MANAGEMENT OF *Ralstonia solanacearum* (POTATO WILT DISEASE) VIRULENCE BY USING BIOACTIVE COMPOUNDS

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Abstract

Potato is an important vegetable crop of Bangladesh which is facing challenges worldwide due to a quarantine pathogen, *Ralstonia solanacearum*. It is a very successful bacterial pathogen against most of the traditional management practices. Suspension of ten bioactive compounds viz. propolis, honey, turmeric powder+oil, turmeric powder, magnesium chloride, boiled rice fluid, boiled rice fluid+iodine, sun dried cow dung powder, honey+iodine and sodium bicarbonate were evaluated and compared with control (sterile water), commercial bactericide (Krosin AG) and farmers practice (stable bleaching powder). *In vitro* assessment was done by comparing the inhibition zones produced on TZC (tetrazolium chloride) solid medium in disc diffusion method. All of those compounds produced larger inhibition zones as compared to control which indicated the effectiveness of the test compounds against the bacteria. To screen out the performances of those compounds *in vivo*, potato seedlings were inoculated in sterilized soil by soil soak method. Later, mature plants were inoculated in unsterilized soil to find the better resulting compound(s) in field soil condition against the disease. Finally, suspension of cow dung (@25%), propolis (@ 6mg/ml) and turmeric powder (@25%) were selected for trial as soil and seed treatment against the pathogen. It was found that, cow dung reduced 28.89% disease severity index which was followed by 26.67% in propolis and 22.22% in turmeric powder as compared to control (84.44%) in artificially inoculated potato plants against *R. solanacearum*.

***Key words*:** Bacterial wilt; Potato; *Ralstonia solanacearum*; Management; Bioactive compounds.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is considered a super vegetable as well as a versatile source of food security. It is the 4th most important crop after wheat, rice and maize in the world (FAOSTAT 2014). Bangladesh is the 7th producer of potato in the world by producing 86.03 lakh tons of potato. Yet, the yield of potato is quite lower than the yield of Ireland and India (FAOSTAT 2017). The reasons behind lower yield of potato include- inadequate supply of quality seeds, poor soil health, different pests and diseases etc. Considering the diseases of potato, soil borne diseases alone can cause a yield loss of as much as 10–20% of potato annually (USDA 2003) and among them wilt or brown rot caused by *Ralstonia solanacearum* is the most destructive one (Yuliar *et al.* 2015). The disease challenges the potato industry by causing yield losses and quarantine concerns throughout the world in temperate, subtropical and tropical regions (CABI 2017, Champoiseau *et al.* 2009). 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). In India, this disease causes 50% crop loss in potato in a regular manner (Mukherjee and Dasgupta 1989) which goes up to 75% losses as reported in some areas of Karnataka (Gadewar *et al.* 1991) and it emerged as a major constraint for potato cultivation from 2007 in Madhya Pradesh (India) by causing heavy crop losses (Sagar *et al.* 2013). In Bangladesh more than 30% of potato crops get affected by *R. solanacearum*, with more than 14% yield reduction (Karim *et al.* 2018). A temporary ban was imposed by Russia on the entry of potatoes from Bangladesh in May 2015 on food safety grounds after detecting this organism in the exported potatoes (Parvez 2017).

Due to the biological nature of "heterogeneous species complex" (capable of showing higher variability in biochemical properties in different type of environment), *R. solanacearum* became very successful to compromise the barriers of traditional management practices (Karim *et al*. 2018). However, several bioactive compounds has been observed to be effective in inhibition of *R. solanacearum* by Chellemi *et al.* 1997 and Narasimha *et al.* 2015. Bioactive compounds are different organic compounds which can exhibit diverse and versatile antimicrobial activities and can enhance different biological activities in any living organism (Shukla 2015, Gupta and Rana 2016). There are several bioactive compounds which show different level of antibacterial competence against both Gram positive and Gram negative type of bacteria (Karim and Hossain 2018). Honey and propolis were found to be very effective against both Gram negative and positive type bacterial management (Miorin *et al.* 2003). A wide range of pharmacological attributes of curcumin from turmeric were documented for antimicrobial and protective properties (Nagabhushan and Bhide 1992). Cow dung was reported to contain antibiotic agents (Waziri and Suleiman 2013). Magnesium salt in some experiments typically showed positive effects as a stress enhancer of Gram negative *Escherichia coli* (Oyarzua *et al.* 2014). Two traditional aromatic rice genotypes, viz. Kalijira and Chinigura, were effectively inhibited a Gram negative *Agrobacterium* species (Mannan *et al.* 2014). Iodine (mixed with a transporter known as iodofore) successfully inhibited aerobic Gram positive and Gram negative bacteria (Estrela *et al.* 2006). Sodium bicarbonate was reported to show antibacterial properties against different types of bacterial and fungal pathogens (Kelly and Kristin 2005). It was also observed that functional diversity of bacteria was influenced by application of organic compounds in farm land due to the positive correlation of pH, organic matter and water content (Chou *et al.* 2017). Little works have been performed to investigate the antibacterial effectiveness of such bioactive compounds against *R. solanacearum*. Therefore, the study emphasizes the effectiveness of such bioactive compounds in managing the disease severity and virulence of wilt bacteria (*R. solanacearum*) in potato.

MATERIAL AND METHODS

Ten bioactive treatments (Table 1) and, a negative control (distilled water), a standard control (stable bleaching powder frequently used by farmers) and a chemical control (Krosin AG - a bactericide containing 9% streptomycin sulphate and 1% tetracyline hydrochloride) were evaluated to find out the effectiveness in managing bacterial wilt (*R. solanacearum*) of potato by different methods (*in vitro, in vivo* and pot house condition). Suspension of the selected bioactive compounds were prepared as follows: (a) T_1/ Control (sterile water)- sterile distilled water was applied as per requirement; (b) T_2/ Propolis (@ 6mg/ml)- propolis was weighed and soaked in 30% ethanol for 24hrs. Then ethanol soaked ball of propolis was blended and mixed with water to prepare 6g/liter (6mg/ml) of propolis; (c) T_3/ Honey (@20%)- 200g honey was freshly mixed per liter of distilled water and kept in room temperature to be applied; (d) T_4/ Turmeric & oil (@25%)- commercial turmeric powder and mustard oil was mixed @ 1:1 and 250g of it was mixed freshly with per liter of distilled water. The thick suspension was applied by using dropper or teaspoon as per required; (e) T_5/ Turmeric powder (@25%)- 250g of commercial turmeric powder was mixed in a liter of distilled water which was used freshly; (f) T_6/ Magnesium chloride (@3%)- magnesium chloride salt was collected from market and prepared as 30g per liter of distilled water and stored in room temperature; (g) T_7/ Boiled aromatic rice fluid (@10% ie. 100ml/liter)- fresh kalojira rice was bought from market. After cleaning 100 g of the rice it was boiled into a thick soup and was mixed @ 100ml per liter of distilled water (@10%) after straining the fluid and stored in 6^0C temperature; (h) T_8/ Boiled aromatic rice fluid + Iodine (@10% ie. 100ml/liter + 2 drops of iodine per 100ml)- it was prepared in the same manner as T_7 and just added 20 drops of iodine solution per 1000ml which was bought from market; (i) T_9/ Cow dung powder solution (@25%)- well decomposed and sun dried cow dung was weighed for 250g and brought to powder form by using

mortar and pestle. It was then mixed with 1000ml distilled water by vigorous shaking and stored in room temperature to be applied as treatment; (j) T_{10}/ Krosin AG (Bactericide @ 0.5 g per liter water)- it was bought from market and applied at the suggested rate by the company; (k) T_{11}/ Honey + Iodine (@2 drops of iodine per 100ml of 20% solution of honey)- honey solution was freshly made as previous and just 20 drops of iodine was added in 1000ml of that 20% solution and well shaken and kept in room temperature; (l) T_{12}/ Sodium bicarbonate (@10%)- it was brought from market and weighed 100g and added per liter of distilled water and stored in room temperature; and (m) T_{13}/ Stable bleaching powder (@ 23mg/kg- as application rate is 30 kg/ha, soil area= 10000m^2, approx. soil volume= 1000m^3/ha as soil depth is 0.1m, soil bulk density= 1.3 t/m^3 and weight of soil=(1000 X 1.3)= 1300t/ha; ie. 30kg/ 1300,000kg)- it was also bought from market, calculated and weighed for 23 mg/kg to make freshly 23mg in 1litre of distilled water to make the suspension to test *in vitro*. To be applied in soil, 23mg of it was mixed with 1kg of powdery sterilized soil. Then it was applied.

Table. 1. Treatments, dosage and bioactive ingredients of the selected compounds for evaluation of effectiveness against *R. solanacearum*.

Treatment and Dosage	Bioactive ingredient	Reference
T_1= Control (sterile water as negative control)	-	
T_2= Propolis (@ 6mg/ml)	Phenolics & flavonoids	Rahman *et al.* (2010).
T_3= Honey (@20%)	Hydrogen peroxide (H2O2), Methylglyoxal (MGO)& different enzymes	Majtan *et al*. (2014); Balan *et al.* (2016).
T_4= turmeric powder & oil (@25%)	Curcumin (diferuloyl methane)	Balan *et al.* (2016).
T_5= Turmeric powder (@25%)	Curcuminoids which are fat soluble	Narasimha *et al*. (2015).
T_6= Magnesium chloride (@3%)	Mg+2 as enzymatic co-factors, as signaling molecules	Oyarzúa *et al.* (2014).
T_7= Boiled aromatic rice fluid (@10% ie. 100 ml/liter)	Rice-fluid contains rice phytochemicals viz. oryzanols, anthocyanins, amino acids, essential oils, phenolics, etc.	Ishizone *et al.* (2007); Kawakami *et al.* (2006); Chakuton *et al*. (2012); Deng *et al*. (2013); Mannan *et al.* (2014).
T_8= boiled aromatic rice fluid + iodine (@10% ie. 100ml/liter + 2 drops iodine per 100ml)	Phytochemicals oryzanols, anthocyanins, amino acids, essential oils, phenolics etc. & disinfectant	Mannan *et al.* (2014); Estrela *et al.* (2006).
T_9= Sun dried cow dung powder (@25%)	Large number of microorganisms produces metabolites, like k, Na, Mg etc. in higher levels acting as cofactors for various enzymes.	Shrivastava *et al*. (2014); Waziri and Suleiman (2013).
T_{10}= Krosin AG bactericide (@ 0.5 g per liter water as chemical control)	Streptomycin sulphate 9.0 % (w/w) & Tetracyline hydrochloride 1.0 % (w/w), broad spectrum antibiotic	Company packet marketed by Krishi Rasayan Export Pvt. Ltd., India.
T_{11}= Honey + Iodine (@2 drops of iodine per 100ml of 20% solution)	Hydrogen peroxide (H_2O_2), Methylglyoxal (MGO)& different enzymes; disinfectants	Secor and Gudmestad (1993); Estrela *et al.* (2006); Majtan *et al*. (2014); Balan *et al.* (2016).
T_{12}= Sodium bicarbonate (@10%)	Nahcolite, acid neutralizing and changes osmotic pressure which causes microbes to lose water and dehydrate	Kelly and Kristin (2005).
T_{13}= Stable bleaching powder (@ 23mg/kg as standard control).	Active chlorine 20-70% compromises the lipid membrane of bacteria	Sharma and Kumar (2000).

Since differences between virulent and avirulent forms of *Ralstonia solanacearum* can be recognized on TZC (Triphenyl teterazolium chloride) semi selective media (Mikhail *et al*. 2017), prepared suspension of bioactive compounds were then screened on TZC solid media in producing inhibition zones (mm) and in colony counts (cfu/ml) of virulent & avirulent colonies of *R. solanacearum* in disc

diffusion method following Bonev *et al.* (2008) and Liu *et al*. (2012), as it shows phenotypic conversion (PC) phenomena (Alvarez *et al*. 2010). The individual colony of virulent and avirulent type was described by Liu *et al*. (2004) and Zheng *et al.* (2014) (Fig. 1) and TZC plated avirulent colony types were documented by Kumar *et al*. (2017). Those were taken into consideration during the study in counting virulent and avirulent forms of colonies (Fig. 1) by using colony counter. Petri plate inoculation was done through disc diffusion method @ four to five colonies of the organism per 4 ml of sterile water which was incubated for 3 to 4 h (Bauer *et al*. 1966). Then, that suspension of *R. solanacearum* was suspended on the petri plates by using sterile cotton swabs. Later, the discs (about 6 mm in diameter) of whatman no. 1 filter paper impregnated with selected bioactive compounds @ 10 microlitres from the prepared doses were placed on to the inoculated TZC plates and incubated at 28^0C for 36-48 hrs which were replicated for three times. Later, measurements were taken on inhibition zones following Balouiri *et al*., 2016. For ease of work, media plates and broth following proper autoclaving were kept ready in the freeze at 6^0C to use in checking for colony counting.

Fig. 1. Virulent and avirulent colonies of *R. solanacearum* TZC solid medium in the study.

To evaluate those compounds as treatment, Ayana *et al*. (2011), Singh *et al*. (2014) and Zhang *et al*. (2015) were followed for inoculation methods and disease scoring of virulence expression. For *in vivo* evaluation of those bioactive compounds, three weeks old potato seedlings were planted in the seedling tray which was filled with treated soils. Sterilized soils were treated with the suspension of bioactive compounds and inoculated with soil soak method (Zhang *et al*. 2015) before planting of potato seedlings (at 3^{rd} week) and freshly prepared *R. solanacearum* culture (@ 10^8 cfu/ml) in sterile water medium containing 10% dextrose (Alvarez *et al*. 2010) was used to inoculate the soil. The inoculated seedlings were later observed up to two weeks post inoculation (4^{th} and 5^{th}) to record the virulence expression. To study the effect on mature plants in unsterilized soil (just solarized), potato seeds were treated with suspension of bioactive compounds. Root trimming inoculation of 5^{th} week old potato plants were done following Kumar *et al*. (2017) to understand the performance of treatments in natural field soil against the wilt bacteria (*R. solanacearum*). The seedlings were observed up to 2 WPI (weeks post inoculation ie. 6^{th} and 7^{th} week of plants) to record the disease response of those treatments in virulence expression in terms of disease severity score. The study was done following Tanaka and Noda (1973) and previous other literatures. Depending on those responses (*in vitro* and *in vivo*) propolis, turmeric powder and cow

dung were selected as seed and soil treatment for trial study. Potato seeds and soils (unsterilized but solarized) of treatment cages were treated with propolis (T_2), cow dung (T_4) and turmeric powder (T_5) along with control (sterile water as T_1) and standard control (stable bleaching powder as T_3) at the specific doses (as described earlier) to compare those compounds in virulence reduction in field soil condition against *R. solanacearum*. Lemessa and Zeller (2007), Ayana *et al*. (2011), Singh *et al*. (2014) and Zhang *et al*. (2015). For treating the soil, 1 part each treatment suspension was mixed with 3 part of sterile soil powder to get a granular context which was then applied per pit of each treatment. Treated soils were soil-soak inoculated at 2nd week after treatment application. For seed treating, selected treatment suspensions were mixed with sterile soil suspension to make slurry like concentration. Seeds were dipped into slurry of treatment before sowing in the treated and inoculated soil cages. Later, treated seeds were pinch inoculated following Lemessa and Zeller (2007). After that, plants were observed up to 7th WPI (week post inoculation) for disease virulence measurement of the treatments. To understand the effect on pathogen colonization in treated soil, virulent and avirulent colonies of *R. solanacearum* were counted (cfu/ml) from those five treated soils in dilution plate method (at two level of soil dilution ie. 10^{-5} and 10^{-7} level). Virulent and avirulent colonies were counted on TZC solid media following Zheng *et al*. (2014) and Kumar *et al*. (2017) methods at 7th WPI.

Disease virulence was measured by scoring in a six point rating scale (0–5) following Swanson *et al*. (2005). Ayana *et al*. (2011) and Zheng *et al*. (2014) modified from Winstead and Kelman (1952) which suggested- 0 = no wilt symptoms, 1 = one/few/one third of whole leaves showing wilted symptoms, 2 = several/more/half of whole leaves showing wilted symptoms, 3 = most leaves/two third of whole leaves showing wilted symptoms, 4 = whole plant showing wilted symptoms and, 5 = death (collapse) of the whole plant. Since all of the plants showed disease symptoms, the incidence was 100% in all the cases due to artificial inoculation. So, percent severity index (PSI) was calculated to differentiate the treatment effects as described by Cooke (2006). PSI = Σ (scores × 100)/ (number of plants rated × maximum scale of the scores) for each scoring date. All those evaluations were performed at least with three to four replications in calculating mean, standard deviation, severity score and PSI. Data were compiled, tabulated and subjected to statistical significance test by using MSTAT-C and *t*-test in data analysis tool Pak software and percent reduction over lowest in excel of windows10 following Gomez and Gomez (1984) and Zhang *et al*. (2015).

RESULTS AND DISCUSSION

For screening (*in vitro*) of bioactive compounds in producing inhibition zone (mm) and colony counts (virulent & avirulent in cfu/ml) of *R. solanacearum* on TZC solid media, it was observed that significantly largest zone was expressed by T_9 which was followed by T_5, T_7 and T_{11} whereas the smallest zone was found in T_1 (control) (Table 2, Fig. 2, **p<0.01). In case of performances of the compounds in producing virulent and avirulent colonies (cfu/ml in per square cm) it was observed that the lowest count of virulent colony was produced by T_2 which was followed by T_5 and T_9 whereas the significant highest virulent colony count was observed in T_1 (control) (Table 2; Fig. 2; **p<0.01).

In case of evaluation (*in vivo*) of those compounds in potato seedlings in sterilized soil it was observed that the lowest significant DSS (disease severity score) was produced by T_7 which was followed by T_{13}, T_9 and T_3 whereas highest significant score of that was produced by T_1 (control) in 2nd WPI of seedlings against *R. solanacearum* (Fig. 3, **p<0.01).

In the evaluation of those compounds in mature potato plants in unsterilized field soil (just solarized) it was found that the lowest significant DSS (disease severity score) occurred at T_9 which was followed by T_2, T_5, T_{13} and T_6 whereas significant highest DSS was observed by T_1 (control) which was followed by the rest treatments in 2nd WPI in potato plants against *R. solanacearum* (Fig. 4, **p<0.01).

Table 2. Inhibition zone and colony counts of *R. solanacearum* in selected bioactive compounds.

Treatment	Mean Inhibition zone (mm)	Mean colony count of *R. solanacearum* (cfu/ml per square cm)	
		Virulent	Avirulent
T_1= Sterile water (negative control)	8.48 ± 0.53	53.10 ± 17.43	4.62 ± 5.49
T_2= Propolis	14.46 ± 0.58	8.23 ± 8.17	13.43 ± 4.03
T_3= Honey	16.24 ± 0.62	37.69 ± 14.70	9.42 ± 17.30
T_4= Turmeric + oil	17.39 ± 2.20	33.59 ± 7.42	42.75 ± 5.61
T_5= Turmeric powder	20.19 ± 1.64	11.72 ± 4.34	16.86 ± 9.58
T_6= Magnesium chloride	15.07 ± 0.69	29.68 ± 11.17	9.89 ± 13.16
T_7= Boiled rice fluid	17.89 ± 0.56	27.66 ± 13.46	5.66 ± 12.85
T_8= Boiled rice fluid + Iodine	14.84 ± 0.77	30.21 ± 6.67	15.56 ± 7.66
T_9= Cow dung powder	20.69 ± 1.10	12.21 ± 5.34	64.12 ± 10.30
T_{10}= KrosinAG (chemical control)	17.18 ± 0.86	47.83 ± 5.67	22.51 ± 7.73
T_{11}= Honey + Iodine	17.73 ± 1.25	36.55 ± 6.73	10.31 ± 7.97
T_{12}= Sodium bicarbonate	13.34 ± 0.24	39.29 ± 8.34	4.86 ± 11.50
T_{13}= Stable bleaching (standard control)	14.21 ± 1.21	21.85 ± 5.74	25.65 ± 7.04
	**P value < 0.01	**P value< 0.01	**P value< 0.01

In the evaluation of propolis, turmeric powder and cow dung as seed and soil treatment against *R. solanacearum* it was observed that at 7th WPI the lowest significant PSI was occurred at T_4 (60.00% in cow dung @ 25%) which was followed by T_2 (62.22% in propolis @ 6mg/ml) and T_5 (66.67% in turmeric powder @ 25%) whereas significant highest PSI was observed in T_3 (88.89% in standard control/ stable bleaching powder @ 23mg/kg) and T_1 (84.44% in negative control) in natural field soil (Fig. 5, **p<0.01). So, highest significant reduction of PSI over lowest (T_3 as stable bleaching powder @ 23mg/kg) was occurred at T_4 (28.89% in cow dung @ 25%) which was followed by T_2 (26.67% in propolis @ 6mg/ml). T5 (22.22% in turmeric powder @ 25%) was in between T_4 and T_2 whereas the lowest reduction was occurred in T_1 (84.44% in control) at the 7th WAI (week post inoculation) (Fig. 6, *p<0.05).

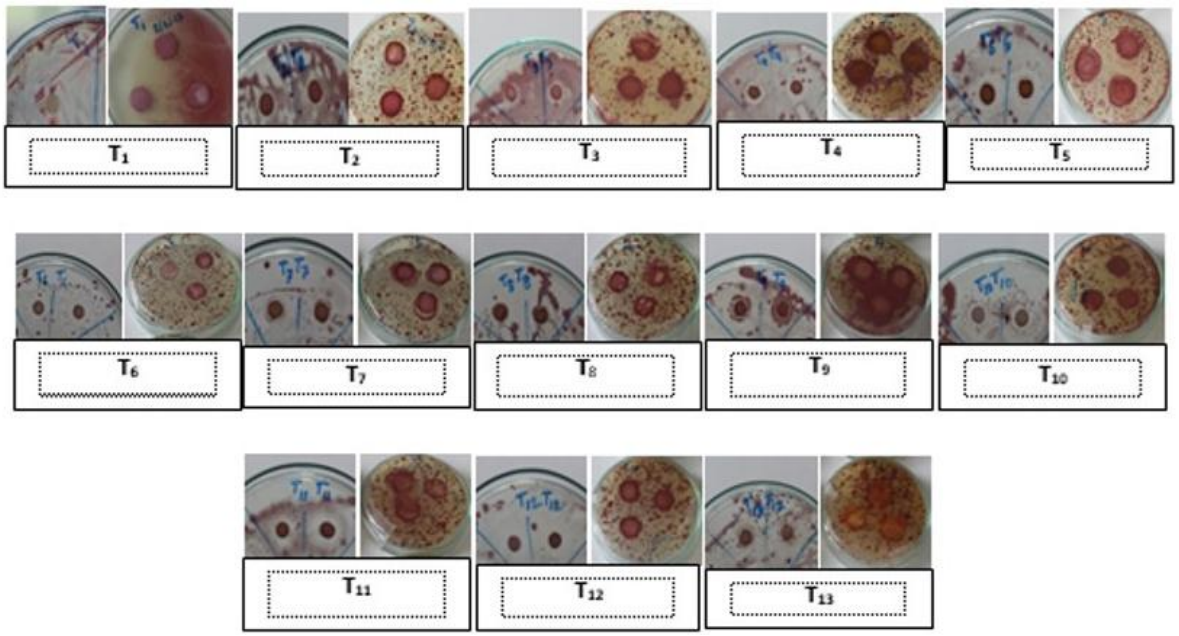

Fig. 2. Bioactive compounds showing inhibition zone (mm) and virulent and avirulent colonies (cfu/ml) in the treatment plates of T_1, T_2, T_3, T_4, T_6, T_7, T_8, T_9, T_{10}, T_{11}, T_{12} and T_{13} against *R. solanacearum*.

For virulent and avirulent colony counts (cfu/ml per cm^2) in soil of different treatments, it was observed that significantly lowest virulent colony was produced in T_4 (26.48 cfu in cow dung @ 25%) which was followed by T_2 (29.44 cfu in propolis @ 6mg/ml, comparatively dry colonies) and T_5 (33.54 cfu in turmeric powder @ 25%) whereas the highest of that was found in case of T_3 (65.71 cfu in stable bleaching powder @ 23mg/kg) which was followed by T_1 (55.10 cfu in control) (Fig. 8, **p<0.01). On the other hand, significant highest avirulent colony count was found in case of T_5 (52.46 cfu in turmeric powder @ 25%) which was followed by T_4 (49.18 cfu in cow dung @ 25%) and T_2 (31.89 cfu in propolis @ 6mg/ml) whereas the lowest of that was occurred in case of T_3 (8.96 cfu in stable bleaching powder @ 23 mg/kg) which was followed by T_1 (39.90 cfu in control) (Fig. 7, **p<0.01). Thus, differences of virulent and avirulent colony counts (Fig. 8) of soil collected from different treatments were reflected in TZC plated solid media at 5th week post inoculation.

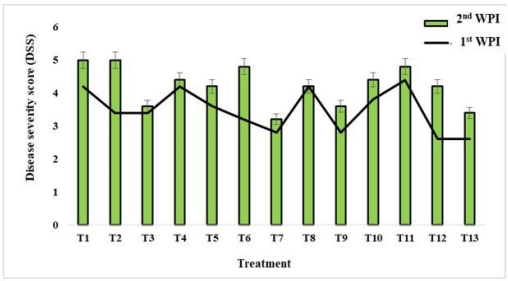

Fig. 3. Disease severity score (DSS) of the bioactive compounds in potato seedlings in sterilized soil against *R. solanacearum* at 1st and 2nd WPI (weeks post inoculation) (**p<0.01).

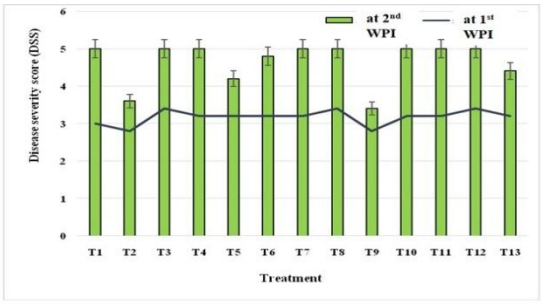

Fig. 4. Disease severity score (DSS) of the bioactive compounds in 1st and 2nd WPI (week post inoculation) of potato plants in unsterilized soil (just solarized) against wilt pathogen (*R. solanacearum)* (**p<0.01).

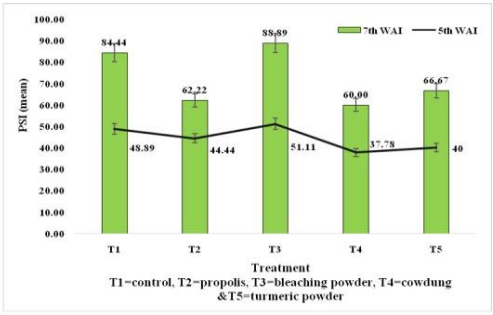

Fig. 5. PSI (Percent severity index) of propolis, cow dung and turmeric powder as both seed and soil treatment compared to controls (negative and standard) against wilt disease (*R. solanacearum*) in 5th and 7th WPI (**p<0.01).

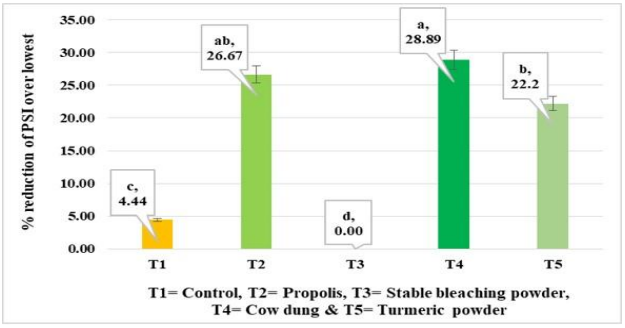

Fig. 6. Percent reduction of PSI of wilt disease (*R. solanacearum*) at 7th WPI in propolis, turmeric powder and cow dung treatment (*p<0.05).

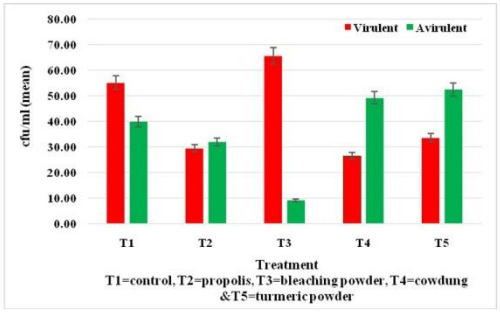

Fig. 7. Virulence expression (virulent & avirulent colony count/square cm) of *R. solanacearum* in soil dilution plates (@ 10^{-7} level) from different treatments on 7th WPI (**p<0.01).

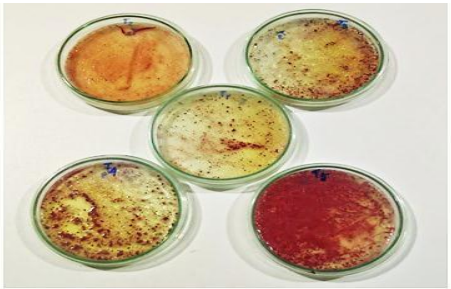

Fig. 8. Virulent and avirulent colony expression of *R. solanacearum* in soil dilution plates (@ 10^{-5} level) of different treatments on 7th WPI.

Several traditional control strategies viz. quarantine, breeding for resistance, biological control and chemical control has been applied worldwide, but no single management practice controlled the wilt disease (*R. solanacearum*) successfully in potato crop (Karim *et al.*, 2018). Nevertheless, some bioactive compounds have been effective in inhibition of many dangerous strains of bacteria (Leksomboon *et al.* 2000) and *R. solanacearum* (Chellemi *et al.* 1997, Narasimha *et al.* 2015). Thus, ten bioactive compounds (viz. propolis, honey, tumeric powder, $MgCl_2$ salt, aromatic rice fluid, iodine, cow dung and sodium bicarbonate) were selected to test against *R. solanacearum* of potato following the review study of Karim and Hossain (2018). In disc diffusion method (*in vitro*) all of the treatments except control produced inhibition zones. But, in unsterilized soil only propolis, turmeric powder and cow dung showed very positive response in severity reduction of wilt disease in artificial inoculation. But, commercial bactericide Krosin AG (a compound containing 9% streptomycin sulphate and 1% tetracyline hydrochloride) found to perform lower during the study which was found parallel to the study of Farag *et* al. (1982 and 1986) in CABI (2017). Based on the performances of *in vitro* and *in vivo* evaluation suspension of propolis, turmeric powder and cow dung were trialed as both soil and seed treatment in artificially inoculated potato plants with *R. solanacearum* as functional diversity of bacteria can be influenced by application of organic compounds (Chou *et al.* 2017). All of the three appeared very much effective in reducing the disease virulence (severity index and colony counts) of pathogen compared to control and stable bleaching powder. The reasons of their antibacterial efficacy were methodically studied by several researchers. Chemical composition and antibacterial activity of propolis were studied by Bosio *et al*. (2000) and Miorin *et al*. (2003). They found that bee propolis is rich in flavonoids and phenolics which is why it exhibits antibacterial properties. Several similar studies were done by Zumla and Lulat (1989), Martos *et al*. (1997), Nieva Moreno *et al*. (1999) and Marcucci *et al*. (2001) and, those studies verified that phenolics of propolis including cinnamic acid derivatives and flavonoids were exhibiting antibacterial properties. Rahman *et al*. (2010) studied the concentration of propolis as antibacterial agent and observed that higher concentration of propolis can produce greater inhibition zones against both Gram negative type *Escherichia coli* and Gram positive type *Staphylococcus aureus*. However, Miorin *et al*. (2003) suggested that the extent of effectiveness of propolis and their chemical composition varies depending on bee species and geographic region. Turmeric (*Curcuma longa* L.) was researched in medicinal context by Eigner and Scholz (1999) and Gupta *et al*. (2012). They found it as an extensively used medicinal plant for numerous pathological research due to the presence of curcumin (diferuloyl methane) with a wide range of attributes, such as antioxidative, antimicrobial and wound-healing properties which was parallel to the findings of Nagabhushan and Bhide (1992), Aggarwal and Harikumar (2009), Frenkel *et al.* (2013), Moghadamtousi *et al*. (2014) and many others. In a study, Narasimha *et al*. (2015) found 10% (w/v) turmeric powder to produce an inhibition zone (*in vitro*) from 15 to 25 mm against several virulent strains of *R. solanacearum* that is in support to present study. However, it was observed that application @ 25% (w/v) was doing better in unsterilized soil in pre-evaluation of the study. In different researches, Sethuraman and Ray (2003) and many others studied the composition of cow dung and their antibacterial properties. Sethuraman and Ray (2003) found it to contain approx. 80% water and a matrix of undigested plant material rich in nutrients, micro-organisms, and their byproducts which was corresponding to the findings of Nene (2003) and Randhawa and Kullar (2011). But, Khanuja (2002), Waziri and Suleiman (2013) and Shrivastava *et al*. (2014) found it 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 at higher levels in cow dung were described by Waziri and Suleiman (2013) in the study. Those elements act as enzymatic cofactors in different biochemical processes in relation to antibacterial activity which was also revealed by Gupta *et al*. (2016). However, in the study

of seed and soil treatment, stable bleaching powder was observed to perform lower compared to control which might be due to the reaction of bleaching powder with organic matters in the soil. Because application of stable bleaching powder reduces the bacterial population on small scale (Saddler 2005) but it can be inactivated by organic matter by releasing a toxic chlorine gas (Kennedy and Bek. 1998).

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