Scientific Papers. Series A. Agronomy, Vol. LXI, No. 1, 2018
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

ANTIFUNGAL ACTIVITY OF NANO CALCIUM POLYSULFIDE AGAINST PATHOGENIC FUNGI ON TOMATO

Şerife Evrim ARICI, Ahmet ŞİMSEK, Mustafa ASKIN

Suleyman Demirel University, Faculty of Agriculture, Department of Plant Protection, Isparta, Turkey

Corresponding author email: evrimarici@sdu.edu.tr

Abstract

Many organic and inorganic compounds are known to exhibit widespread anti-fungal activity with successful usage in agricultural applications. The action mechanism of sulfur agents is that sulfur interacts with organic substances and forms sulfides and pentatonic acid having anti-microbial and anti-parasitic activities. Nowadays elemental sulfur is widely used in plant growing as a fungicide and acaricide. In recent years great considerable attention has been paid to the synthesis methods of antifungal properties of nanoscale sulfur due to the development of nanotechnology. Micronized sulfur and sulfur nanoparticles have antifungal and inbition effects of sporulation against pathogenic fungi. In this study, the antifungal activities in vitro of nano calcium polysulfide were investigated against two pathogenic fungi caused disease on tomato (Fusarium oxyporium lycopersici, Botrytis cinerea). Different concentrations of nano calcium polysulfide (0,0.5, 1, 2%) were tested for growth inhibition of Fusarium oxyporium lycopersici, Botrytis cinerea. Growth inhibitions of tested pathogens were measured in vitro. Nano calcium polysulfide showed low inhibiton against Fusarium oxyporium lycopersici, Botrytis cinerea. Mycelial growth inhibition was observed at a maximum of 2% nano calcium polysulfide application for Botrytis cinerea (34,4%) and 0.5% application for Fusarium oxyporium lycopersici (30.72). Nano calcium polysulfide inhibited only spore germination. Mycelial growth in both fungi was very weak increased in nano calcium polysulfide compared to the control.

***Key words**: calcium sulfide, tomato, Fusarium oxyporium lycopersici, Botrytis cinerea.*

INTRODUCTION

Diseases are a major source of crop and plant damage that can be caused by a number of plant pathogenic (disease-causing) organisms. Fungi are the number one cause of crop loss worldwide. The effective control of plant diseases have been used chemical fungicides. Fungicides play a key role in disease control in resistance management strategies, but equally important are new fungicides with established modes of action with enhanced characteristics such as systemicity, curativity, and longevity of disease control. According to recent studies a new crop protection product takes around 10 years and approximately 260 million USD from discovery to first sales (McDougall, 2010). Many of the pesticides have been associated with health and environmental issues. Residues of pesticides can be found in a great variety of everyday foods and beverages, including for instance cooked meals, water, wine, fruit juices, refreshments, and animal feeds. Taking into consideration the health and environmental effects of chemical pesticides, it is clear that the need for a new concept in agriculture is urgent. This new concept must be based on a drastic reduction in the application of chemical pesticides, and can result in health, environmental, and economic benefits.

One of the promising directions of development of environmentally safe and efficient fertilizers, means of protection and stimulation of plant growth are nanotechnologies.

The use of nanoformulatioms leads to increased resistance to adverse weather conditions and the increase the harvest of almost all products (Fedorenko et al., 2006). Various nanoparticles are employed in plant disease management. Among them are involved micronized sulfur and sulfur nanoparticles which have antifungal and inbition effects of sporulation against pathogenic fungi (Deshpande et al., 2008; Massalimov et al., 2012; Ahmed, Lee, 2015). In this study, Antifungal Activity of Nano Calcium Polysulfide against Pathogenic Fungi on Tomato were investigated. Lime sulfur production usually include calcium sulfite, calcium sulfate, and metal sulfides.

MATERIALS AND METHODS

In this study, the antifungal activities of nano calcium polysulfide were investigated against two pathogenic fungi caused disease on tomato (*Fusarium oxyporium lycopersici*, *Botrytis cinerea*) *in vivo and in vitro.*

Dual culture. Different concentrations of nano calcium polysulfide (0,0.5, 1, 2%) were added into PDA media. After that a 0.6 cm agar plug with *Fusarium oxyporium lycopersici*, *Botrytis cinerea* were placed in the middle of the agar plate one by one. The dual culture plates were incubated at room temperature. The radial growth was measured each day, until the dishes were completely colonized by the fastest mycelium. Radial growth reduction of *B. cinerea*, in presence of nano calcium polysulfide was calculated in relation to the growth of the pathogen, by using the following formula that measures the percentage of the inhibition of the radial mycelial growth:

Inhibition of the mycelial radial growth (%)= C-T/C × 100,

where:

C is the radial growth measurement of the pathogen in control and

T is the radial growth of the pathogen in the presence of nano calcium polysulfide.

All the experiments in laboratory were replicated 2 times.

In vivo experiment. Five pots were allocated for each group. The nano calcium polysulfide were applied against *Fusarium oxyporium lycopersici* and grey mould caused by *Botrytis cinerea* (Table 1). Two control (untreated) groups for each fstudy were used during this study. The nano calcium polysulfide applications were prepared in 1 litre of water and applied at different dosages (0, 0.5, 1, 2%) Tomato plants were dipped in different doses for 2 minute and planted out. Applications were made from soil, soil+foliar and foliar. After 48 hours the spore suspension of *Fusarium oxyporium lycopersici* were adjusted at 10^6 spore/ml and twenty ml were applied to soil. *Botrytis cinerea* was applied on the leaf surface of tomate by counting 10^6spore/ml. After 30 days, the plant symptoms were rated according the following evaluation scale:

For *Fusarium oxyporium lycopersici:*

0 = symptomless plants; 1 = 50% of leaves chlorotic or wilted; 2 = >50% of leaves wilted but plants not dead; and 3 = dead plants.

For *Botrytis cinerea*

0 = no symptoms, healthy plants; 1 = less 10% of infected stems and leaves with lesions for no more 10% of shoot length; 2 = less 20% of infected stems and leaves with lesions for no more 20% of shoot length; 3 = less 40% of infected stems and leaves with lesions for no more 50% of shoot length; 4 = less 80% of infected stems and leaves with lesions for no more 80% of shoot length; 5 = infected areas covering whole the stems and leaves causing wilting and death of.

Table 1. Combination table of applications against *Fusarium oxyporium lycopersici*, *Botrytis cinerea*

Treatment (%)	Concentration of application (%)	Number of plant
Soil application	0.5	4
	1	4
	2	4
Soil+foliar application	0.5	4
	1	4
	2	4
Foliar application	0.5	4
	1	4
	2	4
Control for *Fusarium oxyporium lycopersici*		
Contol for *Botrytis cinerea*		

The disease severity was evaluated using Townsend Heuberger's formula (Towsend, Heuberger, 1943). The percentage effect of the applications was calculated using the Abbott formula (Abbott, 1925).

Statistics. All experiments were carried out independently at least twice. Data were analyzed by analysis of varience (ANNOVA) to detect differences between treatments. Mean comparisons were made using TUKEY's tests; all statistical tests were conducted at a probability level of $P \leq 0.05$. All analyses were performed using the SPSS 21 software.

RESULTS AND DISCUSSIONS

Dual culture. Growth inhibitions of tested pathogens were measured *in vitro*. Nano calcium polysulfide showed low inhibiton against *Fusarium oxyporium lycopersici*, and *Botrytis cinerea.* Mycelial growth inhibition was observed at a maximum of 2% nano calcium polysulfide application for *Botrytis cinerea* (34.4%) and 0.5% application for *Fusarium oxyporium lycopersici* (30.72%). Nano calcium polysulfide inhibited only spore germination (Figure 1). Mycelial growth in both fungi were very weak increased in nano calcium polysulfide compared to the control (Table 2). Ismail et al. (2016) investigated the effect of different chemicals characters (sulfur and silver nanoparticles; micronized particles of tetramethylthiuramdisulfide, tebuconazole, carbendazim and inorganic peroxides - CaO, SrO, BaO; water solvent of miramistin and 22 flucytosine) against some fungal pathogens (*Alternaria alternate, Aspergillus niger, Candida albicans, Fusarium graminearum, Penicillium notatum*). It was reported that two types of elementary inorganic substances nanoparticles (sulfur and silver) had been antifungal effect to *Botrytis cinerea* and *Fusarium oxyporium lycopersici*.

Nano calcium polysulfide provided heavy disease pressure. Foliar application of nano calcium polysulfide (1-2%) reduced *Botrytis cinerea* on tomato plants and severtiy (leaf and stem infected area) wtih 82.46-82.60% effectiveness (Table 3).

Figure 1. Mycelial growth inhibition of *B. cinerea* in presence of nano calcium polysulfide

Table 2. Inhibition of *Fusarium oxyporium lycopersici* and *B. cinerea* growth in presence of nano calcium polysulfide

Doses of nano calcium polysulfide (%)	Mycelial growth inhibition (%)	
	Fusarium oxyporium lycopersici	*Botrytis cinerea*
0.5	20.18±4.8	8±0.0
1	24.12±2.7	22.22±2.2
2	30.72±3.4	34.42±1.1

Table 3. Efficiency of the nano calcium polysulfide used in the experiment against *B. cinerea*

Treatment	Disease severity (%)	Effect (%)
0.5K	28	67.3 f
1K	25	71.83 ef
2K	23	73.53 de
0.5K+Y	20	76.35 cde
1K+Y	16	81.33 abc
2K+Y	16	81.4 ab
0.5Y	19	77.13 bcd
1Y	16	82.46 a
2Y	14	82.60 a
Control	86	

The fungicidal activity of the nano calcium polysulfide were tested against *Fusarium oxyporium lycopersici*. The results presented in Table 4. The nano calcium polysulfide was supressed with concentration to 1-2%. As shown in tthe present study, the soil application of nano calcium polysulfide was very effective for *Fusarium oxyporium lycopersici* control.

Table 4. Efficiency of the nano calcium polysulfide used in the experiment against *Fusarium oxyporium lycopersici*

Treatment (Fus)	Disease severity (%)	Effect (%)
0.5K	18	77.39 b
1K	10	82.56 a
2K	10	83.3 a
0.5K+Y	19	77.35 b
1K+Y	11	81.86 a
2K+Y	8	82.48 a
0.5Y	58	11.28 d
1Y	56	12.93 d
2Y	50	22.4 c
Control	65	

Jamar et al. (2017) investigated new fungicide formulations available for organic pear farming. The study shows that protective applications, at 300 degree-hours (DH) before inoculation, of copper hydroxide (0.1%), wettable sulphur (1%), lime sulphur (2%) and potassium bicarbonate (1%) significantly reduced pear scab severity with more than 96% effectiveness. Biological properties of sulfur nanoparticles such as antifungal and antibacterial activities have been studied, with physical and chemical properties. Antifungal effects of micronized sulfur and sulfur nanoparticles against two types of pathogenic fungi *Aspergillus niger* and *Fusarium* on sporulation, ultrastructural modifications and phospholipid contents of fungal strains have been studied and obtained results revealed to perspectives of using sulfur nanoparticles (Choudhury et al., 2011). Antibacterial activity of sulfur nanoparticles (5.7 nm) was very high against Gram-positive *Staphylococcus aureus*, while this type of nanoparticles has not antibacterial activity against Gram- negative bacteria (Suleiman et al., 2015)

Nanotechnology is an interdisciplinary research field. In recently it has been investigated to improve agricultural yield in nanotechnology. The green revolution resulted in blind usage of pesticides and chemical fertilizers which caused loss of soil biodiversity and developed resistance against pathogens and pests as well. Nanoparticle-mediated material delivery to plants and advanced biosensors are possible only with nanoparticles or nanochips (Prasad et al., 2017; Kim et al., 2017). Nano-encapsulated conventional, pesticides fertilizers and herbicides that help in the slow and sustained release of nutrients and agrochemicals, resulting in precise dosing of the plant.

The use of sulfur-based products has resulted in the suppression of tested pathogenic organisms, particularly in the form of nanoparticles of nano-calcium polysulfide.

The global consumption of pesticides is about two million tons per year. Careless and arbitrary use of pesticides increases resistance to pathogens and pests, reduces soil biodiversity, kills useful soil microbes. Environmentally friendly nano calcium polysulfide may be used to reduction of the presence of pathogens in the soils and on the plants.

CONCLUSIONS

Preliminary results indicated that nano calcium polysulfide supressed *Fusarium oxyporium lycopersici* and *B. cinerea* on tomato plant. Causes organic enlargement of pesticides, pollinators decreases and destroys the natural habitat of farm. The potential uses and benefits of nanotechnology are enormous. We recommend the nano calcium polysulfide as alternative complex fungicide may be useful to reduce the pesticide load on the environment.

REFERENCES

Abbott W.S., 1925. A method of computing the effectiveness of an insecticide. J Econ Entom, 18: 265-267.

Ahmed A.I.S., Lee Y.S., 2015. Nanoparticles as alternative fungicides: Manufacturing, concept and activities. Kor. J. Mycol., 43 (4): 207-215.

Choudhury S.R.,. Mandal A.M.G., Chakravorty D., Pal M., Pradhan S., Goswami A., 2011. Surface-modified sulfur nanoparticles: an effective antifungal agent against *Aspergillus niger* and *Fusarium oxysporum*. Applied Microbiology and Biotechnology, 90 (2): 733-743.

Deshpande A.S., Khomane R.B.,Vaidya O.H., 2008. Sulfur nanoparticles synthesis and characterization from H S gas using novel. Biogdegradable iron chelates in W/O microemulsion. Nanoscale Res., 3: 221-229.

Fedorenko V.F., Buklagin D.S., Golubev I.G., 2006. Use of nanotechnology and anomaterials in the agricultural sector and the challenges of information they provide development Nanotechnology-production, pp: 09-413.

Ismail M., Yuri M., Farit U., Ahmed A.I.S., Muhambetkali B., Bolat U., 2016. Antifungal activity of inorganic micro and nanoparticles against pathogenic fungi compared with some traditional organic drugs, Plant Dis Rep, 27: 340-343.

Jamar L., Song J., Fauche F., Choi J., Lateur M., 2017. Effectiveness of lime sulphur and other inorganic fungicides against pear scab as affected by rainfall and timing application J Plant Dis Prot 124:383–391.

Kim D.Y., Kadam A., Shinde S., Saratale R.G., Patra P., Ghodake G., 2017. Recent developments in anotechnology transforming the agricultural sector: a transition replete with opportunities, Journal of The Science of Food and Agriculture, 98 (3): 849-864.

Massalimov I.A., Medvedev U.A., Zaynitdinova R.M., Mufazalova N.A., Mustafin A.G., 2012. Assessment of antifungal activity of micronized and nanosized elemental sulfurJ. Nanotechnology and Nanoscience, 3 (1): 55-58.

McDougall P., 2010. The cost of new agrochemical product discovery, development and registration in 1995, 2000 and 2005–8. Available at http://www.croplife.org/ view_document.aspx?docId=2478.

Prasad R., Bhattacharyya A., Nguye, Q.D., 2017. Nanotechnology in sustainable agriculture: recent developments, challenges, and perspectives, Front. Microbiol, 8 (1): 1-13.

Suleiman M., Al-Masri M., Al Ali A., Aref D., Hussein A., Saadeddin I., Warad,. 2015. Synthesis of nano-sized sulfur nanoparticles and their antibacterial activities. J. Mater. Environ. Sci., 6 (2): 513-518.

Townsend GK, Heuberger JW., 1943. Methods for estimating losses caused by diseases in fungicide experiments.