

MANAGEMENT OF BACTERIAL WILT (*Ralstonia solanacearum*) OF POTATO: FOCUS ON NATURAL BIOACTIVE COMPOUNDS

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Abstract

The bacterial wilt disease caused by *Ralstonia solanacearum* is an extremely destructive soil borne bacterial pathogen to potato. It appeared as rapid and fatal wilting symptoms in the host. The pathogen entered through different wounds and easily disseminated via infected biological material, soil, contaminated irrigation water, surface water, farm equipment etc. and could survive for many years in association with alternate hosts. It is a widely distributed and very much diversified soil borne pathogen having an unusually broad host range with long-term survivable ability. Direct yield losses caused by the pathogen varied from 30 to 90% depending on different factors such as cultivar, weather factors, soil type, cropping pattern and strain etc. Bacterial wilt continued to be an economically serious problem for field-grown potatoes in many tropical, subtropical and warmer areas of the world including Bangladesh. But the effectiveness of conventional management is limited because of some special biological features of the bacteria. Mostly protective methods and chemical control remain ineffective, antibiotics show hardly any effect, and efficacious biocontrol method has yet to be developed against the organism. However, during the recent decades, some natural bioactive compounds, viz. propolis, honey, turmeric, magnesium chloride, cow dung, aromatic rice extract, iodine, sodium bicarbonate etc. have got attention for their effectiveness in inhibiting a range of serious bacterial pathogens from both Gram positive and Gram negative types. As no conventional method has been found effective alone, such compounds could be tested for their effectiveness against the very successful soil borne bacteria to overcome the traditional management limitations.

Key words: Management, *Ralstonia solanacearum*, potato, natural bioactive compounds.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is cultivated and recognized as a popular vegetable throughout the tropical and subtropical regions (Hayward 1991a). Potato is best known for its carbohydrate content (approximately 26 grams in a medium potato) and it is a high energy food that contains about 80 kcal per 100 grams of fresh product. The potato has vital role to human nutrition (Horton 1987). It produces more carbohydrates than either rice or wheat. It has great economic significance. Its production provides jobs and food security to some 800 million people globally (Hoffler and Ochieng 2008). Bangladesh is the 7th producer country in the world by producing 86.03 lakh tons of potato. However, the yield of potato is comparatively lower in the country to the major potato growing countries like- Ireland and India. Lower soil fertility, inadequate supply of certified seeds, use of low yielding varieties, different pests and diseases etc. are the reasons behind the lower yield. Among those, soil borne diseases are considered to cause a yield loss of as much as 10–20% annually (USDA 2003).

Ralstonia solanacearum (Smith, 1896) is the most destructive soil-borne pathogen (Yuliar *et al.* 2015) that affects potatoes in temperate, subtropical and tropical regions by causing bacterial wilt or brown rot disease (Champoiseau *et al.* 2009). The Gram negative bacterium normally invades plant roots from the soil through wounds or natural openings, colonizes the intercellular space of the root cortex and vascular parenchyma, and eventually enters the xylem vessel and spreads into the stem and leaves (Yuliar *et al.* 2015). Infected potato plants die rapidly within 3-4 days and disease severity mostly increases when it is found associated with root nematodes (Sitaramaiah and Sinha 1984). The tuber carries the pathogen in vascular tissues, on tuber surface and within lenticels as tolerant or latent carrier

(Ghosh and Mandal 2009). Yield losses due to the disease varied from 33 to 90% in the potato growing areas of the world (Elphinstone 2005). Direct yield losses caused by *R. solanacearum* depending on the host, cultivar, climate, soil type, cropping pattern and strain. The total value of Egyptian potato exports fell from a peak of US\$ 102.12 million in 1995 to US\$ 7.7 million in 2000 mainly due to brown rot quarantine, imposed by the European Union (EU) (Kabeil *et al.* 2008). In India, this disease causes 50% crop loss in a regular manner (Mukherjee and Dasgupta 1989) and up to 75% losses are reported in some areas of Karnataka (Gadewar *et al.* 1991). In Bangladesh more than 30% of potato crops affected by *R. solanacearum*, with over 14% reduction in yield (Elphinstone 2005).

Geographic distributions of the pathogen are highly influenced by the factors like availability and abundance of the host(s), and suitability of the climatic conditions etc. Its world-wide distribution and destructive nature over 450 plant species resulted it to be the most important bacterial plant pathogen (Kelman 1998, Prior *et al.* 1998). The bacterial wilt pathogen is very diversified, widely distributed and has an extensively wide host range (over 200 species) with major host crops like potato, tomato, *Musa* spp. etc. and some minors like groundnuts (*Arachis hypogaea*), brinjal (*Solanum melongena*) and ginger (*Zingiber officinalis*) (Denny 2006, Hayward 1991a). Moreover, the bacterium is known to enter in VBNC state (viable but not culturable) under unfavorable conditions (van Elsas *et al.* 2001). Such biological phenomena of the pathogen help it to build up the inoculum potential which lead it to induce a destructive economic impact (Kelman 1998). *R. solanacearum* is a very successful plant pathogen. Due to the speciality in biological features of it, several difficulties are created in effective management through traditional practices which include: i) controlling the pathogen through preventive options is not applicable to infested location; ii) cultural options show limited success because the pathogen is able to survive in a very wide host range along with asymptomatic weed hosts and in soil for a long period of time ((Mbaka *et al.* 2013, Saddler 2005); iii) the complexities due to pathogen strain(s), host and environmental interactions make the resistant breeding extremely difficult (Tung *et al.* 1990); iv) using antibiotics against the pathogen is a challenge. Because the bacteria localize inside the xylem, and antibiotics (*viz.* streptomycin, ampicillin, tetracycline and penicillin) could show hardly any effect; in fact, streptomycin increases the incidence in Egypt (Farag *et al.* 1982); v) soil fumigants show either slight or no effect except chloropicrin among other fumigants like methyl bromide, DD MENCS (a mixture of methyl isothiocyanate, dichloropropane and dichloropropene), and metham (Enfinger *et al.* 1979); but it was used as tear gas and “vomiting gas” during ‘World War I’ and scientists warned about chronic exposure of chloropicrin which might result in “very high” cancer risks (Froines 2010); vi) biological control has been investigated with some positive reports with *Bacillus amyloliquefaciens*, *Ralstonia pickettii* and *Pseudomonas mallei* but efficacious biocontrol agents with easier application method and survivability of the agent(s) remain as a major barrier for large scale application in the field (Yuliar *et al.* 2015).

During the recent decades, many natural bioactive compounds have been extensively tested and a good number of reports have documented the antimicrobial effects of such compounds as effective inhibitors of dangerous strains of phytopathogenic bacteria (Leksomboon *et al.* 2000). A wide range of pharmacological attributes of curcumin from turmeric has been well documented for antimicrobial and protective properties (Nagabhushan and Bhide 1992). Cow dung and urine have been used as insecticides and reported that they contained antibiotic agents (Waziri and Suleiman, 2013). Oyarzua *et al.* (2014) showed that the magnesium salts in the microbiological experiments are associated with positive effects. It focuses on the usefulness of magnesium (in form of $MgCl_2$) as a stress enhancer against *Escherichia coli*. The reduction of wilt has been noted by Chellemi *et al.* (1992) with natural and organic amendments. Two traditional aromatic rice genotypes, *viz.* Kalijira and Chinigura, effectively inhibit the Gram negative type *Agrobacterium tumefaciens* (Mannan *et al.* 2014). Iodine (mixed with a transporter known as iodofore) can inhibit aerobic Gram positive and Gram negative

bacteria (Estrela *et al.* 2006). Sodium bicarbonate has shown antibacterial properties against different types of bacterial and fungal pathogens (Kelly and Kristin 2005, Malik and Goyal 2006, Arslan *et al.* 2009). However, a review work has been performed to investigate the success of such antibacterial properties of different natural bioactive compounds against severe starin(s) of bacterial pathogen as alternative approach of management. Therefore, the present study emphasizes the review of effectiveness and inhibition capabilities of such bioactive compounds as antibacterial agents which may be considered during the management of *R. solanacearum* in potato crops.

MATERIAL AND METHODS

Detection

The bacteria *R. solanacearum* is considered to be a “species complex” due to significant variation within the group (Fegan and Prior 2005). It identified from either symptomatic or asymptomatic plants and from water or soil samples by means of several microbiological and molecular methods (Weller *et al.* 2000). Screening tests can facilitate early detection of *R. solanacearum* in plants or contaminated soil and water samples, but they cannot be used to identify the race or biovar. These screening tests include bacterial streaming, plating on a semi-selective medium, such as TZC medium etc., polymerase chain reaction (PCR) with specific primers, and pathogenicity tests using susceptible hosts, such as tomato seedlings (Elphinstone *et al.* 1996, Weller *et al.*, 2000). Commercially available immunostrips can be used for the rapid detection of *R. solanacearum* in the field or lab. Isolation from symptomatic material can easily be performed using Kelman’s tetrazolium chloride (TZC) medium. In case of secondary infections, the isolation of the pathogen on selective media was necessary. Biovar test is a biochemical assay which can be identified from a panel of disaccharides and sugar alcohols (Hayward (1994b). It was based on their ability to utilize three hexose alcohols (mannitol, sorbitol and dulcitol) and to produce acids from the three disaccharides, lactose, maltose and cellobiose (Table 1).

Table 1. Identification of biovars of *R. solanacearum* based on the utilization of certain carbohydrates (Hayward 1994).

Carbohydrate		Biovar 1	Biovar 2	Biovar 3	Biovar 4	Biovar 5
Hexose alcohol	Mannitol	-	-	+	+	+
	Sorbitol	-	-	+	+	-
	Dulcitol	-	-	+	+	-
Disaccharides	Lactose	-	+	+	-	+
	Maltose	-	+	+	-	+
	Cellobiose	-	+	+	-	+

‘+’ indicates utilization of hexose alcohol and acid production

This test requires specialized media and it may take several days to several weeks. The strains of *R. solanacearum* can be sub-classified into phylotypes and then into sequevars using PCR and gene sequence analysis (Champoiseau *et al.* 2009). Many standard methods for the detection (of latent infection), identification and preparation of media for *R. solanacearum*, used in official EU testing schemes. Detection of latent infection is performed by an immuno-fluorescence test and/or selective plating on SMSA medium eventually combined with optional PCR assays, ELISA or fluorescent *in situ* hybridization tests which can be performed for added sensitivity. A combination of at least two different complementary tests is required to identify the species and biovar unambiguously. Unequivocal identification of R3bv2 must rely on at least two distinct methods of screening and biovar test (Champoiseau *et al.* 2009). SMSA medium as modified by Elphinstone *et al.* (1996) has been used successfully in Europe for latent infection (Elphinstone *et al.* 1998). A presumptive test in the field can be the water streaming test as described under disease symptoms or a serological agglutination test using a field kit in the form of a lateral flow device (Danks and Barker 2000).

Colony character

On solid agar media, individual bacterial colonies are usually visible after 36 to 48 hours growth at 28°C. the colonies of the normal or virulent type are white or cream-colored, irregularly shaped, highly fluidal, and opaque. A tetrazolium chloride (TZC) medium (Kelman 1954) can differentiate the virulent and non-virulent colony types by appearing as white with pink centers (Fig. 1) of virulent colonies and dry, uniform round and dark red of non-virulent/ mutant colonies. However, it has the ability of changing state from virulent to avirulent, termed as “phenotypic conversion” (PC) by reduced production of extracellular proteins and polysaccharides due to some environmental stress (Shekhawat and Perombelon 1991).

RESULTS AND DISCUSSION

Symptom of Bacterial Wilt Disease (Ralstonia solanacearum) in Potato

A plant showing wilting can be suspected to have *R. solanacearum* infection. The symptom starts with slight wilting (Fig. 1a and b.) of the leaves at the ends of the branches during the day which recovers at night; eventually, plants fail to recover which is soon followed by total wilting. Milky or cloudy threads like streaming signifies the presence of *R. solanacearum* of bacterial wilt disease (Fig. 1a) while the cut end of infected stem kept in a glass of water. Streaming is clearly observed when the bacterial population in the vascular bundles is high, due to the blocking of the vessels specifically in the xylem (Kelman 1953); low populations of the pathogen may not be visible to the naked eye. Infected symptomatic tuber shows browning of vascular bundle region (Fig. 1c.).



Fig. 1. Bacterial wilt infected potato stem: a) early symptom, b) cloudy thread like bacterial streaming; brown rot infected potato tubers: c) vascular browning symptom, (d) colonies of *R. solanacearum* on Kelman’s TZC medium.

Diversity of the Pathogen

Ralstonia solanacearum is a heterogeneous species complex comprising of four broad genetic groups. The existence of these four groups was confirmed by Guidot *et al.* (2007). Cook *et al.* (1989), and Cook and Sequeira (1994) divided the species *R. solanacearum* into 46 multilocus genotypes (MLGs) based upon restriction fragment length polymorphism (RFLP) analysis. Several attempts have been made to find a suitable classification system (Table 2). It was historically subdivided into five races based loosely on host range, five biovars, and a phylogenetically meaningful system based on DNA sequence analysis (Fegan and Prior 2005). They classified *R. solanacearum* into four major genetic groups called phylotypes (Table 2). Within each of the races or biovars there are numerous subtypes that can be associated with certain geographical regions (He 1983). Therefore, it affects crops of economic importance in almost all the tropical, subtropical and warmer temperate regions of the world. Biovar 2 presumed to have originated in South America and has a wide spread distribution in many countries of Southern Europe, the Mediterranean area, Argentina, Chile and Uruguay (Hayward 1991a). Biovars 1 and 2 are predominant in the Americas, Biovar 3 predominates in Australia, and Biovars 2, 3 and 4 occurs in India, Indonesia, Papua New Guinea, Sri Lanka and China (together with Biovar 5).

Only Philippines have all of biovars 1-4, and biovar 3 predominates in the lowland regions in Asia and Bangladesh (Ahmed *et al.* 2013).

Table 2. Diversity equivalences among phlotypes, biovars and races of *R. solanacearum* (Fegan and Prior 2005).

Group	Diversity equivalences											
	I			II			III		IV			
Phylotype												
Biovars	3	4	5	2T	1	2	2T	1	2T	1	2	R B
Races	1	4	5	1	2	3						

R= *Ralstonia syzygii*, B= *Pseudomonas celebense*; Origin: I=Asia, II=America, III=Africa, IV=Indonesia.

Economic Impact of R. solanacearum

R. solanacearum is the most serious pathogen of solanaceous plants in tropics and subtrops. It can cause serious losses even in temperate regions (Elphinstone 2005, Ajanga 1993, Coelho and Nutter 2005). The disease affects about 1.7 million hectares of potatoes in 80 countries (Champoiseau *et al.* 2009, Floyd 2008). It is responsible for an estimated \$1 billion in losses each year (Elphinstone 2005). In Bangladesh, the loss caused by *R. solanacearum* was recorded in Munshigonj (22.65%) as compared to Nilphamari (19.98%) and Jamalpur area (9.07%) (Ahmed *et al.* 2013).

Biological strategy of long-term survivability of R. solanacearum

The bacterial wilt pathogen has an unusually broad host range because of being a soil-borne pathogen and host resistance is limited (Denny 2006, Hayward 1991, Saddler 2005). *R. solanacearum* possesses some especial biological features (Table 3). These are i) the pathogen possesses, extensively wide (over 200 species) and worldwide distributed major host crops like groundnuts (*Arachis hypogaea*), *Capsicum annum*, cotton (*Gossypium hirsutum*), rubber (*Hevea brasiliensis*), cassava (*Manihot esculenta*), castor beans (*Ricinus communis*), brinjal (*Solanum melongena*) and ginger (*Zingiber officinalis*) with many weeds as asymptomatic alternate hosts to induce a destructive economic impact (Kelman 1998); ii) disease severity mostly increases if *R. solanacearum* is found in association with root nematodes.

Table 3. Biological features behind management constraints.

Biological feature	Factor	Condition
Mode of entry	i) Wound created by nematodes or other	Especially the root-knot nematode (<i>Meloidogyne</i> spp.) (Johnson and Schaal 1952, Kelman, 1953); the bacterium could enter the host through points of secondary root emergence (Kelman 1953, Buddenhagen and Kelman 1964, Kelman and Sequeira 1965)
	ii) Unwounded root infection is also possible	When relatively large numbers of bacteria are available (Kelman and Sequeira 1965).
Sources of Inoculum and Dispersal	i) Infected plant materials (seeds, plant, tuber etc.); ii) Infected debris, alternate hosts & weeds; iii) Infested soil, irrigation water, equipments etc.; iv) Plant parts (eg. tubers) with no visible symptom.	Bacterial masses can adhere to soil particles enhancing its survival, tubers can carry the bacteria in three manners, namely externally on tuber surfaces, in lenticels and in the vascular tissues (Shekhawat <i>et al.</i> 1992). The plant parts (eg. tubers) with no visible symptom that ensures the uninterrupted dispersal of the pathogen. (Shekhawat <i>et al.</i> 1992). Infected host debris is an important short-term shelter for <i>R. solanacearum</i> in soil (Graham <i>et al.</i> 1979) allowing survival between growing seasons and serves as a transmission agent. Weeds serving as alternate hosts which are about more than 450 species as symptomless carriers (Hayward 1991a, Prior <i>et al.</i> 1998).
Favorable Environment	Soil and storage temperature	Although cannot survive at >40°C, becomes severe between 35~24°C, no visible symptom observed at <16°C (Ciampi and Sequeira 1980, Seneviratne 1988), and could survive long in lower temperature even at 4°C, which makes it capable of dispersal and survival in the soil/plant materials for long period

	Soil moisture and irrigation	(Granada and Sequeira 1983b). Bacteria could be disseminated with the irrigation water in the environment and could make the disease level increased and affected synergically while in the optimum temperature (Shekhawat <i>et al.</i> 1992).
Survival	“PC” phenomena	As a soil-borne pathogen, it can survive in various types of soils worldwide. It has the ability of changing state [from virulent to avirulent termed as “phenotypic conversion” (PC) by reduced production of extracellular proteins & polysaccharides]. It makes them to remain withstand & viable for a very long periods like 2 to 10 years (Denny <i>et al.</i> 1994, Poussier <i>et al.</i> 2003, Nesmith and Jenkins 1985).
Latency	Inoculum level decrease and certain environmental stresses.	Exists when inoculum level decrease and certain environmental stresses occurs. Such as exposure to low temperatures, dryness etc. makes them symptomless & undetectable [termed as- viable but non culturable (VBNC) state] (Devi <i>et al.</i> 1982, Shekhawat and Perombelon 1991). “VBNC” observed as- “not all tubers get infected in the symptomatic plants rather diseased tubers occasionally found on the asymptomatic plants” (Eddins 1941).

Disease severity mostly increases by changing the physiology of the plants and increases the susceptibility if *R. solanacearum* is found in association with root nematodes (Chen 1984). It cannot be detected in seeds with a water content of less than 10% (Zhang *et al.*, 1993). Therefore, seed-borne latent infection may result in severe out-breaks of bacterial wilt and/or brown rot of potato.

Limitation of traditional management practices

The survival strategies of the pathogen successfully created difficulties and limited the management success through traditional management practices *viz.* i) preventive measures, ii) cultural measures, iii) chemical measures, and iv) biological measures (Table 4).

Table 4a. Success and limitations of different management practices against bacterial wilt pathogen.

	Types of Management	Success	Limitation
Preventive measures	Quarantine, phytosanitary practices, disease free certified seeds, disinfected equipment, controlled use of flood irrigation and avoiding overhead irrigation etc.	It is successful where the pathogen is not present.	Not applicable in infested location.
	i. Use of resistant cultivars	Success was limited (Mbaka <i>et al.</i> 2013). It is reported to be the most effective and practical method to control bacterial wilt (Black <i>et al.</i> 2003, Grimault <i>et al.</i> 1994).	Because- i. the pathogen is able to survive in the soil over a long time; ii. it can exist in a very wide range of weeds and volunteer crops (Fajinmi and Fajinmi 2010). Unfortunately the complexities of host-pathogen-environment interaction make breeding for resistance extremely difficult (Tung <i>et al.</i> 1990). Because- i. <i>R. solanacearum</i> is a “heterogeneous species complex” with a wide host range (Kelman and Person 1961); ii. high variability in its biochemical properties (Cuppels <i>et al.</i> 1978, Hayward 1964), serological reactions (Schaad <i>et al.</i> 1978), membrane proteins (Dristig and Dianese 1990) and phase susceptibility (Okabe and Goto 1963) confirming the challenges in breeding for resistance.

Chemical measures	ii. Application of the organic amendments	Reported to reduce the disease (Chellemi <i>et al.</i> 1997).	No limitation has been observed.
	iii. Disinfected equipment, controlled use of flood irrigation and avoiding overhead irrigation use of crop rotation etc.	Limited success has been recorded (Mbaka <i>et al.</i> 2013)	Because- i. the pathogen is able to survive in the soil over a long time in asymptomatic weed hosts within a very wide host range (Saddler 2005).
		It is difficult to control bacterial with chemicals (Grimault <i>et al.</i> 1994).	Because- i. the bacteria localize inside the xylem and its ability to survival in the soil; ii. there are no known eradication bactericides available for chemical control of the bacterial wilt disease (Hartman and Elphinstone 1994).
	i. Fumigants (Enfinger <i>et al.</i> 1979)	Chloropicrin was the only formulation that provided significant control throughout the season among others (methyl bromide, DD•MENCs (a mixture of methyl isothiocyanate, dichloropropane and dichloropropene), and metham) (Enfinger <i>et al.</i> 1979).	Fumigant pesticides pose serious health risks and degrade soil health. One hundred years ago, chloropicrin was used during World War I as tear gas and “vomiting gas.” Scientists have concluded that chronic exposure to chloropicrin results in “very high” cancer risks (Froines 2010) and those are prohibited in some countries due to the risks posed to pesticide operators and aquatic organisms, birds, and bees.
	ii. Some antibiotics (Penicillin, Ampicillin, Tetracycline and Streptomycin) has been	Not successful when tested in both greenhouse and field conditions (Hartman and Elphinstone 1994).	It was has shown too little suppression to be applied against the pathogen (Hartman and Elphinstone 1994).
	iii. Application of stable bleaching powder (Saddler 2005).	Reduced bacterial populations and disease severity on a small scale (Saddler 2005).	Sodium hypochlorite (4-6%) may produce skin and ocular irritation or gastric burns, inactivation by organic matter, and release of toxic chlorine gas when mixed with ammonia or acidic condition (Kennedy and Bek. 1998).

Cultural options has been limited success because the pathogen is able to survive in the soil over a long time with asymptomatic weed hosts and a very wide host range ((Mbaka *et al.* 2013, Saddler 2005). The complexities of host-pathogen-environment interaction make breeding for resistance extremely difficult (Tung *et al.* 1990). There are no known eradication bactericides available for chemical control of the bacterial wilt disease (Hartman and Elphinstone 1994). Antibiotics, streptomycin, ampicillin, tetracycline and penicillin showed hardly any effect (Farag *et al.* 1982).

Biological control has been investigated and gained popularity in recent years. But efficacious biocontrol agents have yet to be developed. Positive results were achieved with the antagonistic bacteria *Bacillus amyloliquefaciens*, *Ralstonia pickettii*, *Pseudomonas mallei* (Yuliar *et al.* 2015). However, different problems (formulation of user friendly application and poor performance due to inconsistent colonization in the field) have been reported challenging to use on a commercial scale (Akira *et al.* 2009).

Environment friendly management options to be considered against bacterial wilt disease of potato

During the recent decades, many natural bioactive compounds have been extensively tested and a good number of reports outlined the antimicrobial effects of those compounds for dangerous pathogenic

strains and documented as effective inhibitors of phytopathogenic bacteria (Leksomboon *et al.* 2000). Turmeric (*Curcuma longa* L.) is a medicinal plant. It is used as a food additive (spice), preservative and colouring agent in Asian countries, including China and South East Asia (Khattak *et al.* 2005). It is effective against different virulent strains of *R. solanacearum* in India (Narasimha *et al.* 2015). The chemical composition, medicinal and antibacterial activity of propolis from bees has been reported by Velikova *et al.* (2000 a,b). Honey contains antioxidants and flavonoids that might function as antibacterial agents. Propolis, a flavonoid-rich product of honey comb, exhibited antibacterial properties (Bosio *et al.* 2000) against both Gram negative and positive type bacteria. Honey inhibited the growth of dangerous bacteria from both Gram negative and positive type such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* etc. (Zumla and Lulat 1989).

Table 4b. Success of biological management practices against the bacterial wilt during 2005-2014 (Yuliar *et al.* 2015).

Microorganisms	**Inoculation method and application rate	Mechanisms	Success in yield increase
<i>Bacillus amyloliquefaciens</i> SQR-7 and SQR-101 and <i>B. methylotrophicus</i> SQR-29	Pouring, 6.8×10^{10} cfu plant ⁻¹ (SQR-7), 7.5×10^{10} cfu plant ⁻¹ (SQR-101), 8.2×10^{10} cfu plant ⁻¹ (SQR-7)	Production of indole acetic acid and siderophores	25–38%
<i>Ralstonia pickettii</i> QL-A6	Stem injection, 10 μ L of 10^7 CFU mL ⁻¹	Competition	Not Applicable
<i>Pseudomonas monteilii</i> (A) + <i>Glomus fasciculatum</i> (B)	Stem cuttings were dipped in A (9.1×10^8 mL ⁻¹), B (53 infective propagules) was added to each cutting, and A was then poured again	Increased plant nutrient uptake (N, P, K) and reduced the pathogen population	54%
<i>Brevibacillus brevis</i> L-25 + <i>Streptomyces roche</i> L-9 + organic fertilizer	Mixed with soil at a density of 7.3×10^7 (L-25) and 5.0×10^5 (L-9) cfu g ⁻¹ of soil	Decreased root colonization by the pathogen	87–100%
<i>Bacillus amyloliquefaciens</i> + bio-organic fertilizer (BIO23) <i>B. subtilis</i> + bio-organic fertilizer (BIO36)	Mixed with soil at a density of 5.5×10^6 (BIO23) and 7.0×10^6 (BIO36) cfu g ⁻¹ of soil	Plant growth promotion	64–65%

** Limitations remained with such inoculation method and rate of application on a large scale infected field.

Shrivastava and Pal (2014) had been evaluated cow dung extract for antibacterial properties against *E. coli*, *Pseudomonas* and *Staphylococcus aureus*. Mannan *et al.* 2014 reported the of fluids of unpolished rice grain of two traditional aromatic rice genotypes viz. Kalijira and Chinigura were effectively inhibited the Gram negative type *Agrobacterium tumefaciens*. Jarvis *et al.* 2001 found that cattle manure could be treated with sodium carbonate to eliminate *E. coli* and Corral *et al.* 2006 found sodium bicarbonate (SB) to inhibit the growth of different bacterial pathogen in agar media. Such alternative antibacterial compounds should be tested for effectiveness against *R. solanacearum* which could be considered as alternative options of effective, biologically safe and environment friendly measures of different plant pathogen management.

Propolis

Propolis (honey bees) consists of about 50 constituents, primarily resins (50%), waxes (30%), essential oils (10%), pollen (5%) and others (5%). Propolis is flavonoid-rich product of honey comb, exhibits antibacterial properties (Bosio *et al.* 2000).

The higher concentration of propolis the greater the inhibition zones against Gram negative type *Escherichia coli* and Gram positive type *Staphylococcus aureus* by disc diffusion method (Fig. 2). It is very powerful natural antibiotic (Miorin *et al.* 2003). The antibacterial activity of propolis may be related to the presence of flavonoids (Bosio *et al.* 2000). The extent of effectiveness of honey or propolis

and their chemical composition varies depending on the bee species and geographic region (Miorin *et al.* 2003).

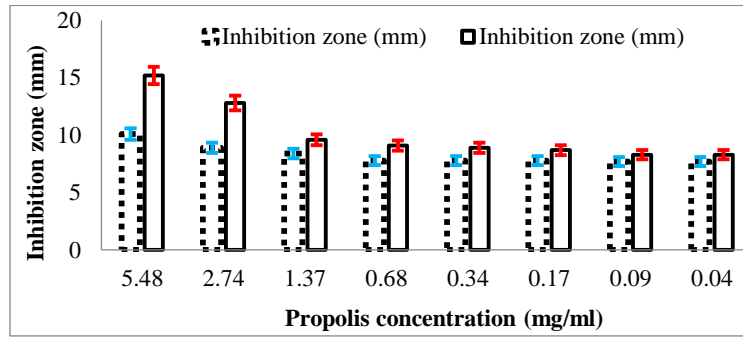


Fig. 2. Inhibition zone of propolis against Gram -ve and Gram +ve by disc diffusion method (Rahman *et al.* 2010).

Honey

The antibacterial activity of honey varies very significantly, and depends on the floral source of the honey. The important antibacterial factor in most honeys is hydrogen peroxide, produced in the honey by the action of glucose oxidase which is added to the honey by the bee, but some antibacterial activity is due to substances which are derived from the flowers (Allen *et al.* 1991).

Balan *et al.* (2016) found effectiveness of manuka honey against a range of serious bacterial pathogens both from Gram positive and Gram negative type and the higher concentration of honey (from 2.5 to 20%) the greater the inhibition was observed. Manuka honey (MH) is produced from the flowers of two New Zealand plants (Fig. 3). The occurrence of high amounts of methylglyoxal (MGO) in New Zealand MH was well documented. MGO was identified as a bioactive compound which is responsible for the antibacterial activity of MH samples (Mavric *et al.* 2008).

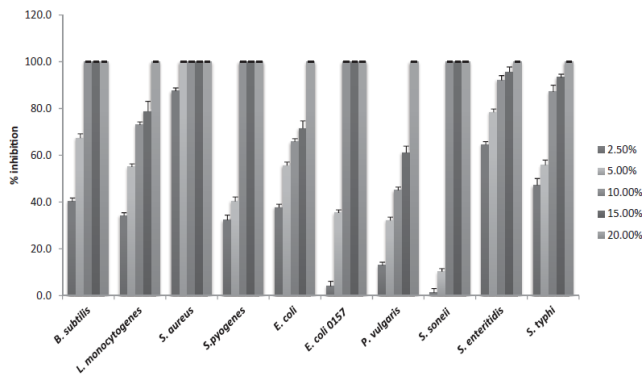


Fig. 3. Antibacterial activity of honey against different bacterial pathogens (after: Balan *et al.* 2016).

Turmeric

Turmeric is used as a food additive (spice), preservative and coloring agent in Asian countries, including China and South East Asia (Khattak *et al.* 2005). It is the source of curcumin (diferuloyl methane), a yellow lipid-soluble polyphenolic dietary compound, produced as the rhizome of turmeric. It is widely used in numerous medicinal benefits. Pathological researchers have shown great interest in curcumin (Gupta *et al.* 2012). A wide range of pharmaceutical attributes of curcumin, such as antioxidative, antimicrobial and wound-healing-protective properties, have been well documented (Aggarwal and Harikumar 2009, Frenkel *et al.* 2013).

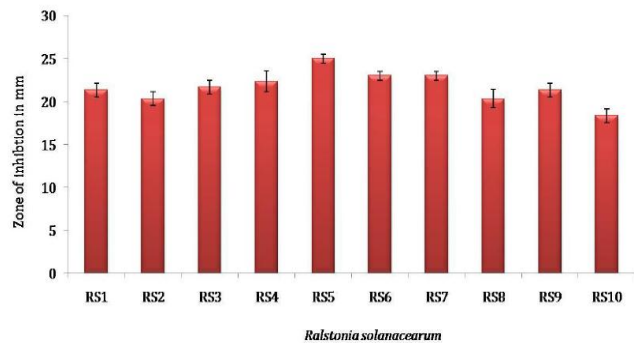


Fig. 4. Inhibition zones of turmeric extracts against ten virulent strains of *R. solanacearum* (after: Narasimha *et al.* 2015).

10% (w/v) turmeric powder extract (Fig. 4) showed an inhibition zone ranged from about 15 to 25 mm against several virulent strains of *R. solanacearum* (Narasimha *et al.* 2015). Curcumin was tested for their antimicrobial activities against both Gram positive (*Bacillus subtilis* NCTC 6276, *Staphylococcus aureus* NCTC 8530) and Gram negative bacteria (*Escherichia coli* NCTC 10863, *Escherichia coli* O157:H7CDC strain G5244, *Salmonella typhimurium* CDC AMO 3398). Curcumin @100 mg/mL against Gram positive type and @250 mg/mL against Gram negative type was required to inhibit 100% growth of those strains (Balan *et al.* 2016) (Fig. 5). However, fat soluble extracts of turmeric and its curcumin component exhibit strong antioxidant activity.

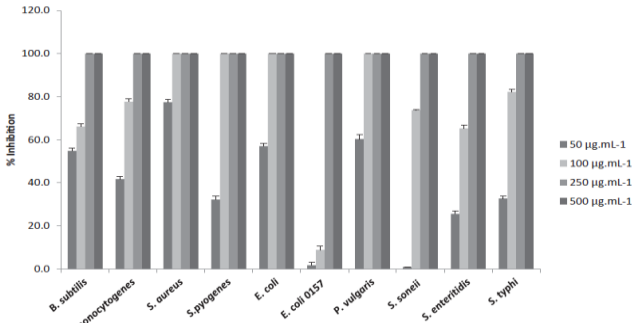


Fig. 5. Inhibition zone of turmeric at different concentrations against different bacterial pathogen (after: Balan *et al.* 2016).

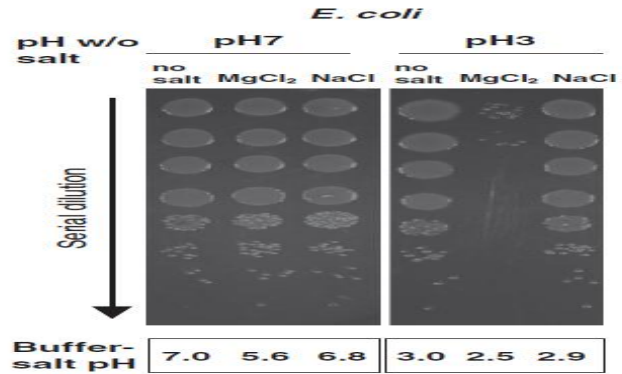


Fig. 6. Antibacterial effect of MgCl₂ salt against *E. coli* (Gram -ve) (after: Oyarzúa *et al.* 2014).

Magnesium chloride

Magnesium is an element essential for life and is found ubiquitously in all organisms. It has the properties in a microbiological context with healing and antiseptic characters. The different cations play important roles as enzymatic co-factors, as signaling molecules, and in stabilizing cellular components. Oyarzúa *et al.* (2014) showed that the magnesium salts in the microbiological experiments typically associated with positive effects (Fig. 6). It focused on the usefulness of magnesium (in form of MgCl₂) as a stress enhancer against *Escherichia coli* (K-12).

Table 5. Antimicrobial activity of cow dung extract against different bacterial pathogens (Waziri and Suleiman 2013).

Extract (mg/mL)	Test microorganisms			
	<i>B. subtilis</i> IZ	<i>C. bacteria</i> IZ	<i>E. coli</i> IZ	<i>S. aureus</i> IZ
0.20	--	--	--	--
0.35	--	7.0±0.32	--	10±0.36
0.70	--	8.0±0.22	--	11±1.54
2.50	0.5±0.01	8.5±0.43	--	13±2.25

Values expressed as mean±SD, n=6, IZ= Inhibition zone in mm

Cow dung

Different parts of plants & oils, animal’s wastes have been used by traditional healers in treatment of different categories of diseases with great success. Cow dung and urine has been used as insecticides and has been reported to contain antibiotic agents (Singh 2001, Khanuja 2002). The use of cow dung in the bioremediation of toxicants in the environment (Randhawa and Kullar 2011). It is referred to as chow chips or cow pit in British English while a deposit of the dung is referred to as cow pie in American English (Perry and Morton 2009). Furthermore, a large number of microorganisms which have biological activities and presently in use as antibiotics and antitumor agents have been reported (Carte 1996).

A study against *E. coli*, *Pseudomonas* and *Staphylococcus aureus* showed that cow dung was highly effective against both of those Gram positive and Gram negative microbes (Shrivastava and Pal 2014). The study revealed that cow dung extract possess antimicrobial properties, which can be used to fight against certain pathogenic diseases and other ailments. Table 5 shows that the cow dung extract has antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus* and was helpful in establishing the antibiotic property of the extract (Waziri and Suleiman 2013).

Aromatic rice

Use of plant-based compound including vegetables, cereals etc. plays a pivotal role in disease prevention. A great deal of recent research has been focused on the development of new bioactive agents from cereals (Wenzig *et al.* 2005, Chung *et al.* 2006, Saikia and Deka 2011). Rice possesses special dietary importance and availability in Asia (WCRF and AICR 1997). Rice-fluid does show an antibiotic effect on Gram negative type *Helicobacter pylori* and their effect (Ishizone *et al.* 2007 and Kawakami *et al.* 2006).

Methanol extract of unpolished grain of two traditional aromatic rice genotypes *viz.* Kalijira and Chinigura were assayed for their activity on the growth and initiation of crown-gall tumors caused by Gram negative type three *Agrobacterium tumefaciens* strains (*A. tumefaciens*- AtSl0105, AtTa0112, and AtAc0114) on potato disks (Fig. 6). The results demonstrated a high correlation between the ability of aromatic rice to inhibit the initiation and growth of *A. tumefaciens* strains on potato disks. It was also observed that tumor inhibition was maximum at higher concentrations (1,000 ppm) of Kalizira and Chinigura rice. Fang *et al.* (2004) suggested that the pure rice phytochemicals were more potent against the pathogenic activity(Duthie *et al.* 2000, Kong *et al.* 2003).

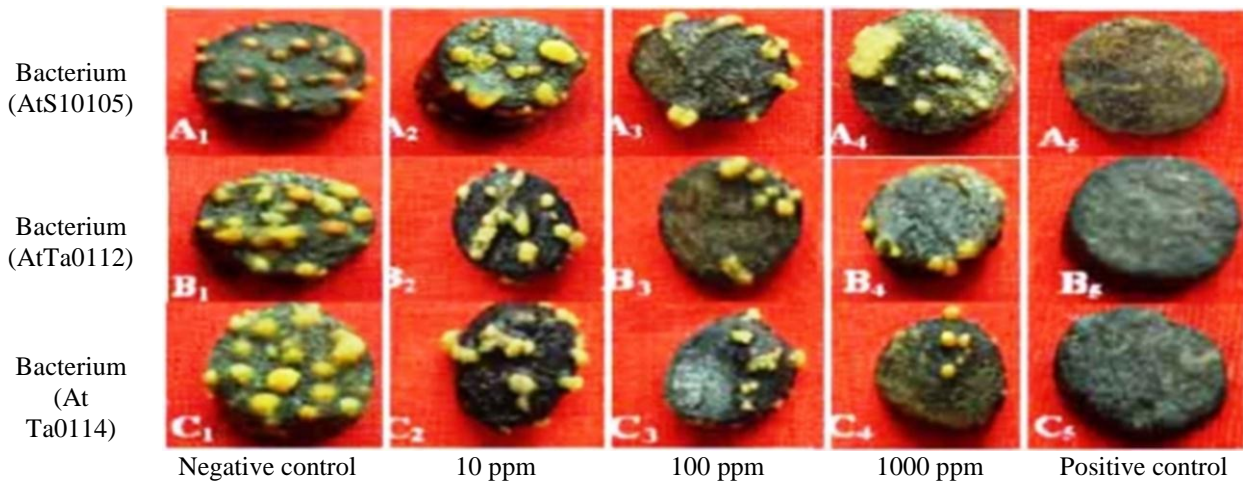


Fig. 6. Tumor inhibition through methanolic extract of Kalijira grain at different concentration caused by Gram negative soil bacteria *A. tumefaciens* strains (after: Mannan *et al.* 2014).

Iodine

Estrela *et al.* (2006) reported that iodoform’s action in releasing iodine gives a higher level of reactivity by precipitating proteins and oxidizing essential enzymes (Table 6). Iodine can be dissolved in aqueous potassium iodide, alcohol or make an assembly with a transporter (known as iodoform) and they are classified as disinfectants (Secor and Gudmestad 1993).

In direct exposure test different pastes with and without iodoform (iodine releasing) shows effective inhibition of different bacterial colonies from both Gram positive and Gram negative type except for *B. subtilis*.

Table 6. Antibacterial effect of different pastes with/ without iodoform (iodine releasing) against different Gram positive and Gram negative type bacteria by direct exposure test (Estrela *et al.* 2006).

Different pastes	<i>S. aureus</i> AGPC	<i>E. faecalis</i> AGPC	<i>P. aeruginosa</i> AGNR	<i>B. subtilis</i> AGPR
CHS	24	---	---	---
	48	---	---	---
	72	---	---	---
CHIS	24	---	---	---
	48	---	---	---
	72	---	---	---
IS	24	---	---	---
	48	---	---	---
	72	---	---	---

(CHS= Calcium Hydroxide + saline; CHIS= Calcium Hydroxide + Iodoform + saline; IS= Iodoform + saline; AGPC= Aerobic Gram-Positive Coccus; AGNR= Aerobic Gram-Negative Rods; AGPR= Aerobic Gram-Positive Rods, spore forming; +++ = presence of growth & --- = absence of growth.)

Sodium bicarbonate

Sodium bicarbonate, NaHCO₃, has particular significance in its ever-growing use for its safety, low cost, low abrasivity, water solubility, acid buffering properties, and, antibacterial properties (McCombs *et al.* 2001, Barnes 1999). The hypertonic (with greater osmotic pressure) sodium bicarbonate solution causes the more hypotonic microbial cell to lose water, consequently dehydrating and eventually killing the cell (Lawrence and Block 1968). Although these are all desirable outcomes, some studies have shown that the sodium bicarbonate must be allowed to interact at least 30 minutes with the bacteria cell to be fully effective. Fletcher *et al.* (1984) proved that sodium bicarbonate had no effect on the viability of *S. mutans* when exposed only for a short time. In many cases, sodium bicarbonate was found to be effective against different microorganisms (Fig. 7).

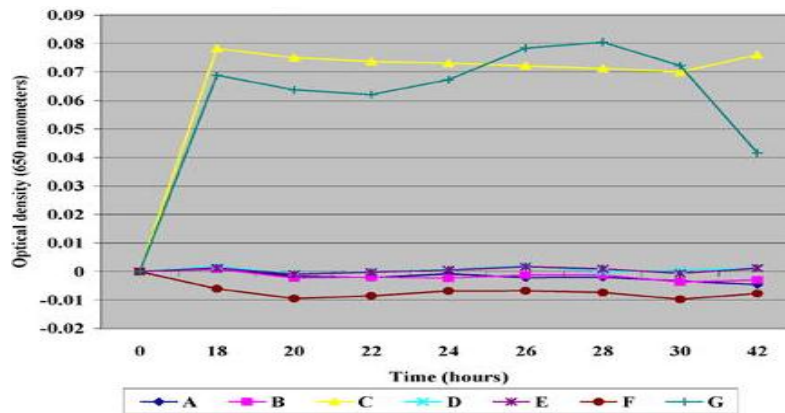


Fig. 7. The effect of sodium bicarbonate and hydrogen per oxide on *S. mutans* (Kelly and Kristin 2005).

Although sodium bicarbonate has been used mostly to formulate toothpaste and cosmetic products for its antibacterial and acid-neutralizing properties (Kelly and Kristin 2005). It has been reported to be virucidal and inhibited the growth of several fungi (Malik and Goyal 2006, Arslan *et al.* 2009). Sodium bicarbonate has also been shown to enhance the effectiveness of other agents for controlling mould growth (Wan *et al.* 2003).

Kelly and Kristin (2005) experimented on the growth of *Streptococcus mutans* where, row A was filled with distilled water without *S. mutans*; row B had 10% sucrose without *S. mutans*; row C had 10% sucrose and *S. mutans* alone; row D had 10% sucrose, sodium bicarbonate and *S. mutans*; row E had

10% sucrose, sodium bicarbonate, 3% hydrogen peroxide and *S. mutans*; row F had 10% sucrose, 3% hydrogen peroxide and *S. mutans*; and row G had *S. mutans* alone (Fig. 7). Rows A (without *S. mutans*), B (without *S. mutans*), C (with *S. mutans*), and G (with *S. mutans*) were used as experimental controls and rows D, E, and F were the experimental groups. Significant statistical differences were observed in Rows D (sodium bicarbonate), E (sodium bicarbonate and hydrogen peroxide), and F (hydrogen peroxide) as compared to row C (*S. mutans* in sucrose) and G (*S. mutans* alone) in the study. However, Rams *et al.* (1985) shows a five-minute exposure to sodium bicarbonate quickly immobilized the motile rods and they also reports that higher concentrations of sodium bicarbonate may not only suppress harmful bacteria, but also lead to increase in healthy bacteria. Barnes (1999) shows *S. mutans* to be susceptible against 4% sodium bicarbonate. Additionally, a four-week study by Legier-Vargas *et al.* (1995) establishes that using a concentration of 65% sodium bicarbonate lowered the level of *S. mutans*.

As no conventional method was found effective in controlling the pathogen, it is urgent to search and test the effectiveness of environment friendly bioactive compounds to overcome the management limitations against the wilt pathogen.

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