

STUDY OF ONION SULFUR-CONTAINING COMPOUNDS AS BIOFUMIGANTS AGAINST SOIL BORNE PLANT PATHOGENS

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ABSTRACT

Soil-borne plant pathogens are a major constraint to sustainable agriculture due to their persistence, adaptability, and resistance to conventional control strategies. Besides fungal pathogens, Gram-negative bacteria such as *Xanthomonas campestris* and *Pseudomonas aeruginosa* significantly contribute to plant disease complexes, largely due to biofilm formation and antimicrobial resistance. The present study evaluated the biofumigant potential of natural sulfur-containing compounds derived from *Allium cepa* (onion) against these soil-borne bacterial pathogens. Sulfur-rich compounds were extracted from fresh onion bulbs through organic solvent extraction following enzymatic activation of S-alk(en)yl-L-cysteine sulfoxides. Antioxidant activity assessed by the phosphomolybdenum assay produced a linear ascorbic acid calibration curve ($y = 0.0009x$). The extract exhibited substantial total antioxidant capacity, with values of 578 $\mu\text{g/mL}$ and 678 $\mu\text{g/mL}$ expressed as ascorbic acid equivalents (AAE),

confirming the presence of redox-active sulfur metabolites. Antimicrobial activity evaluated by agar well diffusion showed inhibition zones of 12.6 mm against *P. aeruginosa* and 14.3 mm against *X. campestris*, comparable to streptomycin (14–15 mm). The contact time assay demonstrated strong time-dependent bactericidal activity, with 96.55% reduction in *P. aeruginosa* and 99.54% reduction in *X. campestris* after 24 hours. In soil-based pot experiments, onion extract significantly reduced pathogen populations compared to infected controls and showed suppression trends comparable to synthetic fumigant treatment.

Although the synthetic fumigant produced slightly stronger immediate reduction, onion-derived sulfur compounds provided sustained suppression under soil conditions.

1. INTRODUCTION

Soil-borne pathogens pose a significant challenge to sustainable crop production due to their persistence, adaptability, and broad host range. Fungal genera such as *Fusarium*, *Rhizoctonia*, *Verticillium*, and *Phytophthora* cause root rots, wilts, and damping-off, while bacterial pathogens like *Xanthomonas campestris* and *Pseudomonas aeruginosa* contribute to black rot, soft rots, and leaf blights. These pathogens survive in soil through resistant structures or biofilms, making them difficult to control using conventional chemicals. Repeated use of synthetic bactericides has led to resistance and environmental concerns, highlighting the need for safer, sustainable alternatives.

Biofumigation using sulfur-rich plant residues offers an eco-friendly solution. *Allium cepa* (onion) is rich in sulfur-containing compounds such as thiosulfinates, sulfides, and polysulfides, which exhibit antimicrobial and antioxidant properties. These compounds can suppress soil pathogens, disrupt microbial membranes, and modify soil microbial communities, promoting disease-suppressive conditions. Analytical techniques like Gas Chromatography Mass Spectrometry (GC-MS), TLC allow identification of these bioactive compounds, while assays such as phosphomolybdenum test help evaluate their antioxidant potential.

This study investigates the antimicrobial and antioxidant activity of onion-derived sulfur compounds against soil-borne bacterial pathogens, aiming to explore sustainable strategies for plant disease management.

MATERIALS AND METHODS

1.1 Extraction of Sulfur-Containing Compounds from Onion

Fresh, unusable onion bulbs (100 g) were peeled, chopped, and homogenized in 100 mL chilled distilled water to activate alliinase and convert S-alk(en)yl-L-cysteine sulfoxides into reactive sulfur compounds. The homogenate was left for 5–10 min before extraction with 60 mL of a cold ethyl acetate–diethyl ether mixture. The lower organic layer was collected, and the extraction repeated once. Combined extracts were dried over anhydrous sodium sulfate, filtered, and evaporated to obtain crude sulfur extract, which was stored at 4 °C or –20 °C.

2.2. Identification of Bioactive Sulfur Compounds by Gas Chromatography

The sulfur compounds in the onion extract were analyzed using a YL6500 GC system. Nitrogen was used as the carrier gas (2.0 mL/min), with a split ratio of 1:20 and injector temperature of 250 °C. Oven temperature was programmed from 45 °C (5 min hold) to 260 °C at 8 °C/min and held for 40 min. Peaks were analyzed for retention time and area to determine relative composition of compounds.

2.3. Determination of Antioxidant Activity by Phosphomolybdenum Assay

Total antioxidant activity of onion extract was measured using the phosphomolybdenum method. Extracts (2000 µg/mL) and ascorbic acid standards (200–1000 µg/mL) were mixed with phosphomolybdenum reagent (0.6 M H₂SO₄, 28 mM sodium phosphate, 4 mM ammonium molybdate) and incubated at 95 °C until colour developed. Absorbance was measured at 695 nm and expressed as ascorbic acid equivalents (AAE). [Judita Bystrická, *et.al*(october2014)].

Nutrient Agar. After incubation, colonies were counted and expressed as CFU/mL. Percentage bacterial reduction was calculated by comparing treated samples with growth control.

2.4. Antimicrobial Activity Assay

Agar well diffusion was used to evaluate antimicrobial activity against *Pseudomonas aeruginosa* and *Xanthomonas campestris*. Nutrient Agar plates were seeded with 0.5 McFarland standard bacterial suspensions. Wells (6 mm) were filled with onion extract and streptomycin (positive control). Plates were incubated at 37 °C for 24 h (*P. aeruginosa*) and 30 °C for 48 h (*X. campestris*), and inhibition zones measured in millimeters.

2.5. Contact Time Assay

The bactericidal effect of onion sulfur extract was evaluated using a contact time assay. A stock solution of the extract (100 mg/mL) was prepared in DMSO. Overnight cultures of *Pseudomonas aeruginosa* and *Xanthomonas campestris* were adjusted to 0.5 McFarland standard ($\sim 1.5 \times 10^8$ CFU/mL) and diluted in Nutrient Broth to obtain $\sim 1 \times 10^6$ CFU/mL.

The extract was added to achieve a final concentration of 4 mg/mL. Streptomycin- treated and untreated cultures served as positive controls, respectively. Tubes were positive controls, respectively. Tubes were (*X. campestris*) with shaking (120 rpm).

Samples were collected at 0 min and 24 h, immediately neutralized with 1% L-cysteine in PBS, serially diluted, and plated on Nutrient Agar. After incubation, colonies were counted and expressed as CFU/mL. Percentage bacterial reduction was calculated by comparing treated samples with growth control.

$$\% \text{ reduction} = \frac{\text{control cfu} - \text{test cfu}}{\text{control cfu}} \times 100$$

2.6. Soil Pot Experiment to Compare with Synthetic Fumigants

Sterile soil (1 kg) in pots was inoculated with *P. aeruginosa* and *X. campestris* (1×10^5 CFU/g). Treatments included: Set1: Onion extract (50 mg/kg soil) with 3-day covering to retain volatiles. Set2: Pathogen-only control. Set 3: Synthetic fumigant (Amistar 250 SC, 0.67 mL/kg) with 7-day covering. Surface-sterilized seeds (10 per pot) were sown, and soil samples collected at 0, 24, 48 h, and 7 days for CFU determination. Seed emergence was recorded after 21 days:

$$\% \text{ reduction} = \frac{\text{control cfu} - \text{test cfu}}{\text{control cfu}} \times 100$$

RESULTS

Extraction of Sulfur-Containing Compounds: The extraction of sulfur-containing compounds from 100 g of fresh onion bulbs yielded a small, oily residue after evaporation of the solvent. The crude extract had a characteristic pungent aroma, indicative of volatile sulfur compounds such as thiosulfinates and sulfides. The extract was stored at 4 °C for further chemical and biological analyses. Appearance: Oily residue, Odor: Strong pungent, characteristic of sulfur volatiles Storage: 4 °C or -20 °C in amber vials. The successful extraction confirmed that enzymatic hydrolysis of S- alk(en)yl-L-cysteine sulfoxides occurred, producing bioactive sulfur compounds suitable for subsequent GC analysis, antioxidant assays, and antimicrobial testing.

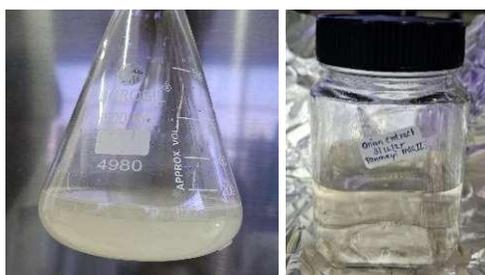


Fig. 1: Extraction of sulfur-containing compounds in onion using diethyl ether and Ethyl acetate solvent.

ANTIOXIDANT ACTIVITY OF ONION EXTRACT BY PHOSPHOMOLYBDENUM ASSAY

The phosphomolybdenum assay confirmed that the *Allium cepa* extract possesses significant total antioxidant activity. The extract showed a concentration-dependent increase in absorbance at 670 nm, indicating effective reduction of Mo(VI) to Mo(V). The antioxidant capacity was expressed as ascorbic acid equivalents (AAE), demonstrating the presence of redox-active sulfur-containing compounds. The observed antioxidant potential suggests that onion-derived sulfur metabolites contribute to oxidative stress modulation and may enhance their antimicrobial and biofumigation efficacy. [[Judita Bystrická, *et.al* (october2014)].

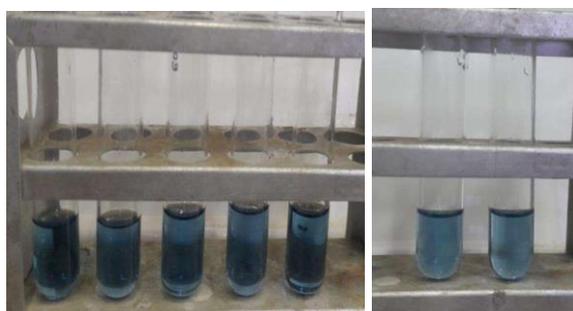
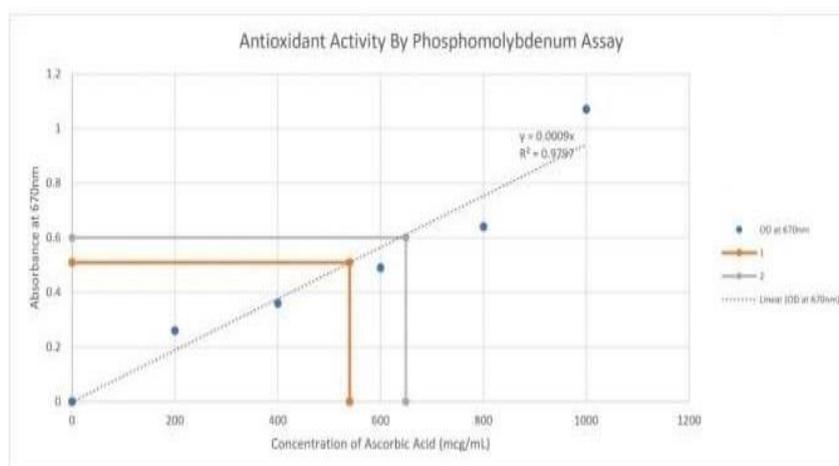


Fig. 2: Phosphomolybdenum assay showing total antioxidant activity of *Allium cepa* sulfur extract compared with ascorbic acid standard. Increasing blue-green colour intensity In standard tubes confirms concentration-dependent antioxidant activity, while ext.



Graph 1: Standard calibration curve of ascorbic acid for phosphomolybdenum assay.

ANTIMICROBIAL ACTIVITIES: [Balouiri *et.al.*(2016), Jhon J Zones (2006)].

Antimicrobial activit of onion extract against *P.aeruginosa* and *X.campestris*. zone of

inhibition by agar well diffusion method.

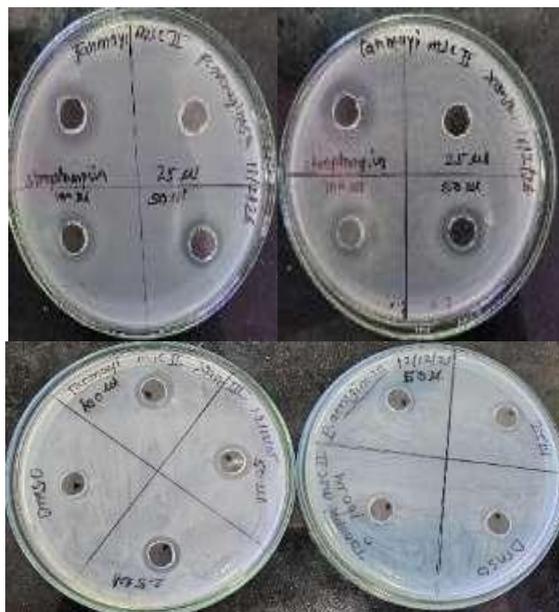
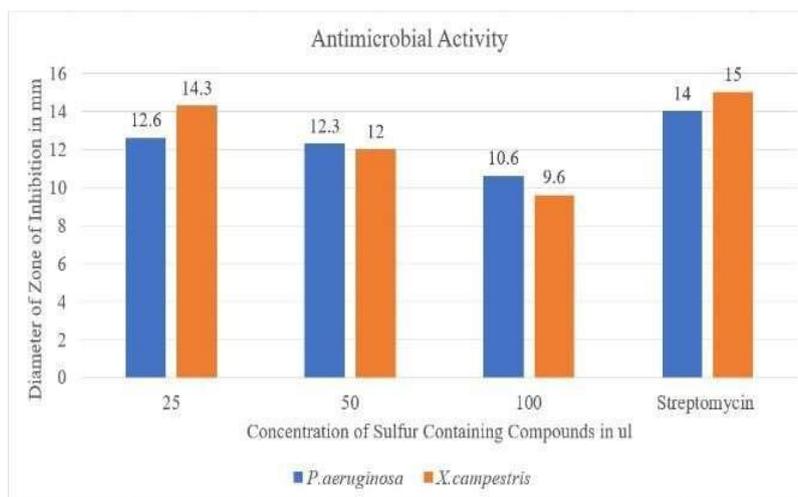


Fig. 3: Antimicrobial activity of onion extract against *P.aeruginosa* and *X.campestris*.



Graph 2: Dose-dependent antibacterial activity of onion extract against *P.aeruginosa* and *X.campestris* by agar well diffusion method.

Table 2: Antimicrobial activity of onion extract against *P.aeruginosa* and *X.campestris*. zone of inhibition observed by agar well diffusion method.

Concentrations	<i>P.aeruginosa</i>	<i>Xanthomonas campestris</i>
100ul	12.6mm	14.3mm
50ul	12.3mm	12mm
25ul	10.6mm	9.6mm
DMSO	-	-
Streptomycin 100ug/mL (control)	14mm	15mm

CONTACT TIME [Arnault et.al.2013].

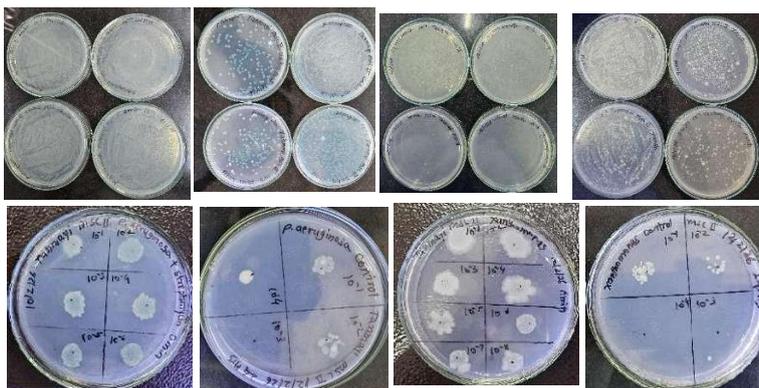


Fig. 4: Contact Time Assay of Onion Extract Against *P. Aeruginosa* and *X. Campestris* (Reduction in Cfu With Increasing Exposure Time.

Table 3: Contact Time Assay of Onion Extract Against *P. Aeruginosa* and *X. Campestris* (Time-Dependent Reduction In Bacterial Viability).

ORGANISMS	TREATMENT	0 min	24hrs	% reduction
<i>P.aeruginosa</i>	Onion extract	5.89×10^7	2.027×10^6	96.55%
<i>P.aeruginosa</i>	Streptomycin 100mg/mL	7.85×10^7	1.1×10^4	99.99%
<i>X.campestris</i>	Onion extract	7.83×10^7	3.573×10^5	99.54%
<i>X.campestris</i>	Streptomycin 100mg/mL	1.05×10^{10}	7.23×10^3	>99.999%

TO COMPARE EFFECTIVENESS WITH SYNTHETIC FUMIGANTS TO CHECK EFFECTS IN SOIL: Xuepeng Fu and Chunxia Li et.al. (2016) Yutong Ji et.al.(2024)].



Fig. 5: Pot experiment showing different soil treatments: control pathogen-only, pathogen +onion extract, and pathogen +synthetic fumigant.

Table 4: Time dependent reduction of *Xanthomonas campestris* in pot experiment.

Time	<i>X.campestris</i> only (control)	<i>X.campestris</i> + synthetic fumigants	<i>X.campestris</i> + onion extract
0 min	-	-	-
24hrs	4.94×10^6	1.89×10^7	1.52×10^8
48hrs	5.19×10^6	2×10^3	1.2×10^3
7 days	4.8×10^8	2.4×10^6	12.1×10^6

Table 5: Time-dependent reduction of *P. aeruginosa* in pot experiment.

Time	<i>P.aeruginosa</i>	<i>P.aeruginosa</i> +synthetic fumigants	<i>P.aeruginosa</i> +onion extract
0 min	<300	-	-
24 hrs	7.32×10^5	1.79×10^8	1.5×10^7
48 hrs	2.55×10^6	7.84×10^5	2.42×10^5
7 days	9.05×10^9	3.81×10^6	4.27×10^6

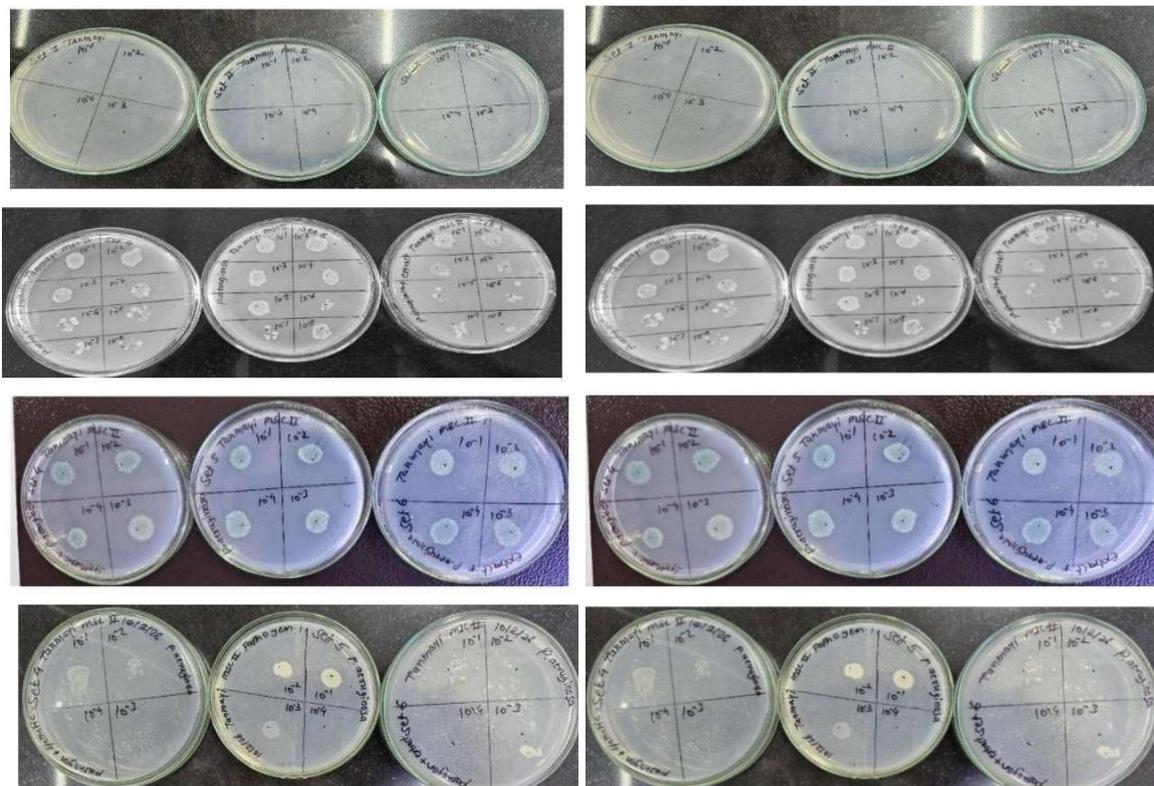


Fig 6: Effect of onion extract and synthetic fumigants on soil population of *X. campestris* and *P. aeruginosa* at different time intervals.

SEED GERMINATION RESULTS



Fig. 7: Comparative tomato seed germination response in pathogen-inoculated soil

treated with onion extract, synthetic fumigant, and untreated control conditions for *Pseudomonas aeruginosa* and *Xanthomonas campestris*.

Table 6: Effect of treatments on tomato seed emergence in soil inoculated with *P.aeruginosa*.

Treatment	No. Of seeds sown	No. Of seeds germinated	% emergence
<i>P.aeruginosa</i> (control)	10	0	0
<i>P.aeruginosa</i> + synthetic fumigants	10	8	80%
<i>P.aeruginosa</i> + onion extract	10	5	50%

Table 7: Effect of treatments on tomato seed emergence in soil inoculated with *X.campestris*.

Treatment	No. Of seeds sown	No. Of seeds germinated	% emergence
<i>X.campestris</i> (control)	10	2	20%
<i>X.campestris</i> + synthetic fumigants	10	8	80%
<i>X.campestris</i> + onion extract	10	6	60%

IDENTIFICATION AND ANALYSIS OF ONION EXTRACT BY GAS CHROMATOGRAPHY: (*Dijkmans et al., 2014; Nwachukwu & Slusarenko. et.al. 2012*)

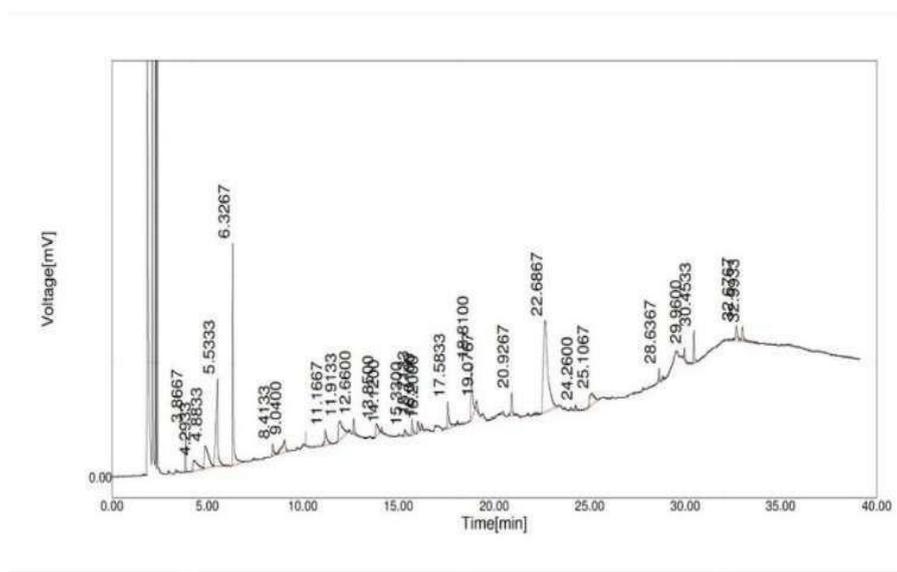


Fig. 8: GC Chromatogram of onion extract showing retention time distribution peak.

Table 8: Retention Time, Peak Area, and Relative Percentage Composition of Compounds Identified in Onion Sulfur Extract by GC.

No.	RT[min]	Area[mV*s]	Height[mV]	Area%
1	3.8667	23.5825	10.6517	1.7078
2	4.2933	43.9717	2.5511	3.1843
3	4.8833	76.4370	5.4468	5.5354
4	5.5333	148.4530	21.1183	10.7506
5	6.3267	162.5542	53.3108	11.7717
6	8.4133	15.4027	2.9523	1.1154
7	9.0400	41.1286	3.0736	2.9784
8	11.1667	33.4096	3.6194	2.4194
9	11.9133	69.6045	4.5168	5.0406
10	12.6600	17.5083	3.6886	1.2679
11	13.8500	27.2307	2.9949	1.9720
12	14.1200	5.5701	1.2470	0.4034
13	15.3300	13.2233	1.4437	0.9576
14	15.7133	16.3733	3.8254	1.1857
15	16.0100	12.3770	2.6221	0.8963
16	16.2000	8.8277	1.5733	0.6393
17	17.5833	39.5142	6.2441	2.8615
18	18.8100	76.8580	13.1214	5.5658
19	19.0767	13.0445	2.2976	0.9446
20	20.9267	31.1982	5.4347	2.2593
21	22.6867	351.7092	22.0090	25.4698
22	24.2600	6.9046	1.2562	0.5000
23	25.1067	45.9074	2.8192	3.3245
24	28.6367	7.7740	2.5231	0.5630
25	29.9600	10.7829	2.7488	0.7809
26	30.4533	29.3714	7.7230	2.1270
27	32.6767	29.7234	3.5085	2.1525
28	32.9933	22.4450	3.3225	1.6254
Sum		1380.8874	197.6439	

4. DISCUSSION

The present study demonstrated that sulfur- containing compounds extracted from *Allium cepa* exhibit significant antioxidant and antimicrobial activity, along with effective suppression of soil-borne bacterial pathogens. Gas chromatography analysis revealed 28 distinct peaks, indicating a complex phytochemical profile dominated by volatile sulfur compounds. The major peak at RT 22.68 min (25.47%) likely corresponds to higher organosulfur compounds such as dipropyl trisulfide, while other peaks suggest the presence of disulfides and low molecular weight sulfur volatiles. These findings are consistent with previous reports (*Dijkmans et al., 2014; Nwachukwu & Slusarenko, 2012*) describing sulfur compounds as principal bioactive constituents in onion extracts. Unlike Brassica-based biofumigation, which relies on isothiocyanates (*Kirkegaard et al., 2000*), the present results highlight thiosulfinates as key functional compounds, suggesting a mechanistically distinct but comparable biofumigation potential.

The phosphomolybdenum assay confirmed substantial antioxidant capacity, indicating the presence of redox-active sulfur metabolites. Similar observations have been reported by *Nwachukwu and Slusarenko (2012)*, who emphasized the role of thiosulfates in oxidative stress modulation and microbial inhibition.

The antimicrobial assays demonstrated clear inhibition zones and significant CFU reduction against *Pseudomonas aeruginosa* and *Xanthomonas campestris*. The time- dependent bactericidal effect observed in the contact time assay aligns with findings by *Arnault et al. (2013)* and *Mihajlović et al. (2017)*, who reported prolonged antimicrobial activity of sulfur-based biofumigants. Statistical analysis confirmed significant reduction in bacterial populations ($p < 0.05$).

In soil-based pot experiments, onion extract significantly reduced pathogen populations and improved seed germination compared to infected controls. Although synthetic fumigants showed slightly stronger immediate suppression, onion-derived sulfur compounds provided sustained pathogen control. These findings are consistent with previous biofumigation studies (*Kirkegaard et al., 2000; Christopher et al., 2010*) and support the potential of onion-derived sulfur compounds as natural, eco-friendly alternatives for sustainable soil disease management.

5. CONCLUSION

The present study demonstrated that *Allium cepa* (onion) extract possesses significant antioxidant and antimicrobial properties against important soil-borne bacterial pathogens, *Xanthomonas campestris* and *Pseudomonas aeruginosa*. The antioxidant assay confirmed the presence of bioactive sulfur-containing compounds with notable redox potential, indicating their role in plant defense related biochemical activity.

The agar well diffusion assay revealed clear zones of inhibition against both test organisms, confirming the antibacterial efficacy of the extract. The contact time assay further established a time dependent bactericidal effect, with substantial reduction in viable cell counts within 24 hours. Percentage reduction analysis showed high suppression levels, indicating strong antimicrobial potential comparable to synthetic bactericides.

In the pot experiment, onion extract significantly reduced pathogen populations over time compared to untreated control. Although synthetic fumigant treatment showed rapid initial

suppression, the onion extract demonstrated consistent and sustained reduction under soil conditions. This suggests that natural sulfur compounds from onion can function as effective biofumigants. The findings support the potential use of onion-derived sulfur compounds as eco-friendly, cost-effective, and sustainable alternatives to synthetic chemical fumigants. The study highlights their applicability in integrated disease management strategies for controlling soil-borne bacterial pathogens while minimizing environmental risks and resistance development.

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