Journal of Agricultural Technology 2011 Vol. 7(2): 423-430
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Effects of sodium and potassium salts on *Colletotrichum capsici* incitant of anthracnose on bell pepper

Ajith, P.S. and Lakshmidevi, N.*

Microbiology, University of Mysore, Mysore, Karnataka State, India.

Ajith P.S. and Lakshmidevi, N. (2011). Effects of sodium and potassium salts on C*olletotrichum capsici* incitant of anthracnose on bell pepper. Journal of Agricultural Technology 7(2): 423-430.

The effects of sodium and potassium salts on conidial germination, mycelial growth and infection of Capsicum fruit were studied for *Colletotrichum capsici*, pathogen responsible for Anthracnose disease in Bell Pepper (*Capsicum frutescence*). Sodium benzoate, Sodium metabisulphite and Potassium metabisulphite at 1000 µg per ml concentration completely inhibited the conidial germination and mycelial growth whereas Potassium nitrate inhibited the conidial germination and mycelial growth by 70% and 12% respectively. When salt solutions with varying concentrations were applied to wounded Capsicum fruit prior to inoculation, 39% to 82% of smaller lesions were observed when compared to control. From the results it is evident that both sodium and potassium salts have ability to control *Colletotricum capsici* and can be used as part of post harvest disease management practice.

Key words: *Capsicum*, Fruit rot, acervuli, Spore germination

Introduction

Bell Pepper (*Capsicum frutescence*) is one of the major spice crops grown in tropics, subtropics and warmer temperate areas of the world, cultivated in an area of about one million hectares (Martelli, G.P. and Quacquarelli, A. 1983). India is one of the largest exporter of capsicum in the world, cultivated in an area of 7, 33, 800 hectares and in Karnataka State it is cultivated in an area of 3,284 hectares (Anonymous, 1995). The fruits are used as a fresh and processed vegetable belongs to the family *Solanaceae*. The chief nutritional value lies in their high vitamin 'C' content and is also well known for its neutraceutical properties. Several diseases, particularly of fungal origin attack the capsicum crop. Among these Anthracnose caused by *Colletotrichum capsici* (Sydow) (Butlerr, E.J. and Bisby, 1918), is more conspicuous as it causes severe damage

*Corresponding author: N. Lakshmidevi;e-mail: lakshmi_devi64@yahoo.co.in

to Capsicum fruits (fruit rot) (Fig. 1) in the field as well as in storage resulting in loss of about 84% (Heggens, 1930, Smith and Crasson, 1959) and accounts for millions of dollars in losses every year. *Colletotrichum* is capable of causing disease on virtually all parts of the pepper plant during any stage of plant growth. However fruit lesions are the most economically important aspect of this disease. On fruit initially symptoms begin as water-soaked lesions that become soft, slightly sunken, and become tan. The lesions can cover most of the fruit surface and multiple lesions occur at later stages of infection. The characteristic feature of anthracnose symptom is formation of acervuli in concentric rings on disease lesion. The conventional approach to control Anthracnose is by the use of synthetic antifungal compounds. However, recently doubts have increasingly been expressed about the safety of many fungicides. It has emerged that, a significant number of commonly used fungicides pose threat to human health and environment. There is a need to provide more biocompatible fungicides which are, by definition, safe in the environment, non-toxic to humans and animals and are rapidly biodegradable. A broad range of organic molecules have been found to have fungicidal properties and are effectively used for postharvest disease control. Sodium salts were shown to inhibit fungal pathogens of fruits, field crops, vegetables and ornamentals (Claudia oliver *et al*., 1999; Melanie *et al.,* 2002). Sodium benzoate and sodium metabislulphite effectively suppressed the potato dry rot caused by *Fusarium sambucinum* (Melanie *et al.,* 2002). Potassium metabisulphite was found to inhibit growth *of Gliocephalotrichum microchlamydosporum, Colletrichum gloeosporioides* and *Botryodiplodia theobromae in-vitro*, the causative fungi of Brown spot, Anthracnose and Stem end rot respectively of Rambutan (*Nephelium lappaceum*) fruits (Sivakumar *et al.,* 2001). Based on previous studies, the present work was undertaken to determine whether the use of Sodium and Potassium salts had any effect on spore germination, mycelium growth and infection of Capsicum fruit by *Colletotrichum capsici* and to develop strategies to control post harvest loss.

Materials and methods

The pathogen *Colletotrichum capsici* was isolated from diseased Capsicum fruits and was maintained on Potato Dextrose Agar (PDA) under refrigerated conditions. Conidia were harvested by adding 10 ml of sterilized distilled water to culture plates, gently swirl the liquid to dislodge the spores and filter the suspension through four layers of sterile cheese cloth. The desired concentration of 10^6 spores /ml was prepared by diluting the suspension after counting them using a Hemocytometer.

Fig. 1. Anthracnose symptoms caused by *Colletotrichum capsici* on Bell pepper fruit.

Effects of sodium and potassium salts on spore germination

The salts viz., Sodium benzoate, Sodium metabisulphite, Potassium nitrate and Potassium metabisulphite were used for the present study. To assess the effects of sodium and potassium salts on germination of conidia, different concentrations ranging from 100µg - 1000 µg/ml of the above said salts were prepared in Sterilized warm Potato Dextrose Broth (PDB) taken in different labeled tubes. A 30 µl freshly harvested *C.capsici* spores were inoculated and the tubes were incubated at 12/12 hour light/dark cycle at 20±2 ^{0}C. After incubation readings were taken for percent germination of spores at 24, 30, 36 and 42 hour time interval (Ricker, and Punja, 1991). A conidium was considered germinated if the length of germ tube was more than half the length of the conidium (Biggs,. 1999). The experiment was conducted by maintaining triplicates (thrice) data were analyzed with analysis of variance, and means were separated with Waller-Duncan k-ratio‘t’ test.

Effects of sodium and potassium salts on mycelium growth

To assess the effects of Sodium and Potassium salts on mycelial growth, salts were prepared in sterile distilled water and were added to autoclaved warm (45 to 55 ^{0}C) Potato Dextrose Broth (PDB) to provide a range of concentration from 100-1000 µg/ml taken in 250-ml stationary flasks. A 30 µl drop (10^{6}spores/ml) of *C.capsici* spore suspension was inoculated to each flask, and incubated in the dark at 20±2 ^{0}C, and growth was assessed after 14 days by determining the dry weight of the mycelium. The experiment was performed by maintaining three replicate per treatment, and results were analyzed with analysis of variance, and means were separated with Waller-Duncan k-ratio‘t’ test.

Effects of salts on Infection of Capsicum fruit

Freshly harvested Capsicum fruit were taken and was treated with 2-3% Sodium hypochlorite solution for 1min to remove surface microflora. Each Capsicum fruit is artificially wounded by piercing the epidermis with a sharpened 7 mm diameter cork borer and remove the disk of epidermis with a sterile scalpel. Later it is dipped in sodium and potassium salt solutions with varying concentration (100 µg - 1000 µg/ml) for 2 mins, and for control it is dipped in sterile deionized water. Treated fruit were then allowed to air-dry, after which a 30 µl drop (10^6spores/ml) of *C.capsici* spore suspension was inoculated to each wound and placed on paper trays in plastic tubs to help maintain a high relative humidity, and the diameter of the resulting lesions was measured 7 and 14 days after inoculation. This experiment was conducted thrice with three replicates of 6 fruit each for each treatment. Data were subjected to analysis of variance, and means were separated with the Waller-Duncan *k*-ratio *t* test.

Results

Effects of sodium and potassium salts on spore germination

At 24h there was 78.5% spore germination of *C.capsici* was recorded in control. Salts viz., Sodium benzoate, Sodium metabisulphite and Potassium metabisulphite completely inhibited the spore germination at the concentration of 1000 µg/ml. Wwhereas, 30% spore germination was observed in Potassium nitrate treatment (Fig. 2). With prolonged period of incubation, 100% spore germination was noticed in control. In all the treated spores the inhibition of spore germination was observed even after 48 hours of incubation except Potassium nitrate.

Effects of sodium and potassium salts on mycelium growth

At 200 µg/ml concentration, Sodium benzoate and Sodium metabisulphite completely inhibited the growth of mycelium, where as Potassium metabisulphite and Potassium nitrate exhibited 58.2% and 93.3% (Fig. 3) of growth when compared to control.

Effects of salts on infection of capsicum fruit

It is observed that treated capsicum fruit at1000 µg/ml salt solution of Sodium benzoate, Sodium metabisulphite and Potassium metabisulphite

exhibited 81% to 82% (Fig. 4, Table 1**)** smaller lesion when compare to control, whereas Potassium nitrate exhibited 39% smaller lesion.

Table 1. Lesion diameter after 7 days on wounded Capsicum fruit inoculated with *Colletotrichum capsici.*

Salt Treatment	Lesion diameter (mm)			
	Salt Concentration (µg/ml)			
	100	**200**	**500**	**1000**
Sodium benzoate	31.3 b[yz]	27.1 b	37.9 b	16.3 b
Sodium metabisulphite	27.8 b	24.9 b	33.8 b	12.1 b
Potassium nitrate	21.3 c	12.9 c	32.4 c	11.1 c
Potassium metabisulphite	7.86 d	7.56 d	28.36 c	7.76 c
Control	43.53 a			

y values are means of 18 measurements.
z letters denote significant differences among means in columns according to the Waller Duncan test ($p = 0.05$).

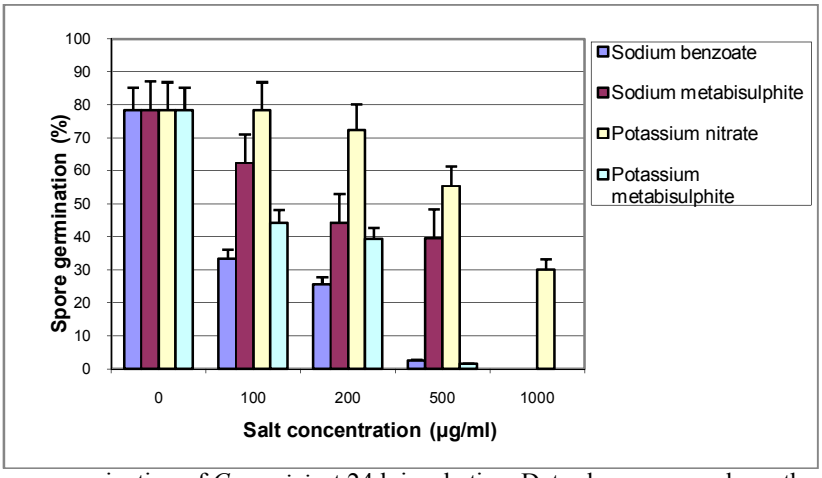

Fig. 2. Spore germination of *C.capsici* at 24 h incubation. Data shown as graph are the means of three replicates of 50 spores each.

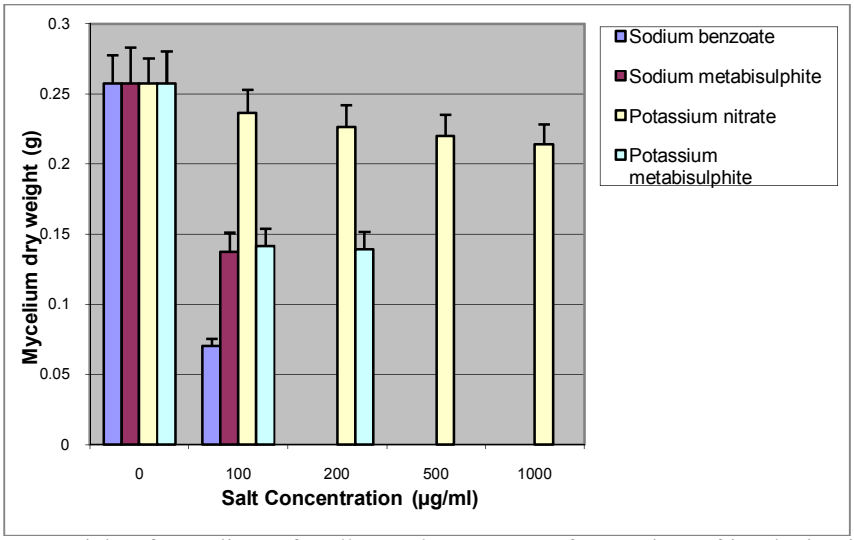

Fig. 3. Dry weight of mycelium of *Colletotrichum capsici* after 14 days of incubation in PDB treated with Sodium and Potassium salts.

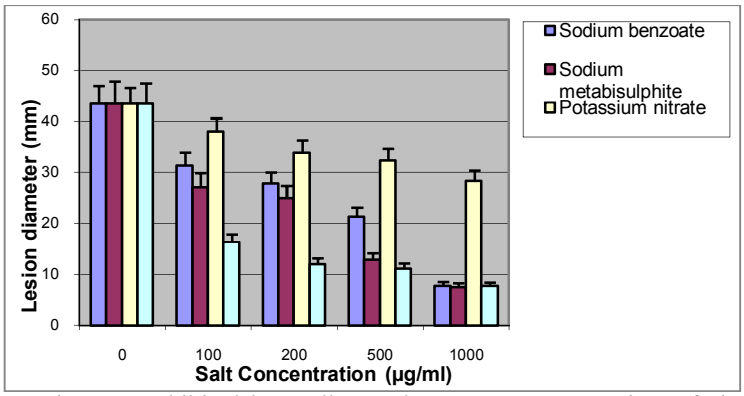

Fig. 4. Lesion Diameter exhibited by *Colletotrichum capsici* on Capsicum fruit treated with Sodium and Potassium salts after 7 days of incubation.

Discussion and conclusions

In the present study it is evident that Sodium and Potassium salts suppress fruit rot on *Capsicum frutescence*, and were found effective. It is observed that inhibition of both germ tube elongation and mycelium growth even at lower concentrations *in-vitro*, and lowers the rate of infection of host tissues pretreated with salts *in-vivo*. Sodium salts were found to be more effective when compared to potassium salts. Sodium salts checked the infection significantly in the inoculated fruits when compared to uninoculated control. The earlier studies revealed that sodium benzoate which, were under acidic

conditions, converts to undissociated benzoic acid and become responsible for its antimicrobial activity (Concise International Chemical Assessment, 2004). Also it is found that sodium benzoate was used as an alternative to synthetic fungicide for the control of post-harvest green and blue moulds of Citrus caused by *Penicillium digitatum* and *Penicillum italicum* (Lluis Palou *et al.,* 2002).Whereas, sodium metabisulphite are being used as food additive and it is mainly used as food preservative having antimicrobial function. The inhibitory effect of sulphites on yeast, molds and bacteria has also been well documented (Lindsay, R.C. 1985, Russell, N.J, and Gould, G.W. 1991). The Postulated mechanism include reactions with protein carbonyl groups, FAD+, RNA and DNA (Russell, N.J, and Gould, G.W. 1991).Although potassium nitrate was not found effective in controlling mycelial growth even at higher concentrations (1000 µg/ml), the potassium metabisulphite effectively suppressed the growth and it was observed that there was no visible growth of *C. capsici* at a concentration of 500 µg/ml *in vitro*.

The effect of potassium metabisulphite against microorganisms was reported earlier (Frazier, W.C. and westhoff, D.C.1998). As it converts itself into sulphur dioxide in aqueous solution which acts as an active antimicrobial compound. The mechanism include the reduction of disulphide linkages, formation of carbonyl compounds, reaction with ketone groups, and inhibition of respiratory mechanisms (Frazier, W.C. and westhoff, D.C.1998, Rengasamy Balasubramanium and Phillip Poole, 1995). Although Sodium and Potassium salts were practiced to control many fungal diseases, no significant work has been done to control post harvest Anthracnose on *Capsicum frutescence* and also the chemicals selected were commonly used as additives or food preservatives and are considered as safe to the environment and human health when treated with suitable doses. From the results it is made clear that both sodium and potassium salts have ability to control *C. capsici* both *in-vitro* and *in-vivo*.

Acknowledgment

The authors acknowledge the support extended by University Grants Commission (UGC-RGNF), Government of India, India for financial assistance.

References

Anonymous, 1995.Statistical Data on Horticultural crops in Karnataka State, Department of Horticulture, Government of Karnataka, Lalbagh, Bangalore. 15pp.

Biggs, A.R.1999. Effects of Calcium salts on apple bitter rot caused by two *Colletotrichum* spp. Plant Dis.83:101-1005.

Butler, E.J. and Bisby, 1918. Fungi and disease in plants. Thacker spink and Co., Calcutta. Pp.547.

Claudia oliver, Carol R. Mac Neil, Rosemary loria. 1999, Application of Organic and Inorganic salts to field-grown Potato tubers can suppress silver scurf during Potato storage. Plant Dis. Vol.83 No.9.

Concise International Chemical Assessment, 2004. Document No.26, Benzoic Acid and Sodium benzoate. International programme on chemical safety. IPCS INCHEM home.

Frazier, W.C. and westhoff, D.C. 1988. Food microbiology. 4th ed. Mc. Graw-Hill, Inc. Singapore. 539p.

Heggens, B.B. 1930. A Pepper fruits rot. Georgia Experiment Station Bull. Georgia, USA. Pp.145-149.

Lindsay, R.C.1985. Food additives. Food chemistry.2nd edition.O.R. Fennema,ed. Marcel Dekker Inc., New york. 629-687p.

Lluis Palou, Joseph Usall, Joseph L Smilanick, Maria-Jose Aguilar, Inmaculada Vinas. 2002. Evaluation of food additives and low-toxicity compounds as alternative chemicals for the control of *Pencillium digitatum* and *Penicillium italicum* on citrus fruit, Pest Management Science, Vol 58, Issue 5, 459-466p.

Martelli, G.P. and Quacquarelli, A.1983.The present status of tomato and peper viruses.Acta Hortic. 127: 39-64.

Melanie R. MECTEAU, Joseph ARUL and Russell J.TWEDDELL. 2002. Effect of organic and inorganic salts on the growth and development of *Fusarium sambucinum*, a Causal agent of potato dry rot. Mycological Research. 106:688-696.

Rengasamy Balasubramanium and Phillip Poole. 1995. Botrytis control-PMS (Potassium metabisulphite) another weapon against botrytis. Hort Research Publication. Vol 37: 10-11p.

Ricker, M.D., and Punja, Z.K.1991. Influence of fungicide and chemical salt dip treatments on crater rot caused by *Rhizoctonia carotae* In long-term storage. Plant Dis. 75: 470-474.

Russell, N.J., and Gould, G.W.1991. Food preservatives Blackie, London.20p.

Sivakumar, D, Wilson wijeratnam, R.S. and R.L.C, wijesundera. 2001. Effect of GRAS Compounds on Mycelial Growth, Pectic Enzyme Activity and Disease Severity of Postharvest Pathogens on Rambutan (*Nephelium Lappaceum*). Phytoparasitica 29(2).

Smith, K.W. and Crasson, D.F. 1959. The taxonomy, etiology and control of *Colletotrichum piperatum* (E&F) and *C.capsici* (Synd) (BB). Plant Disease Report. 42: 1099-1103.

(Received 9 June 2010; accepted 7 March 2011)