Mesopotamia Journal of Agriculture

https://magrj.mosuljournals.com

TESTING THE EFFICACY OF SILICON AND GLUTATHIONE IN INDUCING SYSTEMIC RESISTANCE AGAINST FUSARIUM SOLANI, THE CAUSE OF BROAD BEAN ROOT ROT

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Article information
Article history:
Received:3/3/2023:
Accepted:12/5/2023
Available:30/6/2023

Keywords:
Broadbean, Fusarium, Glutathione, Peroxidase, Silicone, SOD.

DOI:
https://doi.org/10.33899/magrj.2023.138178.1218

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ABSTRACT

Silicone and Glutathione application are including systemic acquired resistance or resistance induced against plant diseases against. It is a successful means to control plant rot disease and it leads to fundamental effects on the physiology of the host, as Silicone and Glutathione use in as induced resistance agent hostile to Fusarium solani causal agent of Broad bean root rot disease .The results of this study appeared that Silicone and Glutathione application have make less root tot incidence and severity in Broad bean plants and increase resistance-Linked enzymes Action of Peroxidase (POD) Polyphenol Oxidase (PPO) superoxide dismutase (SOD) and Total Phenols (TP) in Broad bean infected roots with F. solani treated with Silicone and Glutathione. This indicates the ability of silicon and glutathione to induce resistance against fungus in bean plants by increasing the activity of polyphenol oxidase, peroxidase, and superoxide enzymes. These results are promising for the use of silicon and glutathione to control root rot infection of broad bean caused by F. solani.

INTRODUCTION

Broad bean plant, Vicia fabae L., is a widespread winter annual crop and is among the oldest plants to have been cultivated; around 6000 BC it is believed to become part of the eastern Mediterranean diet. (Kosterin, 2014, Albala, 2017) And large numbers of the remains of the Broad bean plant from the third millennium BC appear in archaeological sites in the Mediterranean basin and Central Asia. Ripe broad bean seeds contain 11% water, 58% carbohydrates, 26% protein, and 2% fat. Every 100 grams provides 1425 kilojoules (341 calories) and contains the highest protein-to-carbohydrate ratio among other common legume crops such as chickpeas, peas, and lentils. It also contains a good percentage of vitamins, such as A, and provides a rate ranging from 52 to 77% of the daily needs of minerals. Like manganese, phosphorus, magnesium, and iron, it contains a moderate to the rich percentage of vitamins B3 and B1, providing 19–48% of the daily requirement (Sharan *et al*., 2021). This crop is exposed to infection by a number of agricultural pests, and the diseases caused by fungi are among the most important problems and determinants facing the cultivation of the bean crop. Among the fungal diseases that the bean crop is exposed to and that accompany the plant in all stages of its growth are seed rot diseases, seedling death, and root rot. These diseases are caused by soil-endemic fungi (Akrami *et al*., 2012). Fusarium solani, which is one of the most

important species of the Fusarium genus, is also one of the most frequent culprits responsible for the death of seedlings and root rot in bean roots. (Dugassa *et al*., 2021). The control of these diseases depends mainly on the use of fungicides, which are successful means to curb most plant diseases, but they have dangerous effects on human health and increase environmental pollution (Lamichhane et.al., 2020), so there is a need to replace them with other environmentally friendly means to control plant diseases Silicone and Glutathione which, including systemic acquired resistance or resistance induced against the causative. It is a successful means to control plant diseases, and it leads to fundamental effects on the physiology of the host, which quickly leads to the activation of defense genes specialized in plants sensitive to infection with pathogens and thus the formation of many chemical and structural compounds in response to an unsuitable external factor such as pathogens and part(Liang *et al*., 2005 and Holler *et al*., 2010).The enzymes Peroxidase (POD), Polyphenol Oxidase (PPO), Catalase (CAT), and superoxide dismutase (SOD) are among these compounds. These enzymes are essential for the formation of phenolic compounds, which are secondary metabolites used in plant resistance against pathogens. moreover, the peroxidase enzyme is one of the enzymes involved in the defense mechanisms, as it works to form compounds that prevent the penetration of the pathogen into the plant tissue. Superoxide dismutase enzyme reduces toxic hydrogen peroxide concentrations accumulated in plant cells when exposed to pathogens (Babitha et.al., 2002 and Attia et.al., 2022). This phenomenon increases plant resistance against pathogens by applying several agents. In biotic and abiotic plants, it is called the "induced resistance system."The focus has been on a number of chemical inducers to induce plant resistance against many root-pathogenic fungi, such as the use of silicon and glutathione, which give new opportunities to control fungal diseases as a protection system for crops. (Kawano, 2003 and Pieterse, 2014)

The aim of the research is to study the effect of silicon and glutathione on the development of resistance in bean plants against root rot.

MATERIALS AND METHODS

Fungal pathogen.

Fusarium solani has been isolated from broad bean plant infected with root rot small pieces taken form plant root, placed on Petry dishes 8.5 cm contain Potato Dextrose Agar (PDA), the dishes have been incubated for seven days, at 25 ± 2 ° C.

Broad bean types.

The following types were used: French, Dutch, Spanish.

Silicone and Glutathione application.

Silicone product (Brricade) and glutathione were used to treat Broad bean seeds after sterilizing them superficially with sodium hypochlorite solution (0.1 %) for 3 minutes, then washed with sterile distilled water, soak for 24 hours in the solution (100 mg/ l) while the seeds of control soak for 24 hours in distilled water only.

Green House Experiment.

To assess the ability of silicon and glutathione to control *F. solani* root rot and getting resistance in Broad bean plants, five kg plastic pots have been sterile and filled with already sterile soil have been used. The sterile soil has been pollinated 2 days before sowing with *F. solani* grown on PDA. Four Broad bean seeds have been sowed

in single Pots five replicate have been used for all treatments. Results have been set down after all seeds were germinated in control treatment. Incidence and severity of root rot have been were calculated after forty five days, root rot severity has been calculated according to an index consisting of 5 degrees (Cong *et al*., 2018) .

Biochemical Estimation.

Sample preparation.

Half gram has been weighted of Broad bean roots and homogenized with ten ml of 0.1M sodium phosphate buffer (pH 7), after that centrifuged at 10,000 rpm for ten minutes, filtrate, and have been used to determination activity of Peroxidase (POD), Polyphenol Oxidase (PPO), Catalase (SOD).

Polyphenol Oxidase (PPO) enzyme activity estimating.

Estimation of Polyphenol Oxidase activity has been done according to (Shi *et al*., 2002)

Peroxidase (POD) enzyme activity estimating.

Estimation of Peroxidase (POD) activity has been done according to (Howell *et al*., 2003)

Superoxide dismutase (SOD) enzyme activity estimating.

Estimation of Superoxide dismutase (SOD)activity has been done according to (Mesa-Herrera *et al*., 2019)

Total Phenols (TP) Determination.

Total phenol in root samples were determined using Folin–Ciocalteu reagent described by (Jain *et al*., 2017). The root sample was measured at 750 nm Gallic acid (0–250 mg/l) was used as standard.

Statistical analysis.

Random Complete Block Design (RCBD) with three replicates means have been compared by using LSD at P = 0.05.

RESULTS & DISCUSSION:

Effect of Silicone and Glutathione on root rot percentage:

Figure (1) depicts the impact of applying Silicon and Glutathione alone or in combination on the percentage of root rot caused by *F. solani* in broad bean types.

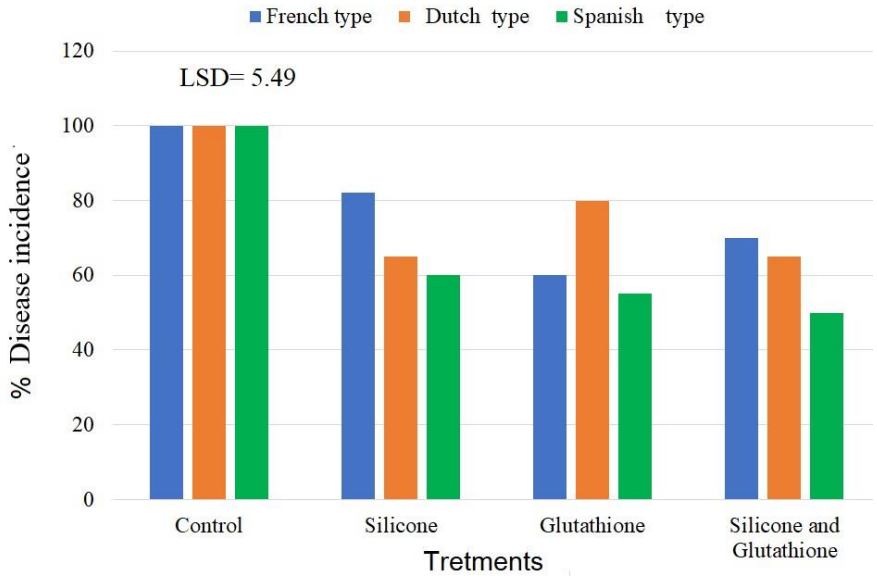

Figure (1) displays the impact of applying Silicone and Glutathione either alone or in combination on the percentage of root rot caused by *F. solani* in different types of broad beans.

The findings demonstrate that soaking the beans in a solution of Glutathione and Silicon resulted in the lowest infection rate of 50% for the Spanish type, which was significantly different. from the control treatment that recorded an infection rate of 100%. Additionally, the use of Silicon treatment for the French type yielded an infection rate of 58.3%.

Effect of Silicone and Glutathione applied on severity of root rot.

Results in Figure (2) show the effect of Silicone and Glutathione applied either alone or in combination on severity of root rot caused *F. solani* in bean species where the lowest value of the severity of root rot infection caused by the fungus *F. solani* in bean species was recorded in the glutathione silicon treatments in the Dutch and Spanish types 0.14, with a significant difference from the control treatment in both types 0.45 and 0.56, then the glutathione treatment in the two types. French type 0.32.

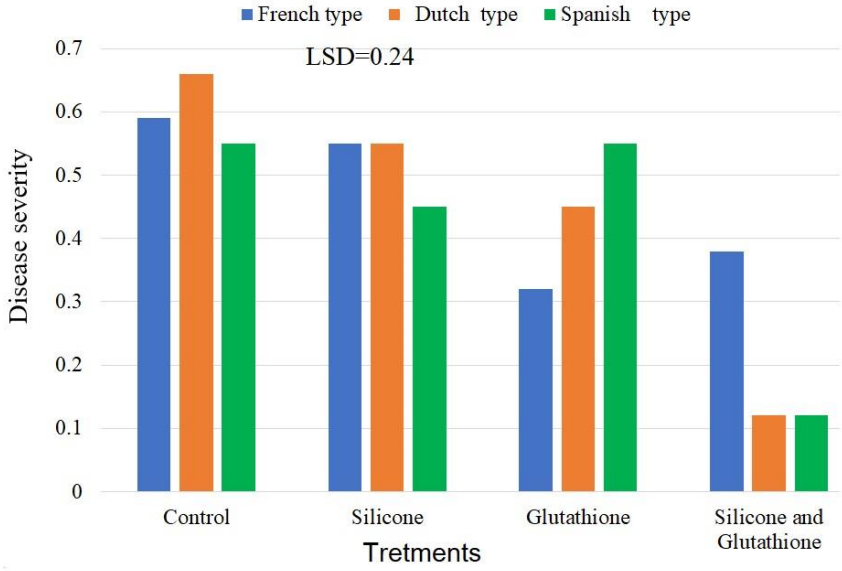

Figure (2) illustrates the impact of applying Silicone and Glutathione either alone or in combination on the severity of root rot caused by *F. solani* in different types of beans.

Effect of Silicone and Glutathione on activity of Polyphenol oxidase.

Means in table (1) show Polyphenol oxidase enzyme activity which increased in Broad bean root in Silicone and Glutathione and *F solani*, treatment compared to Broad bean plants treated with *F solani* only; in Spanish and Dutch type highest averages of enzyme activity have been recorded which treated with Silicone and Glutathione 97 and 93.33 (uint.min g fw $^{-1}$).

Table (1): Effect of Silicone and Glutathione application on activity of Polyphenol oxidase (uint.ming fw^{-1})in broad bean roots.

Treatments	Broad bean types		
	Spanish type	Dutch type	French type
Control	74.00	68.33	70.66
Plosive control *F. solani*	86.33	86.33	84.00
Silicone	92.66	90.33	92.66
Glutathione	89.00	93.12	91.66
Silicone and Glutathione	97.00	93.33	91.66
LSD 5% 3.45			

Effect of Silicone and Glutathione on of Peroxidase enzyme activity.

Means in table (2) show Polyphenol oxidase enzyme activity which increased in Broad bean root in ilicone and Glutathione and *F. solani* treatment compared to Broad bean plants in Spanish type 7.82(uint.min g fw^{-1}) treated with *F. solani* only the highest averages of enzyme activity have been recorded in Dutch type treated with Silicone 7.54 (uint.min g fw^{-1}).

Table (2): Effect of Silicone and Glutathione application on activity of Peroxidase enzyme (uint.ming fw^{-1})in broad bean roots

Treatments	Broad bean types		
	Spanish type	Dutch type	French type
Control	6.01	5.49	5.65
Plosive control *F. solani*	7.00	7,02	6.83
Silicone	7.31	7,28	7.53
Glutathione	7.18	7.54	7.39
Silicone and Glutathione	7.82	7.52	7.39
LSD 5% 0.24			

Effect of Silicone and Glutathione on of Superoxide dismutase enzyme activity

Means in Table (3) show that the activity of Superoxide dismutase enzyme activity which increased in Broad bean root in bean plants in Silicone and Glutathione and *F solani*, treatment compared to Broad bean plants treated with *F solani* only the highest average enzyme activity was recorded in Spanish type 46.93 (uint.min g fw^{-1}) then in French type treated with Silicone 45.20 (uint.min g fw^{-1}).

Table (3): Effect of Silicone and Glutathione application on activity of Superoxide dismutase enzyme (uint.ming fw^{-1})in broad bean roots

Treatments	Broad bean types		
	Spanish type	Dutch type	French type
Control	36.08	32.96	33.98
Plosive control *F. solani*	42.31	42.11	40.11
Silicone	44.84	43.70	45.20
Glutathione	43.06	45,16	44.35
Silicone and Glutathione	46.93	45.16	36.08
LSD 5% 1.43			

Effect of Silicone and Glutathione on Total Phenols.

Means in Table (4) show that the highest level of total phenols was in French type treated with Silicone and Silicone and Glutathione 5.72 ,5.67 (mg. g. fw^{-1})

Table (4): Effect of Silicone and Glutathione applied either alone or in combination on Total Phenols (mg. g. fw^{-1}) Broad bean types.

Treatments	Broad bean types		
	Spanish type	Dutch type	French type
Control	1.90	1.98	2.06
Plosive control *F. solani*	2.88	2.56	3.05
Silicone	4.56	4.39	5.72
Glutathione	5.23	5.23	5.39
Silicone and Glutathione	5.51	3.87	5.67
LSD 5% 0.12			

The infection rate and the high severity of the disease *F. solani* is due to its high pathogenicity and its ability to produce secondary metabolic compounds. It secretes toxic substances that affect the germination process and causes its failure. It also produces a group of enzymes that dissolve the walls of host cells that help penetrate the host, such as Cellulase, Pectin methyl esterase, Pectin transcliminase, Pectinase, Proteinase (El-Sayed *et al.,* 2020 and Perincherry *et al.*,2021).

Studies indicated that *F. solani* infects bean plants and the symptoms of the disease appear according to the stage of development of the host at the time of infection. It infects the seeds before germination and causes them to rot and thus leads to the failure of germination partially or completely, leading to their decomposition. roots and lead to large economic losses with return (Santos *et al.*, 2011 and Jaafar, 2012).

Abd-El-Kareem *et al.,* (2021) tested the effect of four concentrations 0 ,2, 4, 6 gm. L^{-1} of silicon salts and calcium silicate on the mycelium growth of *F. solani* and *R. solani*. Causing black root rot disease in strawberries in vitro. The results were that all tested concentrations significantly reduced the growth of mycelium.

There are many explanations for the role of silicon in curbing the pathogens of putrefaction. Silicon is able to improve the plant's natural defense system, as plants showed increased activity of peroxide enzymes, chitins, polyphenol oxidases, flavonoids, and phytoalexins, which play an important role in plant resistance to fungal pathogens (Fauteux *et al.,* 2005) and the production of phenols, anti-pathogen compounds, phytoalexins, and proline in Silicon treated plants indicates that these compounds may have a role in plant protection. (Rodrigues *et al.,* 2014).

Previous studies indicated an increase in the effectiveness of antioxidant enzymes, including peroxidase, polyphenol oxidase, and superoxide enzymes, as well as an increase in the accumulation of phenols when treated with induction factors, whether biotic or non-biotic, including treatment with silicon and glutathione (Alizadeh- Fortunato *et al.,* 2012).

The treatment with silicon and glutathione also leads to the activation of the antioxidant defense system that protects plants from the damage of oxidative stress during infection with pathogens by inhibiting the production of active oxygen compounds ROS, as well as the production of various types of antioxidants enzymes, including peroxidase and polyphenol oxidase, which contribute to the oxidation of active oxygen compounds ROS (Rajeswari, 2014).

These enzymes constitute one of the elements of the defense system induced when the plant is exposed to infection with pathogens, including fungi, as the peroxidase enzyme removes the toxic effects of active oxygen compounds, including hydrogen peroxide (H_2O_2) and free oxygen, by converting them into water. These rapid transformations prevent the toxic effect caused by the active oxygen compounds in the infected plant. In addition, the affected cells accelerate the final respiratory pathway, which may lead to an increase in the activity of another antioxidant enzyme, the catalase enzyme (Colville and Smirnoff, 2008). Superoxide dismutase is an antioxidant that protects cellular components from oxidation. It catalyzes the breakdown of superoxide oxygen (O-) into molecular oxygen (O2) and hydrogen peroxide (H_2O_2). Hydrogen peroxide is also harmful and is degraded by other enzymes such as catalase (Maurya and Namdeo, 2021). The peroxidase enzyme plays a role in many physiological processes in plant life, the most important of which are

oxidation and reduction reactions, as well as stimulating the synthesis of lignin, a compound necessary to inhibit and curb the activity of pathogens (Dicko, *et al.*, 2006, Al-Noman, Ibrahim ,2020 and Aljuboori, *et al.*, 2022). As for the defensive role of the polyphenol oxidase enzyme in plants, it is represented by the oxidation of existing phenolic compounds. In plant cells to produce quinones, which undergo a series of polymerization reactions leading to the production of polyphenol oxidase melanins, which have anti-microbial activity, preventing these organisms from infecting plants (Glagoleva *et al.*, 2020 and Babitha *et al.*, 2002) indicated an increase in the activity of an enzyme that increased the activity of SOD upon infection with *S. bacteria* and viruses in incompatible interactions between host and pathogen, as in the infection of potato plants, *Phytophthora infestans* and bean plants, with *Pseudomonas syringae* tobacco plants with downy mildew caused by *Peronospora tabacina*, and coffee trees with rust fungusand downy mildew disease in millet plants caused by *Sclerosporagraminicola* as well as increasing the activity of enzymes lipoxygenase, glucanase, 1,3-B, peroxidase, phenylalanine ammonia-lyase.

CONCLUSIONS

The results showed that the use of silicon and glutathione induces systemic resistance in broad bean plants against *F.solani-causingagents* of rotroots in Broad bean, this was represented in increasing the effectiveness of the enzymes peroxidase, polyphenol oxidase, super dismutase and increasing the content of total phenols, this led to a decrease in the incidence and severity of Broad bean root rot disease.

ACKNOWLEDGMENT

The authors extend their sincere thanks to the College of Agriculture and Forestry and at the University of Mosul for providing laboratory facilities to accomplish this work.

CONFLICT OF INTEREST

The authors state that there are no conflicts of interest with the publication of this work.

اختبار فعالية السيليكون والكلوتاثيون في تحفيز المقاومة الجهازية ضد الفطر **Fusarium solani** المسبب لتعفن جذور الباقلاء

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الخلاصة

يعد استخدام مستحضرات السيليكون والجلوتاثيون لاستحثاث المقاومة الجهازية ضد أمراض النبات. من الوسائل الناجحة للسيطرة على أمراض النبات ومن ضمنها الامراض الناجمة عن الفطر *Fusarium sp.* والتي تم استخدامها في هذه الدراسة بثلاثة تراكيز كعامل لاستحثاث المقاومة الجهازية في نباتات الباقلاء تحت ظروف الاصابة بالفطر *Fusarium solani* المسبب لتعفن الجذور أظهرت النتائج أن استخدام السيليكون

والكلوتاثيونبثلاثة تراكيز كعامل لاستحثاث المقاومة الجهازية في نباتات الباقلاء قد قلل وبشكل معنوي من نسبة وشدة الاصابة بتعفن الجذور الناجم عن الفطر F. solani كما زاد من فعالية الإنزيمات المرتبطة بالمقاومة وهي بيروكسيديز (POD) ، بولي فينول أوكسيديز (PPO) ، ديسموتيز الفائق (SOD) ، ومحتوى الفينولات الكلي في جذور نباتات الباقلاء المعاملة بالسيليكون والكلوتاثيون. وهذا يشير هذا إلى ان المعاملة بالسيليكون والكلوتاثيون يعمل على استحثاث المقاومة في النبات من خلال زيادة فعالية إنزيماتبيروكسيديز (POD)، بولي فينول أوكسيديز (PPO)، ديسموتيز الفائق (SOD). تبدو هذه النتائج واعدة فيما يتعلق باستخدام السيليكون والكلوتاثيون لمقاومة الإصابة بتعفن جذو نباتات الباقلاء الذي يسببه الفطر *F. solani*.

الكلمات المفتاحية: باقلاء ، بيروكسيديز، ديسموتيز الفائق ، كلوتاثيون، سيليكون.

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