Journal of Oilseed Brassica, 15 (2) : 294-299, July 2024

Short Communication

Efficacy of alkali salts against *Sclerotinia sclerotiorum* causing stem rot of Indian mustard

Chetan VNP[1], Pankaj Sharma[2], Shelke Saraswati Sudam[1], Pooja Bharmera[1] and PS Sandhu[2]*

[1]*Department of Plant Pathology, Punjab Agricultural University, Ludhiana 141004, Punjab, India*
[2]*Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, Punjab, India*
**Corresponding author:prabhsandhu@pau.edu*
(Received: 18 January 2024; Revised: 26 June 2024; Accepted: 28 June 2024)

Abstract

Acidifying infection area by secreting acids is important mechanism utilized by several necrotrophic organisms. *Sclerotinia sclerotiorum* is a necrotrophic pathogen that causes stem rot of Indian mustard, utilize oxalic acid during infection, leading to the rapid death of host tissues. Thus, the study was undertaken to evaluate the effect of the alkali environment created by organic and inorganic salts (sodium carbonate, potassium silicate, calcium propionate, and sodium propionate) on the growth and development of *S. sclerotiorum* using *in vitro* and *in vivo* assays. The lower area under mycelial growth curve (AUMGC) value for calcium propionate @1.4% (15.5) followed by sodium propionate @1.4% (16.5) against unamended control (118) indicated that mycelial growth rate was reduced with increased concentrations of alkali salts. Detached leaf assay on Indian mustard showed complete inhibition of pathogen development by sodium carbonate @1% and potassium silicate @1.4%. The findings suggest that alkali pH developed due to organic and inorganic salts had an inhibitory effect on the growth and development of *S. sclerotiorum.*

Keywords: *Alkali pH, area under mycelial growth curve (AUMGC), detached leaf assay, per cent growth inhibition, Sclerotinia sclerotiorum*

Introduction

Rapeseed-mustard being an oilseed crop, holds an important position in the world as well as in India. It is being cultivated around the world in more than 70 countries and the production and productivity of rapeseed-mustard in the world was 72.37 million tonnes and 2039 kg/ha respectively, during 2020. Globally, India accounts for 12.6% of the world's total production with a production of 9.124 million tonnes and 1216 kg/ha productivity (FAOSTAT, 2021). In recent years, there has been a considerable decline in the productivity of the crop due to various groups of pathogens, such as fungi, bacteria, viruses, phytoplasma, etc, that cause biotic stress to the crop in collusion with insects and weeds. Sclerotinia rot (also called white stem rot) is one such destructive fungal disease that is gaining importance in recent times due to its occurrence in most areas around the world on a wide host range (Hegedus, 2005). The pathogen infection on the stem during flowering causes blockage in the vascular bundles restricting the movement of nutrients to the inflorescence. This results in shrunken, partially filled pods and the shattering of prematurely ripened pods that adversely affect the seed quality and yield. It causes huge yield losses of up to 80 % in India, Australia, Canada, the United States, China, and European countries (Saharan and Mehta, 2008; Young *et al.,* 2012; Wang *et al.,* 2019; Yu *et al.,* 2020; Bennett *et al.,*2021). Ascospores released from apothecia infect the leaves causing water-soaked necrotic lesions. These lesions expand and reach the stem via the petiole. The disease appears as elongated water-soaked lesions on the stem, arising from the collar region and later spreading rapidly to the upper portions of the stem (Bolton *et al.,* 2006). The plant wilts, topples, and dries when complete girdling of the stem occurs. The formation of sclerotia is seen on the affected plant and in the stem pith. Many agronomic practices have been applied to suppress Sclerotinia wilt so far. These practices include early sowing of the crop (Gupta *et al.,* 2004), optimum spacing, optimum plant density and optimum use of fertilizers (Shukla *et al.,* 2005), and clean cultivation. The application of fungicides to control Sclerotinia, as well as other soil-borne pathogens, is most effective. However, this method has side effects on both environment and public health. It is challenging to control Sclerotinia wilt once a plant has been infected, and there is still a considerable demand for alternative practices for controlling the disease. Previous studies demonstrated that different organic and inorganic salt compounds recognized as 'Safe' (GRAS) were effective in suppressing the growth of many fungal pathogens, including *Fusarium sambucinum* (Mecteau *et al.,* 2002), *Botrytis cinerea* (Türkkan *et al.,* 2017), *Fusarium equiseti*, *F. proliferatum*, *F. semitectum*, *F. solani* f. sp. *phaseoli*, *F. verticillioides*, *Rhizoctonia solani* AG4–HG I, *Macrophomina phaseolina*, and *Sclerotium rolfsii* (Türkkan and Erper, 2015) and *Penicillium digitatum* (Venditti *et al.,* 2018).

To the best of our knowledge, few reports regarding the effects of various organic and inorganic salt compounds in controlling Sclerotinia are presented in the literature. Therefore, the current study aimed to investigate the efficacy of sodium carbonate, potassium silicate, sodium propionate, and calcium propionate to control Sclerotinia stem rot in Indian mustard through *in vitro* and *in vivo* bioassays.

Materials and Methods

Pathogen isolation

The pathogen was isolated from the sclerotia collected from the infected Indian mustard plots of Punjab Agricultural University, Ludhiana, India. The collected sclerotia were surface sterilized with 0.1% $HgCl_2$ solution. The sclerotia were sliced using a sterile blade and placed onto the petri plate containing PDA. The plates were incubated at 25 ± 2°C in a BOD incubator. The actively growing bit of mycelium was used for subculturing. The plates were temporarily stored at 4˚C after the complete growth of the pathogen. Sclerotia formed in plates were collected and packed in small polyethene covers and were stored for a long time at -20˚C in a deep freezer.

Efficacy of salts against *S. sclerotiorum* using poisoned food technique

Four salts [sodium carbonate (SC), potassium silicate (PS), sodium propionate (SP), and calcium propionate (CP)] were evaluated for effect on the mycelial growth of *Sclerotinia sclerotiorum* using the poisoned food technique. The salt compounds were added to the PDA medium after sterilization at 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6 and 1.8 % (w/v). Mycelial plugs (5 mm diameter) of the 3-day-old culture of the fungus were placed on each petri plate and incubated at 25 ± 2°C. PDA plates without salt addition were used as controls. Colony diameters were recorded at two perpendicular points for 24-hour intervals until the mycelial growth reached the edge of the control plates. Mycelial growth inhibition (MGI) and area under mycelial growth curve (AUMGC) were calculated using the formula given by Mueller *et al.* (2002). Each experiment included three replicates per treatment.

$$AUMGC = \sum_{i=1}^{n-1} \frac{(X_{i+1} + X_i)/2}{t_{i+1} - t_i}$$

Where,

X_{i+1} = Radial mycelial growth (mm) at the $i+1^{th}$ observation,

X_i = Radial mycelial growth (mm) at the i^{th} observation,

t_{i+1} = Time after inoculation (day) at the $i+1^{th}$ observation

t_i = Time after inoculation (day) at the i^{th} observation,

n = total number of observations

Efficacy of salts against *S. sclerotiorum* using detached leaf assay

For detached leaf assay, the effective treatments of SC, PS, SP, and CP were tested against *S. sclerotiorum* on leaves of susceptible variety of Indian mustard var. RL 1359. A moist sugar solution-dipped cotton swab was placed on the petiole of the leaf to avoid drying. The leaves were sprayed with alkali salts. The bits were placed on the leaf and kept at room temperature for 3 days. Lesion length was recorded at 24-hour intervals.

Statistical analysis

All statistical analyses were performed using R software (version 4.1.2). Data obtained from the current study were separately subjected to one-way analysis of variance (ANOVA).

Results and Discussion

To evaluate the efficacy of alkali salts against *S. sclerotiorum,* poisoned food technique was performed (Perez-Vicente, 2013). In this assay, various concentrations of different alkali salts such as sodium carbonate, potassium silicate, sodium propionate and calcium propionate were amended into PDA media and was inoculated with the actively growing mycelial bit at the centre. Alkali salts @ 0.2% showed the least growth inhibition of 15.62% in case of PS, 46.25% in case of SC, 12.5% in case of CP and 6.25% in case of SP. Similarly, the highest AUMGC was seen at the lowest concentration of salts i.e. 84.25 in case of PS, 50.4 in case of SC, 105 in case of CP and 112.5 in case of SP. The PDA media amended with SC (0.6% to 1.8%), PS (0.8% to 1.8%), CP (1.6% and 1.8%), and SP (1.6% and 1.8%) showed negligible radial growth which implies complete inhibition of mycelial growth when compared with unamended PDA medium This indicates that increased alkali salts significantly affected the mycelial growth inhibition in terms of radial growth at the various concentrations ($p < 0.05$). (Fig.1 & 2). These treatments had a null AUMGC value against the unamended control, which had 118. The AUMGC values indicate the overall impact of treatments combining both radial growth and duration of inhibition. The reducing AUMGC values with increasing concentration of salts suggests the negative effect on mycelial growth.

To test the efficacy of alkali salts against this pathogen under *ex vivo* conditions, detached leaf assay was performed on the leaves of susceptible variety of Indian mustard, var. RL 1359. *Ex vivo* conditions were evaluated the prophylactic effect of this alkali salts on the pathogen. The effective concentrations under poisoned food technique such as SC (0.6%, 0.8%, and 1%), PS (1%, 1.2%, and 1.4%), SP, and CP (1.4%, 1.6%, and 1.8%) were tested against *S. sclerotiorum.* . The

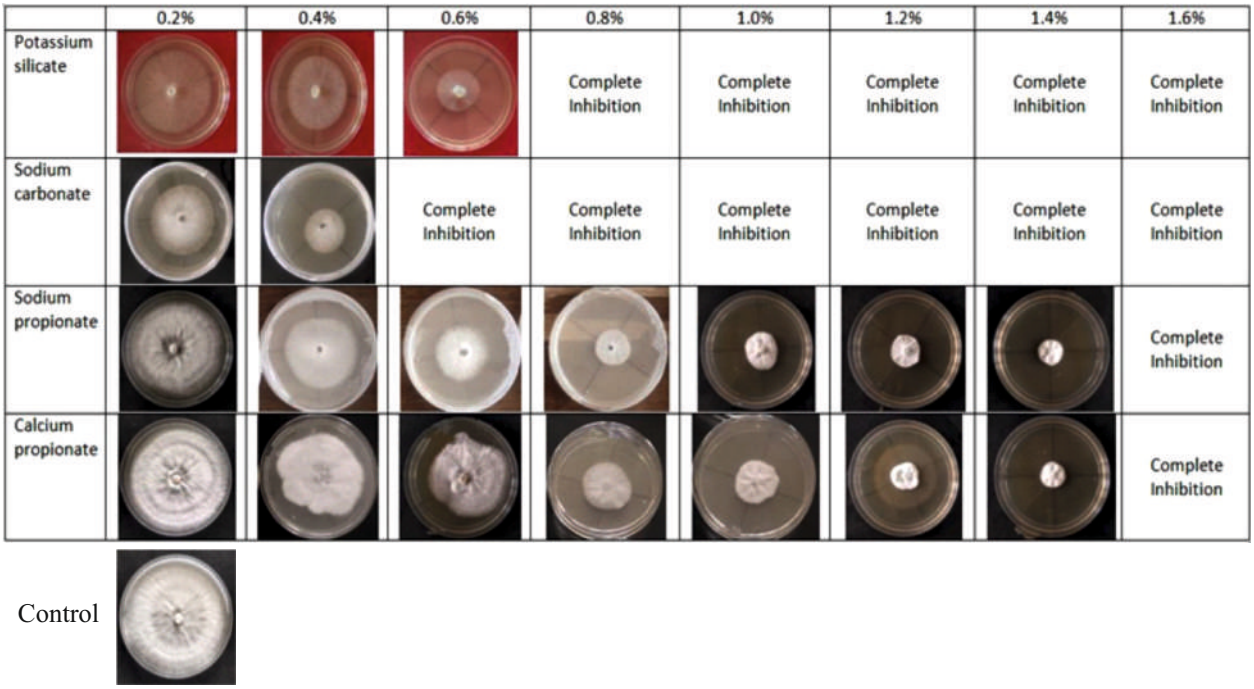

Fig 1: *In vitro* bio-efficacy of alkali salts against *Sclerotinia sclerotiorum* using poisoned food technique

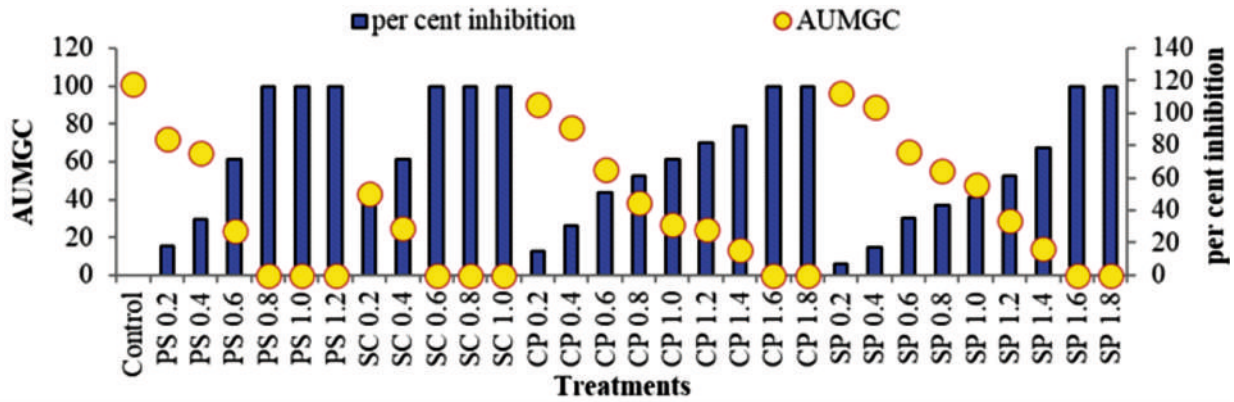

Fig. 2: Graphical representation of AUMGC and per cent inhibition of radial growth against *Sclerotinia sclerotiorum* by alkali salts treatments (Poisoned food technique)

treatments PS @1% showed 21.4% inhibition, SC @ 0.6% showed 9.52% inhibition, CP and SP @ 1.4% showed 61.9% inhibition. SC @ 1%, PS @ 1.4%, SP and CP @ 1.6% were found to be effective in completely inhibiting lesion development of pathogen when compared to untreated leaves (2.80 cm) (Fig. 3 & 4). Sodium carbonate could be considered as the best alkali salt as the lowest concentration in comparison to the other salts displayed higher inhibition. This can be observed both in poisoned food technique and detached leaf assay. Potassium silicate displayed relatively better inhibition in comparison to the other salts i.e. sodium propionate and calcium propionate.

This study emphasized the use of organic and inorganic salts against Sclerotinia stem rot in rapeseed-mustard. Researchers have evaluated the efficacy of different salts against various plant pathogens so far (Turkkan and Erper, 2015). Several studies have revealed that the direct effect of high solution pH was among the main reasons for the effective management of pathogens using salts Palmer *et al.* (1997) evaluated the effects of different salts, including SC on the polygalacturonase (PG) activity of *Botrytis cinerea* and concluded that pH inhibited PG activity. Turkkan *et al.* (2017) found that <10.0mM was the ED50 of SC that would inhibit spore germination of *Botrytis cinera* under *in vitro* conditions.

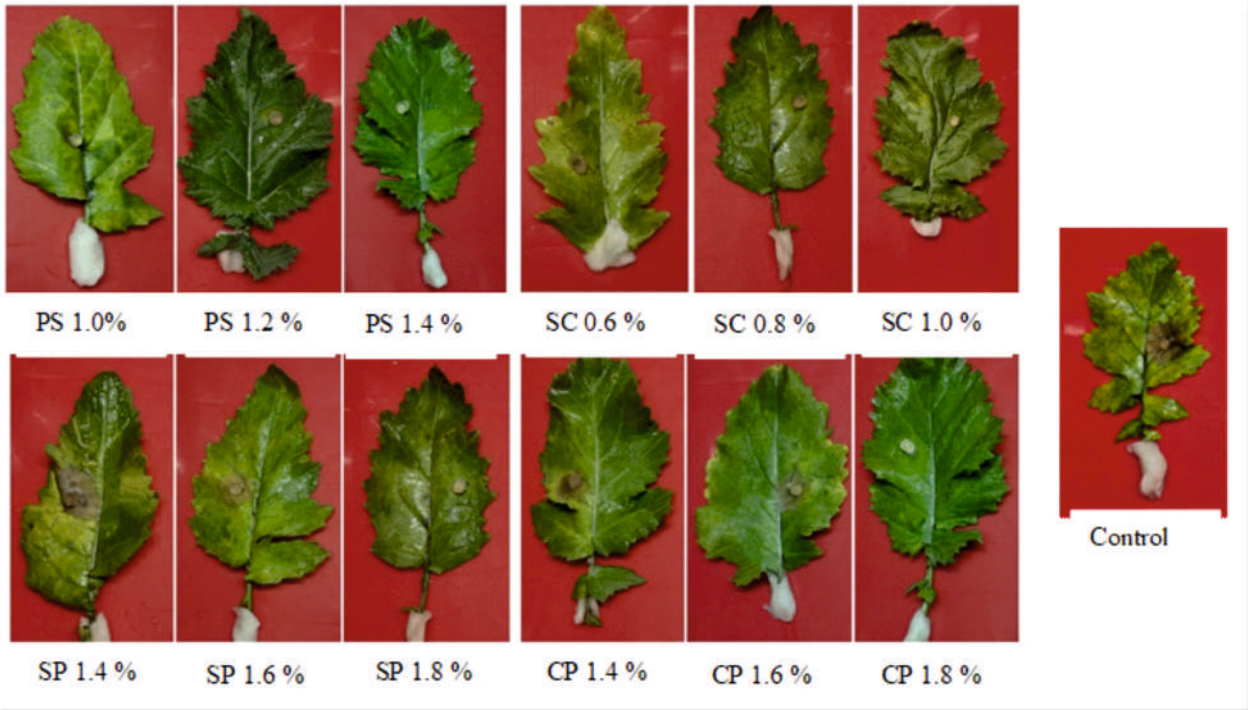

Fig. 3: *Ex-vivo* bio-efficacy of alkali salts against *Sclerotinia sclerotiorum* using detached leaf assay

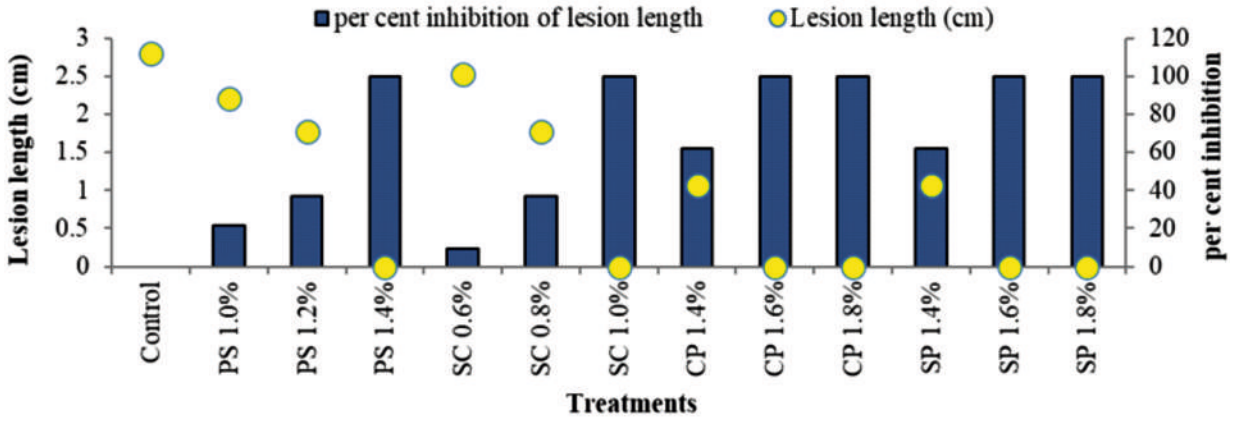

Fig. 4: Graphical representation of lesion length and per cent inhibition of Sclerotinia lesions by alkali salts treatments (Detached leaf assay)

SP and SC showed significant inhibition of various pathogens of bean root rot (Turkkan, 2015). Venditti *et al.* (2018) in their *in vitro* studies, confirmed the fungistatic effect of salt on the spores and the effect of variation in ambient pH on the pathogen's development. CP and SP hindered the mycelial growth of *Geotrichum candidum* under *in vitro* conditions (Turkkan, 2019).

Potassium silicate has a direct suppressive influence on fungal mycelial growth of several phytopathogenic fungi in addition to its ability to strengthen plant cell walls to inhibit infection thereby enhancing the host defence mechanism (Bekker *et al.,* 2009). The study conducted by Shen *et al.* (2010) showed that the growth of fungal pathogens was significantly inhibited on PS-amended PDA plates due to a pH effect. The reduced inhibitory activity of SC in vivo could be due to the alkalinization of the cell wall by the Donnan effect and the speciation of their anions resulted in reduced effectiveness of ions (Yaganza *et al.,*2014). SP @ 0.2 M showed moderate inhibition of soft rot of potato. Turkkan *et al.* (2019) reported that both curative and protective applications of CP and SP @ 2 per cent were

effective in hindering the mycelia growth of *Geotrichum candidum* on potato under *in vivo* conditions. Contrarily, Turkkan and Erper (2015) revealed sodium EDTA at pH 4.67, tolclofos methyl at pH 6.05, sodium bicarbonate at pH 6.26, and SC at pH 10.74 entirely halted the mycelial growth of the isolate of *R.* Sodium bicarbonate and SC activated innate defence mechanisms in citrus against *Penicillium digitatum* by increasing the activity of 1,3-glucanase PAL and peroxidases (Youssef *et al.,* 2014). Further studies must be conducted to decipher the exact role of these salts in disease inhibition and determine their potential to be used as an eco-friendly management strategy.

Conclusion

S. sclerotiorum, being an acidifying fungus, produces oxalic acid and creates an acidic environment during pathogenesis. High pH induces stress in fungus which deprives nutrient uptake and inhibits the production of virulence factors. Thus, to combat this strategy of the pathogen, alkali salts such as sodium carbonate, potassium silicate, sodium propionate, and calcium propionate can be used for disease management. Further studies can provide better insight regarding the mechanism of alkali salts against necrotrophic pathogens.

References

Bekker TF, Kaiser C and Labuschagne N. 2009. The antifungal activity of potassium silicate and the role of pH against selected plant pathogenic fungi in vitro. *S Afr J Plant Soil* **26**: 55-57.

Bennett SJ, Lamichhane AR, Thomson LL, Lui KY and Michael PJ. 2021. Impact of fungicide application and host genotype on susceptibility of *Brassica napus* to Sclerotinia stem rot across the South-Western Australian Grain Belt: A Genotype× Environment× Management Study. *Agron* **11**: 1170.

Bolton MD, Thomma BPHJ and Berlin DN. 2006. *Sclerotinia sclerotiorum* (Lib) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Mol Plant Pathol* **7**: 1-16.

Droby S, Wisniewski M, El Ghaouth A and Wilson C. 2003. Influence of food additives on the control of postharvest rots of apple and peach and efficacy of the yeast based biocontrol product Aspire. *Postharvest Biol Technol* **27**: 127–35.

Epstein E. 2019. Silicon. *Annu Rev Plant Physiol Plant Mol Biol* **50**: 641-64.

FAOSTAT. 2021. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gupta R, Awasthi RP and Kolte SJ. 2004. Influence of sowing dates on incidence of Sclerotinia stem rot of rapeseed-mustard. *Ann Plant Prot Sci* **12**: 223-24.

Hegedus DD and Rimmer SR. 2005. *Sclerotinia sclerotiorum*: when "to be or not to be" a pathogen? *FEMS Microbiol Lett* **251**: 177–84.

Mecteau MR, Joseph ARUL and Tweddell RJ. 2002. Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a causal agent of potato dry rot. *Mycol Res* **106**: 688-696.

Menzies J, Bowen P, Ehret D and Glass AD.1992. Foliar applications of potassium silicate reduce severity of powdery mildew on cucumber, muskmelon, and zucchini squash. *J Am Soc Hortic Sci* **117**: 902-05.

Mueller DS, Dorrance AE, Derksen RC, Ozkan E, Kurle JE, Grau CR and Pedersen WL. 2002. Efficacy of fungicides on *Sclerotinia sclerotiorum* and their potential for control of Sclerotinia stem rot on soybean. *Plant Dis* **86**: 26-31.

Nigro F, Schena L, Ligorio A, Pentimone I, Ippolito A and Salerno MG. 2006. Control of table grape storage rots by pre-harvest applications of salts. *Postharvest Biol Technol* **42**: 142-49.

Palmer CL, Horst RK and Langhans RW. 1997.Use of bicarbonates to inhibit *in vitro* colony growth of *Botrytis cinerea*. *Plant Dis* **81**: 1432-1438.

Punja ZK and Grogan RG.1982. Effects of inorganic salts, carbonate-bicarbonate anions, ammonia, and the modifying influence of pH on sclerotial germination of *Sclerotium rolfsii*. *Phytopathol* **72**: 635-39.

Saharan GS and Mehta N. 2008. Sclerotinia Diseases of Crop Plants: Biology, Ecology and Disease Management. Springer Science and Business Media, BV The Netherlands, 198-285p.

Shen GH, Xue QH, Tang M, Chen Q, Wang LN, Duan CM, Xue L and Zhao J. 2010. Inhibitory effects of potassium silicate on five soil-borne phyto-pathogenic fungi in vitro. *J Plant Dis Prot* **117**: 180-84.

Shukla RK, A Kumar BS, Mahapatra and B Kandpal. 2005. Response of sulphur and nitrogen fertilization on yield, quality and other metrictraits of *Brassica napus*. *J Oilseed Brassica* **7**: 47-51.

Smilanick JL, Mansour MF, Margosan DA, Gabler FM and Goodwine WR. 2005. Influence of pH and $NaHCO_3$ on effectiveness of imazalil to inhibit germination of *Penicillium digitatum* and to control postharvest green mold on citrus fruit. *Plant Dis* **89**: 640-48.

Turkkan M and Erper I. 2015. Inhibitory influence of organic and inorganic sodium salts and synthetic fungicides against bean root rot pathogens. *Gesunde Pflanzen* **67**: 83-94.

Turkkan M, Ozcan M and Erper I. 2017. Antifungal

effect of carbonate and bicarbonate salts against *Botrytis cinerea*, the causal agent of grey mould of kiwifruit. *Akademik Ziraat Dergisi* **6**: 107-14.

Turkkan M. 2019. Effect of various salts on the growth and development of *Geotrichum candidum*, the causal agent of carrot sour rot. *J Phytopathol* **167**: 230-39.

Venditti T, D'hallewin G, Ladu G, Petretto GL, Pintore G and Labavitch JM. 2018. Effect of $NaHCO_3$ treatments on the activity of cell wall degrading enzymes produced by *Penicillium digitatum* during the pathogenesis process on grapefruit. *J Sci Food Agric* **98**: 4928-36.

Wang Z, Ma LY, Cao J, Li YL, Ding LN, Zhu KM, Yang HY and Tan XL. 2019. Recent advances in mechanisms of plant defense to *Sclerotinia sclerotiorum*. *Front Plant Sci* **10**: 1314.

Wisniewski ME, Droby S, El-Ghaouth A and Wilson CL. 1998. The use of food additives to control postharvest decay and enhance biocontrol activity of yeast antagonists. *Proc Int Cong Plant Pathol*: 9-16.

Yaganza ES, Tweddell RJ and Arul J. 2014. Postharvest application of organic and inorganic salts to control potato (*S. tuberosum*) storage soft rot: plant tissue–salt physicochemical interactions. *J Agric Food Chem* **62**: 9223-31.

Young HM, Srivastava P, Paret ML, Dankers H, Wright DL, Marois JJ and Dufault NS. 2012. First report of *Sclerotinia sclerotiorum* on *Brassica carinata* in Florida. *Plant Dis* **96**:1581.

Youssef K, Sanzani SM, Ligorio A, Ippolito A and Terry LA. 2014. Sodium carbonate and bicarbonate treatments induce resistance to postharvest green mould on citrus fruit. *Postharvest Biol Technol* **87**: 61-69.

Yu Y, Cai J, Ma L, Huang Z, Wang Y, Fang A, Yang Y, Qing L and Bi C. 2020. Population structure and aggressiveness of *Sclerotinia sclerotiorum* from rapeseed (*B. napus*) in Chongqing city. *Plant Dis* **104**: 1201-06.