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Evaluation of antibiotics, fungitoxicants and botanicals against *Xanthomonas oryzae* pv. *oryzae*, A cause of bacterial leaf blight of rice

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ABSTRACT

In vitro efficacy of different antibacterial compounds, were evaluated against *Xanthomonas oryzae* pv. *oryzae* (Xoo) causing bacterial leaf blight of rice. Six antibiotics *viz.*, Streptocycline, Streptomycin, Streptomycin sulphate, Plantomycin, Tetracycline hydrochloride and Oxytetracycline hydrochloride were evaluated for their efficacy against the growth of *Xoo* cultures at two levels (50 and 100ppm) of concentration except Plantomycin (500 and 1000 ppm) using inhibition zone assay method. The largest inhibition zone (25 mm) was documented in Tetracycline hydrochloride @ 100ppm, followed by 23 mm obtained in Streptomycin @ 100ppm. Five fungitoxicants comprising Copper Oxychloride 50 % WP (0.15 and 0.25%), Copper hydroxide 77 % WP (0.15 and 0.25%), Carbendazim 50 % WP (0.05 and 0.15%), Validamycin 3L (0.15 and 0.25%) and Propiconazole 25% EC (0.05 and 0.1%) were evaluated for their efficacy against growth of *Xoo* cultures at two levels of concentration. The maximum inhibition zone (19.34mm) was recorded in Validamycin @ 0.25% followed by 18.16mm observed in copper oxychloride @ 0.25%. Effect of combination of antibiotics and fungitoxicants were studied using a set comprising of six commercially available antibiotics *viz.*, Streptocycline (100 ppm), Streptomycin (100 ppm), Streptomycin sulphate (100 ppm), Plantomycin (1000 ppm), Tetracycline hydrochloride (100 ppm), Oxytetracycline hydrochloride (100 ppm), and three fungitoxicants *i.e.* Copper oxychloride 50 % WP (0.25%), Copper hydroxide 77% WP (0.25%), Carbendazim 50 % WP (0.15%) were evaluated for their efficacy against growth of *Xoo*. Single concentration of each antibiotic will be evaluated with combination of three fungitoxicants separately. Maximum inhibition zone (25.8mm) was obtained in case of Streptocycline + Carbendazim which is at par with Tetracycline hydrochloride + Copper oxychloride and Streptomycin sulphate + Copper hydroxide. The cultures of *Xoo* were also screened with plant extracts *viz.*, Neem leaf, Garlic bulb, Onion bulb, Ginger rhizome, Tulsi leaf at three levels (10, 20 and 30%) of concentration for their antibacterial properties against

Xoo. The maximum inhibition zone (9.2 mm) was recorded in Garlic @ 30% followed by 9.14mm resulted in Tulsi @ 30 per cent.

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INTRODUCTION

Worldwide, more than 3 billion people utilize rice as staple food and it accounts for 50 to 80 per cent of their daily calorie intake (Delseny *et al.*, 2001). Over the next 20 years, it is expected that demand of rice will grow by 2.5 per cent per year (Hobbs, 2001). Ultimately, the challenge is to provide food for the increasing population and to ensure food security. To meet this demand, the global rice production needs to be doubled by 2050 (Sheehy *et al.*, 2011 and Skamnioti and Gurr, 2009). Increasing rice yields is one of the few strategies to fight the world's food insecurity and malnutrition. Since the world's population is expected to surge from 6.1 billion in 2000 to 9.2 billion in 2050 (UN, 2005). This prediction in human population requires increasing crop yields to meet the requirements of the rising global demand for food (Fernando, 2006). In Indian context, rice is an important staple food for more than 65 per cent of the population, therefore, national food security hinges on the growth and stability of its production. Efforts for enhancing the productivity of rice are limited by a number of biotic and abiotic stresses. The crop suffers from a number of devastating diseases caused by fungi, bacteria, viruses, nematodes, phytoplasmas and a number of environmental factors (Mew *et al.*, 1993). In India, annual crop losses are estimated about Rs. 6000-7000 crores, losses contributed to diseases are 26 per cent, weeds 23 per cent, insects 20 per cent and rest by birds and nematodes (Raju, 2000). Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae pv. oryzae (Xoo)* is one of the most serious diseases of rice across the world including Asian countries (Ou, 1985 and Mew *et al.*, 1993). Since the introduction and cultivation of new, high-yielding but susceptible rice varieties over a large acreage in recent years, the disease has become one of the most serious problems of rice cultivation in India (Srivastava, 1967). In India, the losses had been estimated to vary from 6-74 per cent (Gnanamanickam *et al.*, 1999; Adhikari *et al.*, 1995 and Rao and Kauffman, 1977). For the management of bacterial leaf blight disease of rice, various control measures are being used but efficient and reliable control measure for BLB is unavailable so far (Singh, 2009). Mega-varieties like MTU-1010, 1001, Sarju-52, Pusa Basmati etc., covering large area are susceptible to BLB. Resistant varieties *viz.*, Improved Pusa Basmati, Improved Samba Masuri, Ajaya etc. are not so popular among the farmers and host resistance is also not stable due to variability and emergence of new races in pathogen. The identification of various fungitoxicants, antibiotics, their combinations, botanicals showing inhibitory effect on *Xanthomonas oryzae pv. oryzae* are in progress for development of better management strategies. Keeping all these points in view, the current study was performed with the firm attitude to evaluate the effectiveness of most efficient antibiotic and fungitoxicants either single or in combination and botanicals against BLB in rice.

MATERIAL AND METHODS

Collection of BLB samples:

The experiments were conducted in laboratory of Department of Plant Pathology, Bihar Agricultural College (BAC), Sabour (Bihar) during *Kharif* 2017. The diseased leaves of rice cv. TN-1 showing typical bacterial blight (BB) symptoms were collected in brown paper bags from Agriculture Experimental Farm, BAC, Sabour and brought to the Laboratory for further processing.

Isolation and pathogenicity test:

Isolation of the bacterium *Xanthomonas oryzae* pv *oryzae* was carried out using infected leaves of rice plant collected from Agriculture Experimental Farm of BAC, Sabour. The sample showing typical leaf blight and bacterial oozing from the cut section during microscopy were used for isolation of bacterium. The diseased portion with healthy tissues was cut into 0.5 to

1 cm pieces. These diseased pieces were disinfected in 1 per cent sodium hypochlorite solution for 30 seconds, followed by three subsequent washing with sterilized distilled water in aseptic condition to remove the traces of NaOCl. The diseased bits were then suspended in a test tube containing 3 ml of sterilized distilled water and squeezed gently with sterilized scalpel. When the water became slightly turbid due to oozing of bacterial cells, the suspension was serially diluted upto 103 dilutions in 9 ml sterile water blanks. This suspension was streaked on nutrient agar (NA) medium with the help of sterilized wire loop. The inoculated plates were incubated at room temperature (27 ± 2^0C) for 48 hrs. After the incubation period, observations were made for the development of well separated, typical, light yellow coloured bacterial colonies resembling *Xoo*. The typical colony of *Xoo* was sub-cultured on NA plates to get pure culture. Cultures on NA slants were preserved for longer duration at 4°C. The isolated *Xoo* proved pathogenic to rice (TN-1) using Koch's postulate, which confirmed that the culture isolated was of *Xanthomonas oryzae* pv. o*ryzae*.

Inhibition zone assay method to assess the efficacy of antibacterial chemicals against *Xoo*:

All these antibiotics were evaluated at different concentrations following inhibition zone assay method. The bacterium was multiplied by inoculating the bacterial culture into the Erlenmeyer's flask containing 20 ml of nutrient broth. The inoculated flasks were incubated at 30^0C for 72 hours. The bacterial suspension (10^8 cell/ml) was then seeded to the lukewarm nutrient agar medium (1000 ml). The seeded medium was poured into the sterilized Petriplates and plates were allowed to solidify. Solution of test antibiotics prepared separately. The filter paper discs (Whatman No. 42) measuring 5 mm in diameter were soaked in the respective chemical solutions of different concentration for 5 minutes and transferred to the surface of the medium seeded with bacterial culture in Petriplates. Six replications of each treatment were maintained. The inoculated plates were kept in the refrigerator at 5^0C for 4 hours to allow the diffusion of chemical into the medium. Then plates were incubated at 28 ± 1^0C for 72 hours. At the end of incubation period these plates were observed for the production of inhibition zone around the filter paper discs. Inhibition zone in each plate was measured in terms of diameter (mm) and the results analyzed statistically.

***In vitro* evaluation of antibiotics, fungitoxicants and their combinations against Xoo:**

In antibiotics, six commonly available antibiotics *viz.*, Streptocycline, Streptomycin, Streptomycin Sulphate, Plantomycin, Tetracycline Hydrochloride and Oxytetracycline hydrochloride (Fig. 1) were evaluated for their efficacy against the growth of *Xoo*. All these antibiotics were evaluated at two different concentrations *i.e.* 50ppm and 100ppm except Plantomycin which was tested @ 500ppm and 1000ppm following inhibition zone assay method. In other experiment, five fungitoxicants (Fig. 2) *viz.*, Copper Oxychloride 50% WP (0.15 and 0.25%), Copper hydroxide 77% WP (0.15 and 0.25%), Carbendazim 50% WP (0.05 and 0.15%), Validamycin 3L (0.15 and 0.25%) and Propiconazole 25% EC (0.05 and 0.1%) were evaluated for their efficacy against the growth of *Xoo* following inhibition zone assay method. However, in combinations of antibiotics + fungitoxicants (Fig. 3), a set of six antibiotics *viz.*, Streptocycline, Streptomycin, Streptomycin Sulphate, Tetracycline Hydrochloride, Oxytetracycline Hydrochloride @ 100ppm in each except Plantomycin @ 1000ppm and three fungitoxicants *i.e.* Copper Oxy-chloride 50% WP (0.25%), Copper Hydroxide 77% WP (0.25%), Carbendazim 50% WP (0.15%) were evaluated for their efficacy against the growth of *Xoo* following inhibition zone assay method.

***In vitro* evaluation of botanicals against *Xoo*:**

Fresh leaves, bulb, cloves and rhizomes of five different plants (Fig. 4) *viz.*, *Neem*, *Tulsi*, Ginger, Onion and Garlic were evaluated against BLB to examine their inhibitory efficacy on growth of the bacteria. Experiment was carried out as per technique described by Meena and Gopalkrishnan (2004). For preparation of plant extracts, fresh plant parts were collected and first they were washed 3-5 times in tap water and then with distilled water. It was processed with sterile distilled water @ 1:2 ratio *viz.*, 1g tissue in 2 ml water and grinded in mortar and pestle, each filtered with separate muslin cloth. The filtrate was centrifuged at 5000 rpm for 15 minutes and clear supernatant was collected. The supernatant was filtered, sterilized to avoid contamination. The obtained extracts formed the standard extract solution (100%). To obtain desired concentration of extracts in the medium, an amount of stock solution to be added in water was calculated by using the formula:

$C_1 V_1 = C_2 V_2$
where,
C_1 = Concentration of stock solution
C_2 = Concentration of aqueous leaf extract desired
V_1 = Vol. (ml) of stock solution to be added
V_2 = Measured vol. (ml) of water in which aqueous leaf extract is to be added.

Remaining procedure was performed similarly as other *in vitro* experiments.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Efficacy of antibiotics against *Xoo*:

In the experiment regarding *in vitro* efficacy of antibiotics against *Xoo*, the largest inhibition zone of 25 mm was documented in case of Tetracycline hydrochloride @ 100 ppm. It was followed by Streptomycin @ 100 ppm with an inhibition zone of 23 mm. The least inhibition zone (11.15mm) against *Xoo* was obtained due to Plantomycin @ 500 ppm (Fig. 1). The findings are quite in conformity with the previous report that best inhibition of virulent isolate of *Xoo* exhibited by Streptomycin followed by Kanamycin, Ampicillin, Sinobionic, Benzylpenicillin and Chloramphenicol. Sensitivity of *Xoo* isolates against antibiotics was progressively increased with increase in the concentration of antibiotics (Khan *et al.*, 2012 and Ashrafuzzaman, 1987). Prasad *et al.* (2018) found that Streptomycin @ 0.05% was most effective against Xoo followed by Streptomycin @ 0.03%. and Streptocycline @ 0.05%. According to Mahto *et al.* (1988), Streptocycline (1000 ppm) exhibited the widest zone of inhibition (27.83 mm) against *Xoo* after 72 hour of incubation. It inhibited *Xoo* growth at all three concentrations (10, 100 and 1000ppm). The present findings are also in agreement with the earlier reports about good antibacterial property of Streptocycline (Mahto *et al.*, 1988 and Chauhan, 1980), Streptomycin Sulphate (Thimmegowda *et al.*, 2012 and Naqvi *et al.*, 2014), Tetracycline Hydrochloride (Balaraman and Rajagopalan, 1978) against *Xoo*. Prophylactic and curative spraying of streptocycline (500 ppm), streptomycin + oxytetracycline (1:9, 250 and 500 ppm),

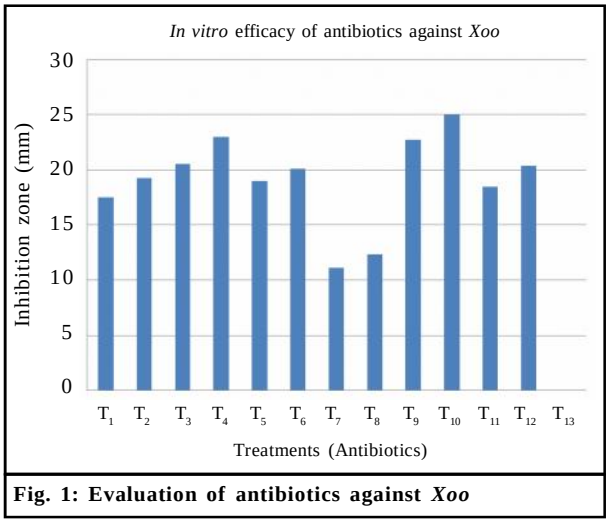

Fig. 1: Evaluation of antibiotics against *Xoo*

Treatment details
T_1: Streptocycline @ 50 ppm
T_2: Streptocycline @ 100 ppm
T_3: Streptomycin @ 50 ppm
T_4: Streptomycin @ 100 ppm
T_5: Streptomycin sulphate @ 50 ppm
T_6: Streptomycin sulphate @ 100 ppm
T_7: Plantomycin @ 500 ppm
T_8: Plantomycin @ 1000 ppm
T_9: Tetracycline hydrochloride @ 50 ppm
T_{10}: Tetracycline hydrochloride @ 100 ppm
T_{11}: Oxytetracycline hydrochloride@50ppm
T_{12}: Oxytetracycline hydrochloride@100ppm
T_{13}: Control

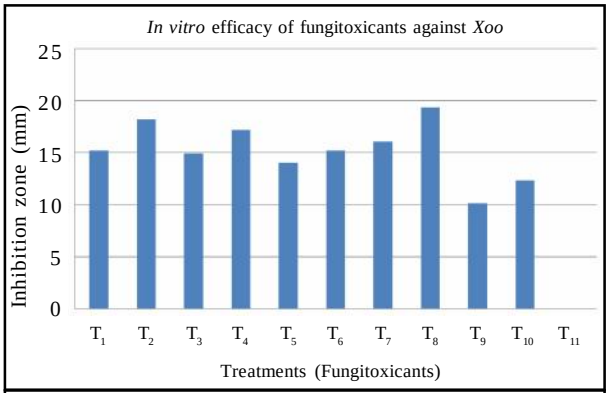

Fig. 2 : Evaluation of fungitoxicants against *Xoo*

Treatment details:
T_1: Copper oxychloride (0.15%)
T_2: Copper oxychloride (0.25%)
T_3: Copper hydrochloride (0.15%)
T_4: Copper hydrochloride (0.25%)
T_5: Carbendazim (0.05%)
T_6: Carbendazim (0.15%)
T_7: Validamycin (0.15%)
T_8: Validamycin (0.25%)
T_9: Propiconazole (0.05%)
T_{10}: Propiconazole (0.1%)
T_{11}: Control

bactrinol-100 (500 ppm) and cow dung extract (20 g/ litre) on rice was found effective against bacterial leaf blight by Mary *et al.* (2001).

Efficacy of fungitoxicants against *Xoo*:

In the experiment conducted, maximum inhibition zone of 19.34 mm was recorded by application of Validamycin @ 0.25% followed by copper oxychloride @ 0.25% (18.16 mm). The minimum inhibition zone was found with Propiconazole @ 0.05% with an inhibition zone of 10.16 mm (Fig. 2). Patel *et al.* (2009) concerned that Blitox-50 is superior treatment and highly effective in reducing bacterial leaf blight intensity and provided better grain yield and 100 g wt. Similar results were also reported by (Munna *et al.*, 2009; Thimmegowda *et al.*, 2012 and Parthasarathy *et al.*, 2014). This results are also in agreement with works of different researchers who proved the efficacy of Carbendazim 50% WP (Swati *et al.*, 2015), Copper hydroxide (Parthasarathy *et al.*, 2014), and Copper oxychloride 50% WP (Patel, 2008 and Khan *et al.*, 2005) against *Xoo*. Fungitoxicants are found to be most effective in combination with antibiotics.

Efficacy of combination of antibiotics and fungitoxicants against *Xoo*:

Maximum inhibition zone was recorded (25.8 mm) in Streptocycline + Carbendazim which is at par with Tetracycline hydrochloride + Copper oxychloride (24.24 mm) and Streptomycin sulphate + Copper hydroxide (24.12 mm) while the minimum inhibition zone was reported Plantomycin + Carbendazim with an inhibition zone of 13.02 mm (Fig. 3). These results coincide with the results produced by Patel *et al.* (2009), they found streptomycin sulphate + copper oxychloride superior over

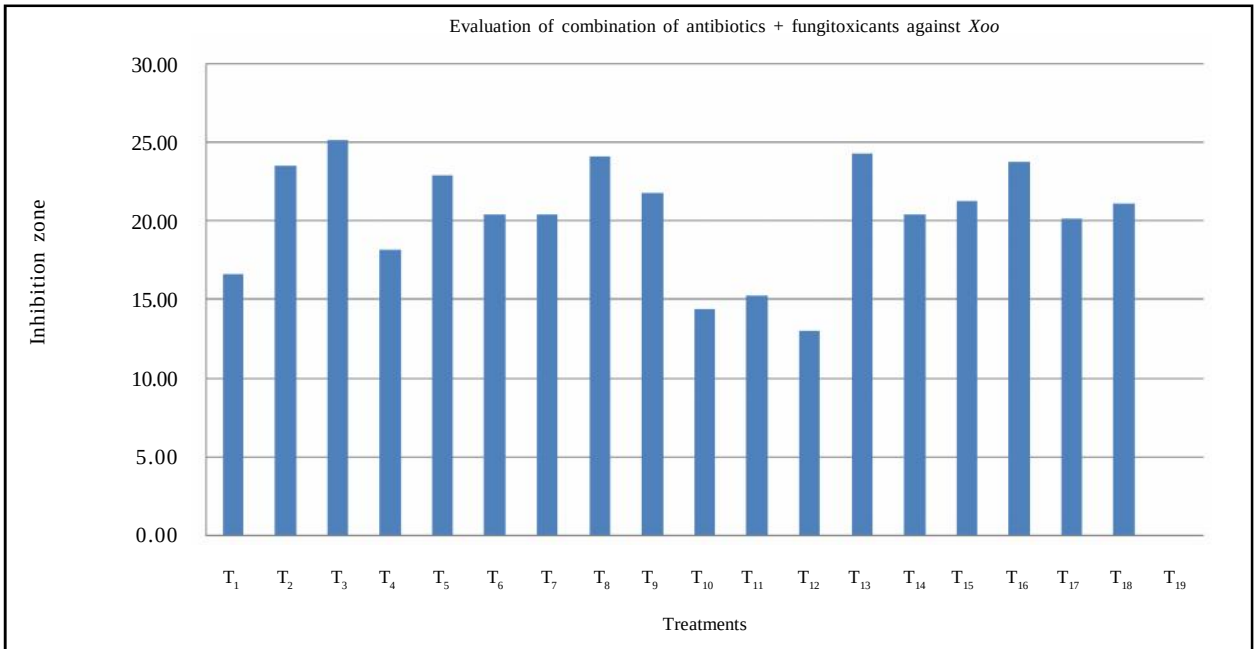

Fig. 3 : Evaluation of combination of antibiotics and fungitoxicants against *Xoo*

Treatment details:

T_1 : Streptocycline (100ppm) + Copper oxychloride (0.25%)
T_2 : Streptocycline (100ppm) + Copper hydroxide (0.25%)
T_3 : Streptocycline (100ppm) + Carbendazim (0.15%)
T_4 : Streptomycin (100ppm) + Copper oxychloride (0.25%)
T_5 : Streptomycin (100ppm) + Copper hydroxide (0.25%)
T_6 : Streptomycin (100ppm) + Carbendazim (0.15%)
T_7 : Streptomycin sulphate (100ppm)+Copper oxychloride (0.25%)
T_8 : Streptomycin sulphate (100ppm)+Copper hydroxide (0.25%)
T_9 : Streptomycin sulphate (100ppm) + Carbendazim (0.15%)
T_{10}: Plantomycin (1000ppm) + Copper oxychloride (0.25%)
T_{11}: Plantomycin (1000ppm) + Copper hydroxide (0.25%)
T_{12}: Plantomycin (1000ppm) + Carbendazim (0.15%)
T_{13}: Tetracycline hydrochloride (100ppm)+Copper oxychloride (0.25%)
T_{14}: Tetracycline hydrochloride (100ppm)+Copper hydroxide (0.25%)
T_{15}: Tetracycline hydrochloride (100ppm) + Carbendazim (0.15%)
T_{16}: Oxytetracycline hydrochloride (100ppm)+Copper oxychloride (0.25%)
T_{17}: Oxytetracycline hydrochloride (100ppm)+Copper hydroxide (0.25%)
T_{18}: Oxytetracycline hydrochloride (100ppm) + Carbendazim (0.15%)
T_{19}: Control

rest of the treatments in inhibition of *Xoo*, which also increased grain and straw yield. Thimmegowda *et al.* (2012) reported the effectiveness of streptocycline and streptocycline + copper oxychloride against bacterial blight under *in vitro* and *in vivo* condition. It was reported that the streptocycline + copper oxychloride was found best with least per cent disease index and highest grain yield returns. Tandan and Chaliganjewar (2016) reported that among the chemicals, copper oxychloride @ 0.25% + streptomycin sulphate (200 ppm) was found most effective with highest per cent inhibition against *Xoo* as compared to other chemicals. Singh *et al.* (2015) observed that at 1 per cent concentration of oxytetracycline + copper oxychloride produced maximum (2.83 cm) inhibition zone of 2.83 cm followed by streptomycin + copper oxychloride (2.23 cm). Prasad *et al.* (2018) reported that maximum inhibition zone (24.42 mm) against *Xoo* was exhibited by Streptocycline @ 0.3% + Carbendazim @ 0.15% and it was at par with 24.25 mm and 23.67 mm zones of inhibition exhibited by Streptomycin Sulphate @ 0.03% + Copper hydroxide @ 0.25% and Streptocycline @ 0.03% + Copper hydroxide @ 0.25%, respectively.

Efficacy of botanicals against *Xoo:*

The maximum inhibition zone (9.2 mm) was recorded in Garlic @ 30% followed by 9.14mm resulted in *Tulsi* @ 30 per cent (Fig. 4). Various plant extracts were reported to possess differing level of antibacterial activities *in vitro* against phytopathogenic fungi, yeast, and bacteria. However, only few limited data available on antibacterial activity due to plant extracts in plant pathogenic bacteria. Narasimhan *et al.* (1995) and Kagle *et al.* (2004) have reported antibacterial activity and management of bacterial diseases with use of different plant extracts in other crops. Kumar *et al.* (2009) also reported that at 25 per cent concentration, minimum number of colonies development were observed in case of *Allium sativum* (18.67) which showed production of maximum inhibition *i.e.*, 41.06 per cent. Present research finding is also showing similarity with the findings of Kagale *et al.* (2004); Meena and Gopalakrishnan (2004) and Sunder *et al.* (2005). They also reported the inhibitory effect of *Azadirachta indica* and *Zingiber officinale* against *Xathomonas oryzae* pv. *oryzae*. Kagle *et al.* (2004); Kumar *et al.* (2009) and Narasimhan *et al.* (1995) recommended the use of Garlic, Ginger as well as *Neem* for control of seed borne bacteria, *Xanthomonas oryzae* pv. *oryzae* and showed reduction in development of disease.

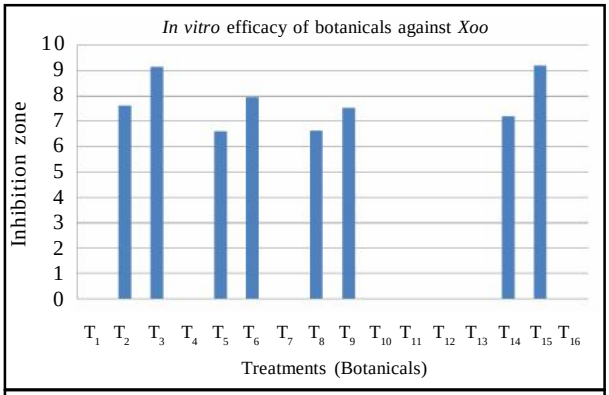

Fig. 4 : Evaluation of botanicals against *Xoo*

Treatment details:
T_1: *Tulsi* leaf extract @ 10%
T_2: *Tulsi* leaf extract @ 20%
T_3: *Tulsi* leaf extract @ 30%
T_4: *Neem* leaf extract @ 10%
T_5: *Neem* leaf extract @ 20%
T_6: *Neem* leaf extract @ 30%
T_7: Ginger rhizome extract @ 10%
T_8: Ginger rhizome extract @ 20%
T_9: Ginger rhizome extract @ 30%
T_{10}: Onion bulb extract @ 10%
T_{11}: Onion bulb extract @ 20%
T_{12}: Onion bulb extract @ 30%
T_{13}: Garlic bulb extract @ 10%
T_{14}: Garlic bulb extract @ 20%
T_{15}: Garlic bulb extract @ 30%
T_{16}: Control

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