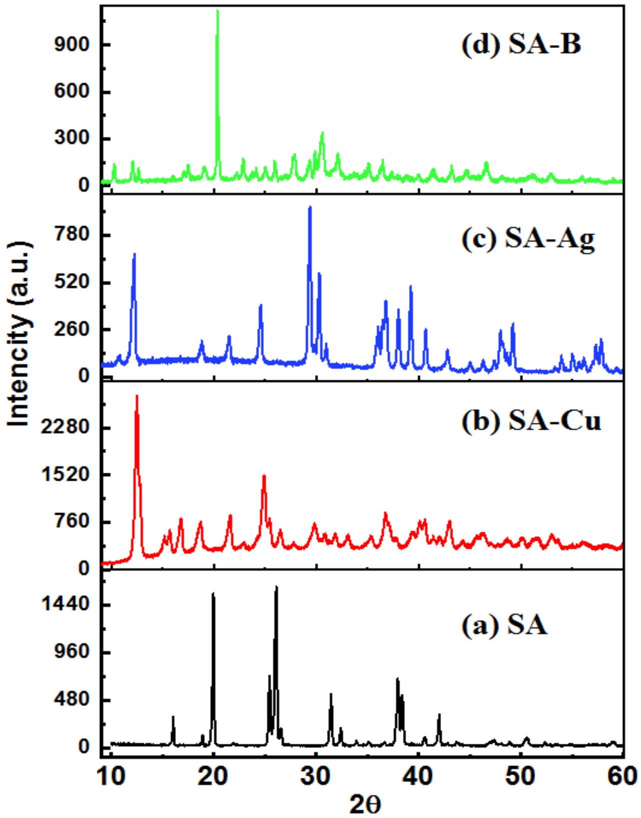


Fig. 4 XRD analysis of: (a) succinic acid; (b) SA/Cu; (c) SA/Ag; and, (d) SA/B complexes.

3.3 FTIR analysis

FTIR analysis was carried out in order to determine the chemical bonds of succinic acid formed during complexation with copper, silver, and boron ions." is already grammatically correct. FTIR analysis shows the formation of new bonds between the metal ions and carboxyl groups in succinic acid. In the IR spectrum of succinic acid, a large O–H valence band is observed in the range of 2900-3300 cm^{-1}, because carboxylic acids usually exist in the form of dimers as a result of their hydrogen bonds (Fig. 5a). The next change is observed in the stretching and bending vibrations of the C=O, C–O and O–H groups in the region of 1300-1800 cm^{-1}. The C=O absorption band of succinic acid is at 1690 cm^{-1}, O-H bending is seen at 910 and 1414 cm $^{-1}$, while stretching of the C-O group is observed at 1310 cm $^{-1}$. The difference between COO^{-} asymmetric and COO^{-} symmetric stretching vibrations depends on the type of coordination between the metal and the carboxylate. The carboxylate group retains C_2 symmetry when coordinated as a bridging or bidentate group. The metal atom is equally bonded to the two oxygen atoms in succinic acid.

The major peaks of the SA/Cu complex appears at 628, 1278, 1387, 1500, 2940 and 3482 cm^{-1} (Fig. 5b). The FTIR spectra of samples with copper, following adsorption tests, did not present additional peaks or changes in range positions when compared to those of other samples.

The IR spectrum of the SA/Ag complex shows major peaks attributed to the interaction between the carbonyl group of the succinic acid and Ag, as confirmed by the appearance of prominent peaks around 580, 1403, 1430, 1500, 1612, 3367, 3477 and 3578 cm^{-1} (Fig. 5c). This confirms the multi-functionalization of Ag ions. Some absorption bands of the IR spectrum of succinic acid can be seen to have undergone significant changes. Further, in the spectrum of this complex, the absorption bands of functional groups (C-H and C=H) of the succinic acid can be seen in the range of 500-800 cm^{-1}. As a result, a shift would be expected in the carbonyl region of succinic acid-functionalized Ag. With regards to this complex, the COO^{-} ions form a strong asymmetric stretching vibration at 1497 cm^{-1} and a weak symmetric stretching vibration at 1388cm^{-1}.

The major peaks of the SA/B complex appear at 745, 1077, 1295, 1403, 1547, 1634 and 3285 cm^{-1} (Fig. 5d). The typical peak of the formation between succinic acid and boron, usually observed at 1077 cm^{-1}, and was slightly shifted after complex formation. Moreover, the reduction in the absorption intensity of the OH group within the absorption range of 2900-3300 cm^{-1} signifies the creation of a complex between metal ions and succinic acid, as reported in Ref. [33].

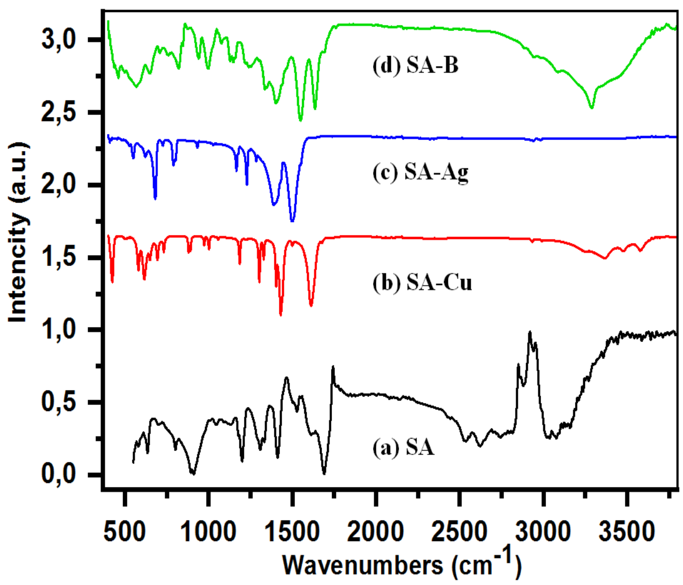


Fig. 5 FTIR analysis of complexes.

3.4 Biological efficiency

The germination of soybean seeds in control samples averaged 70.5%, with a tolerance of ±3% (Table 1 and Fig. 6). On the cotyledons of the control samples are visible signs of infection, including deep, rounded, brown ulcers from the upper and lower sides, which consist of softened tissue, bordered by a roller-shaped rim, covered with ash-black pads (Figs. 6a-6c).

Fig. 6 Germination of soybean control samples, at a) 2 days, b) 10 days and c) 20 days, with visible signs of infection by *Alternaria tenuis Nees*.

Table 1. Information on soybean seed samples, including germination and infection.

No	Samples	Germination, %	Dwarfism	Detected pathogens, %	
				Fusarium	Alternaria tenuis Nees
1	Control	70.5	10.5	20	23.4
2	Pre-treated with SA/Ag	94.9	1.5	5.5	2.7
3	Pre-treated with SA/Cu	93.5	1.8	5.9	4.3
4	Pre-treated with SA/B	90.2	7.3	6.4	8.5

This is due to the presence of the *Fusarium* and *Alternaria tenuis Nees* pathogens (Table 1). The control soybean seeds were attacked by the following pathogens: *Fusarium spp.* – 20%, *Alternaria tenuis Nees* – 23,4%. Besides, 10,5% of control seeds have dwarfism. Such mycotoxins can negatively affect human health, such as acting as contributing factors to liver cancer and reduced bone strength.

The control samples of soybeans over the period from shoot to cotyledon seedlings are affected by diseases that often lead to severe reduction in the number of plants, as well as their weakening and overall reduced yields (Fig. 6c). On the stems of the controls, weeping brownish spots were found, covered with a white, dense, flaky or cottony coating, on which large, black sclerotia with a white core formed, which affects soybeans in all phases of plant development. Found in high percentages in the control samples, *Alternaria*, which *causes* rot*,* leaves traces that are mainly preserved in seed materials, on plant residues and in soils, and is a pathogenic microorganism that is characterized by its olive or blackish-brown color and back club-shaped, with three transverse longitudinal septa (Fig. 7a).[34] Seeds can be affected by *Alternaria*, which is a facultative parasite, causing a black *germ*. Figs. 7a and 7b show the *Alternaria* samples that were found in soybean controls.

Generally, soybeans are affected by *Fusarium* over the entire growing season. Fungi of this genus cause root rot of seedlings and adult plants, wilting, and rotting of beans and seeds. The shoots of control samples during the experiment were unevenly thickened, deformed, and had brown, rounded, deep ulcers on their cotyledons along the upper and lower sides, covered with yellow-orange blooms (Figs. 8a-8c). This is one of the most harmful diseases that affect soybean plants. Manifestations of *Fusarium* depend on the physiological state of a plant, the degree of its resistance, infectious load, and the specific physiological activity of the pathogen, inclusive of growth rate, and formation of toxins and enzymes, among others.

The germination of soybean seeds pre-treated with SA/Cu, SA/Ag or SA/B complexes averaged 92.8%, with a tolerance of ±2%. The highest germination level was 94.9%, which

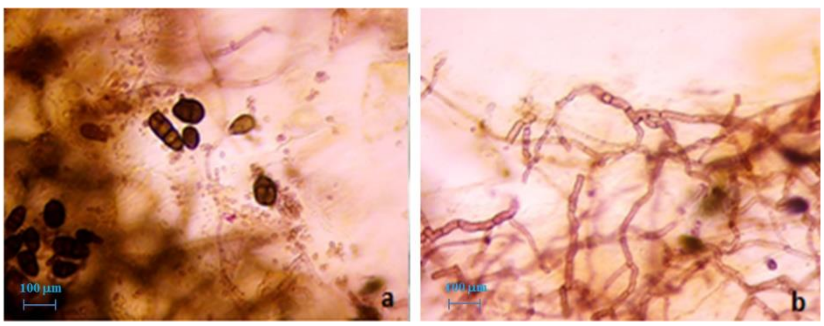


Fig. 7 Control sample pathogens: (a) *Altenaria* spores; and, (b) conidiophores of *Altenaria*, magnified *to* 400x.

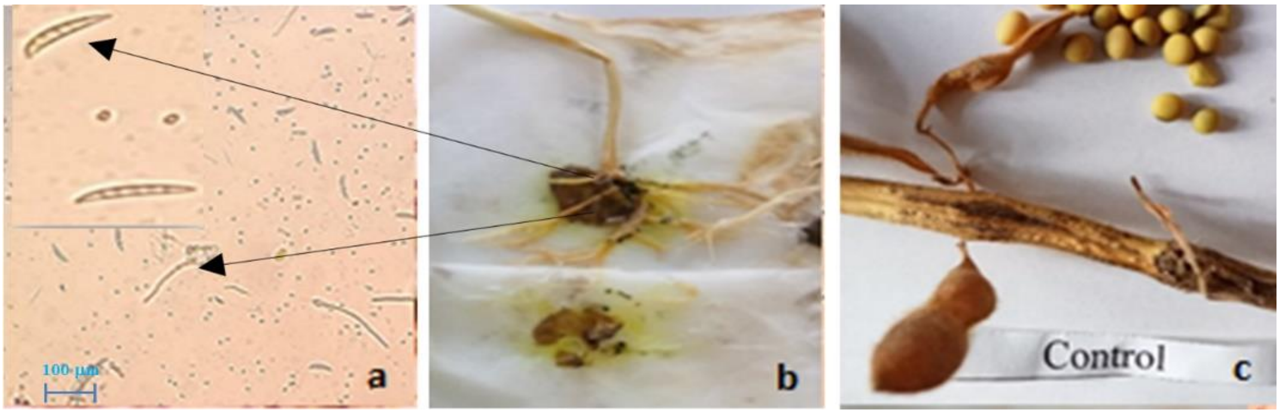


Fig. 8 Soybean control samples attacked by *Fusarium*: (a) enlargement of the macroconidia by 200 x; (b) the roots of a seedling; and, (c) the stem of a seedling.

was 24% higher than that of the control sample (Table 1). Infection by *Fusarium* and *Alternaria* of the samples treated with the SA/Ag complex was the lowest, at 5.5% and 2.7%, respectively, which demonstrates significant decreases compared to the control group, by 4 and 10 times, respectively. The SA/Cu complex was only slightly less effective, though the SA/B one was somewhat less so, especially as regards protection against *Alternaria*. Further, dwarfism was reduced by around 10 times compared to the control in the cases of the SA/Ag and SA/Cu complexes, but was only slightly less for the SA/B complex (Table 1). Outwardly healthy seedlings have well-formed roots, stems and cotyledons (Figs. 9a-9c), and are absent any darkening or spots.

The investigation revealed that these complexes have a stimulating impact, promoting both seed germination and the growth of soybean seedlings. Figs. 10a-10c depict the influence of these complexes on these biological processes. The most substantial activity against the *Alternaria tenuis Nees* pathogen was exhibited by the complexes of succinic acid with silver and copper, as evidenced by the absence of infection in the seedlings (Figs. 10a and 10b). However, in the case of SA/B-treated seedlings, a distinct thickening was observed, along with the development of circular, brown ulcers on both the upper and lower surfaces of the cotyledon leaves. This observation strongly suggests the presence of infections caused by both *Alternaria tenuis Nees and Fusarium* (Fig. 10c).

Our research involving SA/Ag, SA/Cu, and SA/B complexes highlighted that the response of various pathogenic and non-pathogenic organisms to these complexes is not uniform. Pathogenic microorganisms exhibit a considerably higher sensitivity to the actions of SA/Ag and SA/Cu-based complexes. The ranking of antifungal activity among these complexes is as follows: SA/Ag > SA/Cu > SA/B. Notably, the monoligand complex of succinic acid with silver demonstrated remarkable efficacy across all tests, displaying a robust biostimulatory and bactericidal effect.

The results of the study show that pre-sowing treatment of soybean seeds with complexes containing succinic acid with silver, copper or boron has both a biostimulating and fungicidal effect, the latter of which was confirmed by a significant decrease in the growth and germination of *Fusarium and Alternaria.*

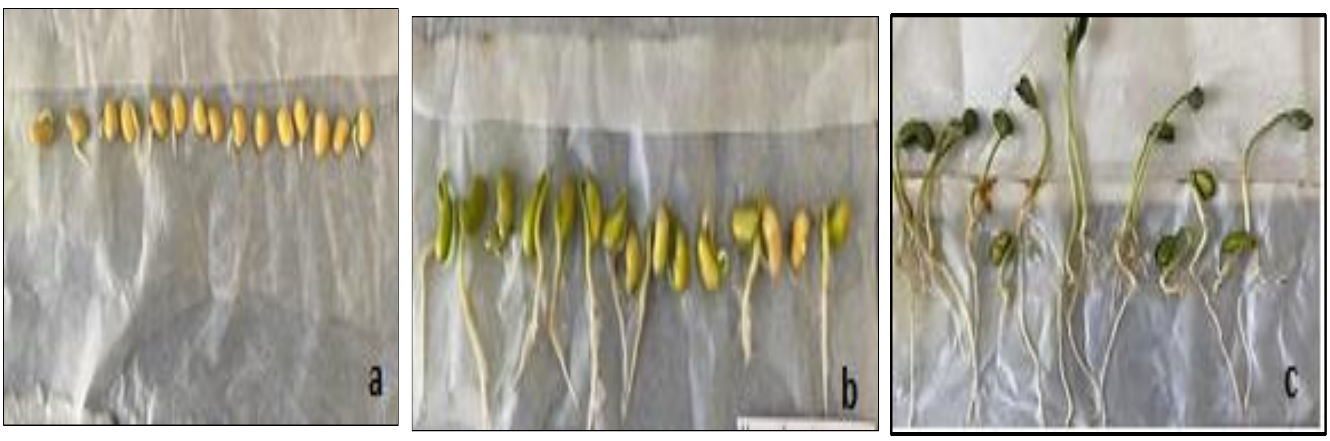


Fig. 9 Mycological analysis of seeds grown using the SA/Cu, SA/Ag or SA/B complexes at (a) 3 days, (b) 11 days and (c) 21 days.

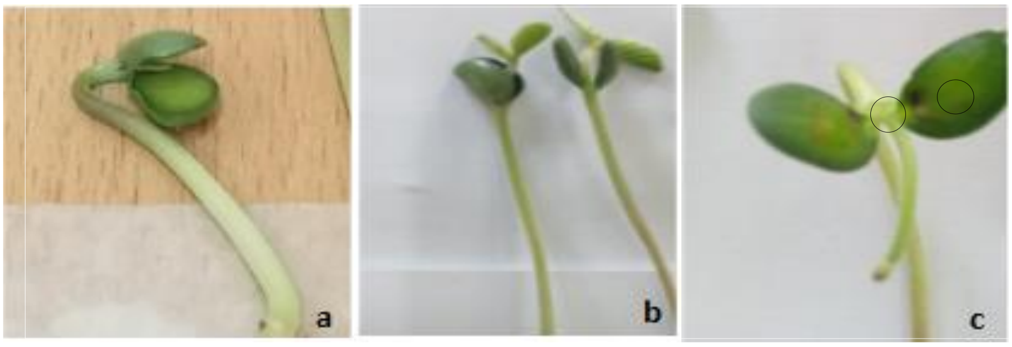


Fig. 10 Vegetative period of plants treated using the complexes: *(a)* SA/*Ag*; (*b)* SA/Cu; and, (*c) SA/*B.

4. Conclusion

Monoligand complexes involving succinic acid and Ag^+, Cu^{2+}, and B^{3+} ions were prepared through reactions in aqueous solutions. Copper carbonate and silver nitrate were employed for the synthesis of the SA/Ag and SA/Cu complexes, while boric acid and sodium succinate were utilized to create the SA/B complex. Examination of the IR spectra suggests that the bond between the ligand and metals is established through the interaction of the carboxyl group in the acid with the metal ions. Specifically, the bond between the carboxyl group of succinic acid and silver is characterized by absorption bands at 580 cm^{-1}, while with copper, it is marked by absorption at 628 cm^{-1}. The research findings confirm that boron ions create a complex compound by binding to two molecules of succinic acid through the interaction of the carboxyl group of the acids with B-OH. This interaction is distinguished by absorption bands observed at 2900-3300 cm^{-1}, 745 cm^{-1}, and 1690 cm^{-1}. The complexes were all established to contain a typical single-crystal structure, with a space group and monoclinic crystal lattice.

The 0.1% solution of the synthesized complexes demonstrated a notable antibacterial effect against pathogenic fungi like *Fusarium* and *Alternaria*, while concurrently promoting seed germination during the early stages of development. So, after presowing treatment of soybean seeds under laboratory conditions, germination increased by 23%, 24.4% and 19.7%, respectively, compared to the control samples. Of the three complexes, SA/Ag was determined to possess the greatest overall stimulating effect and have high efficiency against the fungi pathogens *Fusarium and Altenaria*. The outcomes achieved suggest the substantial promise of employing these complexes for the pre-sowing treatment of agricultural crop seeds.

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Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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