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Effects of Silicon on Development and Controlling of Powdery Scab in Irish Potato *(Solanum tuberosum)*

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Abstract: Effects of different silicon levels on plant height, stem diameter, disease incidence and severity and its activity on polyphenol oxidase and peroxidase enzymes on powdery scab caused by *Spongospora subterranean* on Irish potatoes (BP1) was assessed. This was a greenhouse study at the University of Zimbabwe Crop Science department. A mixture of 50% sterilised vermiculite and 50% sterilised sand was used as growing media. 0, 250, 500 and 750ppm silicon concentrations were used. Irrigation water, other fertilizers and insecticides applied were made uniform across all treatments. Variations among treatments were monitored from three weeks after planting then on weekly basis for the coming eighty weeks. Silicon concentrations showed significance difference ($p<0.001$) on plant height and stem diameter of Irish potatoes. It proved to play a significant role in the growth of the potato plants. Silicon levels showed no significant difference on powdery scab incidence with all plants getting diseased. Silicon reduced the infestation severity at high concentrations. It also increased the activity of polyphenol oxidase and peroxidase enzymes in potatoes at different response rates. Results from the study indicates great potential of silicon based fertilizers in controlling powdery scab as well as growth rate of Irish potatoes.

Keywords: *Spongospora subterranea*, Irish potato, powdery scab, silicon based fertilizers, activity.

Research Paper

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1.0 INTRODUCTION

1.1. Potatoes and Fungal Infections in Agronomy

Irish potato is a solaneceous crop which has increased popularity and demand on the market in Zimbabwe. Reason for this is due to an increase in market and industrial processing sectors. It have been known to be grown by commercial farmers in Zimbabwe in the past but now small scale farmers in areas such as Nyanga, Mutasa and Domboshava have ventured in its production according to ZFCU, 2010. The crop originated in South America and has well established in Zimbabwe with seed production and breeding activities concentrated in the Eastern highlands according to Makoni, Tsikirayi, Urombo and Mandisodza, 2013.

Although the number of farmers venturing in production of potatoes is increasing, the quantity produced is still below demand levels. Poorly adapted varieties, poor agronomic practices, drought during the production season, poor soils as well as pest and diseases are some of the causes of low quality and quantity potatoes. High incidences of fungal diseases especially powdery scab are the main causal agents of the depleting quantities and quality of potatoes in the country. Powdery scab is caused by a fungus called *Spongospora subterranean*. Tubers affected by the fungus fail to find market as well as the tubers for seed. Once potatoes are affected by the disease, their susceptibility to other diseases also increases. The fungus is said to be linked with the vectoring of Potato Mop Top Virus (PMTV) according to Agrios, 2005.

Sources of inoculum for the powdery scab disease are alluded to infected seed potatoes, manures and contaminated soils though studies show limited relationship amongst these. *Spongospora subterranea* is an obligate biotroph. Its resting spores can still be detected in the soil. Apart from potatoes the disease is said to have a wider horst range mainly in solaneceous crops such as tomatoes and nightshade. Effective control method for the disease has not yet been developed although farmers and researchers are attempting to fight the disease by developing other regulating strategies according to Wale *et al*, 2005.

Only four potato varieties are grown mainly for commercial purposes. Amethyst, an average sized bulbs

occupy the greatest area, followed by BPI, early maturing medium sized bulbs, Mont-Claire variety which produces large flattish tubers good for making chips and Pimpernel, a yellow fleshed variety, which is grown under contract production for crisp manufacturers. There was an increase in the demand for potatoes in Zimbabwe from 2002 up to 2013 by 45 percent according to Makoni *et al*., 2013 though its contribution to global population is very small according to Table 1. Potatoes are grown for several uses which include potato chips production, mashed potato production, boil with skin or mix with other vegetables. Potatoes are a rich source of Vitamin A, Vitamin C and carbohydrates. Potatoes are also used for alcohol, chemurgical application, flour production, starch, dextrin and livestock feed as fresh feed, in the form of tubers or as silage or in a dried form according to Falloon, Merz, Lister, Wallace and Simon, 2011.

Table 1: Zimbabwe's contribution in potato production in the world

	1980	**1990**	**2000**	**2010**
World total	240464520	266624520	327349600	324181889
Zimbabwe	20441	31000	32000	58000
Contribution %	0.0085	0.012	0.0098	0.018

Source: FAO STAT, 2012

Potatoes do well in areas where temperatures do not exceed 32 degrees according to Gong, Chen,Wang and Zhang, 2003. They explained that below temperature of 15 degrees Celsius (ºC), potatoes are susceptible to frost injury, which may result in poor yields and quality. They also highlighted that the optimum temperatures for potato production ranges from 25 to 30(ºC). The crop does well on a wide range of soils. Best soils for production are the medium textured loam soils with good drainage and high organic matter content. Heavy soils are discouraged because they may become hard when dry and may affect the shape of the tubers. Soil pH of around 5 to 5.5 is said to be highly favorable and water logging conditions should be avoided. Potatoes are a summer crops and their growing season starts from around November to March in Zimbabwe. For high yields, farmers are recommended to plant in November with the first rains.

The pathogen, *Spongospora subterranea* is said be found worldwide wherever potatoes are regularly grown. Despite affecting Irish potatoes, *Solanum tuberosum*, *Spongospora subterranea* is said to have a wide horst range. Harrison, Searle and Williams, 1997, reported that solaneceous plants are more susceptible. Volunteer potatoes may also be important as a host. Root infections have been observed on wheat, barley, oats, perennial ryegrass, oilseed rape, turnips, chickweed, and clover. However, these are dead end infection plants because the pathogen cannot multiply on these crops.

Van De Graaf *et al.*, 2007, stated that some soils suppress powdery scab severity though the mechanism is not well understood. Disease inoculum can come from infected or contaminated seed tubers, soil contamination where infected tubers have been grown, contaminated manure or slurry spread on field, field to field movement of soil and as wind-borne particles. They also elaborated that powdery scab is more prevalent in cool, wet climates, but the exact effects of temperature and moisture on disease incidence have no clear evidence yet. The causative agent of powdery scab disease is a fungus, *Spongospora subterranean* which reduce yield to some extent, renders the tubers unmarketable with scabbed appearance. There is also rejection of tubers for seed which help prevent infestation of clean fields according to Ueli Merz, 2008. The pathogen produces naked plasmodia and is considered a water mold. *Spongospora subterranea* is regarded an obligate parasite.

Detection of the pathogen in the soil and on tubers is based on the presence of sporosori, the resting spores of the pathogen. These spores can be detected through bioassays, using Polymerase Chain Reactions (PCR), and antibody methods as recommended by Falloon *et al.*, 2011. They noted that zoospores in the presents of water helps the pathogen to swim but can only travel very short distances, a few millimeters at the most. This pathogen is a member of the family Plasmodiophoridae, which has recently been shifted from the fungi to the protozoa kingdom. The pathogen has a resistant resting stage that enables it to survive in soil even up to six (6) years or more. *Spongospora subterranea* is also said to transmit potato mop-top virus according to Van De Graaf *et al.*, 2007.

1.2. Disease cycle and common control mechanisms

Merz, 2008, alluded that not much is known particularly for *Spongospora subterranean* life cycle hence is centered on the basis of other plasmodia. The life cycle takes at most 2 weeks as summarized in Figure 1. Time from zoospore release, from a spore ball, to root infection is very short, about one hour. Spore balls are relatively large, 20-100 mm in diameter, and comprises of hundreds of cysts. These spore balls are resistant to many hash environmental conditions. Spore balls can keep on releasing swimming spores for up to 20 years. These spores are short lived as most die in dry soils.

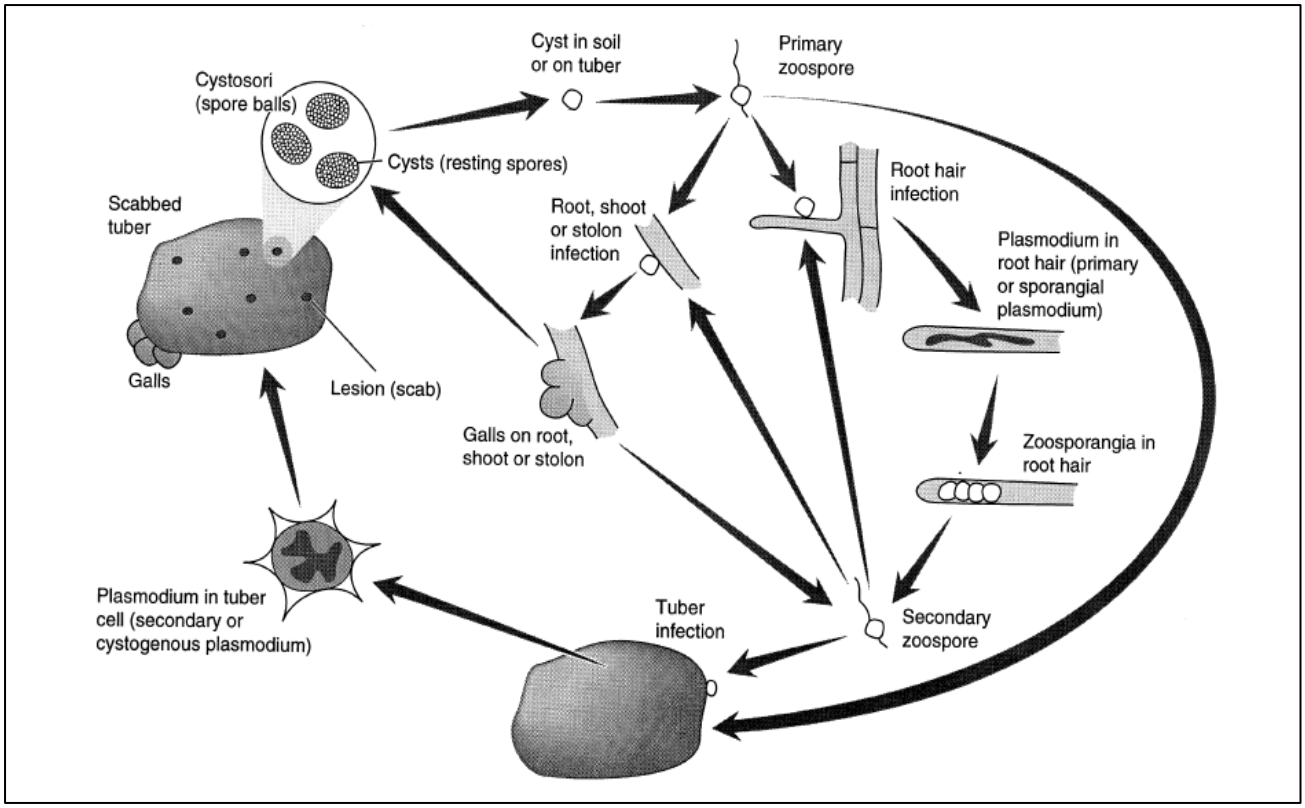

Figure 1: The summary of the life cycle of powdery scab
Source: Merz, 2008

Symptoms of this disease are only confined to below ground parts like tubers and roots. Infected roots and stolons may have white gall like tumors, which turn brown as they mature. These galls are the results of enlargement and proliferation of diseased cells. The pathogen enters the tuber through lenticels, wounds and sometimes potato eyes. Powdery scab affected tubers symptoms roughly resemble those affected by common scab. When stored, infected tubers are susceptible to other disease problems such as fusarium dry rot, bacterial soft rot and other opportunistic invaders. Symptoms can develop after harvest. Infected tubers may shrink in storage. Tuber to tuber contamination in storage has never been well documented according to Falloon *et al.*, 2011. The pathogen also affects the stems near the soil line.

Currently, no control method completely eradicates powdery scab according to Fallon, 2008. He stressed that a good control measure should reduce the inoculum level in the soil and the seed. Late planting is reported to be a cultural practice that exposes the pathogen to conditions which are less favorable for development and spread. Avoiding soil compaction is also a cultural practice that reduce powdery scab build up and infestation. Understanding of soil inoculum levels may be beneficial in determining which cultivar to plant. Reducing inoculum spread in fields can be done by the use of clean, treated manure. Spore balls were reported to survive the alimentary canal of cattle which have fed on powdery scab infected potatoes according to Van De Graaf *et al.*, 2007. Long rotations are useful in reducing the amount of inoculum in the soil. Planting clean certified seed assists in control of inoculation. Composting of infected material kills the pathogen by building high temperatures though a few spore balls may survive.

Other cultural control possibilities include the use of resistant cultivars although no cultivar has yet been efficient in proving complete resistance according to Brown *et al.*, 2007. Chemical control have been effected through use of zinc, fluazinam (Omega), mancozeb, and meta sodium as the most common pesticides. Sulphur has shown little control according to James and Crowe, 2008. Soil with high levels of zinc have been shown to suppress powdery scab although the mechanism have not been clearly documented as alluded to by Pieter Van De Graaf and Scri, 2005. Fluazinam has also shown great efficacy in reducing powdery scab though phytotoxicity has been observed with its application in tubers. Incorporation in soil has shown to be the best way to apply the chemical. Biological control has been seen used especially by use of trap crops. Only drawback of this method is that the field is taken out of production for the whole year. Merz and Falloon, 2009, stated that green manures with high levels of glucosinolate may be effective in controlling the fungi according to its life cycle in Figure 1.

1.3. Incorporating silicon in potato fertilizers as a remedy

Silicon is reported to be the second most abundant element in the earth's crust. Most common used silicon fertilizers include calcium silicate slag, calcium silicate and sodium metasilicate. The element is taken up by plants and often present in moderately high concentrations in plant tissues according to Gong *et al*., 2003. They also added that silicon concentration in plant tissues sometimes exceed the concentration of some major nutrients like nitrogen and potassium. Deposition of silicon in the epidermal cell layer enhances the resistance of plant tissues to fungal penetration. Within a given plant species or cultivar, tissue levels of silicon varies depending to soil type and soil silicon availability to plants.

Apart from inhibition of fungal diseases, silicon has also been reported to enhance certain mineral imbalances as well as other diseases triggered by abiotic stresses in plants according to Ma, 2004. Several studies have found out that, silicon can reduce or prevent manganese and iron toxicity and has positive effects on aluminum toxicity. Study also pointed out that silicon does not affect manganese uptake, but rather its distribution in plant tissues. Weiss and Schwerdtfeger, 1994, stressed that, when silicon levels are low in the tissue, manganese tend to distribute unevenly and accumulates to toxic levels in spots, in leaves. However, sufficient levels of Si seemed to cause even distribution of manganese in plant tissue, thereby preventing accumulating to toxic levels in leaves. They also stressed that silicon cause detrimental nutrient imbalances between zinc and phosphorus. Brown *et al*, 2007, noted that silicon can be used to reduce salinity stress as well as to reduce transpiration rate in plants as well as protecting leaves from ultraviolet radiation damage.

Ning *et al.,* 2014, observed that silicon accumulates around fungal hyphae as well as on infection pegs in diseased host plant cells. They also noted that phenolic materials and chitinases also promptly accumulate in these diseased host cells. Silicon amended plants have also been observed to have a higher significant percentage of diseased cells which accumulate phenolics. Fungal hyphae penetration on the phenolic-laden cells of silicon amended plants was found to be seriously damaged by the accumulated phenolics according to Ueli Merz, 2008. These phenolics were observed to be fungi toxic. Therefore, this proved that silicon fertilization can reduce disease susceptibility primarily by stimulating host-plant defenses. The activity of peroxidase enzymes had been said to increase in infected resistant cultivars. Peroxidases are associated with inducing resistance, directly through production of inorganic ions and phenolic compounds that inhibit fungal growth, and indirectly through production of reactive oxygen species that mounts at infection sites destroying the pathogen. Polyphenols are also defence related enzymes which works in the same manner as peroxidases, with activity also being high in resistant cultivars site of infection.

The purpose of this study was aimed at developing an effective control method for powdery scab caused by *Spongospora subterranea* on potatoes using silicon. Appropriately recommended silicon concentration application was assumed to help control the effects of powdery scab. There was need to assess the effect of silicon on development of powdery scab and its induction of peroxidase (POD) and polyphenol oxidase (PPO) activity on Irish potato (*Solanum tuberosum).*

2.0. METHODOLOGY

This study was conducted at the University of Zimbabwe in Harare. The experiment was laid out in a greenhouse as a Randomized Complete Block Design (RCBD). Four treatments of different silicon concentrations were done with each treatment being replicated randomly 3 times in one block. Four blocks were used for the experiment as depicted in Table 2.

Table 2: Silicon concentrations and variety used on treatments

Treatment	Potato variety	Silicon Level (ppm)
1	BP1	0
2	BP1	250
3	BP1	500
4	BP1	750

Source: Authors data

Sprouted potato seed tubers were planted in black polythene 15 kg bags. The bags were half filled with 50 % sterilized vermiculite and 50% sterilized sand. Sterilization was done by boiling water to hundred degrees then immersing the medium in the boiling water for 30 minutes. Sterile pockets were then half filled with a mixture of 50% sterilized vermiculite and 50% sterilized sand. Sprouted tubers were then planted at a depth of 7cm. Compound S was applied as a basal fertilizer at a rate of 30g/plant. Planted bags were arranged in a RCBD. Replicates were in a randomized map generated by mini tab 16. Silicon treatments were added 2 weeks after planting as a solution. 250mg of silicon was put into 1L of distilled water to produce a 250 parts per million (ppm) solution. Solutions of 500ppm and 750ppm concentrations were made accordingly.

Stem diameters where measured using the venier calipers starting from 3 weeks after planting and on weekly basis for the coming eight (8) weeks. The incidence of powdery scab on the tubers was obtained by counting the number of powdery scab infected tubers then expressing this as a fraction of the total number of tubers produced on that plant. This was then converted to percentage by multiplying by 100. Thus, Incidence = (Number *of infected tubers/Total tuber number per plant*) * 100.

Severity scoring was done according to the method described by Scottel, Wu, Goktepe and Charlton, 2005. Severity scale used for rating powdery scab was as follows:

0-healthy, no lesions on the potato tubers
1-less than 1% area covered by scab lesions or not more than 5 scab lesions
2-(1~10%) area covered by scab lesions or 6-25 scab lesions
3-(10~25%) area covered by scab lesions
4-(25~50%) area covered by scab lesions
5-50% or more area covered by scab lesions

For assessment of polyphenol oxidase, method described by Ngadze and Sciences, 2012 was used. 5g of fresh tuber tissue crushed in liquid nitrogen to powder using pestle and mortar. 2.5g of the powder, homogenate, was mixed with 5ml of 0.05M sodium phosphate buffer (pH 6) which contained 5% polyvinyl polypyrolide. This solution was then filtered using muslin cloths then the filtrate centrifuged at 13.00 rounds per minute (rpm) for 5 minutes at 4ºC. 1ml from the centrifuged solution, supernatant, was taken to another tube where it was mixed with 2.9ml of 0.05M sodium phosphate buffer at pH 5.5 and with 1ml of 0.1M catechol (sigma). 3 equal parts were separated for polyphenol oxidase assay. 1ml of phosphate buffer was substituted for tuber tissue in the control experiment. Absorbance was then measured at 546nm for 4 minutes at 20second interval and readings obtained converted to per minute basis.

For assessment of peroxidase, 5g of fresh tuber tissue was crushed in liquid nitrogen to powder using pestle and mortar. 2.5g of the powder, homogenate, was mixed with 5ml of 0.05M sodium phosphate buffer (pH 6) which contained 5% polyvinyl polypyrolide. This solution was then filtered using muslin cloths then the filtrate centrifuged at 13.00 rpm for 5 minutes at 4ºC. 1ml from the centrifuged solution, supernatant, was taken to another tube where it was mixed with 2.9ml of 0.05M sodium phosphate buffer at pH 5.5 and with 1ml of guaiacol (sigma) and 1ml of 2% hydrogen peroxide. 3 equal parts were separated for polyphenol oxidase assay. 1ml of phosphate buffer was substituted for tuber tissue in the control experiment. Absorbance was measured at 470nm for 4 minutes at 20 seconds rest. The enzyme activity was then expressed in units per gram of protein.

Minitab 16 was used to test for assumptions. Data was analyzed using Genstat 14 and Fisher's 5% LSD was used to do mean separations.

3.0 RESULTS AND DISCUSSION

3.1 Effects of silicon on plant height

Plant heights in all silicon levels increased with progress in time from week 1 to week 8 as shown in Figure 2. Treatment 1 produced lowest mean heights from week 1 to week 8 while treatment 4 produced the highest mean heights on the same period as depicted in Figure 2.

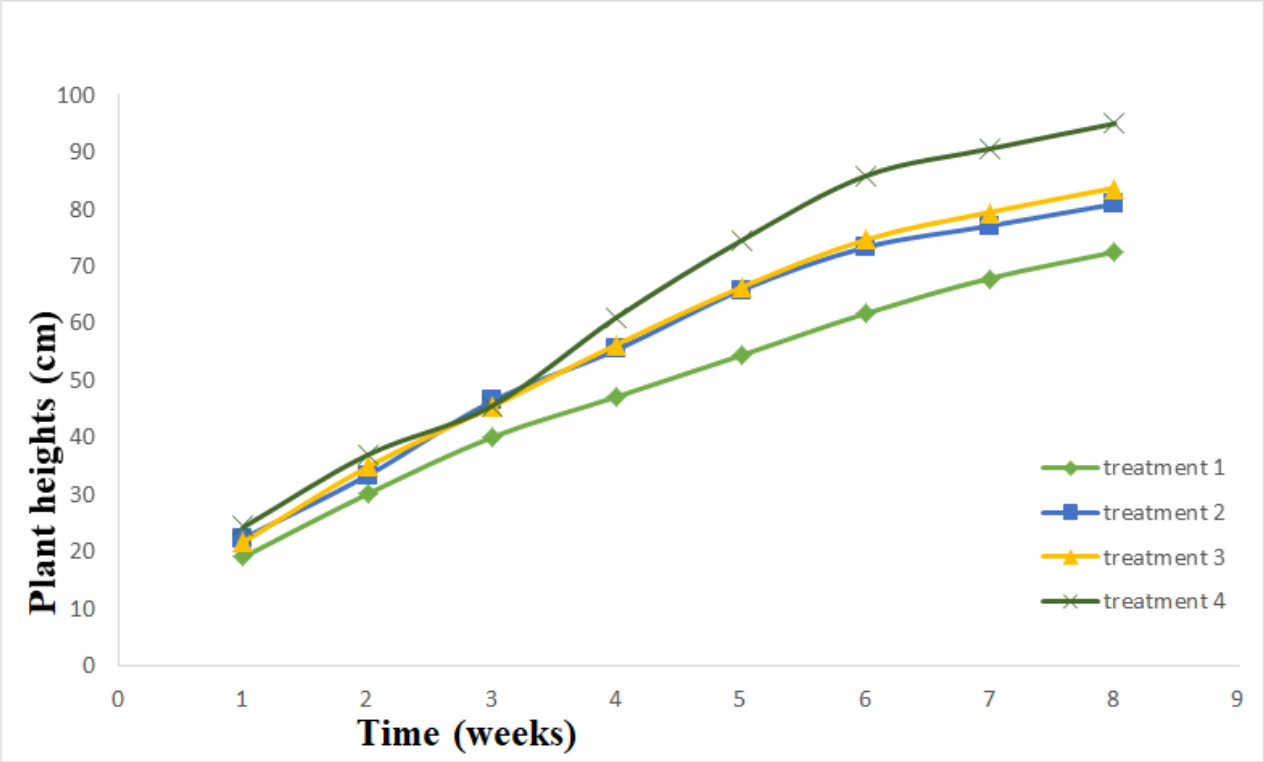

Figure 2: Effects of different silicon concentrations on Irish potato height for the time under study
Source: Authors data

There was no significant difference ($p>0.01$) in the effects of silicon concentration on height from week 1 to week 4 however significant difference ($p<0.01$) were noted from week 5 to week 8 as depicted in Figure 2. Simple statistics (5% LSD) were used to compare the significant treatment means of different silicon levels. From week 5, treatments 4 and 1 means were significant from all other treatment means, treatment 2 and 3 means were not significantly different from each other but different from treatments 1 and 4.

3.2 Effects of silicon on stem diameter

Stem diameter in all silicon levels increased with increase in time from week 1 to week 8 as shown in Figure 3. Treatment 4 produced the highest mean stem diameter from week 1 to week 8 as depicted in Figure 3.

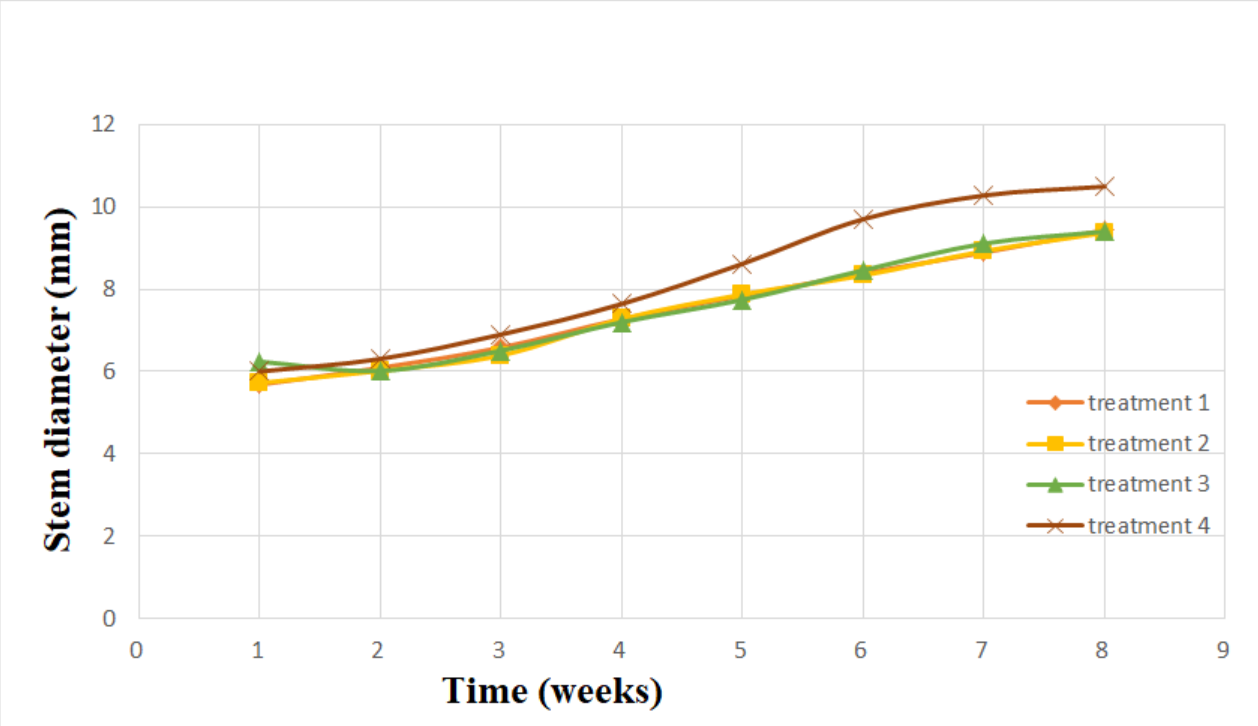

Figure 3: Effects of silicon concentrations on stem diameters of potato plants
Source: Authors data

Silicon concentrations showed no significant difference ($p>0.01$) on their effect on stem diameter from week 1 to week 4, however significant from week 5 to week 8. Simple statistics (5% LSD) were used to compare the significant treatment means of different silicon levels of stem diameter. From week 5 to week 8, means of treatment 1, 2 and 3 were not significant different from each other but rather, all were significantly from treatment 4. The response of plants diameter to silicon treatments was slow but proved to be significant ($p<0.01$) from week after application. Those plants that had high silicon treatments (500ppm and 750ppm), proved to produce strong stems that resisted lodging. This observation showered to go hand in hand with the finds by Fauteux *et al.*, 2005, who reported that if silicon is abundantly available to plants, it can improve the physical characteristic of plants like helping in anchoring the plant from lodging conditions.

3.3. Effect of silicon on powdery scab disease incidence

Silicon concentration did not show any significant differences in controlling the incidence of powdery scab caused by Spongospora subterranea. All the treatments were affected by the disease with incidence reaching 100% in silicon levels of 0ppm, 250ppm and 750 ppm while a 98% incidence was recorded on plants treated with 750ppm as depicted in Figure 4.

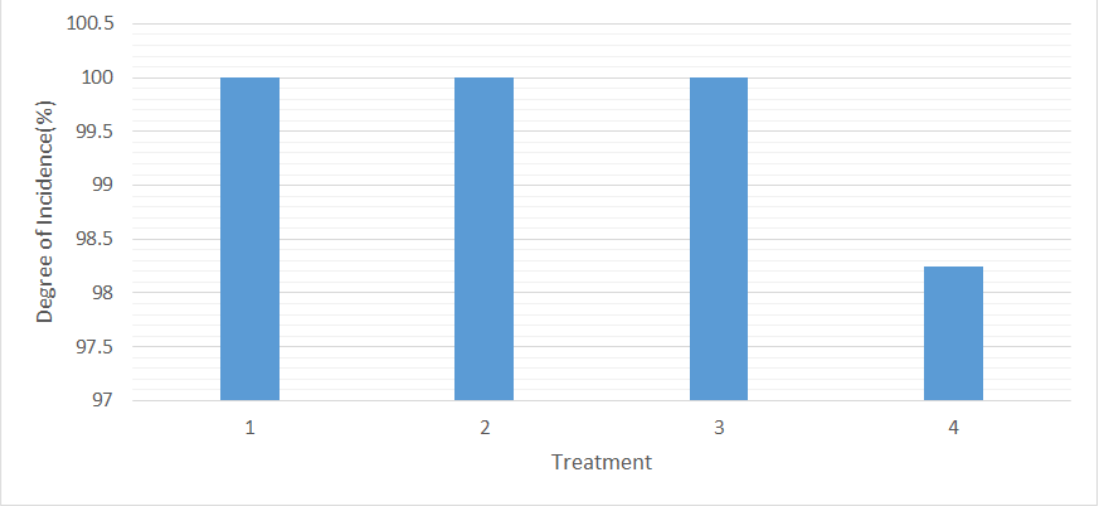

Figure 4: Effects of silicon levels on the incidence of powdery scab
Source: Authors data

3.4. Effects of silicon on severity

The increase in silicon levels showed to reduce the severity of powdery scab as depicted in Figure 5. The effect of silicon concentration was significant ($p<0.01$) on the reduction of powdery scab severity.

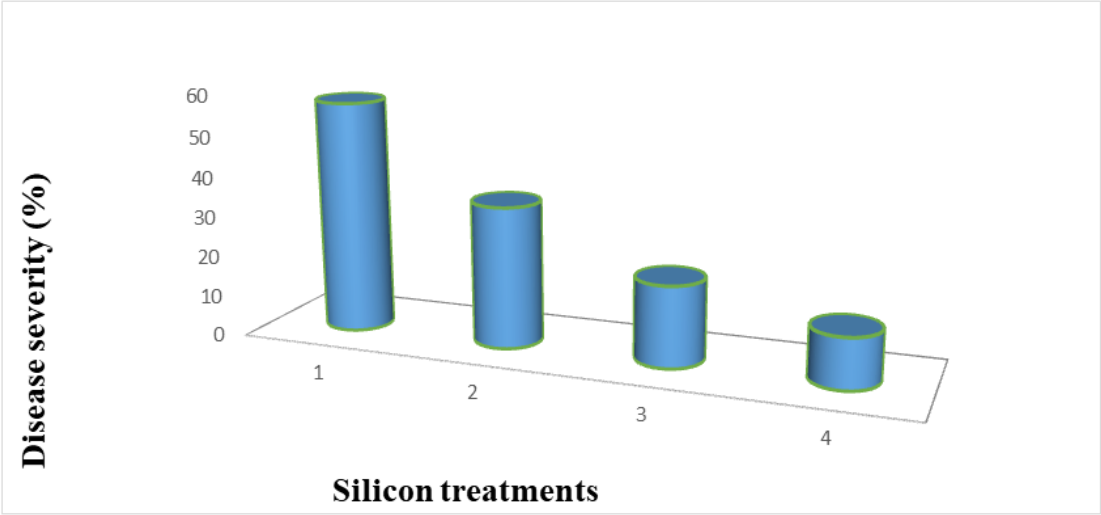

Figure 5: Effects of silicon levels on disease severity scores (%)
Source: Authors data

Mean severity score (%) of treatment 1 was significantly different from all other treatment means. Treatment 3 and 4 means were not significantly from each other but were significantly different from treatment 1 and 2 as depicted in Figure 5. Severity scoring (percentage) decreasing with increase in concentration, thus 0ppm tubers severely affected with 500ppn and 750ppm not severely affected. However on the current study with diatomaceous earth with 73% silicon, silicon concentration of 500ppm and 750ppm per plant, significantly reduced powdery scab severity. This is because, silicon if abundant to the plant, it is deposited in the epidermal cells of cells (tubers) thus acting as a physical barrier to entry, build up and spread of the pathogen inside the host in agreement with Datnoff *et al.*, 1997.

3.5 Effects of silicon concentration on PPO activity

The activity of PPO increased as the time progressed from week 1 to 3 with treatment 4 having the highest enzyme activity. Significant differences were noted between different silicon concentrations ($p<0.001$) as highlighted in Figure 6.

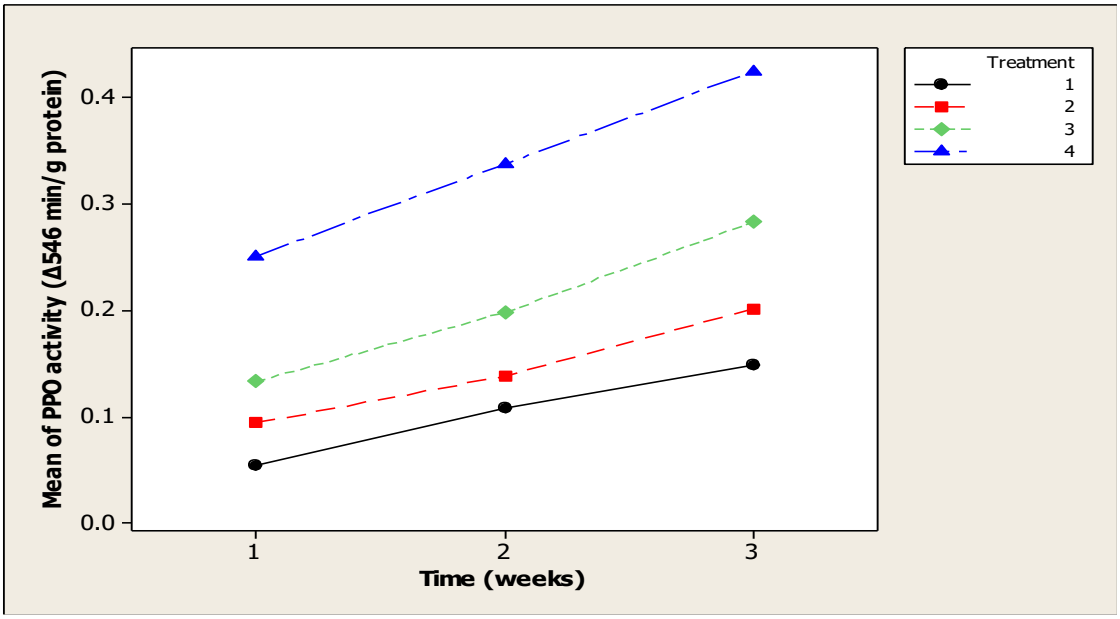

Figure 6: Effect of silicon concentration on PPO activity
Source: Authors data

3.6 Effect of silicon concentration on POD activity

The activity of POD increased as the time progressed from week 1 to 3 with treatment 4 having the highest enzyme activity. Significant differences were noted between different silicon concentrations ($p<0.001$) and mean separations for significant means are depicted in Figure 7.

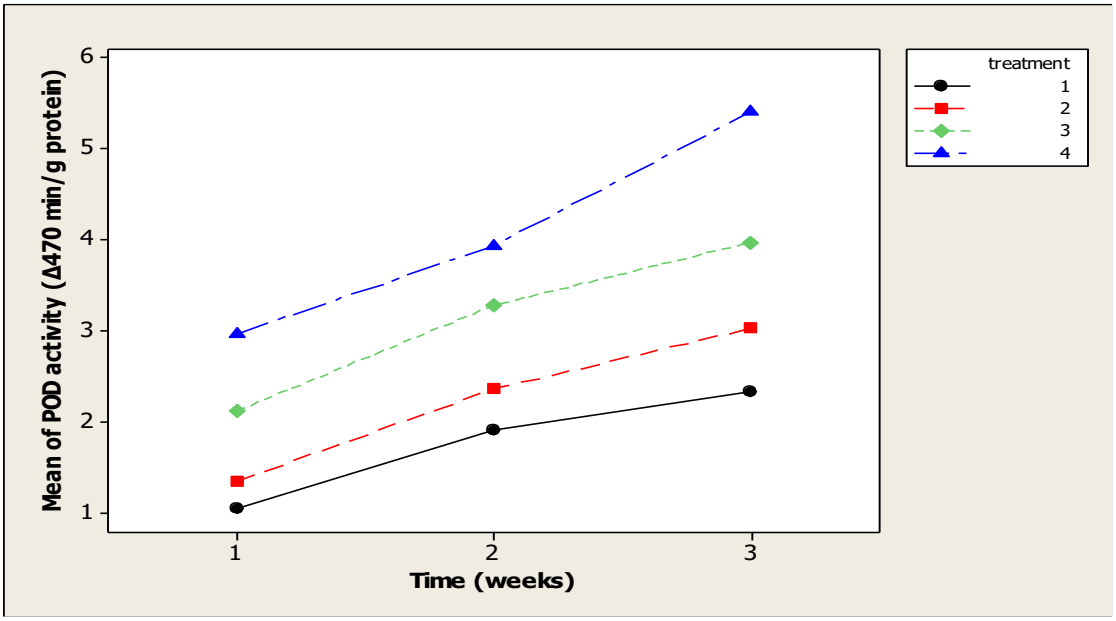

Figure 7: Effect of silicon concentration on POD activity
Source: Authors data

What is interesting from the data gathered in this study of growth parameters, plant height and stem diameter, is that significant differences caused by treatment levels were noted from week 5 to week 8, also an increase in silicon concentration reduced the severity of powdery scab. Silicon concentrations did not show any significant difference in their effects on powdery scab disease incidence on potatoes. All the potatoes tubers from the current study were affected by *Spongospora subterranea*. However, the degree at which theses tubers expressed symptoms differed with the silicon levels.

4.0. CONCLUSION

From the current study in Irish potatoes, silicon has proved to increase their growth rate with a slow response in the first 4 weeks and significant changes occurring in the latter weeks. This study found out that

application of high silicon levels can result in increased stem height and stem width of potato plants. Silicon concentrations have proved not to significantly reduce the incidence of powdery scab but rather indicated to reduce the severity of the disease when applied at higher concentrations. Silicon can increase the activity of PPO and POD enzymes in potatoes which have direct influence on severity, thus high activity results in low severity.

5.0 RECOMMENDATIONS

Silicon based fertilizers in adequate quantities boost growth of potatoes. Farmers and researchers should look at integrated measures of controlling *Spongospora subterranea* rather than always relaying on use of chemical. They is also need for more research on the effects of silicon on chlorophyll production as well as root growth and movement. More is also needed to be done on the same research in different conditions such as field or natural conditions than relying on greenhouse results.

Conflict of Interest

The authors declare that there is no conflict of interest with regard to date here presented and final presentation of the study hence any criticisms both positive and negative can be nailed to the author's door.

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