Research Article

Biochemical Characterization and Management of Tomato Stem Rot with Boron and Plant Biocides

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Abstract | Current study was conducted for biochemical characterization of pathogen of tomato stem rot and to evaluate the effect of different doses of boron, plant extracts and a bactericide in different combinations on tomato plants. This research study was performed at Agricultural Research Institute, Tarnab, Peshawar-Pakistan. Different biochemical tests were conducted to confirm stem rot pathogen. Boron was applied at the rates of 1.97, 2.96 and 3.95 g seedbed^{-1}. Plant extracts were neem (*Azadirachta indica*) and ghwaraskay (*Dodonaea viscosa*). Morphological traits such as plant height, maximum number of fruits plant^{-1} and yield were observed and severity of disease was investigated for supplement efficacy. The biochemical results obtained were all positive, which confirmed stem rot pathogen as *Erwinia carotovora* sub spp. *chrysanthemi* (gram-negative bacteria). Highest plant height (75.3 cm) and maximum number of fruits plant^{-1} (14.3) were recorded with *A. indica* when applied at 15ml L^{-1} along with highest dose of boron at 3.95 g seedbed^{-1}. The severity was observed 0.0% in supplement of boron of high dose with addition to neem extract and bactericide. Maximum yield (106.7 tons ha^{-1}) was recorded with *A. indica* at the rate of 15ml L^{-1} and boron at the rate of 3.95 g seedbed^{-1}. It was concluded from this study that boron is beneficial at high dose (3.95 g seedbed^{-1}) along with neem extract for controlling the disease severity and enhancing yield of tomato.

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Introduction

Tomato (*Solanum lycopersicum* L.), being an important greenhouse and field-grown vegetable crop, is native to South America (Olaniyi et al., 2010). Tomato plant has weak stem due to which it mostly sprawls over the ground and sometimes its production has many obstacles such as early blight, late blight and soft rot as well as stem rot disease induced by bacteria and viruses (Agrios, 1997). In Pakistan, stem rot disease poses huge losses to tomato production annually ranging between 05-20% (Maqbool et al., 1988). Punjab province is reported with 75-100% incidence of stem rot disease on tomato (Shahid et al., 2007). In Khyber Pakhtunkhwa, the most significant disease is also stem rot caused by two *Erwinia* species that is *E. carotovora* sub spp. *carotovora* and *E. carotovora* sub spp. *chrysanthemi* (Aysan, 2001).

Excessive use of synthetic pesticides has always produced potential risks to human health, beneficial organisms and environment, which prompted people

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for harmless alternatives in the form of plant based biocides (Adams and Garcia, 2005). Several plants are rich in bioactive compounds, like polyacetylenes, alkaloids, terpenoids, flavonoids and tannins (Ahn et al., 1998) that are at an advantage since these compounds are often toxic to limited species including specific target pests, can degrade quickly and thus suitable in integrated pest management programs for sustainable agriculture (Park et al., 2002). Neem (*Azadirachta indica*) tree in the form of neem oil extracts and seed water extracts is a good candidate for disease management. *Dodonaea viscose* commonly known as ghwaraskay, is a shrub and its crude extract has inhibitory effects against Gram positive and Gram negative bacteria (Gertie et al., 2003).

Plants utilize soil nutrients for its growth and when available in balanced amount leads to healthy production. An essential among the micronutrients, which is deficient in most soils, is boron (Graham and Webb, 1991). Boron develops and strengthens the plant cell wall; therefore, it is vital to plant health and low-level results poor growth of young tissues and plant development (Brown et al., 2002).

Being an important crop of Khyber Pakhtunkhwa, severe losses by stem rot in tomato and the lack of research in this aspect in the area, lead to the initiation of current research study in order to investigate biochemical characterization of the pathogen of tomato stem rot disease and to study the effectiveness of boron nutrient, plant extracts and synthetic bactericide against the pathogen.

Methods and Materials

Pathogen culture

Tomato plants were sown in Agricultural Research Institute (A.R.I), Tarnab, Peshawar in completely randomized block design with three replications and all agronomic practices were kept constant. Nutrient agar media was prepared by mixing nutrient agar (2.8 g L^{-1}), K_2HPO_4 (2 gm), NaCl (5.0 gm), and bactopeptone (1 gm) in distilled water and poured into conical flasks. From the suspension, culture of pathogen loopful was taken and inoculated to the nutrient agar media (Hafiz et al., 2012). Selected individual colonies (circular, shiny, raised, creamy white and transparent) grown on nutrient agar media were incubated at 28°C for another 24 h for pure culture of pathogen (Hafiz et al., 2012).

Biochemical characterization techniques

The culture were further directed to various tests such as 3% Potassium Hydroxide (KOH), catalase reaction, anaerobic growth, growth at 36°C, yeast dextrose culture (YDC), tolerance to 5% Sodium Chloride (NaCl) and mucoid growth for verification of stem-rot pathogen (Schaad, 1980; Suslow et al., 1982; Dickey, 1984; Lelliot and Stead, 1987).

Management of Pathogen, Erwinia species

An artificial inoculation of healthy plants with bacteria was done with tooth pick method (Keeling, 1982). Boron at different levels and plant biocides, either alone or in combination, were used in the management of tomato stem rot disease. Boron was applied to soil after two weeks of transplantation of tomato plants while plant extracts were applied to tomato plants infected with *Erwinia chrysanthemi*.

Utilization of boron

According to the area, recommended doses of boron fertilizer (1.97, 2.96 and 3.95 g $seedbed^{-1}$) were used. Boron supplement standards for plant were recorded to be 3 kg $acre^{-1}$, whereas for its optimum height, fruit and yield, 2 kg $acre^{-1}$ and 4 kg $acre^{-1}$ were used. These amounts of boron were converted to present study area that resulted as the above mentioned doses.

Preparation of biocide extract

Plant leaves of *A. indica* and *D. viscosa* were collected from University of Peshawar. The collected leaves were brought to pathology section of ARI Tarnab, washed with distilled water to remove dirt and were left to dry at room temperature for about a week. The leaves were then converted into fine powder using electric grinder. Fine powder of leaves was boiled for 15-20 minutes. The solution was left for two hours to cool down and then filtered with Whattman No. 1 filter paper. Filtered extract were left at room temperature and stored at 4 °C for avoiding inactivation of bioactive compound (Sukhdev et al., 2008).

The two plants extract of *A. indica* and *D. viscosa* were utilized at 15 ml L^{-1} and 20 ml L^{-1} respectively. The application ratio of plant extracts was in association with control treatment, CuS (copper sulphate) that amount generally as 3-5 ml L^{-1}.

Data collection

In our present study, a total of sixteen treatments (P1B1, P1B2, P1B3, P1, P2B1, P2B2, P2B3, P2, CuSB1, CuSB2, CuSB3, CuS, B1, B2, B3 and a control, where P1: *Azadirachta indica*, P2: *Dodonea viscose*, B1: Boron @ 1.97 g. seedbed^{-1}, B2: Boron @ 2.96 g. seedbed^{-1}, B3: Boron @ 3.95 g. seedbed^{-1}, CuS: Bactericide) were used that were replicated three times. Each treatment consisted of a single row having seven plants. During this study, plant height was recorded in centimeters. Numbers of fruit per plant were recorded randomly from each seedbed and then averaged. The disease incidence percent was recorded in accordance with the scale formulated by Wright et al. (2005). Picking of fruits from the seedbed tomato plant was done twice and then the overall calculation of the fruit yield in kg seedbed^{-1} was recorded (Naz et al., 2011). This was later converted into tons ha^{-1}.

Statistical analysis

The collected data were analyzed by carrying out the analysis of variance using Statistix 8.1 version computer software. Separation of means was done by least significant difference test at $P \leq 0.05$ (Steel and Torrie, 1984).

Results and Discussion

Characterization of stem rot, Erwinia species

Samples from tomato were observed for stem rot disease symptoms that were later used in various tests for identification. Table 1, shows the positivity in 3% KOH test revealing the samples to be gram-negative. Bacterial colonies produced threads, which is an indication of KOH to lyse the cell and extract the threads. Results of other tests were positive such as catalase test, growth at 36 °C, anaerobic growth, yellow pigment on YDC, tolerance at 5% NaCl and mucoid growth. These observations justified *Erwinia* specie bacteria responsible for causing stem rot disease in tomato plants.

Table 1: *Characterization of tomato stem rot, Erwinia species (Ech) where positivity is indicated by (+) while negativity by (–) signs.*

Test	Result	Laboratory result
3 % KOH	+	Ech
H_2O_2	+	Ech
Anaerobic growth	+	Ech
Growth at 36°C	+	Ech
Yellow pigment on YDC	+	Ech
Tolerance 5% NaCl	+	Ech
Mucoid growth	+	Ech

Effect of different treatments on various parameters of tomato plants

Plant extracts with the combination of boron were observed to have a significant implication on all the subjected parameters. Results indicated that plant extracts have a profound effect on plant height, fruits production per plant, reduction in disease severity and over all yields as compared to the untreated plants. The most significant effect was observed by neem plant extract with high dose (3.95 g seedbed^{-1}) of boron, which produced maximum height (75.3 cm), maximum number of fruits plant^{-1} (14.3), minimum level of disease severity (0-25%) and maximum yield (106.7 tons ha^{-1}). However, least significant effects were recorded for untreated plants for their poor height (32 cm), minimum number of fruits per plant (2.7), maximum level of disease severity (75-100 %) and minimum yield (20.1 tons ha^{-1}).

For plant height (cm) the effect of different treatments is shown (Figure 1), revealing that maximum mean plant height (75.3 cm) was achieved by neem plant extract when in combination with high dose (3.95 g seedbed^{-1}) of boron followed by combination of boron (3.95 g seedbed^{-1}) and bactericide producing 65.0 cm plant height, while the lowest plant height (32.0 cm) was observed in non-treated plots (control).

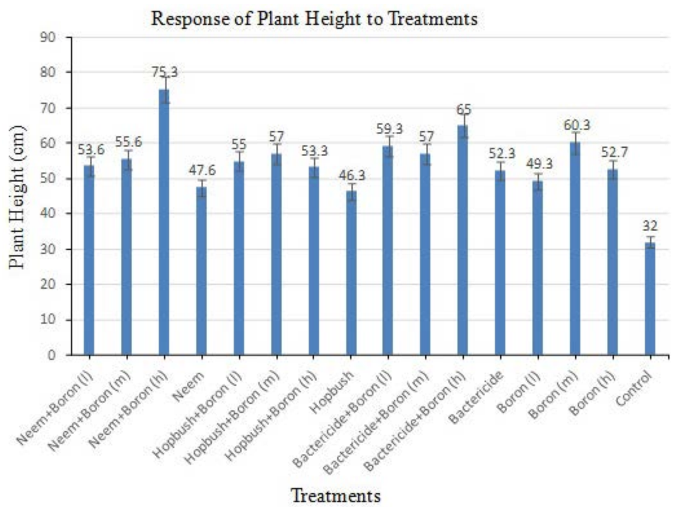

Figure 1: *Effect of different treatments on the mean tomato plant height (cm).*

The effect of treatments on number of fruits produced was similar (Figure 2); indicated overall fruits plant^{-1} was significantly different with increased fruit production. Maximum mean numbers of fruits plant^{-1} were obtained in treatment combination of neem extract and high dose of boron (3.95 g seedbed^{-1}) producing 14.3 fruits plant^{-1}. This was followed by combination boron (3.95 g seedbed^{-1}) with bactericide, boron (1.97 g seedbed^{-1}) with bactericide, boron (2.96 g seedbed^{-1}) with bactericide, and boron (2.96 g seedbed^{-1}) with *D. viscosa* plant extract, attaining 11.7, 9.3, 9.3 and 9.7 fruits plant^{-1} respectively, but these treatments were significantly not different from each other. Minimum values of fruits plant^{-1} were observed in control plots producing 2.7 fruits plant^{-1}.

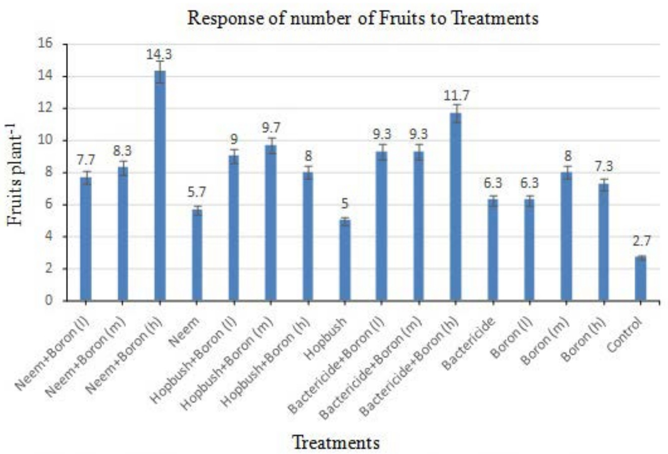

Figure 2: *Effect of different treatments on the mean of fruits per plant.*

Treatments and their combinations also showed a profound impact on the disease severity of the tested plants (Figure 3). Most plants with stem rot symptoms were seen to have major numbers lesser than 50%. Less disease severity was recorded in combination of neem plants extract with high dose of boron (3.95 g seedbed^{-1}), but this was not significantly different from combination of high dose of boron (3.95 g seedbed^{-1}) with bactericide where 0.0 % disease severity was recorded. Further, disease severity was found maximum in control plants (>75% disease severity).

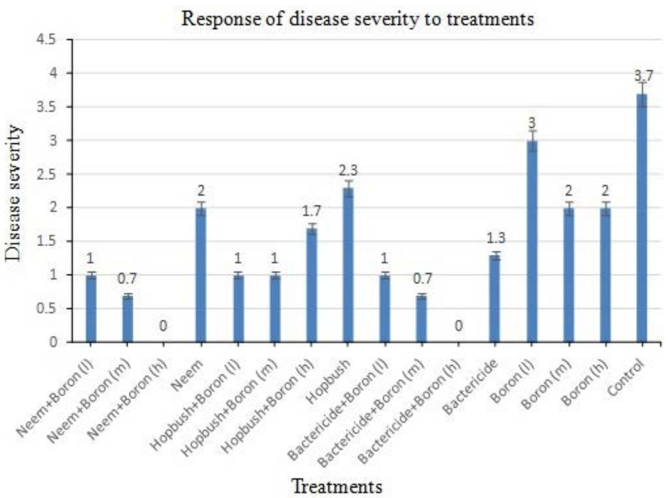

Figure 3: *Effect of different treatments on the mean disease severity of tomato stem rot Erwinia specie.*

For yield in tons ha^{-1} produced by plants, treatments and their combinations showed significant differences (Figure 4). Maximum yield per hectare was produced by plots with combination of neem plants extract and high dose of boron (3.95 g seedbed^{-1}) yielding mean 106.7 tons ha^{-1} of tomatoes. The general trend of the test is arbitrary and has similar impacts on the crop yields. Minimum figures were observed in untreated plots yielding mean 20.1 tons ha^{-1} of tomatoes.

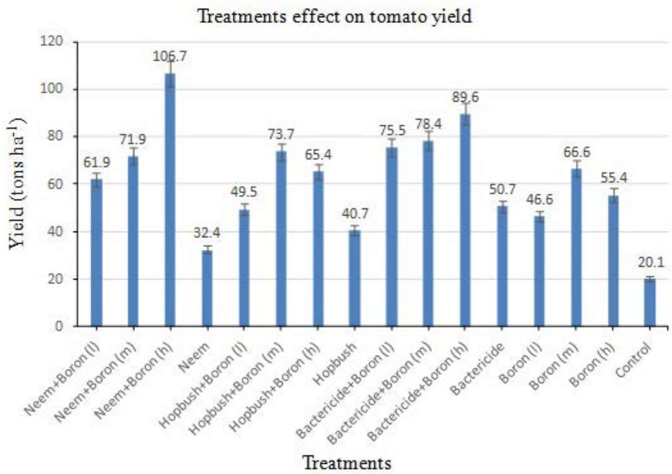

Figure 4: *Effect of different treatments on the mean yield (tons ha^{-1}).*

The overall results of plants extract and their combinations have produced statistically significant differences that were observed with a profound effect on general as well as specific agronomic parameters of plants, as well as on the plant health that resulted in more fruits and ultimately more yields.

This study was conducted for the identification of *E. chrysanthemi* and various treatments were used against the pathogen of tomato stem rot disease. The tests had all positive results declaring the species to be gram negative bacteria. These results are in line with Dicky (1979) and Manafi and Kneifel (1990), where these were recommended rapid methods for the identification of gram positive and gram-negative bacteria. Stem rot disease caused in tomato were of bacteria *Erwinia* species that mostly depends upon the environmental conditions. Dicky (1979) reported similar results, by identifying various *Erwinia* species from different sources. These tests have limitation, as various strains of *E. Chrysanthemi* have been analyzed for various tests, in which some might not give similar results through these biochemical tests, such as in rice *E. chrysanthemi* differentiate in characterization from that of stem rot disease (Goto, 1978). Thus, PCR analysis can be used for specificity identification of various strains of *E. chrysanthemi*.

The results indicated that boron supplement with plant extract or synthetic bactericide enhanced the plant height. These findings are exactly similar to that of Kostas and Dordas (2006) relating boron and manganese to plant physiology, which showed that boron had highly affected the height of plants such as in case of present study. Other than the three main components of fertilizer, nitrogen (N), potassium (K) and phosphorous (P); boron (B) can also be used as an enhancer for plant quality and stability with respect to the environment (biotic and abiotic factors).

The numbers of fruits observed in plants were due to boron alone as well as in combination with plant extracts, especially the neem extract along with boron yielded more number of fruits. The antimicrobial activity of plant extract controlling the disease plus boron in support of fruits resulted in higher fruit number. These findings were supported by Almas (1999).

Disease severity was high in plants with low doses of boron compared to high dose mixed with plant extracts as well as synthetic bactericide. This showed the significance of boron on disease resistance rather than the mixed supplement with boron dose varied. These results were supported by Christos (2009) indicating boron with an adverse effect on cell wall and cell membrane hence supporting the defense against external stimuli. Thus, with productivity, boron also play crucial role in resistance to biotic factors in modulating cell organelles.

The yield of tomato was observed with all those supplement that were in combination with boron in which neem extract and high dose of boron had the highest yield. Current data were supported by earlier findings of Kostas and Dordas (2006) and Almas (1999). The plant extract or the bactericide decrease the severity whereas, boron not only support the cell, but also enhance the production level of plant.

Conclusions and Recommendations

Based on findings of current study, the stem rot pathogen was declared *Erwinia carotovora* sub spp. *chrysanthemi* as gram negative bacteria by different proposed tests. Moreover, extracts of *A. indica* in combination with highest dose of boron was highly effective in reducing disease incidence and increasing the height, fruits produced per seedbed as well as overall yield of tomato comparative to all other treatments.

It is recommended that natural products isolation from plants and their bioassay should be done for antimicrobial activities. Awareness should be created among farming community regarding dosage rate of boron and its different combinations with different plant extracts for better management of *E. chrysanthemi*. To the farmer community where they are dependent on the fruits rather than plants, such supplement of boron can enhance the productivity of tomato.

Further research is required for the molecular study of boron and its influence on the cell mechanism and also PCR analysis should be done for the specificity of various strains of *Erwinia* species identification.

Novelty Statement

This study focused on the importance of boron in enhancing the productivity of tomato crop. Further, the use of a natural plant extract, *A. indica* greatly reduced stem rot incidence in tomato fields that set a great breakthrough into using chemicals against such bacterial disease.

Author's Contribution

AURS designed the idea and supervised this research work, while AB assisted in manuscript writing and research conduction. MZK collected the data and wrote manuscript. MSK analyzed the data and helped in results interpretation and BA helped in technical writing and reviewed the manuscript. All authors read and approved the final manuscript.

Conflict of interest

Authors have declared no conflict of interest.

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