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Effects of chitosan on seed germination, anthracnose control and yield parameters of capsicum (*Capsicum annum* L.) under field condition

RGAS Rajapakse[1], P Premarathna[2], JK Kahawatta[3], Shyamalee Kohombange[4*], ERSP Edirimanna[5], AK Rathnayake[6], KRC de Silva[7], DCKK Dissanayake[8]

[1-4] Horticultural Crop Research and Development Institute, Gannoruwa, Sri Lanka
[5] Fruit Crop Research and Development Institute, Horana, Sri Lanka
[6-8] Atomic Energy Board, Colombo, Sri Lanka

Abstract
Anthracnose disease is major constraints to profitable cultivation of Capsicum in Sri Lanka. Currently fungicides are used to control this disease at alarming scale. Some chitosan molecules have been proven to control some plant diseases and simultaneously increase yield of crops. Two type of chitosan compounds i.e. chitosan oligomer and chitosan fungicide were used to study the effects of chitosan formulations on seed germination, anthracnose control and yield parameters of capsicum (*Capsicum annum* L.) under field conditions. Causal agent of the anthracnose disease of capsicum was identified as *Colletotrichum gloeosporioides* based on microscopic observation. Results revealed that higher seed germination in oligochitosan (300ppm) treated seeds. Seed treatment by oligochitosan (300ppm) or formulation of oligochitosan and chitosan fungicide mixture (300ppm) and spraying of formulation of oligochitosan and chitosan fungicide mixture (600ppm) or alternative spraying of oligochitosan (300ppm) and chitosan fungicide (600ppm) formulations in two times at nursery stage and six times after transplanting in 7 days intervals reported low anthracnose incidences, higher number of capsicum pods and higher yield similar to conventional treatment i.e. seed treatment by fungicide (Thiram 70WP) solution (1000ppm) and spraying of fungicide (Mancozeb 70WP) at the rate of 2g/l two times at nursery stage and six times after transplanting in 7 days intervals.

Keywords: anthracnose, chitosan, seed germination

Introduction

Anthracnose disease is one of the major constraints to the profitable cultivation of capsicum in rainy period. Recently, a serious incidence of anthracnose of capsicum was observed in many areas of Sri Lanka. Losses occurr45ed in the field and during the transport. In addition to direct losses, the disease impairs quality. As a result of this, even saleable fruit commands a lower price than would be obtained in the absence of the disease. It has been reported that apart from the pre-harvest losses, pod quality deterioration of capsicum due to anthracnose range from 20 - 50% in Sri Lanka (Rajapakshe *et al.*, 2008) [12]. To date, research on anthracnose disease in Sri Lanka was mainly directed towards the development of control methods using fungicides. Application of agro-chemicals are the major plant protection method over decades even though they are associated with many disadvantages including their expensive applications, environmental pollution and human health hazards due to excessive usage. This has emerged a worldwide huge trend to explore other environmental friendly alternative methods for plant protection.

Chitosan, a given name to a de-acetylated form of chitin, is a natural biodegradable compound derived from crustaceous shells such as crabs and shrimps (Sandford 1989, Tahtal *et al.*, 2007) [14, 18]. Chitosan has been proven to control numerous pre and postharvest disease on various horticultural commodities (Aziz Aziz *et al.*, 2006, Kareem *et al.*, 2006, Wang *et al.*, 2007, Bautista-Banos *et al.*, 2005) [4, 8, 20, 5].

Oligochitosan induces a series of defense reactions in plants correlated with enzymatic activities and chitosan fungicide compounds has a direct effect on microbes by fungistatic or fungicidal potential (Meng *et al.*, 2010, Raafat and Sahl, 2009, Tikhonor *et al.*, 2006) [10, 11]. Therefore, the objectives of the study were to investigate the effect of commercial formulations of chitosan on seed germination, anthracnose control and yield parameters of capsicum crop.

Materials and Methods

Experiments were conducted in IM_{3a} agro ecological zone at Matale in Central province. Two types of chitosan, one compound is oligochitosan with selected molecular mass (Viscosity Average Molecular Mass, M_V = 3000 – 10000 Da), possess plant growth promoter/elicitor properties, while other one is low molecular weight Chitosan in M_V range 10000 – 100000 Da possess significant fungicidal activity for many common diseases in plant-pathology were prepared by Atomic Energy Board, Colombo, Sri Lanka and used for this study program. Fungicidal potential of chitosan fungicide against some pathogens was identified by in vitro test (Rajapakse *et al.*, 2014) [13].

Commercial formulation of chitosan i.e. mixture of chitosan oligomer and chitosan fungicide were developed using selective chitosan molecules. Seed treatments were done by dipping seeds in chitosan formulations, fungicide solution (Thiram 70WP) and water for 90 minutes of respective treatments. Seed germination (%) was tested by placing 100 seeds of each treated seed an even distance on a moistened papers towel and place in a plastic boxes and leave in room temperature (28±2^{0}C) for five days. Then number of germinated seeds on each paper towel was counted and percent seed germination of each treatment was calculated.

In field experiment, three weeks old nursery raised capsicum seedlings of variety 'CA 8" were transplanted in beds of 2 m x 2 m at a spacing of 80 cm x 50 cm as recommended by Department of Agriculture, Sri Lanka (Anonymous, 1990) [2]. Hand weeding was done at 3 and 6 weeks after transplanting. Imidacloprid 200 g/l SC was applied two times at nursery stage and six times in the field to control insects. The experiments were laid out in randomized complete block design with five replicates. Foliar spraying was done using a knapsack sprayer at 7 day intervals.

Table 1

Treatments	Treatment Combination
T1	Seed treatment by dipping seeds in formulation of oligochitosan and chitosan fungicide mixture (300ppm) for 90 minutes and spraying of formulation of oligochitosan and chitosan fungicide mixture (600ppm) in two times at nursery stage and six times after transplanting in 7 days interval.
T2	Seed treatment by dipping seeds in formulation of oligochitosan (300ppm) for 90 minutes and alternative spraying of formulation of oligochitosan (300ppm) and formulation of chitosan fungicide (600ppm) in two times at nursery stage and six times after transplanting in 7 days intervals.
T3	Seed treatment by dipping seeds in 1000 ppm fungicide (Thiram 70WP) solution for 90 minutes and Spraying of fungicide (Mancozeb 70WP) at the rate of 2g/l two times at nursery stage and six times after transplanting in 7 days intervals.
T4	Control- Seed treatment by dipping seeds in water for 90 minutes and foliar spraying of water.

Anthracnose incidence in each plot of capsicum was recorded as percent disease infection. Fruit number and yield of each plot was recorded at harvest. Data were analyzed using the SAS statistical package.

Pathogen isolation and identification

Anthracnose affected capsicum fruits were collected from experimental plots and pathogens were isolated from anthracnose lesions of disease affected fruits on Potato Dextrose Agar (PDA). Single spore isolates of pathogen were prepared from mycelia of single conidia cultures grown on PDA. Pathogens were identified on the basis of morphology of sporulating acervuli and conidia by microscopic observations and colony morphology on PDA culture medium. Pathogenicity of all isolates was tested by wound inoculation with conidia suspension *i.e* pin prick method and subsequent anthracnose development on fruit surfaces.

Results

Anthracnose of capsicum, in common with similar disease of other crops, is caused by species of the genus *Colletotrichum* (Agrios, 1988) [1]. Several species of *Colletotrichum*, *C. capsici*, *C. gloeosporioides, C. acutatum, C. coccodes*, *C. graminicola* have been implicated in the anthracnose disease of capsicum from various part of the world (Hadden & Black 1988, Sariah, 1994, Sutton, 1992) [7, 15, 17]. Isolates of anthracnose pathogen of capsicum were identified by comparison of their colony morphology on PDA with published data. According to the colony morphology on PDA medium and the shape and size of conidia, isolates were identified as *C. gloeosporioides* (Table 1).

Table 1. Characters of fungal isolates of *C. gloeosporioides* collected from anthracnose affected capsicum pods

Characters of fungi isolates	Morphology and culture characters of isolates C. gloeosporioides
Colony colour on PDA	Grey initially then turned light brown or brown, colony margin wavy
Reverse colony colour on PDA	Brown
Acervuli	Brown colour masses, conidia present inside.
Setae	Absent
Conidia	Cylindrical with round end shaped, aseptate, Fat globules present inside

Isolates tested for their ability to induce lesions in mature fruits using the pin prick method indicated that tested isolate of *C. gloeosporioides* had the ability to develop anthracnose lesions on capsicum fruits. The disease was identified by large 1-1.5 cm diameter, brown, circular depressions and the fungus appeared as brown acervuli on the surface of inoculated capsicum fruits. The pathogen of anthracnose disease in the experimental area of central region of Sri Lanka was *C. gloeosporioides*.

Table 2: Effect of different treatments on seed germination, anthracnose incidence, number of pods and yield of capsicum

Treatment	Mean percent seed germination	Mean anthracnose incidence/plot	Mean number of pods/plot	Mean pod yield (Kg)/plot
T1	75.2	7.3 ^{b}	2556 ^{a}	48.3 ^{a}
T2	75.8	8.9 ^{b}	2356 ^{a}	45.1 ^{a}
T3	70.5	5.3 ^{b}	2434 ^{a}	47.2 ^{a}
T4	68.3	36.7 ^{a}	365 ^{b}	7.3 ^{b}

Note: Means with the same letter(s) on the column are not significantly different at P≤0.05 and ns: Statistically not significant at P≤0.05.

Higher seed germination was observed in oligochitosan treated seeds. Results revealed that significantly lower anthracnose incidences and significantly higher pod number and pod yield were recorded in chitosan formulation treated plots as well as fungicide (Thiram 70 WP and Mancozeb 70WP) treated plots compared to control treatment. It has been reported that chitosan has a direct effect on the morphology of the chitosan – treated microorganism reflecting its fungistatic or fungicidal potential (Bautista-Banos *et al.*, 2005, Ben-Shalom *et al.*, 2002, Seyfarth *et al.*, 2008) [5, 6, 16]. In addition to its direct microbial activity, other studies strongly suggest that chitosan induces a series of defense reactions correlated with enzymatic activities and synthesis of specific phytoalexins with antifungal activity (Aziz Aziz *et al.*, 2006, Awadalla and Mohmond, 2005) [4, 3]. Chitosan induces structural barriers for example inducing the synthesis of lignin- like material (Bautista-Banos *et al.*, 2005) [5]. For some horticultural and ornamental commodities, chitosan increased harvested yield (Aziz Aziz *et al.*, 2006, Mawgoud *et al.*, 2010, Zakarial *et al.*, 2009) [4, 9, 21]. As a nontoxic biodegradable fungicide, as well as an elicitor, chitosan molecules has the potential to become a new class of plant protectant in eco-friendly agricultural systems.

Conclusion

Causal agent of the anthracnose disease of capsicum in the location was identified as Colletotrichum gloeosporioides. Seed treated with formulation of oligochitosan and chitosan fungicide mixture (300ppm) or only oligochitosan (300ppm) for 90 minutes increase seed germination of capsicum over seed treated with fungicide (Thiram 70WP) solution (1000ppm) and water treatment.

Seed treatment by dipping seeds in formulation of oligochitosan and chitosan fungicide mixture (300ppm) for 90 minutes and spraying of formulation of oligochitosan and chitosan fungicide mixture (600ppm) in two times at nursery stage and six times after transplanting in 7 days interval or seed treatment by dipping seeds in formulation of oligochitosan (300ppm) for 90 minutes and alternative spraying of oligochitosan (300ppm) and chitosan fungicide (600ppm) formulation in two times at nursery stage and six times after transplanting in 7 days intervals reported low anthracnose incidence and higher number of pod and pod yield of capsicum similar to pesticides treatment i.e. seed treatment by dipping seeds in fungicide (Thiram 70WP) solution (1000ppm) for 90 minutes and spraying of fungicide (Mancozeb 70WP) at the rate of 2g/l two times at nursery stage and six times after transplanting in 7 days intervals.

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