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ORIGINAL ARTICLE

Sustainable management of bacterial wilt of tomato using dried powder of *Xanthium strumarium* L.

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Abstract

Because of its known anti-bacterial properties, we explored the potential of *Xanthium strumarium*, an invasive, enormous mass-producing weed, for the control of *Ralstonia solanacearum* which causes bacterial wilt (BW) of tomato. Both in-vitro and *in-planta* experiments were conducted, using different concentrations of the dried powders of the plant parts applied to infested soil at different times. Addition of a 20% (w/v) aqueous extract of leaf powder or succulent shoot powder to wells cut in nutrient agar inhibited growth of *R. solanacearum*. In *in-planta* experiments, 4.5% (w/w) leaf powder applied to artificially infested soil 10 days before transplant (DBT), produced the best effect and enhanced root length, shoot length, and plant fresh bio-mass by 64%, 37%, and 42%, respectively, as compared to inoculated control. Leaf powder also lowered the area under disease progress curve (AUDPC) by 38%, and the pathogen counts (g^{-1} dry soil) by 1.202 $\log_{10}$ units. Succulent shoot powder (4.5% w/w) applied 20 DBT proved to be better than other application times and increased root length, shoot length, and plant fresh bio-mass by 55%, 42%, and 57%, respectively, as compared to inoculated control. Succulent shoot powder also decreased AUDPC by 35%, and the pathogen counts (g^{-1} dry soil) by 1.294 $\log_{10}$ units. Our data strongly suggest that 4.5% (w/w) of leaf or succulent shoot powder, applied 20 DBT, can be an effective component of the integrated disease management (IDM) against BW.

Keywords Integrated disease management · Area under the disease progress curve · Soil amendment · Organic agriculture · *Ralstonia solanacearum*

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most valuable vegetable crops. Besides being a major source of vitamins, minerals and other nutrients, it is considered as a cash crop for medium and small-scale land holders of Pakistan. It has diverse uses including raw, cooked or processed. Originally being a tropical plant, it is now grown worldwide. China, USA, India and Turkey are the top producers of tomato. In Pakistan, tomato consumption frequently exceeds its production forcing the country to import the commodity from India and Afghanistan. Pakistan's average tomato yield is much lower as compared to the world's, i.e., 10.7 tons/ha vs. 36 tons/ha (MINFAL 2009). Among many other factors responsible for low yield, the occurrence of bacterial diseases is one major cause (Laterrot 1998). Bacterial wilt (BW) of tomato, caused by *Ralstonia solanacearum*, is one of the most destructive diseases of the crop. The initial symptoms of this disease include wilting of leaves during the hot part of the day and recovering during night. Later, the whole plant permanently wilts and dies. The presence of long, dark brown streaks in vascular bundles of young infected stem is another prominent symptom. The distinctive sign of the disease, however, is the release of white milky exudates by freshly cut infected stem. The disease quickly progresses after infection, especially if the temperature ranges between 29 °C and 35 °C (Champoiseau et al. 2009).

The pathogen is highly diversified and is considered as a species complex. It consists of a large number of strains which differ from each other in terms of biochemical properties, pathogenicity and geographical distribution (Hayward

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1994). Race 3, biovar 2 of this species complex is the most important causing huge annual losses in as many as 80 countries of the world, including Pakistan (Floyd 2007). Yield losses range from 10 to 30% in tobacco, 0–90% in tomato, 80–100% in banana, 20% in groundnut and up to 90% in potato (Elphinstone 2005). In Pakistan, Punjab and Sindh provinces are significantly affected by this disease. The frequency of the disease in these provinces ranges from 5 to 25%, indicating a potential threat to the cultivation of many solanaceous vegetables (Burney et al. 1999). Multiple vegetable crops have been shown to be seriously vulnerable to this disease. The incidence of the disease was found to be 22% in sweet peppers, 17% in hot peppers, 13% in tomatoes, 10% in potatoes and 5% in brinjals (Begum et al. 2012). Furthermore, biovar 3 is the most dominant strain of BW pathogen found in Pakistan (Begum et al. 2012; Shahbaz et al. 2015).

Control of plant bacterial diseases in general and BW in particular, is not easy. The main hurdles include the ability of the pathogen to survive in weeds, in multiple hosts, deeper in soil, in water, and its high variability (Wang and Lin 2005; Nguyen and Ranamukhaarachchi 2010). Chemical control is not feasible because of the non-availability of efficient commercial antibacterial chemicals, the ability of the pathogen to develop resistance against them and environmental hazards caused by them (Oguwike et al. 2013). The efficacy of biological control is limited by the unreliable colonization, narrow range and the high inoculum requirement of the biocontrol agents (Whipps and Gerhardson 2007). The use of resistant varieties to control the disease is rendered ineffective because of the high variability of the pathogen and the breakdown of resistance under conditions favorable for disease development. This situation necessitates the use of integrated disease management (IDM) which is considered as the best measure for the control of diseases like BW. IDM approach has the potential to reduce BW up to 100% under in-vitro or under field conditions (Anith et al. 2004).

One effective component of IDM against plant diseases including BW could be the use of medicinal plants having antibacterial properties. Most plant products are reportedly (Tripathi and Dubey 2004) safe, non-phytotoxic, biodegradable and could be used in different formulations such as green manure, dried powder organic amendments (OAs) (Naz et al. 2015a, b) or aqueous extract treatments (Askarne et al. 2012) for the control of plant diseases caused by different pathogens. Many plants have been found to have strong anti-bacterial properties (Lo Cantore et al. 2004). The use of such plants could provide an effective component of IDM against different bacterial plant diseases including BW. One-month-old potted tomato plants (cv. Pullrex) sprayed with aqueous extracts of fresh *Allium sativum* (1%, w/v) and *Ficus carica* (30%, w/v) were protected against *Pseudomonas syringae* pv. *syringae* (bacterial speck of tomato), *Xanthomonas vesicatoria* (bacterial spot of tomato), and *Clavibacter michiganensis* subsp. *michiganensis* (bacterial canker of tomato) (Balestra et al. 2009). The extracts controlled the diseases up to 65% (*A. sativum*) and 38% (*F. carica*) of that of the standard copper treatment (Balestra et al. 2009). Similarly, when cakes of *Brassica juncea* L. or neem (at 4 q ha^{-1}) were incorporated into soil, a 50% reduction in the incidence of soft rot disease (caused by *Pectobacterium chrysanthemi* Burkholder) of *Aloe barbadensis* Miller and *Aloe vera* (L.) Tourn and a four-fold increase in rhizome yield was achieved (Sharma et al. 2010). Aqueous extracts and dried powders of the medicinal plant weed, *Adhatoda vasica* were found to restrict the in-vitro growth of *R. solanacearum* as well as control BW under screen-house conditions (Din et al. 2016). It was hypothesized that the residues of plants suppress pathogen population either by improving chemical, physical and biological properties of soil or by releasing anti-bacterial compounds (Cardoso et al. 2006).

In order to make use of, on a large scale, an effective plant residue for the control of a plant bacterial disease, it is imperative that the plant in question has good anti-bacterial properties, produces a huge vegetative bio-mass in a short time, and is available at a low cost. Cocklebur, *Xanthium strumarium* L. (Asteraceae) is one such plant. It is an annual weed ranging in height from 20 to 150 cm. The stem is hairy and profusely branched giving the plant a bushy appearance (Saha et al. 2012). In Pakistan, the plant grows in waste places and along road sides producing huge biomass right after spring rains and is available free of cost. Because of its long vegetative period (March to September) and ability to quickly produce huge biomass, it is a good candidate for plant disease control through soil organic amendments. The plant possesses anti-bacterial properties and has many bio-active compounds such as alkaloids, flavonoids, quinone and others (Farooq et al. 2014). However, the ability of this plant to control plant diseases, particularly plant bacterial diseases, to the best of our knowledge, has not been reported so far. Therefore, we explored the possibility to use this plant as soil organic amendment to eliminate or reduce soilborne phase of the BW pathogen. The aim of the study was to test several hypotheses such as (i) is there any difference between the disease-suppressing ability of the residues from different parts (stems, leaves, or succulent shoot) of the plant?; (ii) is there any influence of the application time of the residue in terms of disease control?; (iii) is there a dose effect of the residue in controlling bacterial wilt?

Material and methods

Procurement of plants, preparation of plant extracts and inoculum

Plants of *X. strumarium* were collected from waste places, roadsides, fields in the outskirts of Peshawar, and authenticated by a weed scientist of the department of Weed Science, The University of Agriculture, Peshawar, Pakistan. Plant parts such as leaves, succulent shoots, and stems were separated,

rinsed with tap water and shade dried for several weeks. The brittle-dried plant parts were separately ground to fine powders. To prepare different concentrations (5%, 10% and 20% *w/v*) for in-vitro use, powders of different plant parts were separately soaked (using sterilized distilled water) in dark for 48 h. To obtain particle-free aqueous extracts, the soaked powder suspensions were filtered through clean muslin cloth, and the solid residues were discarded.

To prepare the bacterial inoculum to be used in various experiments, a pre-identified, characterized and −80 °C-preserved pure culture of the pathogen (Din et al. 2016; Khan et al. 2019; Najeeb et al. 2019), obtained from the pathogenic plant bacterial culture collection of the department of Plant Pathology, The University of Agriculture, Peshawar, was grown on nutrient agar (NA) plates, at 27 °C for 48 h. The surfaces of the NA plates were then flooded with 0.85% sterilized saline solution, scraped with rubber spatula and the resulting suspension was adjusted to 10^8 cfu/ml (OD_{600} = 0.3; Lin et al. 2014). This suspension was used as inoculum for subsequent experiments.

In-vitro studies

To test the extent of the in-vitro growth inhibition of the bacterial pathogen by different concentrations of the water extracts of the dried powders of leaves, succulent shoots and stem of *X. strumarium*, agar well diffusion method (Perez et al. 1990) was used. To prepare bacterial lawns for the in-vitro experiment, 100 μl of the bacterial suspension (10^8 cfu/ml, i.e.OD_{600} = 0.3; Lin et al. 2014) were placed per NA plate and spread uniformly using sterilized bent glass rod. Using sterilized cork borer (9 mm diameter), four wells along the circumference and one in the center were punched in the NA medium. Four of the five wells of each plate were filled with water extracts of three different concentrations (100 μl each), one (positive control) with 100 μl (100 ppm) streptomycin and one (negative control) with 100 μl sterilized distilled water (SDW). The plates were incubated at 27 °C overnight. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition (mm). The relative antibacterial potency of the given preparation was calculated by comparing its zone of inhibition with that of the SDW and the antibiotic. Completely randomized design with factorial arrangement having four replications per treatment was used.

Screen house studies

Growing tomato plants, inoculation of the potted soil and transplantation

To grow pathogen-free tomato plants, large earthen pots were filled with pasteurized (100 °C for 6 h; Naz et al. 2015a) field soil (sand: clay: silt; 30%:23%:47%) and tomato seeds (cv. Rio Grande) were directly sown in. Irrigation and fertilizer requirements were fulfilled as per horticultural recommendations. Small plastic pots (15 cm-diameters; each having I kg soil) were filled with field soil (Aysan et al. 2003; Khan et al. 2019; Najeeb et al. 2019). The soil of each pot was mixed with the respective treatment powder, pre-moistened and poured with 35 ml of the bacterial suspension (as described before). One-month-old, vigorous and healthy tomato seedlings were then transplanted to these plastic pots. Each pot received one transplant.

Influence of dried powders of leaves, stems, succulent shoots and their doses

Dried powders (0 g, 15 g, 30 g, and 45 g/kg potted soil) of leaves, succulent shoots and stem of *X. strumarium* were thoroughly mixed with the field soil of each pot before inoculation and transplantation. Total of 12 treatments (4 × 3 = 12) were used and each treatment was replicated eight times using factorial Completely Randomized Design. Total of 96 pots were divided into three sets of 32 pots each. One set (32 pots) received leaves powder, the second set (32 pots) received succulent shoot powder and third set (32 pots) received stem powder. Among the set of 32 pots, eight pots were amended with 0 g, eight with 15 g, eight with 30 g and eight with 45 g plant powder. The weather was rainy (frequent spring rains) during the whole duration of the experiment, so, potted plants were watered (irregularly) as needed. Plants were fertilized with 100 ml (per pot) 0.1%, 20-5-32 + micronutrients hydrosol fertilizer (Engro Crop Ltd) only once at the beginning of the experiment (Kokalis-Burelle et al. 2005). The experiment was terminated 60 days after transplanting. Data were recorded on plant shoot and root length (by measuring the length of the main shoot and root using transparent plastic ruler), plant fresh bio-mass (taking the weight of the entire plant using electronic balance), and disease severity. The experiment was repeated concurrently in another location a few km away in March–April; 28 ± 5 °C (maximum temperature) with no modifications.

Influence of time of application and different doses of dried powder of succulent shoot

To test the influence of time of application of the dried powder of succulent shoots of *X. strumarium* on the control of BW, four doses (as in experiment 1) of dried powder of succulent shoots were applied to potted soil 0 days, 10 days and 20 days before transplanting (DBT). Mixing of the doses (powder) with potted soil was done before pre-moisting and artificially inoculating the soil of each pot. There were total of twelve (3 × 4 = 12) treatments and each treatment was replicated eight times using factorial CRD. The 96 pots of the experiment were divided into three sets of 32 pots each. One set (32 pots) received succulent shoot powder 20 DBT, second set (32 pots)

received succulent shoot powder 10 DBT and the third set (32 pots) received succulent shoot powder 0 DBT. Among the set of 32 pots, eight pots were amended with 0 g, eight with 15 g, eight with 30 g and eight with 45 g plant powder. The timing and incubation of the experiment were the same as described in experiment 1.

Assessment of disease severity

To assess disease severity over the growing season, data were recorded four times (at 15 days interval) using 1–5 disease rating scale (Wai et al. 2013). Values for each treatment were converted to disease index (%) as per Abdel-Monaaim et al. (2011)

$$DS\% = \frac{\sum n}{5N} \times 100$$

Where ∑n = summation of all categorized values (each value = category number x number of plants present in that category) for each treatment. N = number of plants per treatment; 5 = highest category of rating scale. For each treatment, area under disease progress curve (AUDPC) was calculated as per Madden et al. (2007):

$$AUDPC = \sum_{i=0}^{n} \left(\frac{X_{i+1} + X_i}{2} \right) (T_{i+1} - T_i)$$

n = total number of observations; Ti = time at i^{th} observation; $X_{i=}$ quantity of infection at i^{th} observation.

Monitoring changes in population of *R. solanacearum* in artificially inoculated soil

Soil samples were taken from all treatments and analyzed for the presences of the culturable bacteria, using 10-fold serial dilution pour-plate method. Two soil cores (12 × 1.1 cm) per pot were taken (using sterilized cork borer) from the vicinity of roots of tomato plants. The 16 soil cores of each treatment were thoroughly mixed together to make a composite sample (Schonfeld et al. 2003; Gruter et al. 2006). Three sub-samples were then taken from each composite sample. Each sub-sample was serially (10-fold) diluted up to 10^{-7}. To do this, 5 g soil was added to 45 ml of SDW, stirred and thoroughly mixed for a few minutes, using a magnetic stirrer. Using the 10^{-7} dilution, 100 μl per plate were spread on TZCNA selective medium followed by an overnight incubation at 27 °C. Four plates per sub-sample were used. To prepare TZCNA, tetrazolium chloride or TZC (0.5% w/v) was added (1 ml/100 ml) to the molten autoclaved NA medium (Goszczynska et al. 2000) before pouring into Petri plates. Bacterial colonies recovered on all four 10^{-7} plates for each sub-sample were counted, averaged and cfu g^{-1} dry soil calculated (0 days and 60 days after soil inoculation). Bacterial counts g^{-1} soil, were converted to $\log_{10}$. Decrease in the soil population of the bacterium was calculated by subtracting the final $\log_{10}$ values from the initial $\log_{10}$ values.

Statistical analysis

Treatments such as various concentrations of aqueous extracts prepared from finely ground powders of the plant parts and different doses of the ground powders applied to soil at different intervals of time were considered as independent variables. Plant yield contributors such as shoot and root length, plant fresh biomass and other parameters such as inhibition zones, disease severity and cfu g^{-1} dry soil were regarded as dependent variables. The data of the two experiments were pooled together. Pooling of data was validated by pre-t-test (showing no statistical difference) performed for the growth rate of tomato plants grown in two nearby locations with similar environment. To find out the influence of the different treatments on the control of BW, data recorded on disease severity, AUDPC (area under disease progress curve) and other parameters were subjected to analysis of variance using Statistix (Campbell and Madden 1990). Treatment means were compared using Fisher's protected least significance difference (LSD) test at $p = 0.05$ (Gomez and Gomez 1984).

Results

In-vitro studies

The results (in-vitro bacterial growth inhibition) of aqueous extracts prepared from the dried powders of plant parts (leaves, succulent shoots and stem) as well as those of different concentrations of each plant part differed significantly ($P \leq 0.05$) from each other (Fig. 1). Water extracts prepared from dried powder of leaves inhibited the in-vitro bacterial growth more than the extracts of the other parts. The least growth inhibition was obtained by extract prepared from dried powder of stem. In case of comparison of concentrations of each plant part, it was found that 20% (w/v) concentration achieved the biggest zone of inhibition, followed by 10% and 5%. Among parts-concentration combinations, the largest zone of inhibition was recorded for extract prepared from leaf powder at 20% concentration followed by 20% concentration of extract prepared from succulent shoot powder. Extract prepared from stem powder at 5% concentration gave smallest zone of inhibition.

Screen house studies

Influence of finely ground dried powders of plant parts and their doses of on plant growth parameters

The results of the dried powders of plant parts as well as their doses on all yield-contributing factors were found to be

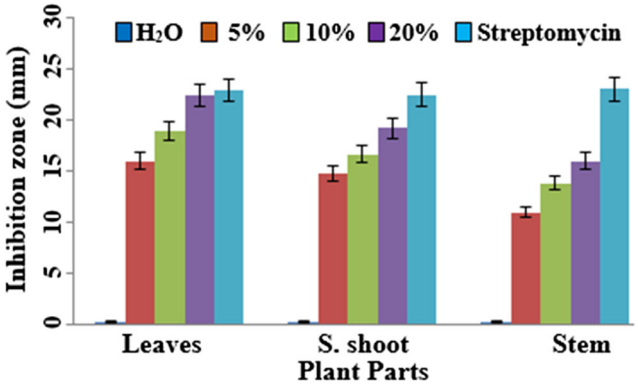

Fig. 1 Inhibition zones of in-vitro bacterial growth: Different concentrations (5%, 10%, 20% w/v) of finely ground dried powders of leaves, succulent shoots and stems of *X. strumarium* were used to prepare aqueous extracts. One hundred μl of each extract/SDW/streptomycin were placed per well, and the inoculated NA plates were incubated overnight at 27 °C. The experiment was repeated once and data were pooled

significantly ($p \leq 0.05$) different from each other. Leaf powder at the rate of 45 g/kg potted soil gave the best results followed by succulent shoot powder at the same rate. The least influence was observed on growth parameters when soil was amended with 15 g powdered stems (Table 1). Soil amended with 45 g of leaf powder improved root length (cm) by 64%, shoot length (cm) by 37%, and fresh biomass (g) of tomato plants by 42% as compared with the values of inoculated control. The lower dose (30 g/kg soil) of leaf powder also improved root and shoot lengths and plant fresh bio-mass. The corresponding percent increases for these parameters were 54%, 27%, and 31%, respectively.

Influence of times of application and doses of finely ground dried powder of succulent shoot on plant growth parameters

Both the times of application of the dried powder of succulent shoot of *X. strumarium* as well as its different doses significantly ($p \leq 0.05$) affected the yield-contributing plant growth parameters such as plant fresh biomass, shoot length and root length (Table 2). It was found that generally, the increase in doses and time of application increased the plant growth parameters. As compared to control, root length, shoot length, plant fresh biomass of the plants were enhanced by 55%, 42%, 57%, respectively, when treated with 45 g/kg soil applied 20 days before transplanting. The lowest values for plant growth parameters were recorded when 15 g/kg soil of succulent shoot powder was applied to potted soil 0 days before transplanting.

Influence of application times, plant parts and finely ground dried powder doses of *X. strumarium* on population dynamics (cfu g^{-1} dry soil) of *R. solanacearum*

The higher dose (45 g kg^{-1} soil) applied 20 DBT, significantly ($p \leq 0.05$) reduced the pathogen population (a reduction of 1.29 log $_{10}$ units) in artificially infested soil as compared to 15 g kg^{-1} soil treatment and the control (Table 3). The same dose applied 10 DBT, also achieved significant reduction in pathogen population in comparison to other doses and the control. However, the results displayed by all lower doses, applied 10 DBT and all doses applied 0 DBT were non-significant. Regarding the influence of plant parts in reducing the pathogen population in infested soil, the higher dose (45 g kg^{-1} soil) of leaf powder (applied 10 DBT) significantly ($p \leq 0.05$) reduced the pathogen population (a reduction of 1.20 log $_{10}$ units) as compared to lower doses and control. Likewise, the higher dose (45 g kg^{-1} soil) of succulent shoots powder (applied 10 DBT) also differed significantly ($p \leq 0.05$) from lower doses and the control. The results of the stem powder doses were non-significant.

Influence of application times, plant parts and finely ground dried powder doses of *X. strumarium* on AUDPC (area under disease progress curve)

It was found that, in general, higher doses and prolonged times of application decreased the AUDPC more than the lower doses and shortened application times. Treatment of 45 g/kg soil applied 20 DBT gave the lowest AUDPC values and reduced the AUDPC values by 35% when compared to those of control (0 g/kg soil) (Table 4). This treatment was followed by 45 g/kg soil applied 10 DBT which reduced the AUDPC value by 33%. The treatment 15 g/kg soil applied 0 DBT proved to be the least effective. It allowed the highest AUDPC value. Moreover, AUDPC values decreased with increase in doses. The effects of the dried powders of plant parts and those of the different doses differed significantly ($p \leq 0.05$) from each other. The treatment combination of dried powder of leaves powder at 45 g/kg soil gave the lowest (1432.5) AUDPC value (Table 4). In comparison to control, it was 38% lower. The treatment combination of dried powder of succulent shoot at 45 g/kg soil reduced AUDPC value by 28% as compared to control. Stem powder at 15 g/kg soil proved to be the least effective and gave the highest AUDPC value.

Discussion

The ability of bacterial wilt (BW) pathogen to use multiple survival mechanisms in the absence of its live host makes its management elusive. The pathogen can survive in soil, water,

Table 1 Effect of finely ground dried powders of different parts of *X. strumarium* and their doses (applied 10 DBT) on growth parameters of inoculated tomato plants

Plant Parts	Doses (g)	Plant Growth Parameters		
		Root length (cm)	Shoot length (cm)	Fresh biomass (g)
Leaves	0	14.2 ± 2.40 ghi	41.0 ± 3.94 cde	34.0 ± 2.97 cdef
	15	24.8 ± 4.29 de	47.0 ± 4.89 bcd	42.1 ± 2.92 bcd
	30	31.2 ± 2.81 bc	56.2 ± 2.47 ab	49.5 ± 4.79 ab
	45	39.8 ± 2.52 a	65.7 ± 2.75 a	59.0 ± 3.29 a
Succulent shoots	0	11.3 ± 2.10 hi	35.7 ± 1.99 de	30.9 ± 3.22 ef
	15	19.6 ± 4.41efg	40.2 ± 4.24 cde	35.5 ± 2.17 cdef
	30	25.2 ± 3.20 cde	47.7 ± 3.32 bc	42.7 ± 4.96 bc
	45	32.2 ± 3.16 b	55.5 ± 5.55 ab	52.8 ± 3.92 ab
Stems	0	10.0 ± 3.65 i	31.7 ± 4.99 e	24.5 ± 4.29 f
	15	16.4 ± 3.60fgh	35.0 ± 3.64 e	31.4 ± 2.75def
	30	21.2 ± 2.98 ef	42.7 ± 2.63 cde	37.8 ± 4.45 cde
	45	27.7 ± 2.84 bcd	49.0 ± 4.96 bc	44.8 ± 7.30 bc

Mean ± standard deviation. Means in a column sharing the same letter are not significantly different ($p \leq 0.05$) from each other (Fisher's protected LSD test). For shoot and root lengths data, main shoot and root of each plant were measured using clear plastic ruler and for fresh biomass data, weight of the entire plant was taken using electronic balance. DBT = Days before transplantation. The experiment was repeated once at the same time at a different location and the data were pooled together

seeds (Huet 2014), as well as in xylem vessels of weeds (Wenneker et al. 1999). Lack of the effective disease control chemicals (Saddler 2005; Denny 2007) and the instability of BW-resistant varieties (Hayward 1991) make the management of this disease even harder. Therefore, the use of multi-component-based IDM is the only solution to this problem. Besides other components, dried powders and green manures of different plants could be an effective (Bonanomi et al. 2007; Naz et al. 2015a, b; Din et al. 2016), environment-friendly (Qasem and Abu-Blan 1996) and affordable component of IDM against different diseases including BW. Using *Brassica juncea* L. or neem cakes as soil organic amendments (OAs), Sharma et al. (2010) increased rhizome yield of *Aloe* by four fold and reduced soft rot (*Pectobacterium chrysanthemi*) of *Aloe barbadensis* Miller and *Aloe vera* (L.) Tourn by 50%. Brassica species, if mulched into soil at flowering time, release anti-microbial substances such as isothiocynates, nitriles and thiocynates. These chemicals

Table 2 Effect of different application times and doses of finely ground dried powder of succulent shoot of *X. strumarium* on growth parameters of inoculated tomato plants

Application Times	Doses (g)	Plant Growth Parameters		
		Root length (cm)	Shoot length (cm)	Fresh biomass (g)
20 DBT	0	17.2 ± 3.58 f	38.5 ± 3.06 de	27.4 ± 4.54 ef
	15	24.7 ± 3.85 cde	50.0 ± 4.97 bc	36.3 ± 3.41cde
	30	30.4 ± 2.34 bc	58.0 ± 2.25 ab	48.6 ± 4.54 b
	45	38.4 ± 0.61 a	67.0 ± 1.69 a	64.1 ± 3.7 a
10 DBT	0	15.2 ± 4.36 f	35.5 ± 2.02 de	23.7 ± 1.78 fg
	15	21.5 ± 2.65 def	43.5 ± 3.43 cd	30.1 ± 1.36 dfe
	30	26.4 ± 3.28 bcd	50.3 ± 2.26 bc	38.4 ± 3.21 cd
	45	32.3 ± 3.75 ab	57.2 ± 3.55 ab	52.0 ± 2.86 b
0 DBT	0	14.7 ± 2.59 f	31.7 ± 2.1 e	17.1 ± 3.80 g
	15	18.5 ± 1.93 ef	37.0 ± 1.3 de	24.9 ± 2.07 fg
	30	25.2 ± 3.16 cde	43.5 ± 3.4 cd	31.5 ± 3.79 def
	45	28.2 ± 5.53 bcd	51.7 ± 4.91 bc	43.0 ± 2.11 c

Mean ± standard deviation. Means in a column sharing the same letter are not significantly different ($p \leq 0.05$) from each other (Fisher's protected LSD test). For shoot and root lengths data, main shoot and root of each plant were measured using clear plastic ruler and for fresh biomass data, weight of the entire plant was taken using electronic balance. DBT = Days before transplantation. The experiment was repeated once at the same time at a different location and the data were pooled together

Table 3 Effect of different application times and doses of finely ground dried powder prepared from leaves, stems and succulent shoots of *X. strumarium* on population dynamics (cfu/g dry soil) of *R. solanacearum* 60 days after soil inoculation

Doses (g)	Population Dynamics (cfu/g dry soil)					
	Application Timings (S. Shoot)			Plant Parts (10 DBT)		
	20 DBT	10 DBT	0 DBT	Leaves	Succulent shoot	Stem
0	0.78 ± 0.02d	0.79 ± 0.04d	0.77 ± 0.03d	0.69 ± 0.08ef	0.71 ± 0.01def	0.68 ± 0.02f
15	0.99 ± 0.03bc	0.84 ± 0.01 cd	0.81 ± 0.02d	0.89 ± 0.03 cd	0.75 ± 0.04def	0.72 ± 0.01def
30	1.08 ± 0.08abc	0.97 ± 0.02bcd	0.88 ± 0.05 cd	0.98 ± 0.07bc	0.87 ± 0.04cde	0.79 ± 0.04 def
45	1.29 ± 0.09a	1.19 ± 0.04ab	0.97 ± 0.03bcd	1.20 ± 0.09a	1.09 ± 0.05ab	0.88 ± 0.02 cd
LSD	0.26			0.19		

Mean ± standard deviation. Means in a column sharing the same letter are not significantly different ($p \leq 0.05$) from each other (Fisher's protected LSD test). DBT = Days before transplantation. Each value is an average cfu/g dry soil (initial $\log_{10}$-final $\log_{10}$). The experiment was repeated once at the same time at a different location and the data were pooled together

significantly reduce soil populations of *R. solanacearum* (Arthy et al. 2005). Likewise, the addition of *Thymus* spp. to soil and its decomposition released the volatile compound thymol which effectively controlled BW of tomato (Pradhanang et al. 2003; Ji et al. 2005; Ji et al. 2007).

The results of our studies indicated that the application of dried powder of *X. strumarium* to soil at different times and rates significantly improved plant growth characters, reduced bacterial counts g^{-1} soil and decreased AUDPC values. Higher doses of finely ground dried powders of leaves, when applied 20 days before transplanting (20 DBT), were found to be superior to the dried powders of other plant parts used at lower rates and applied fewer days before transplanting. Naz et al. (2015a, b) obtained similar results when green manures or dried powders of *Fumaria parviflora* were applied to soil. The organic amendment suppressed root-knot nematode (*Meloidogyne incognita*) populations in tomato crop as well as improved plant growth parameters. The most obvious reason for the ability of *X. strumarium* to control BW would be the presence of anti-microbial, bio-active secondary metabolites. Common bio-active compounds present in this plant include alkaloids, flavonoids, terpenoids, saponins, tannins (Devkota and Das 2015), phenolics like chologenic and ferulic acids, thiazinediones (Han et al. 2006), triterpenoid saponin (Yadava and Jharbade 2007), and xanthanolide sesquiterpene lactones (Kim et al. 2003). These anti-microbial substances, released on the decomposition of organic matter, kill pathogens (Philogène et al. 2005) by different mechanisms such as rupture of cell membrane, coagulation of cell proteins, and interference with other vital functions (Sikkema et al. 1994). Flavonoids usually affect proteins such as extracellular proteins, cytoplasmic proteins or enzymes (Al-Obaidi 2014). Alkaloids damage DNA or inhibit enzymes or both (Tanaka et al. 2006). Similarly, saponins have been reported to react with the sterol component of cell membrane (Wang et al. 2000).

Dried powders of plants, in addition to having antimicrobial substances, could contain natural elicitor compounds

Table 4 Effect of various doses of finely ground dried powders prepared from leaves, succulent shoots and stems of *X. strumarium* and their application times on disease severity (AUDPC)

Doses (g)	AUDPC					
	Plant's Part Powdered (applied 10 DBT)			Application Timing (S. shoot powder)		
	Leaves	S. shoot	Stem	20 DBT	10 DBT	0 DBT
0	2340.1 ± 4.35b	2377.5 ± 4.35a	2370.0 ± 8.70a	2550.0 ± 4.21b	2565.1 ± 6.92a	2572.5 ± 6.08a
15	1920.0 ± 4.35f	2062.5 ± 1.73d	2137.5 ± 5.10c	2182.5 ± 2.64e	2325.2 ± 3.60d	2400.0 ± 8.88c
30	1695.2 ± 4.35i	1867.5 ± 3.46 g	2025.0 ± 8.80e	1972.5 ± 3.60 h	2002.5 ± 3.60 g	2130.0 ± 7.00f
45	1432.5 ± 1.73j	1710 ± 8.88 h	1875.0 ± 5.13 g	1635.0 ± 4.08 k	1717.5 ± 4.35j	1837.5 ± 4.35i

Mean ± standard deviation. Means in a column sharing the same letter are not significantly different ($p \leq 0.05$) from each other (Fisher's protected LSD test). For disease severity, data were recorded four times (at 15 days interval) using 1–5 disease rating scale (Wai et al. 2013). Area under disease progress curve (AUDPC) was calculated as per Madden et al. (2007). DBT = Days before transplantation. The experiment was repeated once at the same time at a different location and the data were pooled together

which act as SAR activators to trigger the inactive defense systems of plants (Kagale et al. 2004; Walters et al. 2005; Hassan et al. 2009; Mitra and Paul 2017). For example, aqueous extracts of *Hibiscus sabdariffa*, *Punica granatum* and *Eucalyptus globulus* were found to have both the antimicrobial compounds which inhibited the in-vitro growth of the BW pathogen as well as SAR-eliciting compounds, which elicited strong systemic resistance in potato plants against bacterial wilt (Hassan et al. 2009). Aqueous leaf extract of *Datura metel* was also reported (Kagale et al. 2004) to have both anti-bacterial compounds and SAR-activating compounds. It inhibited the in-vitro growth of *Xanthomonas oryzae* pv. *oryzae* (Xoo) and activated SAR against bacterial leaf blight of rice. Moreover, the addition of finely ground dried powders of plants, when added to soil, improve chemical and physical attributes of soil including water holding capacity, compactness, ion adsorption and soil pH buffering (Brady and Weil 1999; Mazola 2002). These factors influence pathogen's viability and distribution in soil, nutrient availability and the release of bio-active substances from crop residues as well as soil microbes (Huang et al. 2006). More importantly, the addition of organic amendments to soil enhances the activities and abundance of decomposers. Obviously, an OA left in soil for longer time would result in enhanced activities and numbers of soil microbes. This might explain why our results of 20 DBT were significantly better than other application times. The effects of the amendments are more pronounced in the top 0–5 cm soil but can be extended to deeper layers via mixing (Treonis et al. 2010). The soil microbes whose activities and numbers increase as a result of addition of organic amendments to soil may have a general mechanism of action against the pathogen or be pathogen-specific, having a particular mechanism of action. The enhancement of a select group of microbes is usually because of the host or the pathogen (Mazola 2002; Huang et al. 2006). For example, certain wheat genotypes selectively enhance the populations of specific 2,4-DAPG (antibiotic)-producing pseudomonads, resulting in the control of take-all disease of wheat (Mazola, 2002). Similarly, in comparison to control treatment, there was a significant increase in the number of rhizosphere microorganisms in amended soils particularly antagonistic to *Verticillium dahlia* (Huang et al. 2006).

Our results indicated that the effect of the finely ground dried powders of *X. strumarium* was dose-dependent; greater doses produced better effect against BW of tomato than smaller doses. These results are in agreement with those of other researchers. Naz et al. (2015a), for example, proved that increasing doses of *Fumaria parviflora* accordingly decreased nematode galls, GI, egg masses and females present in the roots of infected tomato plants. The higher dose of 30 g/kg potted soil controlled root-knot nematodes and increased plant growth parameters more than the lower doses under both green house and natural field conditions. Our results also showed that the application time of 20 DBT was better than 10 DBT. We speculate that in case of 20 DBT, plant powders decomposed for relatively longer time, thus releasing more bio-active secondary metabolites. Moreover, the pathogen got exposed for relatively longer time to these metabolites, resulting in better disease control. Also, the activities and numbers of antagonistic microbes were more pronounced in this treatment combination than others, resulting in lower AUDPC and enhanced yield-contributing parameters. Aliyu et al. (2011) reported similar results. When they amended soil with a relatively bigger dose (12.5 g kg^{-1} soil) of neem leaf powder, they achieved more reduction in disease severity of cowpeas and more increase in plant growth parameters.

The ability of this weed to produce a very large bio-mass in a short time which is available cost-free, its long vegetative phase (March–September), the long shelf-life of its finely ground powder at room temperature (unpublished data) and its effectiveness against BW of tomato and other economic crops, makes it a low cost disease management tool for our poor local farmers. Several additional steps could be taken to further enhance the disease management efficacy of this tool. For example, the soil-borne inoculum of the pathogen could be effectively reduced by plastic mulching of the dried powder-mixed moistened soil during hot summer days before tomato transplantation. The pathogen is heat-sensitive and is killed at soil temperature of 45 °C or above for 2 days (Kang et al. 2007). The temperature of many tomato-growing areas of Pakistan is quite high and this target temperature of 45 °C could be easily achieved through plastic mulching. The extra heat produced by plastic mulching will also enhance the decomposition of organic matter and trap the volatile compounds released (Bonanomi et al. 2007). To reduce the input cost, the powder could be target-applied to individual tomato plants as cheap local labor is available. Our data suggested that the disease suppressiveness of the dried powder was dose-dependent. The maximum safe dose that we used was 45 g/kg soil. However, after doing phytotoxicity studies, this dose could be further increased to achieve a better disease control. Powder particle size seems to be important for its bioactivity. Our preliminary results indicated (unpublished data) that the in-vitro bacterial growth inhibition zones produced by aqueous extracts of very fine powders were significantly bigger than those produced by aqueous extracts of relatively coarse powders. This suggests that more complete mechanical disruption of the plant material probably releases more antibacterial substances resulting in bigger inhibition zones. So, use of very fine powder could further enhance its disease-control ability. Additionally, the incorporation of this medicinal weed as a dried powder or green manure in seed-bed soils could produce disease-free tomato transplants. And pathogen-free transplants would ensure a healthy crop resulting in higher yield. To control many rice diseases, rice farmers in Pakistan routinely incorporate neem (*Azadirachta indica*)

leaves in seed-bed soils to produce disease-free seedlings. To explore the possibility of synergistic effect, the finely ground powders of *X. strumarium* could be combined with the powders of other medicinal plants or with small amounts of commercial bactericides such as copper fungicides. This strategy would also discourage the development of chemical resistance in the pathogen. Thus, our findings suggest that *X. strumarium* as an OA used alone or in combinations with other treatments could prove to be an effective, low-cost disease management tool for BW in tomato and possibly other crops.

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