*Journal of Applied and Natural Science* 8 (3):1146 - 1151 (2016)

JANS

# Evaluation of management of bacterial stalk rot of maize (*Dickeya zeae*) using some chemicals and bio-agents

**Adesh Kumar*, Mandeep Singh Hunjan, Harleen Kaur[1], Rajwinder Kaur and P.P. Singh**

Department of Plant Pathology; [1]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141 004 (Punjab), INDIA
*Corresponding author. E-mail: adeshpp@gmail.com

*Received: October 20, 2015; Revised received: April 07, 2016; Accepted: July 02, 2016*

**Abstract:** A virulent strain (M-13) of *Dickeya zeae* causing bacterial stalk rot of maize, isolated from Hoshiarpur district of Punjab was tested against different antimicrobial agents. Five antibacterial chemicals *viz.,* stable bleaching powder, streptocycline, cristocycline, blitox, kocide and one bioagent (*Pseudomonas fluorescens)* were evaluated against *D. zeae* under *in vitro* and *in vivo* condition. The chemicals were tested with different concentrations i.e. 50, 100, 200, 500, and 1000 and >1000 ppm and bioagent supernatant used as such without any dilutions. All the test chemicals and bioagent (*P. fluorescens* strains) reduced the log cfu/ml of *D. zeae.* Amongst chemicals, stable bleaching powder with 100 ppm concentration showed most effective antibacterial activity which reduced the population of *D. zeae* with 6.35 log cfu/ml and amongst *P. fluorescence* strains, MPF-5 strain showed effective result with 8.07 log cfu/ml. In field condition bleaching powder also showed good result to reduce the disease severity with increased the crop yield when used as both methods (drenching and dusting). However, post inoculation drenching with 100ppm concentration showed minimum mean of disease severity (7.13%) with high yield (7.4 kg) in all three maize cultivars (Dekalb Double, Punjab Sweet Corn-1 and PMH-1). The increase yield per cent as compared to control was 52.4% in Dekalb Double, 64% in Punjab Sweet Corn-1 and 57.9 % in PMH-1 maize cultivars.

**Keywords:** Bacterial stalk rot, Cultivars, *Dickeya zeae*, Maize management

## INTRODUCTION

Maize (*Zea maize*) is the most staple and leading cereal crops in the world. Bacterial stalk rot (BSR) caused by *Erwinia chrysanthemi* pv. *zeae*; presently known as *Dickeya zeae* (Samson *et al.* 2005) is the most devastating disease of maize. This disease is widespread worldwide in Asia, Africa, North America, Central America, South America and Oceania. In India, bacterial stalk rot caused by *D. zeae* is a challenging problem and most destructive disease of maize in the outer Shivaliks of North Western Himalayas covering states like Himachal Pradesh, Uttaranchal, Jammu & Kashmir and adjoining plain areas of states like Punjab, Haryana and Uttar Pradesh etc. The production of maize is hampered by a number of abiotic and biotic factors such as unfavorable climate, nutritional imbalance, diseases caused by fungi and bacteria. Among the biotic factors, the diseases caused by fungi and bacteria are economically more important because they cause heavy yield losses. Bacterial BSR of maize is a major hurdle in quality production and high yield in the crop causing 21 to 98 % yield loss during epidemics (Thind and Payak, 1978). In Punjab, this pathogen is most dominant and becoming a serious concern for maize cultivation in *kharif* sown crop. In severe conditions, the infected plant topples down causing significant reduction in grain yield.

Fungicides and antibiotics solution inhibits the growth of bacterium under *in vitro* and *in vivo* condition (Jones, and Schnabel 2000; Raghuwanshi *et al.* 2013, *et al.* 2008; Abeer and Rehab 2014). Bleaching powder and combination of antibiotics with cupper fungicides found effective to checks the development of *Erwinia* spp. (Lal *et al.*, 1970; Thind and Payak, 1972; Adaskaveg *et al.* 2011 ). Realizing the importance of this disease, the present studies were initiated to isolate, identify and characterize the pathogen with respect to its aggressiveness and also to evaluate different antibacterials and bioagent under *in vitro and in vivo condition* against this pathogen.

## MATERIALS AND METHODS

**Isolation and identification of the pathogen:** Infected samples were collected from different maize growing area of Punjab in the month june-July, 2013 (Kumar *et al.* 2015). The pathogen isolation was done on CVP (Crystal violet pectate) semi selective medium (Kumar *et al.* 2015). Single isolated colonies of the bacterium were further purified, preserved in silica gel and multiplied on the King's B medium, whenever necessary. Sixty two strains of the bacterial stalk rot

ISSN : 0974-9411 (Print), 2231-5209 (Online)  

pathogen were isolated from different locations.

**Pathogenicity test:** Pathogenicity tests were performed on one month old maize plant cv. Punjab Sweet Corn-I. Inoculum was prepared by growing all the isolates in liquid King's B media for 24 hrs at 27 ° C and bacterial concentration was adjusted to approximately $10^8$-$10^9$ cells/ml. Inoculation was done with help of hypodermic needle method (Rangarajan and Chakravarti, 1967). Disease severity was observed after four days of inoculation and final observation was made after 15 days. Infected plant was scored on 1-5 scale (Lal, 1981).

***In vitro* evaluation of different chemicals and bioagents against** *D. zeae*

**Preparation of standard growth curve:** For deciphering the log phase of the pathogen, standard growth curve using M-13 isolate was studied. In the ELISA plate 90µl of Peptone Sucrose Broth (PSB) and 10µl of pure bacteria culture of *D. zeae* (@$10^8$-$10^9$ cells/ml) was dispensed into individual wells. Thirty replications were conducted in each ELISA plate, and growth of bacteria was measured by taking OD of culture after 0, 2, 4, 6, 9, 13, 17, 22, 27, 30, 33, 39 and 43 hours of inoculation with the help of spectrophotometer (Tecan infinite M 200) simultaneously counting cfu/ml by spreading 100 µl of bacterial culture on King's B medium. Regression equation was derived from the slope taken from the graph with optical density value (OD) plotted against cfu/ml.

***In vitro* evaluation of chemicals and bioagents:** *In vitro* evaluation of different chemicals (stable bleaching powder, blitox, kocide, streptocycline and cristocycline) was done using the protocol standardized in Plant Bacteriology Laboratory, PAU Ludhiana. Each chemical was tested at 50, 100, 200, 500, and 1000 and >1000 ppm concentration by optical density method. Simultaneously, culture filtrate of seven *P. fluorescens* strains isolated from maize rhizosphere (designated as MPF-2, MPF-4, MPF-5, MPF-6, MPF-7, MPF-9 and MPF-10) was also tested for their bio-efficacy against *D. zeae* (M-13). The stock solution of standard agrochemicals was prepared on the active ingredient basis and the required concentrations were subsequently made from stock solution by adding required amount of sterilized distilled water. *P. fluorescens* bioagent extract was used as such without any dilution.

In the ELISA plate 90µl of Peptone Sucrose Broth (PSB) and 100µl each test concentration (double strength) was added into individual wells. It was overlain by pipetting in 10 µl of 12 hrs old bacterial culture and mixed by shaking the plate on the plate shaker (Tecan Inc.). Suitable positive and negative controls were also kept in the same plates. Initial OD value was recorded using the Spectrophotometer, Tecan infinite M 200 at 600 nm. The plates were incubated at 28°C temperature for 27 hrs. The OD reading of each well was recorded during the exponential phase of bacterial growth. The OD value was transformed into the log cfu using regression equation.

$$\text{Inhibition of bacterial growth} = \frac{\text{Log cfu/ml growth in control} - \text{Log cfu/ml growth in test concentration}}{\text{Log cfu/ml growth in control}} \times 100$$

Inhibition of bacterial growth was calculated as follow:

The efficacy of the agrochemicals was expressed in terms of ED90 values calculated by plotting log cfu inhibition curve against concentrations of different agrochemicals.

**Evaluation of different chemicals and bioagents against *D. zeae* under field conditions:** The experiment was conducted at experimental field area of the Department of Plant Pathology. Three maize cultivars (Punjab Sweet Corn-1, Dekalb Double and PMH-1) were grown in experiment field area with plot size 4×3 square meters. The virulent isolate of *D. zeae* (M-13) was inoculated in the second internode of maize varieties after 40-50 days of sowing using hypodermic syringe. The talc based powder formulation (600 ml broth culture +1 kg talk powder) of most effective strain of *P. fluorescens* (MPF-5) was used as seed dresser at a concentration of 20 gm $kg^{-1}$ seed. Disease incidence and severity was observed after 15 days of inoculation using 1-5 scale (Lal, 1981). The yield data was recorded at the time of harvesting in terms of kg/ plot. Nine treatments were applied in this experiment viz; T1-Added bleaching powder during sowing time @ 16 Kg/ha; T2-Preinoculation dusting with bleaching powder, followed by irrigation @ 16 Kg/ha; T3-Post inoculation dusting with bleaching powder, followed by irrigation @ 16 Kg/ha; T4-Pre inoculation drenching with bleaching powder solution @ 100ppm; T5-Post inoculation drenching with bleaching powder solution @ 100ppm; T6- Spray of Blitox (0.25%) + 100ppm Streptocycline; T7- Spray of Kocide (0.25%) + 100ppm Streptocycline; T8- Seed treatment with 20g *P. fluorescens* treatment per Kg seed, followed by pre-inoculation foliar spray; and T9-Control (no chemical used)

**Statistical analysis:** Analysis of variance (ANOVA) was conducted to determine significant variation between different treatments with respect to disease severity and yield in three maize cultivars by using "R" software.

## RESULTS AND DISCUSSION

**Isolation and identification of pathogen:** Sixty two isolates of the bacteria were recovered from diseased stalks. Individual isolates were identified on CVP (Crystal violet pectate) medium on the basis of cavity formation and these cultures were further purified on King's B Medium. The bacterium showed off-white, slimy and shiny colonies on the medium.

**Pathogenicity of *D. zeae*:** Out of sixty two isolates, seven isolates of *D. zeae* (M-7, M-9, M-13, M-14, M-29, Chilli-2 and Rice-1) showed highly virulent and toppled down the inoculated plants of cv. Punjab sweet

**Table 1**. *In vitro* efficacy of different chemicals against *D. zeae.*

| Chemicals | Log cfu/ml Concentration in ppm | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **50** | **100** | **200** | **500** | **1000** | **2000** | **2500** | **Control** | **ED50 (ppm)** |
| Stable bleaching powder | 7.43 (0.09) | 6.35 (0.14) | 6.36 (0.03) | 6.33 (0.02) | 6.22 (0.03) | 6.21 (0.05) | 6.18 (0.07) | 9.86 (0.20) | <50 |
| Blitox | 8.64 (0.06) | 8.66 (0.06) | 8.24 (0.09) | 8.24 (0.05) | 8.11 (0.05) | 7.19 (0.13) | 7.05 (0.02) | 9.86 (0.03) | <50 |
| Blitox + streptocycline | 7.71 (0.06) | 7.69 (0.11) | 7.41 (0.14) | 7.40 (0.2) | 7.08 (0.06) | 6.72 (0.08) | 6.68 (0.02) | 9.86 (0.20) | <50 |
| Kocide | 7.82 (0.07) | 7.80 (0.12) | 7.80 (0.12) | 7.78 (0.03) | 7.71 (0.09) | 7.60 (0.14) | 7.50 (0.06) | 9.86 (0.1) | <50 |
| Kocide+ streptocycline | 7.81 (0.08) | 7.71 (0.09) | 7.39 (0.16) | 7.69 (0.07) | 7.67 (0.09) | 7.01 (0.15) | 7.01 (0.12) | 9.86 (0.40) | <50 |
| Cristocycline | 7.94 (0.02) | 6.90 (0.04) | 6.80 (0.15) | 6.20 (0.07) | 6.10 (0.05) | NT | NT | 9.86 (0.15) | <50 |
| Streptocycline | 7.9 (0.02) | 6.47 (0.03) | 6.33 (0.02) | 6.22 (0.18) | 5.9 (0.05) | NT | NT | 9.85 (0.24) | <50 |

Figures in parentheses are standard deviations from mean of seven replications, NT (concentration not tested)

**Table 2**. *In vitro* evaluation of different strains of *P. fluorescens* against *D. zeae*

| **Strain** | **MPF2** | **MPF4** | **MPF 5** | **MPF 6** | **MPF 7** | **MPF 9** | **MPF 10** | **Control** |
|---|---|---|---|---|---|---|---|---|
| **log cfu** | 8.20 (0.10) | 8.35 (.08) | 8.07 (.06) | 8.34 (.015) | 8.60 (.05) | 8.81 (.04) | 8.80 (.10) | 9.62 (.31) |

Figures in parentheses are standard deviations from mean of seven replications

**Table 3.** Evaluation of different chemicals and bioagents against *D. zeae* under field conditions on Dekalb Double, Punjab Sweet Corn -I and PMH-1 variety at Plant Pathology experimental field.

| **Treatments** | **Dekalb Double** | | **Punjab Sweet Corn-1** | | **PMH-1** | | **Mean** | |
|---|---|---|---|---|---|---|---|---|
| | **Disease severity (%) After 15 days** | **Yield (kg)** | **Disease severity (%) After 15 days** | **Yield (kg)** | **Disease severity (%) After 15 days** | **Yield (kg)** | **Disease severity (%) After 15 days** | **Yield (kg)** |
| T1-Bleaching powder at sowing | 11.3 | 7.81 | 10.0 | 2.4 | 14.4 | 5.2 | 11.9 | 5.1 |
| T2-Preinoculation dusting of bleaching powder, followed by irrigation | 12.3 | 8.03 | 8.6 | 2.3 | 11.8 | 6.2 | 10.9 | 5.5 |
| T3-Post inoculation dusting of bleaching powder, followed by irrigation | 8.4 | 7.47 | 9.8 | 2.7 | 11 | 8.2 | 9.73 | 6.1 |
| T4-Pre inoculation drenching of bleaching powder | 10 | 8.67 | 8.38 | 2.9 | 8.5 | 8.2 | 8.96 | 6.6 |
| T5-Post inoculation drenching of bleaching powder | 8.4 | 9.42 | 6.5 | 3.3 | 6.5 | 9.5 | 7.13 | 7.4 |
| T6- spray of Blitox+ Streptocycline | 10.8 | 6.66 | 11.0 | 2.2 | 11.5 | 7.2 | 11.1 | 5.4 |
| T7- Spray of Kocide+ Streptocycline | 12.5 | 7.48 | 9.8 | 2.2 | 10.7 | 5.9 | 11 | 5.2 |
| T8- *P. fluorescens* seed treatment and spray | 19.7 | 5.28 | 10.8 | 2.2 | 14.9 | 5 | 15.13 | 4.2 |
| Control (no chemical used) | 21.15 | 4.48 | 21 | 1.2 | 21.7 | 4.0 | 21.28 | 3.2 |

**Table 4.** Analysis of variance for disease severity of stalk rot and grain yield.

| **Source of variation** | **Df** | ***In vivo* trail** | |
|---|---|---|---|
| | | **Disease Severity** | **Yield** |
| Treatment | 8 | 53.57*** | 19.3*** |
| Replication | 2 | 0.62 | 6.28** |

Level of significance; ***<0.001, **<0.01 and *<0.05

Corn-1 within 4 to 5 days of inoculation. Whereas, another's remaining 55 isolates were not able to topple down the inoculated plants. The most virulent isolate M-13 was used for *in vitro* and *in vivo* testing the

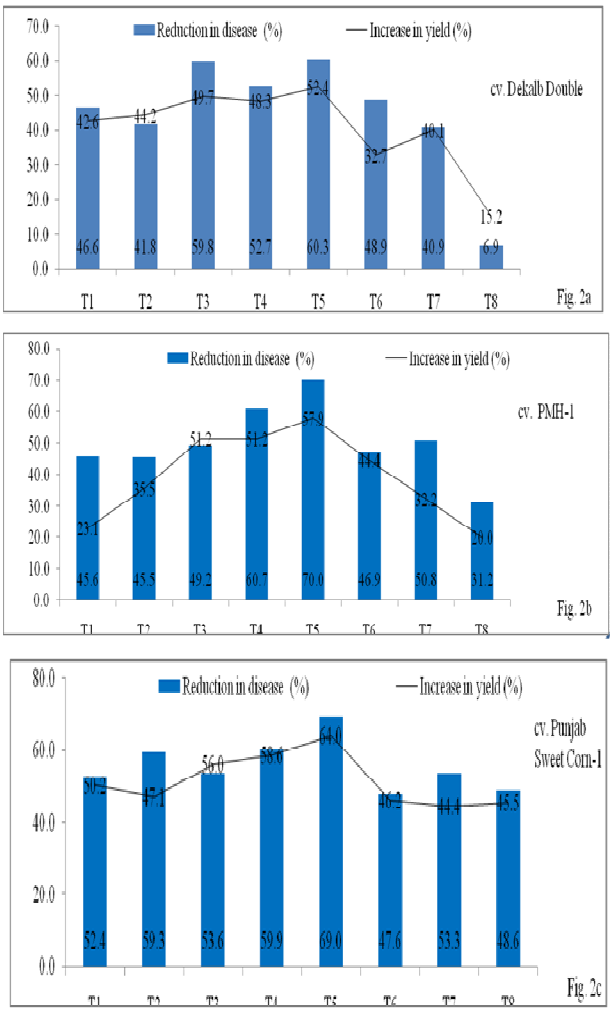


**Fig. 2.** *Treatments effect on percent reduction in bacterial stalk rot disease and increase yield in three maize cultivars (2a Dekalb Double, 2b PMH-1 and 2c Punjab Sweet Corn-1).*

chemicals effect on *D. zeae.* Standard curve studies revealed that the pathogen had log phase at 27hrs under controlled favorable conditions.

***In vitro* efficacy of chemicals against *D. zeae:*** All the chemical treatments as well as bio-agent treatment inhibited the bacterial growth under *in vitro* condition to a varying degree as compared to control. All the chemicals were found effective with ED90 <50 ppm (Table 1). Amongst all, bleaching powder was found to be most effective and gave log cfu value of 6.35/ml. Chlorine has been shown to be having antibacterial properties against phytobacterial such as *Erwinia* spp., *Ralstonia solanacearum* (Parashar *et al.* 1986; Shekhawat *et al.* 1990; Sharma and Kumar 2009; Yuliar *et al.* 2015). These findings are in agreement with Thind and Payak (1972), they used 1 ppm concentration of bleaching powder to check the growth of *E. chrysanthemi* . However, Randhawa (1977) tested eight chemical against this pathogen and found, combination of streptocycline + copper sulphate proved most inhibitory results whereas stable bleaching powder and potassium permanganate were slightly effective. Furthermore, Shekhawat *et al.* (1990) was confirmed most sensitive activity of bleaching powder for the control of bacterial stalk rots of maize. The second best treatment was that streptocycline giving log cfu value 6.47/ ml @ 100 ppm (safest dose for avoiding antibiotics resistance) reducing the bacterial population *in vitro* by manifold. It was also observed that cristocycline showed approximately similar result like streptocycline. Chakravarti and Rangarajan (1966) also found that streptocycline was effective *in vitro* against 6 species of *D. zeae*. Rangarajan and Chakravarti (1969) also suggested that various antibiotics namely streptomycin, terramycin and streptocycline were effective against *D. zeae.* at 100 ppm. In case of cupper based fungicides, combination of blitox (.25%) and kocide (.25%) with streptocycline (100ppm) showed good antibacterial activity with 6.68 and 7.01 cfu/ml respectively as compared to alone. Out of seven strains of *P. fluorescens* tested MPF-5 was found most effective against *E. chrysanthemi* pv. *zeae* and was further tested under field conditions (Table 2).

**Evaluation of different chemicals and bioagents against *D. zeae* under field conditions:** *In vivo* evaluation of different chemicals revealed significant variation with respect to disease severity and subsequent yield in maize. The trails were revealed that application of bleaching powder either as dusting (T2 and T3) or drenching (T4 and T5) was very effective in controlling the disease (Table 3, Fig. 2). Post inoculation drenching with stable bleaching powder (T5) was most effective where mean disease severity was observed to be 7.13 percent in all three maize cultivars (Table 3). It was at par with the treatment (T4) where pre inoculation drenching was done (8.96%) and post-inoculation dusting (T3) with bleaching powder (9.73%). T5 also showed significant increase in mean yield of the all three cultivars i.e. Dekalb Double, Punjab Sweet Corn-1and PMH-1 with 7.4 kg. The yield increase percent as compared to control of all individual cultivar i.e. Dekalb Double, Punjab Sweet Corn-1and PMH-1 was 52.4%, 64% and 57.9% respectively (Fig. 2). However, application of bleaching powder during sowing time (T1) was not effective to control disease. Cupper fungicides Blitox and Kocide with combination of Streptocycline (T6 and T7) were not found significantly effective to control disease. Mean disease severity and yield in T6 was 11.1% and 5.4 kg respectively, similarly T7 also have approximately same response to control disease and yield production.

It was observed that although *P. fluorescens* was effective under *in vitro* condition, it did not show significant control over the disease in field conditions. This treatment (T8) showed 15.13% mean disease severity which was highest after control (no chemical used).

Disease severity values and the yield for each of the antibacterial compounds were compared by two-way ANOVA (Table 4). All treatments showed significantly differences amongst each other, post inoculation drenching of bleaching powder given significantly good result as compared to all other treatments.

Several authors have also reported the field effectiveness of different formulation of chlorine against plant pathogenic bacteria (Sharma and Kumar 2000; Yamasaki *et al.* 2006; Ghosh and Mandal; Yuliar *et al.* 2015). Lal *et al.* (1970) reported that 3-4 surface irrigations (one before maize planting) with chlorinated water containing 100ppm chlorine reduced the incidence of maize stalk rot. Similarly, Thind and Payak (1972) also found that bleaching powder was effective in checking the growth of *E. chrysanthemi* pv. *zeae in vitro* as well as *in vivo*. Shekhawat *et al.*, 1990 were used bleaching powder @ 25 kg $ha^{-1}$ in potato crop and found effective for control of bacterial wilt of potato under glass house and field condition.

Limited studies on biological control of bacteria pathogen have also been reported. Karkouri *et al.*, 2010 observed that *E. chrysanthemi* can be controlled by actinomycete strain. Nagaraj *et al.*, 2012 studied tip-over disease of banana that caused by *E. carotovora* subsp. *carotovora* and *E. chrysanthemi* can be controlled by antagonistic bacteria *viz. Bacillus subtilis* and *P. fluorescens* and VAM fungi (*Glomus fasciculatum*). However in our studies *P. fluorescens* could not provide significant disease control *in vivo* condition.

## Conclusion

In present study, the virulent isolates of *D. zeae* showed best sensitivity reaction with use of bleaching powder as compared to other chemical agents. It was observed *in vitro* condition that 100ppm conc of bleaching powder reduced the cfu value of bacterium with (6.35 log cfu/ml) and same doses of this chemical showed good result to reduce the mean disease severity (7.13%) in all three cultivars of maize with increased in yield. However, *P. flourescens* was found effective under *in vitro* conditions but under field conditions their activity drops down due to prevalence of unfavorable abiotic conditions. The information generated here could also be used to address the issues regarding disease control and ecology of this bacterium vis-à-vis maize crop and further exploration of pathogenic populations management.

## REFERENCES


Abeer, H.M. and Rehab, A. (2014). Investigation on the Effect of Chemical and Biological Control of Bacterial Soft Root Disease of Potato in Storage. *J.Bio. Agri. Health.* 4: 31-44.

Adaskaveg, J. E., Förster, H., and Wade, M. L. (2011). Effectiveness of kasugamycin against *Erwinia amylovora* and its potential use for managing fire blight of pear. *Plant Dis.* 95: 448-454.

Chakravarti, B.P. and Rangarajan, M. (*1966). Streptocycline, an effective antibiotic against bacterial Plant pathogens. Hindustan Antibiot. Bull.* 8: 209-211.

Ghosh, P. P. and Mandal, N. C. (2009). Some disease management practices for bacterial wilt of potato. *The Journal of Plant Protection Sciences* 1 : 51-54, 2009.

Jones, A. L., and Schnabel, E. L. (2000). The development of streptomycin resistant strains of *E. amylovora*. In: Fire Blight: The disease and its causative agent, *E. amylovora*. J. L. Vanneste, ed. CABI Publishing, New York, pp 335-351.

Karkouri, A.E., Hassani, F.Z. E., Mzibri, M.E., Benlemlih, M.E. and Hassouni, M. (2010). Isolation and Identification of an Actinomycete Strain with a Biocontrol Effect on the Phytopathogenic *Erwinia chrysanthemi* 3937VIII Responsible for Soft Rot Disease. *Ann. Microbiol.* 60: 263-68.

Kumar, A., Hunjan, M.S., Singh, P.P. and Kaur H. (2015) Status of bacterial stalk rot of maize in Punjab. *Plant. Dis. Res.* 30: 97-99.

Lal S (1981) Bacterial stalk rot of maize. In:Techniques of scoring for resistance to important diseases of maize. All India co-ordinated maize improvement project, IARI, New Dehli, pp 82-86.

Lal, S., Thind, B.S., Payak, M. and Ranfro, B.L. (1970). Bacterial stalk rot of maize: Resistant breeding and chemical control. *Indian Phytopath.* 23:150-7.

Nagaraj, M.S., Umashankar, N., Palanna, K.B. and Khan, A.N.A. (2012). Etiology and management of tip-over disease of banana by using biological agents. *Int. J. Adv. Biotechnol. Res.* 2: 483-86.

Parashar, R.D., Sindhan, G. S. and Singh J. P. (1986). Field application of Kloricin for the control of soft rot of potato in field and storage. *J. Indian Potato Assoc.* 13**:** 81-82.

Raghuwanshi, K.S., Hujare, B. A., Chimote, V.P. and Borkar, S.G. (2013). Characterization of *xanthomonas axonopodis* pv. *punicae* isolates from Western Maharashtra and their sensitivity to chemical treatments. *The Bioscan* 8: 845-850.

Randhawa, P.S. (1977). Control of bacterial stalk rots and stripe disease of Maize (*Zea mays* L.). M Sc thesis submitted to Punjab Agricultural University Ludhiana.

Rangarajan, M. and Chakravarti, B. P. (1967). A strain of *Pseudomonas lapsa* isolated from corn seed causing stalk rot in India. *Plant. Dis. Rep.* 51:764-766.

Rangarajan, M. and Chakravarti, B.P. (1969). Efficacy of antibiotics and fungicides against corn stalk rot bacteria. *Hindustan Antibiot. Bull.* 2:177-79.

Samson, R., Legendre, J.B., Christen, R., Fischer, M., Achouak, W. and Gardan, L. (2005). Transfer of *Pectobacterium chrysanthemi* (Burkholder *et al* 1953) Brenner *et al* 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *Int. J. Sys. Evol. Microbiol.* 55:1415-27.

Sharma, J. P., Kumar S. (2000) Management of Ralstonia wilt through soil disinfectant, mulch lime and cakes in tomato (*Lycopersicon esculentum*). The Indian Journal of Agricultural Sciences 70.17-19.

Sharma, J.P. and Kumar, S. (2009). Linear reduction of propagules of *Ralstonia solanacearum* in soil by cake and chemicals. *Indian Phytopath.* 62: 49-53.

Shekhawat, G.S., Bhal, B.K., Kishore, V., Sangar, R.B.S., Patel, R.L., De, B.K., Sinha, S.K. and Pani, A.K. (1990). Control of bacterial wilt of potatoes by agro-

nomic practices. *J. Indian Potato. Assoc.* 17: 52-60.

Thind, B.S. and Payak, M.M. (1972). Antibiotics and bleaching powder in the control of bacterial stalk rot of maize. *Hindustan Antibiot. Bull.* 15: 73-74.

Thind, B.S. and Payak, M.M. (1978). Evaluation of maize germplasm and estimation of losses to *Erwinia* stalk rot. *Pl. Dis. Rep.* 62: 319-23.

Yamasaki, M., Kusakari, S., Narita, K., Osamura, K. and Nagai, M. (2006). Control of root rot disease of tomatoes by using weak acidic electrolyzed water (WAEW) in the hydroponic culture solution. *J. Antibac. Antifung. Agents* 34:543–549.

Yuliar, Nion, Y. A. and Koki Toyota, K. (2015). Recent Trends in Control Methods for Bacterial Wilt Diseases Caused by *Ralstonia solanacearum. Microbes Environ.* 30:11.