ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 11, pp. 21405-21408, November, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

SCREENING OF SOME ANTIBIOTICS AGAINST '*RALSTONIA SOLANACEARUM*' CAUSES BACTERIAL WILT IN BRINJAL (*SOLANUM MELONGENA L.*) PLANTS OBTAINED FROM DIFFERENT REGIONS OF RANCHI DISTRICT

Rupa Verma[1]., Ashok Kumar Choudhary[2]., Abhijit Dutta[3] and Sudarshan Maurya[4]

[1,2]Department of Botany, Ranchi University, Ranchi 834001, Jharkhand, India
[3]Department of Zoology, Ranchi University, Ranchi 834001, Jharkhand, India
[4]ICAR–Research Complex for Eastern Region, Research Centre, Plandu, Ranchi 834010, Jharkhand, India

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0810.1067

ARTICLE INFO

Article History:

Received 06th August, 2017
Received in revised form 14th September, 2017
Accepted 23rd October, 2017
Published online 28th November, 2017

Key Words:

Bacterial wilt, zone of inhibition, disk diffusion technique, *Ralstonia solanacearum*,

ABSTRACT

Ralstonia solnacearum causes bacterial wilt in solanaceous crops. *Due to this soil borne bacteria results* enormous crop losses. The brinjal or eggplant or aubergine (*Solanum melongenaL.)* represents the non-tuberous group of Solanum species. Brinjal is the most common, popularand widely grown vegetable crop of both tropics and sub-tropics of the world. In this study, different antibiotics were screened through disk diffusion technique in laboratory, based on the assumption that it might decrease and diminish the severity of wilt disease at field level also. Brinjal isolates at various fields of Ranchi, Jharkhand have been isolated and their results were tested with different antibiotics by using disk diffusion technique. In vitro activities of antibiotics. (ceftriaxon, gentamycin and ambistryn) against five isolates of brinjal strains of *R.solanacearum* were tested and their effectiveness were analyzed by observing their diameter of zone of Inhibition (ZOI).The best result was observed with the ceftriaxone and ambistryn. with ZOI arround 37 mm at 40ppm in all brinjal isolates. However, gentamycin showed ZOI 27mm to 28 mm at 40ppm. It is expected that these antibiotics formulation and application in field against resistant microorganisms may be a step in the right direction for treating the wilt diseases in brinjal plants.

INTRODUCTION

Bacterial wilt is one of the key hindrances for vegetables production. Management *of Ralstonia solanacearum* has become difficult due to wide host range, vascular in nature. Its is a devastating disease distributed all over the world including tropical, subtropical and temperate regions (Hayward, 1991). Formerly known as *Psedomonas solanacearum*, bacterial pathogen *Ralstonia solnacearum*, which is an important soil borne and devastating bacterial phytopathogens, is aerobic in nature, non-spore former, Gram-negative, motile by a polar flagellar tuft and the causative agent of bacterial wilt of solanaceous crops (Yabuuchi *et al*.,1995). The disease is also known as southern blight and *R. solanaceous* wilt and by other names at the various places of its occurrence (Kelman *et al*., 1954). The pathogen infects roots of susceptible plant, usually through wounds (Pradhanang *et al*.,2005) colonizes the xylem, prevents water movement into upper portion of plant tissues (Kelman,1954) and causes bacterial wilt in a very wide range of host plants (AgriosGN., 2008). As the disease develops, all leaves may wilt quickly and desiccate although they remain green (Champoiseau *et al*., 2009). Brinjal is highly productive and usuallyfinds its place as the poor man's vegetable (Som and Maity, 2002). Brinjal or eggplant (*Solanum melongena L.*) is severely affected by bacterial wilt caused by *R. solanecearum* in Ranchi district of Jharkhand. In brinjal, the disease can bring about total destruction of the crop during rainy season in all brinjal growing areas of Karnataka. In India, the loss in yield due to the disease in brinjal and tomato is as high as 80 and 90 per cent, respectively (Rao 1976, Kishun (1987)

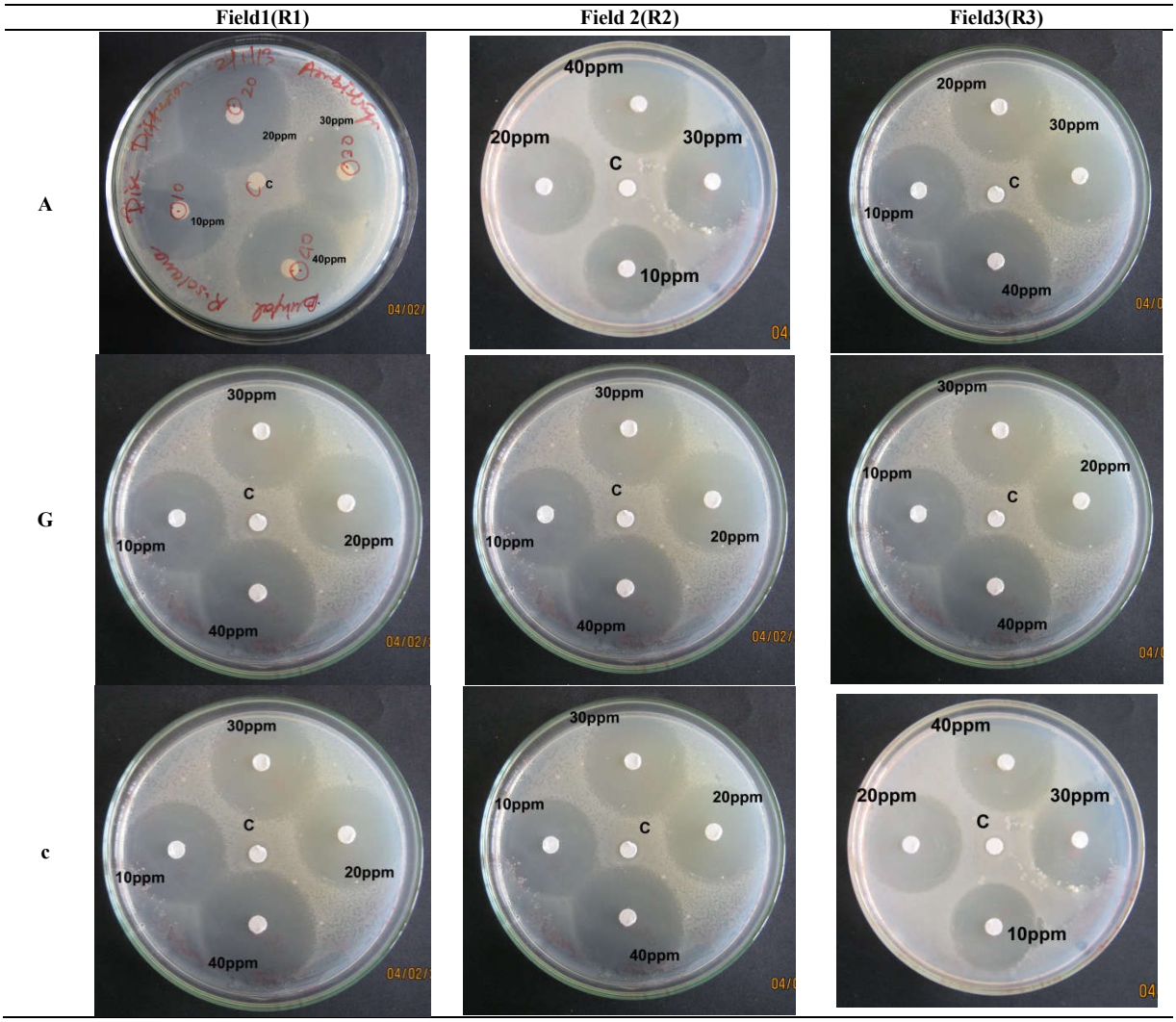
Control of infection has been a major challenge due limited possibility for their chemical control, higher capacity of their survival in diverse environments, their high variability and their existence with an extremely wide host range (Nguyen & Ranamukhaarachchi, 2010The most commonly used chemical treatment has been fumigation of contaminated soil/portion of farm with methyl bromide (Champoiseau *et al*.,2010) which is

Corresponding author:* **Rupa Verma
Department of Botany, Ranchi University, Ranchi 834001, Jharkhand, India

not only very expensive and tedious but applying these on large areas is difficult. Sodium hypochlorite is the another appropriate product which is used at field level for spot treatment of the holes left behind after rogueing of the wilting plants and for general field sanitation but its use is also expensive and tedious). Few antibiotics and peptides have also been used in the past to get rid of bacterial wilts.(Rupa verma *et al*.)

Hence with an aim to develop effective antibacterial agent few antibiotics (Ambistryn, Ceftriaxone, Gentamycin) could be used.antibacterial potential, anti-bacterial activities of antibiotics were assayed using the disc diffusion technique. The bacterial wilt pathogen of brinjal was isolated by serial dilution technique and characterized through morphological and cultural characteristics.

Figure 1 Result of disc diffusion technique using antibiotic-Ambistrynn(S),gentamycin(G) and ceftriaxone(c)against three isolates from field 1,2 and 3)



Despite a number of studies on control strategies of bacterial wilt by means of plant resistance and cropping system but a rigorous and complete protocol to control the disease caused by *R. solanacearum* in various geographical regions is still lacking (Dalal *et al*., 1999). Hence it is imperative to develop various antimicrobial agents for managing and containing *R. solanacearum* other than solvent extract. As amino glycosides and tetracycline interfere with essential steps of protein synthesis and as most of the antibiotics interact with ribosomal RNA (Satoko Yoshizawa *et al.,*1998), the ribosome happens to be the central target of many important antibiotics for inhibiting the protein synthesis of the bacterium and for consequently ceasing its growth and propagation.

The bacterium was Gram negative, rod shaped, noncapsulated and the colonies on TZC medium were white with light pinkish centre and highly fluidal producing copious slime. Growth of bacterium at different temperature and pH levels indicated significant differences and the temperature of 30oC and pH 6 to 7 were *R. solanacearum.* In vitrostudies carried out to find the effectiveness of antibiotics and antagonists in inhibiting the growth of *R. solanacearum* the antibiotics.

METHODS AND MATERIALS

Isolation and identification of R. solanacearum from infected brinjal plants

The pathogen isolation by serial dilution technique yielded *R. solanacearum* colonies which were fludial, dull-white colored with slight pink to red colored centers. The results of morphological studies revealed that the pathogen was Gram-negative rod and non-capsulated. Several workers have confirmed the morphological of *R. solanacearum* as rod shaped and non-capsulated (Smith, 1896; Khan, 1974). The pathogen colonies on TZC medium was highly fluidal with copious slime and appeared white with light pinkcolored centre. Pathogenic and biochemical characteristics viz., positive to nitrate reduction test; negative to starch hydrolysis and gelatin liquification also confirmed the identity of the pathogen as *R. solanacearum* (E. F. Smith, Yabuchi *et al*., 1995). Khan *et al.* (1974) and Shobha (2002) observed similar colony characters in brinjal isolate on TZC medium and further also identified that, the bacterium as *R. solanacearum* based on standard morphological procedures. These results are in accordance with other reports of Kelman, 1954; Buddenhagen et a., 1962; Singh and Hussain (1991).

For a quick field diagnosis, the streaming of milky white masses of bacterial cells (ooze) confirmed the disease is bacterial wilt caused by *R.solanacearum* and to distinguish bacterial wilt from vascular wilts caused by fungal pathogen and nematode. Samples of the diseased plants were collected from each of the surveyed district and were brought to the laboratory for the isolation of different group of isolates of *R. solanacearum*. The symptoms of bacterial wilt were observed and labeled properly.

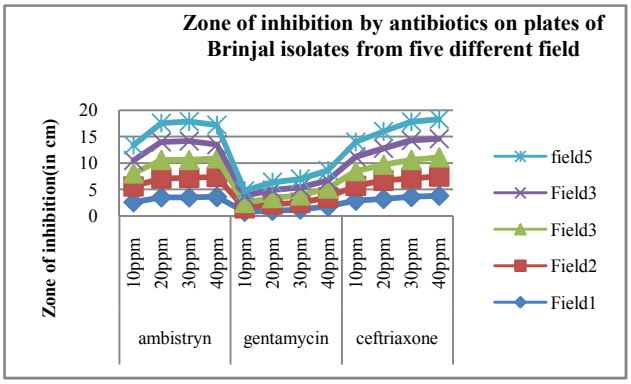


Table 1 Zone of inhibition showed by different antibiotics (ambistryn, gentamycin and ceftryaxone) at various concentrations in brinjal isolates from five different fields (F1,F2,F3,F4 andF5).

solution was prepared for each of the three antibiotics (ceftriaxone, ambistryn and gentamycin). Stock solutions are prepared using the formula (1000/P) X V X C=W, where P+potency of the anitibiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Microbiological screening by disk-diffusion method

Antimicrobial activity of different antibiotics were analysed by the disk diffusion method (Murray *et al*;1995, modified by Olurinola *et al*;1996).Petri plates, test tubes and Nutrient agar (Agar-16gm, nutrient broth-13gm, Distilled water-1000ml) were sterilized at 121^{c} for 15minutes and media was poured on all plates of each isolates and allowed to solidify. These plates were swabbed (sterile cotton swabs) with fresh grown bacterial culture of dilution (10^{-3}cfu/ml). Inoculums were allowed to dry for 5 minutes. Preparation of dried filter paper discs Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven. others for 10, 20, 30 and 40 µg mL^{-1} of antibiotics solution, All disks were placed on the plates as per the marked places. After sealing all plates they were kept for incubation in incubator at 25-29^{0}C.After the incubation period of 24 hours, the diameter of the zone of inhibition (in mm) was measured. The diameter of the zone of inhibition (in mm) of all twelve sets of experiment was measured. Data, thus obtained, was analyzed using the stat graphics software.

RESULTS AND DISCUSSION

In vitro evaluation of antibiotics on the growth of *R. solanacearum*. Three antibiotics were evaluated at different concentrations (Table 1) to test their efficacy to inhibit the growth of *R. solanacearum*, by inhibition zone assay method. Among the antibiotics tested (Table1, Figure1), Ceftriaxone and ambistryn were found to be highly effective with the maximum inhibition range of 36 to 37.00 mm at 40 ppm respectively and this was also on par with gentamycin which recorded inhibition zone of 28mm.(Figure 1.Table1) The analysis of variance with respect to three antibiotics againsed five different isolates is presented in Table 2.

CONCLUSION

Present, there is no effective control measure to manage the bacterial wilt. However, several antibiotics are being used in the field and several other non-antibiotics which are effective against other Gram negative bacteria are being tested in different concentrations against *R. solanacearum.*

Table 2 ANOVA of ZOI At Different Concentrations on different brinjal isolates obtained from five fields(F1,F2,F3,F4and F5)

	ANOVA result									
	Brinjal(F1)		Brinjal(F2)		Brinjal(F3)		Brinjal(F4)		Brinjal(F5)	
	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value	F-ratio	P-value
Gentamycin	42.95	0	45.73	0	19.81	0.002	0.91	0.4277	42.95	0
Ambistryn	139.64	0	32.64	0	32.09	0	47.89	0	139.64	0
Ceftriaxone	112.89	0	106.89	0	45.48	0	5.65	0.0256	112.89	0

Preparation of antibiotic stock solutions

Three antibiotics viz ceftriaxone, ambistryn and gentamycin were selected for screening against the isolations of the bacterium from brinjal (Rupa verma *et al*).500 ppm stock

These antibiotics are effective under in vitro may be also used for agricultural field application Future line of work should be need investigation to be carried out under field conditions to arrive at a more reliable data and performance assessment of antibiotics and bio-agents. Further there is need to identify new

generation of antibiotics (bactericides) and potential bio control agents viz., bacteriophages and other Grampositive and Gram negative bacteria for effecting management of bacterial wilt of brinjal.

References

Hayward, a.c., 1991, Biology and Epidemiology of bacterial wilt caused by Pseudomonas solanacearum. *Ann. Rev. Phytopathol*., 29: 65-87.

Yabuuchi *et al*.1995.Transfer of two Burkholderia and an Alcaligens species to Ralstonia Genus nov; Proposal of Ralstonia picketti (Ralstone, Palleroni and Douderoff (1973)comb nov; Ralstonia solanacearum (Smith1896) comb nov, and Ralstonia eutropha (Davis1969)comb nov, *Microbiology and immunology*.39,897-904.

Kelman A.1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on atetrazolium medium. *Phytopathology*.44, 693-695.

Pradhanang PM *et al*.2005. Application of acibenzolar-S-methyl enhances host resistance in tomato against *Ralstoniasolanacearum*. *Plant disease*, 74, 13-17

Agrios GN. 2008. Plant Pathology. Amsterdam: Elsevier Academic press.647-649.

Champoiseau PG, JonesJB, AllenC.2009. Ralstonia Solanacearum race3 biovar2causes tropical losses and temperateanxieties(online).availableat http;/www.apsnet.org/online/feature/Ralstonia(Accassed 25June 2010). American phytopathological society. Madison,WI

Som, M.G. and Maity, J.K.(2002). Brinjal – Vegetable Crops, 3rd revised edition, edited by Bose, T. K., Kabir, J., Maity, T. K., Parthasarthy, V. A. and Som, M. G., Naya Prokash Publishers, Kolkatta, pp. 265-344

Rao, m.v.b., 1976, Bacterial wilt of tomato and eggplant in India. In: Proc. 1stPlanning Conf. and Workshop on Ecology and Control of bacterial wilt caused by Pseudomonas solanacearum(Eds.) L. Sequerianad A. Kelman, pp.92-94, North Carolina State Univ., Raleigh, pp 94.

Kishun, r., 1987, Studies on bacterial wilt of solanaceous crops. *Anu. Sci. Reptv.,* IIHR, Bangalore, pp65

Nguyen MT, Ranamukhaarachchi SL. 2010. Soil-borne antagonists for biological control of bacterial wilt disease caused by Ralstonia solanacearum in tomato and pepper. *Journal of Plant Pathology*. 92(2): 395-406.

Champoiseau PG *et al*.2010. Ralstonia solanacearum Race 3 biovar2 causing brown rot of potato, Bacterial wilt of tomato and southernwilt of geranium(online).available at http://plantpath.ifas.ufl.edu/rsol/NRI-project/project summary.html(Accessed 25 June (eds.)2010), American phytopathological society. Madison,WI.

Rupa Verma, Ashok Kumar Choudhary, Abhijit Dutta and Sudarshan Maurya 2015. Antimicrobial potential of an animal peptide and some antibiotics against a dreaded soil born phytopathogen *Ralstoniasolanacearum*. *International Journal of Current Research*.Vol. 7, Issue, 10, pp23015-23022.

Dalal NR *et al*.1999. Studies on grading and prepackaging of some bacterial wilt resistant brinjal (*Solanummelogena* L.) Varieties. *J.Soils and Crops*, 9(2), 223-226.

Satoko Yoshizawa1, Dominique Fourmy and Joseph D. Puglisi.2008. Department of Structural Biology, Stanford University School of Medicine, Stanford, CA94305-5400.

Smith, 1896, A new bacterial disease of the tomato, egg plant and Irish potato (Bacillus solanacearum nov. sp.), US *Dept. Agri. Div Veg. Phys. And Path Bull*.,2:1-28.

Khan, *et al* 1974, Studies on Pseudomonas solancearum(E.F. Smith) causing wilt in brinjal, potato and tomato in Mysore state. *Mysore J. Agric. Sci.,* 8: 478-479.

Yabuchi, E., Kosako, Y., Yano, I., Hotta, H. and Nishiuchi, Y.(1995).Transfer of two Burkholderia and Alcaligenesspecies toRalstoniagen nor proposal of Rolstonia picketti (Ralston, Palleroniand Doudoroff, 1973). comb. nov. Ralstonia eutropha(Smith, 1896) comb. nov. and Ralstonia eutropha(Davis, 1969) comb. nov. *Microbiol. & Immunol*.,39: 897-904

Khan *et al*. (1974), Studies on Pseudomonas solancearum (E.F. Smith) causing wilt in brinjal, potato and tomato in Mysore state. *Mysore J. Agric. Sci.,* 8: 478-479.

Shobha (2002), Molecular and biochemical studiesand Pathogenicity treatment in the detection of races/ strains of Ralstonia solancearum causing wilt of solanaceous plants and their prevalence in Karnataka. Ph.D Thesis, UAS, Bangalore. pp-199.

Kelman, 1954 The relationship of pathogenicityof Pseudomonas solanacearum to colony appearance in Tetrazolium medium. *Phytopathol*., 44: 693-695.

Buddenhagen I, Sequeira L, kelmanA.1962. Designation of races in *pseudomonas solanacearum. Phytopathology*. 52, 726.

Singh and Hussain (1991). Singh, B AN Hussain, S.M., 1991, Bacterial wilt of groundnut a new record from India. *Indian Phytopath*.,44: 369-370.

Rupa Verma, Ashok Kumar Choudhary, Abhijit Dutta and Sudarshan Maurya 2015. Antimicrobial potential of an animal peptide and some antibiotics against a dreaded soil born phytopathogen *Ralstoniasolanacearum*. *International Journal of Current Research*.Vol. 7, Issue, 10, pp23015-23022.

Murray PR, Baron EJ, Pfaller MA, Tenover FC, YolkenHR.1995. Manual of clinical microbiology, sixth Edition. ASM Press, Wshington. DC, 15-18.

Olurinola *et al*.1996. A laboratory manual of pharmaceutical icrobiology. Idu, Abuja, Nigeria, 1996, 69-105.

****