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RESEARCH ARTICLE

ANTIMICROBIAL POTENTIAL OF AN ANIMAL PEPTIDE ANDSOME ANTIBIOTICS AGAINST A DREADED SOIL BORN PHYTOPATHOGEN *RALSTONIASOLANACEARUM*

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ABSTRACT

Ralstonia solanacearum, a known dreaded soil borne phytopathogenic bacteria, has been causing enormous crop losses in tropical, subtropical and temperate regions across the world including the eastern plateau and hill regions of India, where soil is mostly acidic. In this study, honey bee derived peptide apidaecin and some antibiotics observed to be exhibiting antimicrobial efficacy against the bacteria *R.solanacearum* isolated from infected tomato, brinjal and capsicum plants. Effectiveness of apideacin was compared with three potential antibiotics likeceftriaxone, ambistryn and gentamicin towards containing the bacterial phytopathogen. Ceftriaxone showed the strongest antibacterial efficacy with zone of inhibition 17.94mm, 17mm and 15.88 mm at 40 µgmL^{-1}respectively in three isolates of *R.solanacearum* vizbrinjal, capsicum and tomato. Ambistryn, compared to gentamicin, was next best in exhibiting antibacterial efficacy with zone of inhibition ranging from 10.2 mm to14.8 mm. Apidaecin exhibited antibacterial effectiveness onbrinjal and capsicum isolates at the concentration of 20 to 40 µgmL^{-1}with zone of inhibition varying from4.1 mm to 8.4 mm at 40 µgmL^{-1}. Apideacin, compared to gentamicin, was effective in capsicum and nearly potent to gentamicin in tomato at higher concentrations. Apideacin, compared to the antibiotics, was moderately effective on selected plant isolates.

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INTRODUCTION

Bacterial wilt disease caused by the pathogen *R.Solanacearum* affects numerous important crops and other ornamental plants worldwide (Elphinstone, 2005, French and Sequeira, 1970). This soil borne pathogen has a large number of hosts, which spans to almost 200 solanaceous plant species and isspread throughout the world. *R.solanacearum,* formerly known as the *Pseudomonas solanacerum* (Smith, 1896), is a causative agent of bacterial wilt of solanaceous crops (Yabuuchi *et al*., 1995) which chiefly attacks potato, tomato, geranium,eggplant, capsicum and some solanaceous weeds such as *Solanumnigram* and *solanumdulcamara* (Martin and French, 1985 and French, 1994). Few antibiotics and peptides have been used in the past to get rid of bacterial wilts .Till now, it is not well understood whether antibiotics can indeed cure the disease caused by *R. solanacearum* and whether animal derived peptides can be a step in this direction. Hence it is imperative to develop various antimicrobial agents for managing and containing *R. solanacearum*.

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The objective of the present study is to understand whether apidaecin, a honey bee derived peptide, can be effectively used towards controlling the wilt disease caused by *R. solanacearum* in brinjal, tomato and capsicum and its comparison with the few potential antibiotics (vizceftriaxone, ambistryn and gentamicin) for the same purpose. In this paper, we address the following question: is apidaecin an effective antimicrobial agent for containing and managing wilt diseases caused by *Ralstoniasolanacearum* as compared to other potential antibiotics? *R. solanacearum* is generally classified into phylotypes and biovars according to their molecular and biochemical characteristics respectively (Fegan and Prior, 2005 and Hayward, 1991). The bacterium is typically soil and water borne, while water ways being a major dissemination route in the environment (Elphinstone, 2005). It enters the plant through injuries of roots, colonizes the vascular system and produces severe disease conditions (Hayward, 1991 and Pradhanang *et al*., 2005). Colonization by the bacterium with in thexylemprevents water movement in to the upper portion of planttissues (Kelman, 1954). All leaves in the plant, with the growth of the disease, may wilt quickly and desiccate even though they may mostly remain green (Champoiseau *et al*., 2009). Expression of virulence factors in *R. solanacearum* is generally controlled by a complex regulatory network that responds to a variety of environmental conditions, the presence of host cells, and bacterial density (Schell, 2000). Aqueous extracts of some plants having medicinal properties have been utilized in the past to control the disease caused by *R. solanacearum* but they have not been found to have significant inhibitory effects on the growth of *R. solanacearum* (Sangoyomi *et al*., 2011). The most commonly used chemical treatment, on the other hand, is to fumigate the contaminated soil or a portion of farm with methyl bromide but it is also not fully efficient for the management of the disease at the field level. There have also been a number of studies on control strategies such as plant resistance and cropping system buta rigorous and complete protocol to control of the disease caused by *R. solanacearum* in various geographical regions is still lacking (Dalal *et al*., 1999).

Keeping these in view, we performed a number of experiments to ascertain the potential antimicrobial agents that can be used for containing and managing the *R. solanacearum*. In this context, the important role of peptides and small proteins isolated from a number of organisms ranging from insects to humans including plants defense, have been established. Use of these peptides and proteins for managing the bacterial wilts, in agriculture, were proposed soon after their discovery. Anti-microbial peptides (AMP) represent a broad class of peptides, which possess significant antimicrobial activity against microorganisms. Conclusions drawn from previous reviews on the identification, characterization and activities of AMP of interest in medicine may be extrapolated to plant pathology (Brogden, 2005, Hancock and Sahl, 2006, Jenssen *et al*., 2006, Zasloff, 2002, Peschel and Sahl, 2006).

In the present study, three antibiotics ambistryn, ceftriaxone and gentamicin and animal derived peptides apidaecin, were used against the isolates of brinjal, tomato and capsicum infected by *R. solanacearum*. Apidaecin isolated from honey bee *Apismellifera,* is a proline rich antibacterial peptide which exhibits antibacterial activity against Gram-negative bacteria through the well– knownbiostatic process (Casteels *et al*., 1993and Li *et al*., 2006). The cationic peptides, such as apidaecin, cecropin and others, are probably the oldest of the host defense systems of different unicellular and pluricellular organisms, ranging from bacteria to fish, plants, insects, birds and mammals includinghumans (Oren and Shai, 1998). Though the mechanism of action of various AMPs has been widely debated, there is a general consensus that these peptides selectively disrupt the cell membranes, whereas the amphipathic structural arrangement of the peptides is believed to play an important role in the actual mechanism. Furthermore, the phospholipids head group charge on cell membranes and the peptide charge distribution has an important role in the peptide–membrane interactions (Oren and Shai, 1998; Cudicand Otvos, 2002).

In this study, antibacterial activity of antibiotics has been compared with the animal derived peptide by using well–known agar well diffusion technique. Antibiotic components of amino glycosides and tetracycline generally interfere with essential steps of protein synthesis. As most of the antibiotics interact with ribosomal RNA, the ribosome naturally becomes the central target of many important antibiotics and inhibits the protein synthesis of the bacterium and consequently ceases its growth and propagation. In a nutshell, in can be summarized, that synthetic peptide apidaecin may be an effective alternative to chemical routes in order to control and manage wilts in solanaceous plants caused by *R. solanacearum* bacteria.

MATERIALS AND METHODS

In what follows, we first discuss the isolation and purification of different strains of *R. solanacearum* from affected brinjal, tomato and capsicum plants. Next, is the process of media sterilization, preparation of bacterial suspension and stock solution of antibiotics and peptide. Thereafter, the process of microbiological screening has been presented by using the agar well-diffusion technique.

Isolationand Purification of *R. solanacearum*

Extensive field survey was conducted in order to determine/ascertain the prevalence of bacterial wilt in solanaceous crops such as brinjal, tomato and capsicum, being grown in different areas of the study. Typical symptoms of bacterial wilted plants were observed and it was found that infected plant leaves initially drooped and turned yellow and the area between leaf veins died and became brown even though plants themselves remained upright. Vascular flow test or ooze test through stem streaming methods is a diagnostic test of wilting caused by this bacterium is used to avoid confusion with symptoms induced by other pathogens (Allen *et al*., 2001). Stem segments from collar region of wilted plants were rinsed with sterilized distilled water containing 1% clorox. Athinthreadofooze was observed when an in fected stem cut sacross and cut end sheld together for a few seconds in the test tubes contain ingsterilized distilled water. Sticky oozeformedtan-white to brown beads. Allt hree strains were isolated from corresponding wilted host plants by streaking loop full of turbid bacterial suspension onto sterile 2,3,5-triphnyltetrazolium chloride media (TTC) and incubated at 30^0c for 2 days. Reddish fluidal colonies were again streaked on TTC plates and the processes were repeated till purified bacterial cultures were obtained with the homogeneity in the colony morphology. For further purification, these three isolations were streaked in King's B media, as shown in Figures 1a–1c and were maintained in the slant of King's B medium.

Media sterilization and preparation of bacterial suspension

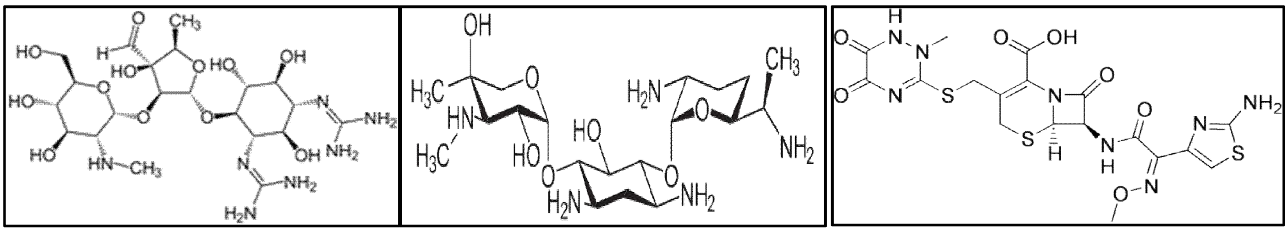
Four set of experiments were performed for each of the three isolates (Brinjal, Tomato and Capsicum). Three antibiotics and a honeybee-derived synthetic peptide were taken. 60 Petri plates, 12 test tubes and 2000 ml nutrient agar (agar-16gm, nutrient broth-13gm and distilled water-1000 ml) were prepared and sterilized (121ºc for 15 min) for each set of experiments. All the three isolates were serially diluted up to third dilution. Five replications of three isolates were kept in all of the four sets of experiments.

a. Brinjalb. Tomatoc. Capsicum

Figure 1. Isolations of different isolates of *R. solanacearum* from the host of brinjal, tomato and capsicumin King's B media

Preparation of antibiotics and peptide stock solution

Three antibiotics and antimicrobial peptide were selected against three isolations of the bacterium *Ralstoniasolanacearum*. Ceftriaxone, which is a sterile, semi-synthetic, broad spectrum cephalosporin antibioticis known inhibitor of cell wall synthesis. Ambistryn, which contains streptomycin is an aminoglycoside antibiotic (procured from Himedia Pharmaceuticals, India). The primary mechanism of action of streptomycin is to bind irreversibly the bacterial ribosomes and thereby inhibiting protein synthesis. Gentamicin is a broad spectrum antibiotic and irreversibly binds 30S-sub unit and inhibits protein synthesis. Apidaecinforms the largest group of prolin rich antimicrobials and induces humoral immunity in honey bee lymph upon bacterial infection. Apidaecinwhich was used in the present investigation is a synthetic peptide in white powdered dry form (in appearance) and procured from Phoenix Pharma Inc. USA and used in the same form. It was stored at 0 to 25^0 C in the refrigerator and used as required for the preparation of stock solution. 500 ppm stock solutions using three antibiotics (ceftriaxone, ambistryn and gentamicin) and synthetic peptide apidaecin were prepared. The chemical structures of these antibiotics and peptide are shown in Figure 2.



(a) Structure of streptomycin (ambistryn) (b) Structure of ceftriaxone (c) Structure of Gentamicin

Gly-Asn-Asn-Arg-Pro-Val-Tyr-Ile-Pro-Gln-Pro-Arg-Pro-Pro -His-Pro-Arg-Leu

(d) Structure of Apidaecin

Figure 2. Structure of antibiotics and a peptide

Microbiological screening by agar well-diffusion method

Antimicrobial activity of different antibiotics and honey bee derived peptide were evaluated by the agar well diffusion Method (Murray *et al*., 1999), as modified by Olurinola and co-workers to determine the antimicrobial activity (Olurinola *et al*., 1996). Plates were sterilized and nutrient agar media was poured on all the sixty plates 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. Four sets of experiments were performed for each of the isolates of *R. solanacearum*. Five replications were maintained for each set of experiments. By using 5mm cork- borer, five wells were made in each of the 60 plates and labeled, one for control at the center and others for 10,20, 30 and 40 µg mL^{-1} of antibiotic and peptide solution. Sterile distilled water was used as control. With the help of micro pipette 10, 20,30and 40µg $mL^{-1}$of antibiotic and peptide solutions were added in each of the 60 plates (having three isolations) and allowed to incubate for 24 hrs at 29-30^0c.The diameter of the zone of inhibition (mm) was measured. Data, thus obtained, was analyzed using the stat graphics software.

RESULTS

The present investigation established that chosen three antibiotics ceftriaxone, gentamicin, ambistryn andanti-microbial peptide apidaec in showed anti-bacterial properties, to a varying extent, against three isolates of *Ralstoniasolanacearum.* All three antibiotics were strong in exhibiting anti-microbial property in case of isolates of brinjal as ceftriaxone showed maximum zone of inhibition (17.94±1.845mm) followed by gentamic in (16.05±0.381mm) and ambistryn (14.198±0.431mm) (Fig. 4). For controlling the bacterial wilts in brinjal, either of three antibiotics was found fully effective and potent at any concentration ranging from 20 to 40 µg mL^{-1}in comparison to apidaecin. Effectiveness of apidaecin, which showed zone of inhibition of 8.4±0.890mmat 40 µg mL^{-1}, was that of similar to gentamicin at 10 µg mL^{-1}concentrations (Fig.3). Hence apidaecincan be used as an antimicrobial agent in brinjal at higher concentration to get the desired result.

In the case of capsicum isolate of *R.solanacearum*, ceftriaxone was highly effective among three antibiotics and apidaecin as it showed zone of inhibition of 17±1.697mm at 40 µg mL^{-1}(Fig. 5). While ambistryn (streptomycin) was highly effective at 20 µg mL^{-1}, apidaecin was found to be more effective in capsicum in comparison to gentamicin in all other concentration (that is, 10, 30 and 40 µg mL^{-1}). Gentamicin showed the least effect in capsicum among all antibiotics and apidaecin.

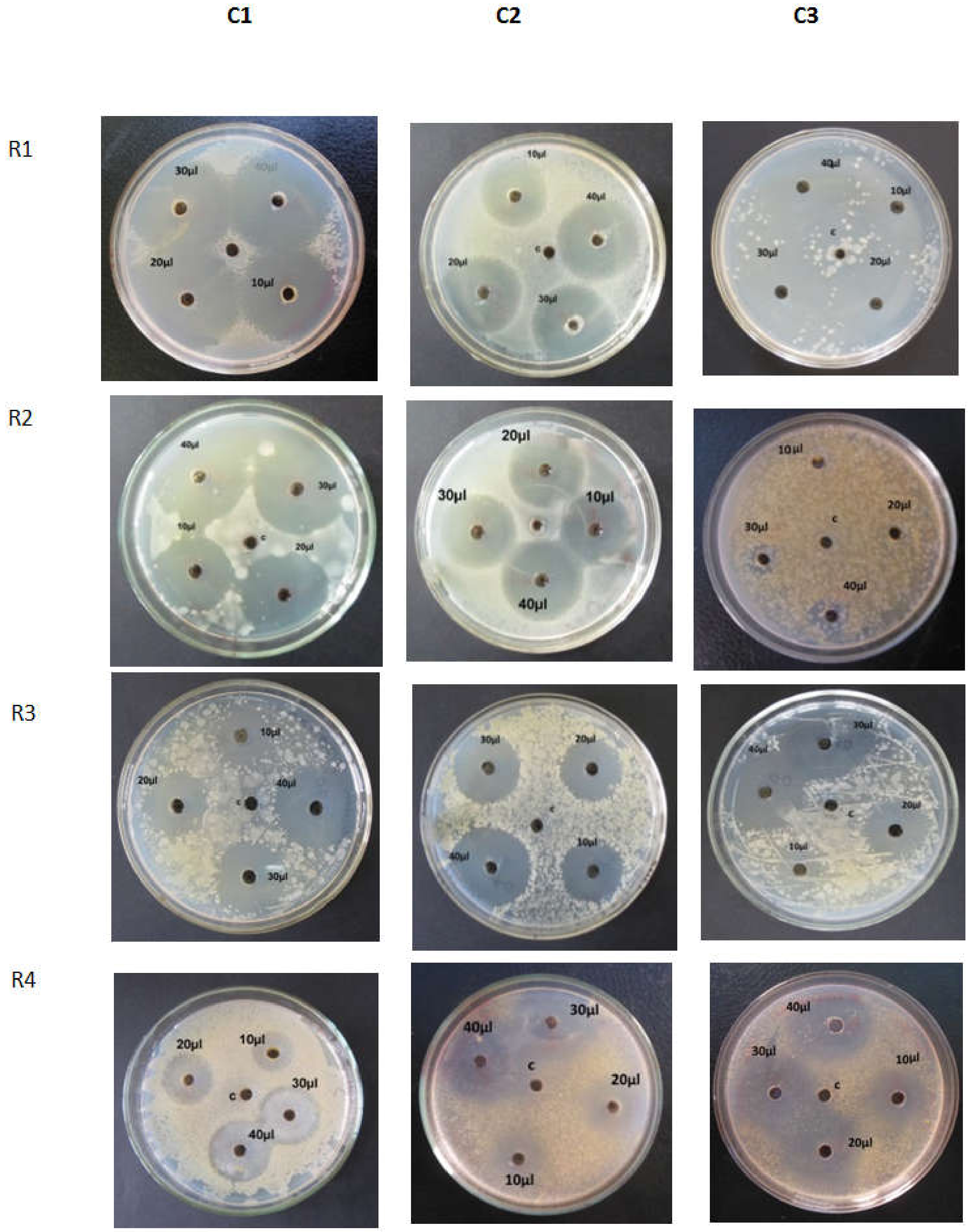


Figure 3. Antimicrobial efficacy, represented by way of zone of inhibition at 10,20,30and40 µgmL^{-1} or ppm of streptomycin (R1), gentamicin (R2) ceftriaxone (R3) and apidaecin (R4) against brinjal (C1), tomato (C 2) and capsicum (C3) isolates of *R.solanacearum*

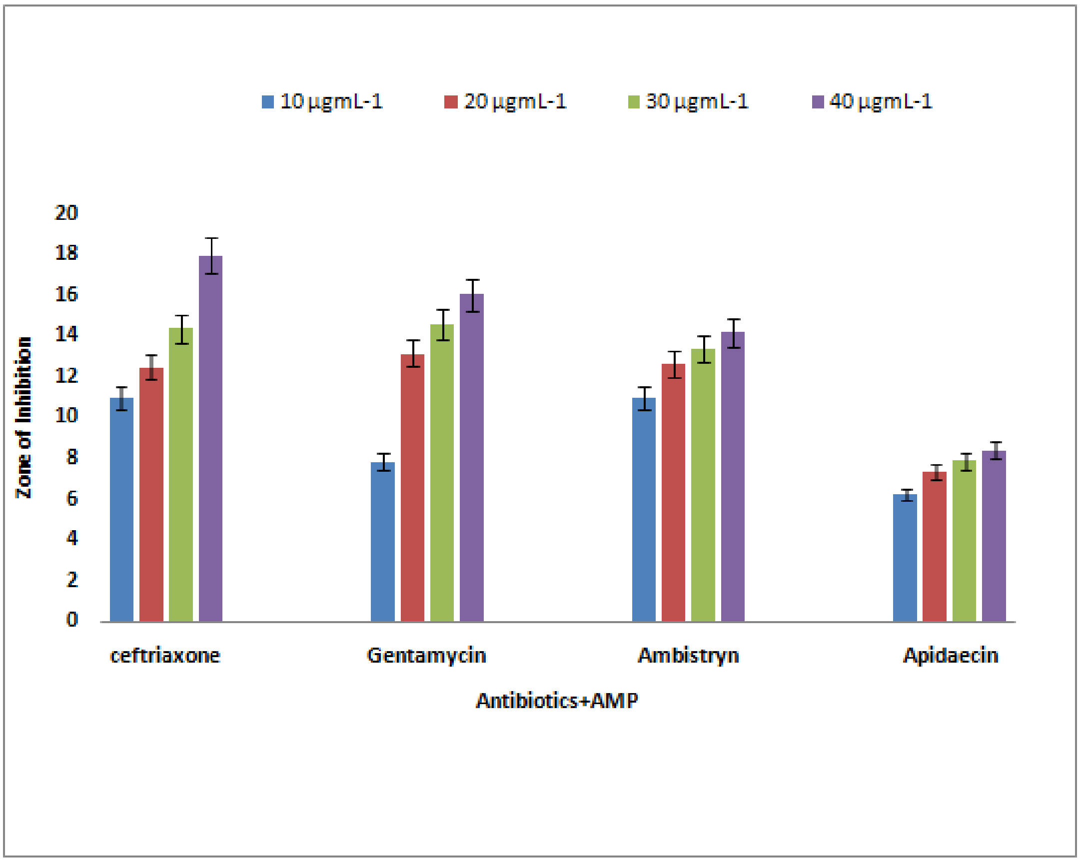


Figure 4. Antimicrobial efficacy (in term of zone of inhibition) of selected antibiotics and an apidaecin at different concentrations against *R. solanacearum*isolated from brinjal

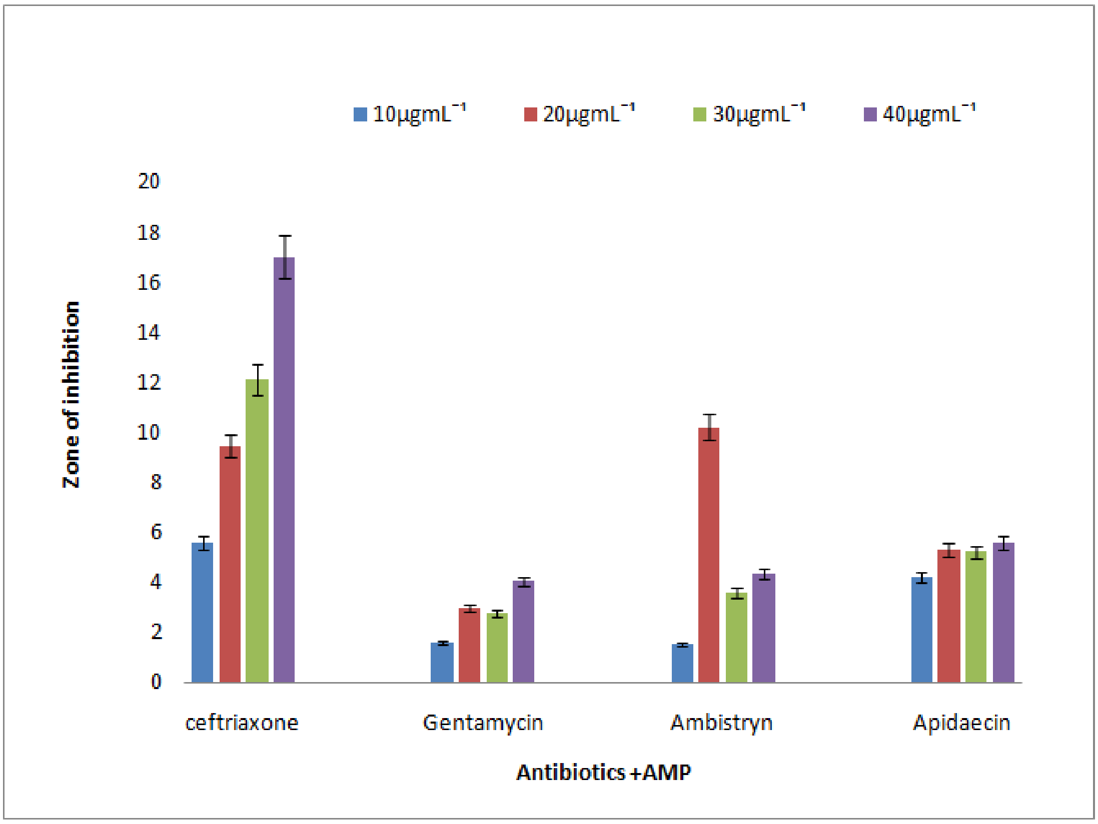


Figure 5. Graphical representation of the standard deviations of various diameters (in mm) of zone of inhibition of capsicum isolates by three different antibiotics and a peptide at 4 different concentrations (in µgmL^{-1})

In the case of tomato isolates, ceftriaxone and ambistryn were found to exhibit strong effectiveness as they showed zone of inhibition of 15.88±0.639mm and14.82±0.253mm respectively at 40µg mL^{-1} and also at other lower concentrations. Gentamicin, on the other hand, showed maximum zone of inhibition (11.7±0282mm) at 20µg mL^{-1}same as ceftriaxone and ambistryn at equivalent concentrations and hence observed to be more effective at this concentration (Fig. 6). The effectiveness of apidaecin was observed as similar to that of gentamicinat 40µg mL^{-1}concentration and was less effective in comparison to other antibiotics at other concentrations (10, 20 and 30 µg mL^{-1}).

Apidaecin has shown moderate effect against these three isolates as compared to antibiotics. It showed 8.4±0.890mm and 5.56±0.161mm zone of inhibition in brinjal and capsicum at 40 µg mL^{-1}respectively and was found least effective in tomato (Fig. 5 to 7).Hence, apidaecin, at the concentrations ranging from 20 to 40 µg mL^{-1} concentrations is an appropriate alternative in the case of capsicum and brinjal.

3.1. Statistical analysis

Various statistics, for each of the three columns of data with different concentrations of antibiotics and peptide, has been displayed in Figures 5–7.

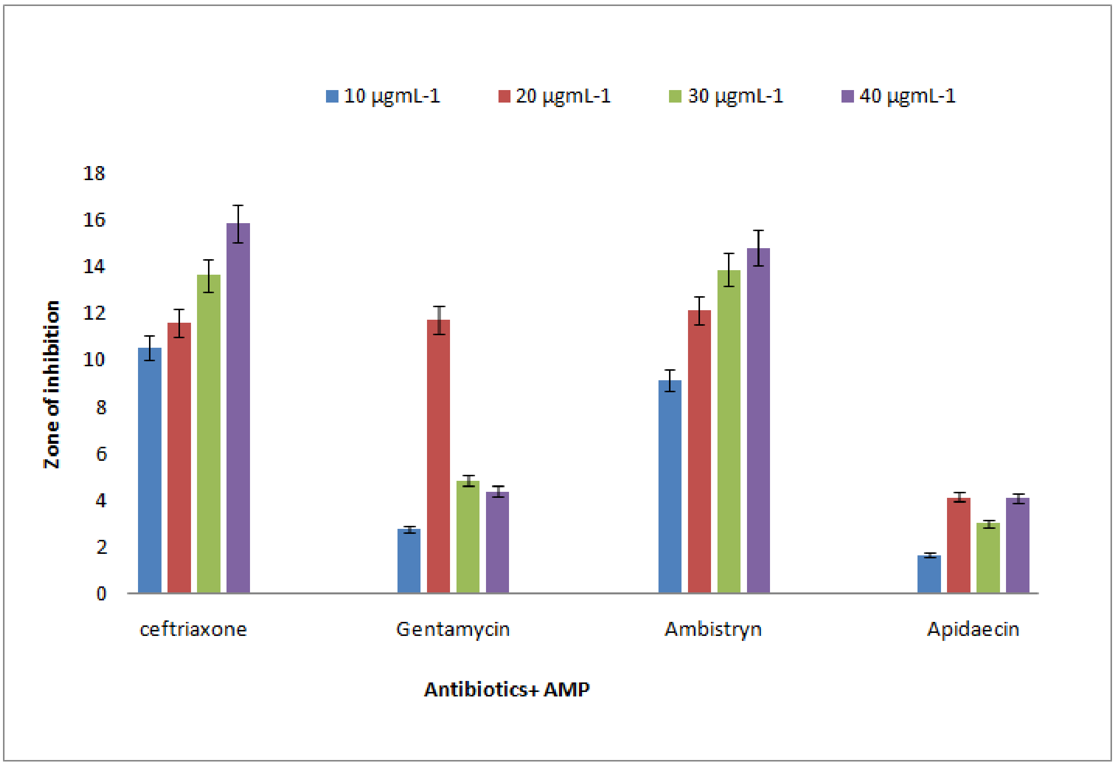


Figure 6. Graphical representation of the standard deviations of various diameters (in mm) of zone of inhibition of tomato isolates by three different antibiotics and a peptide at 4 different concentrations

Table 1. ANOVA of zone of inhibition of different drugs and antimicrobial peptide

	Brinjal		Capsicum		Tomato	
	10ppm-40ppm		10ppm-40ppm		10ppm-40ppm	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
Ceftriaxone	14.1	0.0001	42.95	0	33.99	0
Gentamicin	185.94	0	17.95	0	167.19	0
Ambistryn	33.09	0	139.4	0	81.12	0
Apidaecin	4.69	0.0156	12.66	0.0002	3.78	0.0319

The present study has thrown the light on the fact thatantibiotic ceftriaxone was found to be fully effective in all three isolates of *R.solanacearum* vizbrinjal, tomato and capsicum, as it exhibited strong antibacterial efficacy. Ambistryn worked beston the isolates of brinjal and tomato but was moderately effective in Capsicum. Gentamicin, on the other hand, worked wonders in the case of brinjal only. Thus, antibiotics have emerged as the main basis for controlling the bacterial infection in brinjal as compared to apidaecin (Fig. 3).

Analysis of Variance was performed for testing of significant differences amongst the column mean. The ANOVA table, as displayed in Table 1, decomposes the variance of the data into two components: (a) between-group component and (b) within-group component.

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the four variables at the 95.0% confidence level.

DISCUSSION

Efficient alternatives, to control the disease bacterial wilt caused by *Ralstonias* olanacearum, have been difficult to implement due to a number of factors such as (a) the survival of the pathogen in the soil even in the absence of the host, (b) lack of resistant hosts and (c) the presence of latent infections. Through the present study, we have described the antibacterial activity of three general antibiotics and chemically synthesized peptide antibiotic, apidaecina gainst the bacterial isolates. Ceftriaxone, which is a cephalosporin antibiotic, was found to exhibit strong efficacy against all three isolates (brinjal, tomato and capsicum) at 40 µg mL^{-1} concentration, followed byambistryn, gentamicin and apidaecin. Exceptapidaecin, all the drugs studied here showed better results even at lower concentrations; that is, at 10, 20 and 30 µg mL^{-1}.An interesting and promising approach therefore relies on antibacterial peptides for the simple reason that the bacteria do not develop resistance to these antimicrobial peptide families. Apidaecin, being an antimicrobial peptide may be used in the place of antibiotics, albeit at higher concentrations, in some situations. Use of modified form of apidaecin may be a step forward for improving its antibacterial efficacy in order to enhance its activity even at lower concentrations. As other chemical means for containing/managing of the *R. solanacearum* is not completely known at present, the use of antibiotics may be an effective ways against this dreaded disease. Apidaecinmay act better against the different isolates of *R.solanacearum* above 40 µg mL^{-1} of concentration in vitro.

Conclusion

As antibacterial efficacy of three discussed antibiotics and chemically synthesized peptide against the bacterial isolates is established now, desired result can be achieved by using them at varying concentrations. During the treatment process and by gradually adjusting the concentration of the peptide on different isolates, pathogen may be controlled *in vitro,* which opens new era of the applications at field level. Future lies at identifying, redesigning and/or modifying the sequence of existing apidaecin and finally developing novel antimicrobial agents against the wilt disease for effectively using them in agriculture for disease management. Transfer and expression of isolated antimicrobial peptide genes in plants may result in the generation of transgenic bacterial resistant crop varieties towards the control of the disease and for greater yields of such solanaceous crops at a lower cost. Use of combination of antibiotics is another area for avoiding antibiotic resistance, as field level implications of chemical means or antibiotics not fully known.

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REFERENCES

Allen, C., Kelman, A., French, E. R., 2001. Brown rot of potatoes. *Compendium of potato disease, seconded*. W.R. Stevenson, R.Loria, G.D. France, and D.P. Weingarten, *Eds. APS Press, St. Paul, MN.*, pp. 11-13.

Brogden, K. A. 2005. Antimicrobial peptides: Pore formers or metabolic inhibitors in bacteria? *Nat. Rev. Microbiol.*, 3, 238–250.

Casteels, P., Ampe, C., Jacobs, F., Tempst, P. 1993. Functional and chemical characterization of hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee apismellifera. *J. Biol. Chem.,* 268(10), 7044 - 7054

Champoiseau, P.G., Jones, J. B., Allen, C., 2009. *Ralstoniasolanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. *Plant Health Progress doi*:10.1094/PHP-2009-0313-01-RV.

Cudic, M., Otvos Jr, L. 2002. Intracellular targets of antibacterial peptides. *Curr.Drug Targets*, 3, 101– 106.

Dalal, N. R. *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.

Elphinstone, J.G. 2005. The current bacterial wilt situation: a global review in bacterial wilt disease and the *Ralstoniasolanacearum* species complex (Eds. Allen C., Prior P, Hayward A. C.), *American Phytopathological Society*, St. Paul, MN, USA, pp. 9–28.

Fegan, M. and Prior, P. 2005. How complex is the´ *Ralstoniasolanacearum* species complex? In bacterial wilt disease and the *Ralstoniasolanacearum* species complex., pp. 449-461 (edited by Allen, C., Prior, P., Hayward A.C.). APS Press, St. Paul, MN.Gen. Nov. *Microbiology and Immunology,* 39 (11), 897–904.

French, E. R. and Sequeira, L. 1970.Strains of *Pseudomonasolanacearum* from Central and South America: A comparative study. *Phytopathology,* 60, 506–512.

French, E. R. 1994. Strategies for integrated control of bacterial wilt of potatoes (in Bacterial wilt: The disease and its causative agents, *Pseudomonas solanacearum*, A.C. Hayward and G.L. Hartman, eds), *CAB International,* UK, 98-113.

Hancock, REW, Sahl, H. G., 2006. Antimicrobial and host-defence peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol*., 24, 1551–1557.

Hayward, A. C. 1991.Biology and epidemiology of bacterial wilt caused by *Psedomonassolanacearum.Ann. Rev. Phytopathol*., 29, 65-87.

Jenssen,H., Hamill P., Hancock, REW. 2006. Peptide antimicrobial agents. *Clinical Microbiol. Rev*., 19, 491–511

Kelman, A.1954.The relationship of pathogenicity in *Pseudomonassolanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*, 44, 693-695

Li, W. F., Ma, G.X., Zhou, X. 2006. *Peptides,* 27, 23-50.

Martin, C., French, E.R., 1985. Bacterial wilt of potatoes caused by *Pseudomonas solanacearum, Technical Information Bulletin No. 13*, International Potato Center, Lima, Peru.

Murray, P. R. *et al*. 1999. *Manual of clinical microbiology*. 7th ed (ASM Press, Washington D.C.).

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

Oren, Z. and Shai, Y. 1998. Mode of action of linear amphipathic α helicalantimicrobial peptides. *Biopolymers,* 47, 451– 463.

Peschel, A. and Sahl, H. 2006. The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat. Rev. Microbiol*., 4,529–536.

Pradhanang, P. M. *et al*. 2005. Applicationofacibenzolar-S-methylenhances hostresistance in to mato against *Ralstoniasolanacearum*.*Plantdisease*, 74, 13-17.

Sangoyomi, T. E., Oweseni, A. A., Adebayo, O. S., Omilani, O. A. 2011.Evaluation of some botanicals against bacterial wilt of tomatoes.*Int. Res. J. Microbiol*., 2(9), 365-369.

Schell. M. A. 2000. Control of virulence and pathogenicity genes of *Ralstoniasolanacearum* by an elaborate sensory network. *Annu. Rev. Phytopathol*., 38, 263–292.

SmithE. F. 1896. A bacterial disease of the tomato, eggplant, and Irish potato (Bacillus solanacearumnov. sp.). Bulletin, *Division of Vegetable Physiology and Pathology*, USDA, 12,1–28.

Yabuuchi, E. *et al*. 1995. Transfer of two Burkholderia and an Alcaligenes species to RalstoniaZasloff, M. 2002. Antimicrobial peptides of multicellular organisms. *Nature*, 415,389–395.

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