EFFECTIVENESS OF BIOACTIVE COMPOUNDS IN AMENDING THE SOIL INFESTED WITH SOIL-BORNE *Ralstonia solanacearum* OF POTATO

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Abstract

Three natural bioactive compounds, *viz.* cow dung, bee propolis and turmeric powder were applied to amend the *Ralstonia solanacearum* infested soil of potato. Sun dried cow dung @ 30 t/ha, aqueous extraction of turmeric powder @ 30 kg/ha and propolis @ 5 lit/ha were applied to the inoculated soil by mixing with field soil in SAU (Dhaka) and BARI (Gazipur). Data on pH, organic matter, total cfu/ml and avirulent cfu/ml of the treatment applied soil and per cent disease index (PDI) of bacterial wilt of potato were recorded. Significant difference in soil pH and per cent organic matter (OM) occurred in all the amended soil as compared to control. A decreased total cfu/ml of *R. solanacearum* occurred in all the treated soil, but propolis (8.2 X 10^7) and cow dung (1.1 X 10^8) showed the lowest count. However, in increasing the avirulent count of *R. solanacearum*, turmeric powder (1.8 X 10^9) showed the highest count compared to other amended soil. Significant disease reduction over control was also observed in all the treatments, but propolis (45.65%) and turmeric powder (43.48%) showed the best results in wilt disease reduction.

***Key words*:** Bacterial wilt; Bioactive compound; Potato; *R. solanacearum*; Soil amendment.

INTRODUCTION

Among the soil-borne pathogens, the bacterial species *Ralstonia solanacearum* (Smith 1896) is the most destructive one that affects potatoes in temperate, subtropical and tropical regions throughout the world by causing wilt or brown rot disease. It is responsible for an estimated loss of US $1 billion each year and, the disease has been estimated to affect three million farm families for about 1.7 million hectares of potatoes approximately in 80 countries (Champoiseau *et al*. 2009). Yield losses due to the disease vary from 33 to 90% in the potato growing areas of the world (Elphinstone 2005). Direct yield losses caused by *R. solanacearum* depend on the host, cultivar, climate, soil type, cropping pattern and strain. In India, this disease causes 50% crop loss in a regular manner and up to 75% losses in some areas of Karnataka (Gadewar *et al*. 1991). Bangladesh is the 7th top producer of potato in the world by producing 86.03 lakh tons (Karim *et al.* 2020). More than 30% of potato crops are affected in this country by *R. solanacearum*, with over 14% yield reduction (Elphinstone 2005).

In Bangladesh, *R. solanacearum* incidence was recorded 9.07% in Jamalpur area, 19.98% in Nilphamari area and 22.65% in Munshigonj area (Ahmed *et al*. 2013). Nonetheless, Russia has imposed a temporary ban on the entry of the potatoes from Bangladesh in May 2015 on food safety grounds after detecting the bacteria, *R. Solanacearum* (Karim *et al.* 2018). Controlling the pathogen with chemicals is difficult; antibiotics is not a safe option to be considered, because it hardly showed any effect (Farag *et al*. 1982). Moderate to highly resistant potato varieties have been released, but, the high frequency of latent infection in tubers is still a major problem. Further, the resistant cultivars are not adapted to different agro-climatic zones and are not effective against all the strains of the pathogen (Virupaksh *et al.* 2012). Due to the biological nature of “heterogeneous species complex” (capable of showing higher variability in biochemical properties in different type of environment), efficient transmissibility (overwinters in diseased plant debris, propagative organs, such as tubers, rhizomes, ginger rhizomes or banana suckers, or on the seeds of some crops like capsicum and tomato, also in the rhizosphere of weed hosts e.g. *Solanum dulcamara*, *S. nigrum* etc., and in aquatic habitats and contaminated irrigation water), extensively wide host range (over 200 species) and world-wide distribution, *R. solanacearum* became very successful to compromise the barriers of traditional management practices (Karim 2017). So, in management of bacterial wilt, the main components remained- the use of healthy seed and planting in clean soils (Choudhary *et al*. 2018). But, there is no 100% effective method massively practiced by the farmers in the world and suppression of this soil-borne pathogen is an important consideration for exporting potatoes.

However, soil amendment with antimicrobial compounds (organic) has found effective against soil borne pathogens (Chen *et al.* 2020) and bioactive compounds were observed to be effective in controlling *R. solanacearum* virulence (Sharma and Kumar 2009, Narasimha *et al*. 2015). Narasimha *et al*. (2015) found that 10% (w/v) turmeric powder produced an inhibition zone from 15 to 25 mm against several virulent strains of *R. solanacearum*.

The concentration of bee propolis was studied as antibacterial agent and higher concentration of propolis was found to produce greater inhibition zones against both Gram negative type and Gram positive type bacteria (Rahman *et al*. 2010). Bee propolis is rich in flavonoids and phenolics that is why it exhibits antibacterial properties (Miorin *et al*. 2003). Cow dung was found to contain antibiotic agents showing higher effectiveness against both Gram negative and Gram positive type bacteria; the presence of K, Na, Mg and many other elements were observed at higher level in cow dung (Waziri and Suleiman 2013, Shrivastava *et al*. 2014). Therefore, the study was conducted to manage the soil-borne *R. solanacearum* of potato by using those elements as soil amendment in infested soil.

MATERIAL AND METHODS

To observe the effect of natural bioactive compounds as soil amendment against bacterial wilt of potato- turmeric powder, propolis and cow dung were used following Karim *et al.* (2020) in Sher-e-Bangla Agricultural University (SAU), Dhaka and Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur. The study was conducted in Sher-e-Bangla Agricultural University (SAU) and Plant Pathology Laboratory, Bangladesh Agricultural Research Institute (BARI) during the robi season of 2020-2021. Bioactive compounds were prepared comparing the studies of Silva *et al*. (2005) and, Ibanez and Blazquez (2021). Treatments were applied to inoculated soil as soil amendment and data on per cent disease index (PDI) was recorded. Host reactions were recorded at every 4th day starting from 4th to 28th day after inoculation using the 0–5 scale of Ateka *et al*. (2001). The degrees of infection were recorded as 0 = no visible symptom, 1 = one leaf wilted, 2 = two or three leaves wilted, 3 = all except top two or three leaves wilted, 4 = all leaves wilted and 5 = dead (collapsed).

Soil test was performed to record pH and OM (%) to understand the effect of bioactive compounds on soil health. For soil test, treatments were applied to the potato field soil and wet virgin soil of BARI and, newly transferred composted soil of SAU. After that soil samples were taken (0-15cm depth) from those treated and non-treated plots. Then the samples were analyzed in the Soil Science Laboratory, BARI. Cfu/ml per g of soil was counted for total cfu count and avirulent cfu count to understand the bacterial response to those compounds. Soil was inoculated @ 10^8cfu/ml of *R. solanacearum* in the sick plot. Counting cfu/ml of bacteria in per g of soil was done following Kelman (1954). PDI, total and avirulent cfu/ml (in colony counter) was calculated by using the following formulae (Ayana *et al*. 2011, Pradhanang *et al*. 2000):

$$PDI = [\frac{(Disease\ class * X\ no.of\ plant\ in\ that\ class) + (Disease\ class * X\ no.of\ plant\ in\ that\ class) + \cdots \ldots}{(No.of\ plant\ assessed\ X\ Highest\ score\ of\ disease\ class)} X\ 100]$$

$$* Disease\ class = 0, 1, 2, 3, 4, 5.$$

$$No.of\ cfu\ in\ colony\ counter = (no.of\ colonies\ per\ sq.cm X\ 5\ sq.cm\ X\ 4\ excision).$$

$$Measurement\ of\ cfu\ per\ ml = no.of\ cfu\ X\ dilution\ factor\ /\ (volume\ plated\ in\ ml)$$

Commercially purified turmeric powder and propolis solutions soaked overnight in hot water containing a limited amount of NaCl were used in the experiment for better release of alkaloids and flavonoids following Mukophadhyay *et al*. (1982), Araujo and Leon (2001), Miorin *et al*. (2003), Deb *et al.* (2013) and Saponjac *et al.* (2019). Because Deb *et al.* (2013) reported from the laboratory test that aqueous extract of turmeric (*Curcuma longa*) was found to contain alkaloid, flavonoids and amino acids, whereas the ethanolic extract of it showed just flavonoids and amino acids except alkaloids. And Mukophadhyay *et al*. (1982) studied the activity of curcumin and its analogues, *viz*. sodium curcuminate, diacetyl curcumin, triethyl curcumin, and tetrahydro curcumin. They revealed that sodium curcuminate was more water-soluble than other curcumin analogues; and it was supported by Araujo and Leon (2001).

Preparation and application of the bioactive compounds

Aqueous extraction of turmeric powder (T_4) was prepared @ 30 kg/ha and mixed with recommended cow dung manure @ 30 ton/ha which was then mixed with field soil @ 2:1 ratio to be applied in the *R. solanacearum* inoculated pit of potato field. Aqueous extraction of propolis (T_3) was prepared @ 5 lit/ ha and mixed with recommended cow dung manure @ 30 ton/ha which was then mixed with field soil @ 2:1 ratio to be applied to the *R. solanacearum* inoculated pit of potato field. For cow dung treatment (T_2), it was applied @ 30 ton/ha which was previously sun dried and mixed with field soil (@ 2:1 ratio) to be applied to the inoculated pit of potato field in addition with the recommended cow manure dose to achieve good microbial activity following Manyi-Loh *et al.* (2016) and a control treatment (T_1) was kept as no amendment of soil.

Aqueous extraction preparation

To soak the compounds overnight, turmeric powder (T_4) and propolis (T_3) (@3g/lit and 0.5ml/lit, respectively) were mixed in hot water containing a limited amount of NaCl (compounds: NaCl @ 3:1 ratio w/w). Those prepared compounds were incorporated with the cow dung manure on the next day after soaking and mixed with field soil (@ 2:1 ratio) and applied as soil amendment to the inoculated potato field. Data were subjected to ANOVA by using Analysis Tool Pak and significant difference among the treatments was done by Least Significant Difference Test (LSD) at probability levels of P=0.05 following Arsham (2020).

RESULTS AND DISCUSSION

A significant difference was observed in case of soil pH and per cent organic matter among treated and non-treated soils. In potato field soil at BARI, the highest pH was observed in propolis-T_3 (7.4) amended soil which was followed by turmeric powder-T_4 (7.3) and cow dung-T_2 (7.1), and the highest per cent of OM was also recorded in propolis-T_3 (2.12) which was followed by cow dung-T_2 (1.75) and turmeric powder-T_4 (1.52) as compared to control-T_1 (6.7 and 1.4, respectively). In the wet virgin soil at BARI, it was also found that the highest pH was occurred in propolis-T_3 (8.3) which was followed by both turmeric powder-T_4 (8.2) and cow dung-T_2 (8.2), and the highest percent of OM was also recorded in propolis- T_3 (1.9) which was followed by turmeric powder-T_4 (1.8) and cow dung-T_2 (1.7) whereas the lowest of both (8.0 and 1.4, respectively) was recorded in non-treated control (Table 1). However, no difference in pH and percent of OM was observed to occur among the treated, and between the treated and control of newly transferred composted soil of Sher-e- Bangla Agricultural University, Dhaka (Table 1).

In case of total cfu/ml per gram of amended soil, the highest total cfu/ml was recorded in control-T_1 (2.7×10^8) whereas the lowest of that was in propolis-T_3 (8.2×10^7) followed by cow dung-T_2 (1.1×10^8) and turmeric powder-T_4 (1.7×10^8). So, the propolis and cow dung treatments were comparatively better in decreasing the total cfu count of *R. solanacearum* infested soil. But, in case of avirulent cfu/ml of per gram soil, amendment with turmeric powder-T_4 (1.8 X 10^9) showed the highest cfu/ml as compared to the lowest case in control-T_1 (5.9 X 10^8).

Table 1. Effects of natural bioactive compounds on soil pH and organic matter.

Treatments	pH			OM (%)		
	Potato field soil	Wet virgin soil	Newly transferred composted soil	Potato field soil	Wet virgin soil	Newly transferred composted soil
T_1- Control	6.7	8	8.6	1.4	1.4	1.7
T_2- Cow dung (@ 30 t/ha)	7.1	8.2	8.6	1.75	1.7	1.7
T_3- Propolis (@ 5.0lit/ha)	7.4	8.3	8.6	2.12	1.9	1.7
T_4- Turmeric powder (30kg/ha)	7.3	8.2	8.6	1.52	1.8	1.7
P-value (0.05)*	0.00012	0.00013	--	5.7E-05	0.00063	--

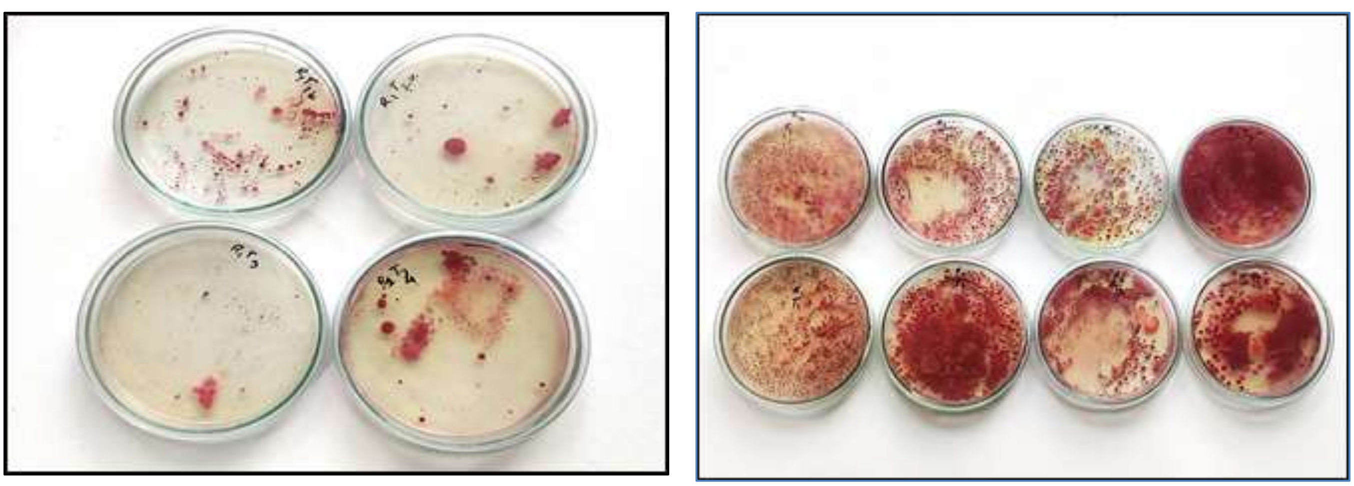
Treatment with turmeric powder-T_4 followed by propolis-T_3 (1.2 X 10^9) and cow dung-T_2 (1.0 X 10^9) in the same count which were statistically similar to each other. Thus, turmeric powder was the best in increasing the avirulent cfu count of those amended soil with bioactive compounds (Table 2 and Fig. 1).

Table 2. Effects of natural bioactive compounds on cfu per ml per gram of treated and control soil.

Treatment	Total cfu/ml (per g of soil)	Avirulent cfu/ml (per g of soil)
T_1- Control	2.7 X 10^8 a	5.9 X 10^8 d
T_2- Cow dung (@ 30 t/ha)	1.1 X 10^8 c	1.0 X 10^9bc
T_3- Propolis (@ 5 lit/ha)	8.2 X 10^7 cd	1.2 X 10^9 b
T_4- Turmeric powder (@ 30 kg/ha)	1.7 X 10^8 b	1.8 X 10^9 a
p-value (0.05)	0.000024*	0.00009*
LSD value	0.16	0.16
% CV	26.71	38.35

Here, *= p value for within treatment (0.000024) is significant at 5% level.

The soil application of the sun dried cow dung-T_2, propolis-T_3 and turmeric powder-T_4 showed a significant reduction of PDI in different treatment amended soils as compared to control (T_1).



a b

Fig. 1. Different petri dishes: **a**. Control-T_1, Cow dung-T_2, Propolis-T_3 and Turmeric powder-T_4 showing cfu/ml of per gram treated and control soil without inoculation; and **b**. with inoculation of *R. solanacearum* @ 10^8 cfu/ml.

The highest PDI was recorded in control-T_1 (61.33%) whereas the lowest of that was observed in propolis-T_3 (33.33%) which was followed by turmeric powder-T_4 (34.67%) and cow dung-T_2 (40.00%) and the last two were statistically insignificantly in respect of percent disease index. So, the highest disease reduction occurred in case of propolis-T_3 (45.65%) which was followed by turmeric powder-T_4 (43.48%) and the lowest was recorded in cow dung-T_2 (34. 78%) over control (Table 3).

Table 3. Effect of natural bioactive compounds on PDI and percent disease reduction of treated and control soil.

Treatment	PDI (Percent Disease Index)	% Disease Reduction
T_1- Control	61.33 a (51.85)	0.00
T_2- Cow dung (@ 30 t/ha)	40.00 b (39.19)	34.78
T_3- Propolis (@ 5 lit/ha)	33.33bcd (35.22)	45.65
T_4- Turmeric powder (@ 30kg/ha)	34.67bc (35.88)	43.48
P- value (0.05)	0.0028*	
% CV	22.79	

Here, *= p value for within treatment (0.0028) is significant at 5% level.

In various studies, *viz.* Michel and Mew (1998), Vudhivanich (2002), and Ayana *et al.* (2011) it has shown that using different soil amendments would minimize bacterial wilt disease caused by *R. solanacearum*; it was because no single strategy has shown 100% efficiency in controlling the disease so far. In the present study, 45.65, 43.48 and 34.78% disease reductions were observed occuring in bee propolis, turmeric powder and sun dried cow dung amended soil @ 5 litre/ha, @ 30 kg/ha and @ 30 ton/ha, respectively. In the experiment turmeric powder, propolis and cow dung containing soil amended the *R. solanacearum* infestation of potato soil and observed an increase in the avirulent count of *R. solanacearum* (1.8×10^9, 1.2×10^9 and 1.0×10^9 cfu/ml, respectively) which revealed the cause of *R. solanacearum* suppression in soil. In another study, Narasimha *et al*. (2015) also found that turmeric powder (10% w/v) produced an inhibition zone from 15 to 25 mm against several virulent strains of *R. solanacearum*.

Bee propolis was also studied as antibacterial agent by Rahman *et al*. (2010) and it was found to produce greater inhibition zones against both Gram negative type and Gram positive type bacteria. Propolis is rich in flavonoids and phenolics that is why it exhibits antibacterial properties (Miorin *et al*. 2003).

Cow dung was also found to contain antibiotic agents showing higher effectiveness against both Gram negative and Gram positive type bacteria by Waziri and Suleiman (2013). The presence of K, Na, Mg and many other elements were observed at higher level in cow dung (Shrivastava *et al*. 2014). Nevertheless, pathogen inactivation was observed to occur in cow manure/slurry when stored in low or moderate temperature over an extended period of time in the influence of UV radiation from sunlight by Maule (2000).

Results of the present experiment are in agreement to the above findings. In another study Sharma and Kumar (2009) applied plant extracts of asafoetida+turmeric, onion and garlic against *R. solanacearum* wilt of tomato and observed to perform well in reducing the primary inocula which also supported the present findings. Ji *et al.* (2005) suggested the use of thymol (a plant-derived chemical) to reduce the disease incidence of bacterial wilt. Akter *et al.* (2021) reported that *Trichoderma harzianum* individually or in combination with Furadan 3G and poultry manure reduced the disease well when applied to soil against the bacterial wilt complex of eggplant. Liu *et al.* (2015) also observed bio-organic fertilizer to enhance the soil suppressiveness against bacteria wilt of tomato. The authors also found an increase in soil pH in amendment treated soil which was parallel to the findings of Michel and Mew (1998) who used soil amendment composed of urea (200 kg of N per ha) and CaO (5,000 kg/ha) against the survival of *R. solanacearum* in four types of soil and, found an initial decrease in a soil with higher pH which resulted a significantly (P < 0.001) lower pathogen population at one week after amending the soil.

However, it was also found that within three weeks after application of the soil amendments, either it caused an initial decrease, or a final decline, or no change in the pathogen population depending on the particular soil type among the four types of Philippine soil. Application of the treatments showed a significant reduction of *Ralstonia solanacearum* cfu/ml of soil and enhancement of soil organic matter and pH.

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