Research Article

Impact of Organic Soil Amendments Using *Nerium oleander* Powder on Bacterial Wilt Severity in Tomato

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Abstract | This study evaluated the efficacy of dried *Nerium oleander* powder in controlling bacterial wilt (BW) in tomatoes. The experiment applied the powder to pathogen-contaminated soil using mulching and mixing techniques, testing two particle sizes (fine and coarse) at four doses (0 g, 14 g, 28 g, and 42 g). Laboratory results showed that all powder concentrations significantly inhibited pathogen growth compared to the control, with the 15% powder extract achieving the highest inhibition zone (16.25 mm), followed by the 10% extract (12.47 mm). The implementation of this approach resulted in significant enhancements in the length of tomato plant roots (98.54%), shoot length (23.11%), and fresh biomass (18.97%) as compared to the control group that did not get any treatment. In addition, the smaller particle size at the higher dosage (42 g/kg soil) functioned better than the larger particle size, leading to increases of 50%, 33.04%, and 31.26% in root length, shoot length, and fresh biomass, respectively. The finely ground powder's effective integration with soil facilitated the rapid breakdown of organic matter, thereby enhancing the release of volatile and non-volatile compounds with bactericidal properties. Consequently, this process effectively suppressed pathogen populations in the soil, contributing to the control of bacterial wilt. Based on these findings, adding 42 g of dried N. oleander powder per kilogram of soil has the potential to effectively suppress bacterial wilt in tomatoes and potentially other vegetables. These findings suggest that dried *Nerium oleander* powder, particularly at a dosage of 42 g/kg soil, holds promise as an effective organic solution for controlling bacterial wilt in tomatoes.

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Introduction

Tomato (*Solanum lycopersicum*) is a globally significant vegetable (Aldubai *et al*., 2022). It ranks sixth in the world's vegetable consumption, with about one-fourth of the 159 million tonnes of fresh tomatoes produced annually being used for processing (Huang, 2004).The remaining tomatoes are consumed either raw or as important in numerous recipes (Gentilcore, 2010). Tomatoes are renowned for their nutritional value, containing important minerals such as iron, phosphorus, and magnesium, as well as antioxidants like lycopene, provitamin A (-carotene), and vitamins E and C (Kumar *et al*., 2012). Brazil, China, Egypt, USA, Turkey, Iran, Italy, and India are the leading tomato-producing nations, collectively accounting for more than 74% of global tomato production (Robertson and Labte, 2006).

In Pakistan, tomatoes are grown twice a year in all four provinces, during the spring and autumn seasons (Ali *et al*., 2020). However, Pakistan's tomato yield remains insufficient compared to other countries worldwide (Noonari *et al*., 2015). Pakistan cultivated 60.5 thousand hectares of tomatoes, yielding 561,900 tonnes of tomatoes overall (Burfisher, 1984). During periods of tomato shortage, the country imports tomatoes from Afghanistan, Iran, and India to meet the high demand (Mesgaran and Azadi, 2018). Pakistan currently produces 10 tonnes of tomatoes per acre, in contrast to other major tomato-producing nations. The low production of the crop can be attributed to various infections that occur during the growing season, including nematodal, bacterial, fungal, and viral diseases (Hussain and Abid, 2011).

This study focuses on a pathogen, a type of bacteria capable of long-term survival in soil (Nesme and Simonet, 2015). It causes significant wilting and poses a serious threat to several important crops, including bell peppers, chili peppers, peanuts, potatoes, tomatoes, and eggplants (Kurabachew and Ayana, 2017). This pathogen is known by various names, including Southern bacterial blight, *Ralstonia solanacearum* wilt, and Southern bacterial wilt. Infected tomato plants exhibit symptoms such as vascular browning, necrosis, and wilting (Champoiseau *et al*., 2009). The foliage of infected plants initially shows signs of bacterial wilt (Sarkar and Chaudhri, 2016). The youngest leaves near the tips of branches are most commonly affected by wilting symptoms, especially during the hottest parts of the day (Billing, 2011). This leakage suggests the possible presence of bacterial cells in the damaged vascular bundles, particularly in the xylem (Reusche *et al*., 2012).

Biological control measures can be integrated with other strategies or utilized to facilitate the development of alternative methods for managing diseases at the field level (Heydari and Pesarakli, 2010). Incorporating biological management is essential to minimize environmental contamination from pesticides, as it not only aids in disease control but also enhances crop yields (Popp *et al*., 2013). Moreover, attempts to combat the ailment through host-plant resistance using cultivars resistant to bacterial wilt have been made, albeit with varying levels of success (Sahu *et al*., 2017).

Plant extracts, owing to their lower environmental impact compared to synthetic pesticides, represent a superior approach to disease management, particularly for bacterial infections (Thakur *et al*., 2021). The utilization of plant extracts as a sustainable alternative to synthetic pesticides in plant disease control, especially for bacterial infections, is gaining popularity (Khursheed *et al*., 2022). The inherent antibacterial properties of plant extracts provide a practical avenue for combating bacterial infections while reducing chemical residues in crops (Srivastava *et al*., 2022; Rahman *et al*., 2022).

Continued research aimed at identifying and optimizing effective compounds derived from plants will continue to shape the future of environmentally conscious disease management in agriculture (Ahmed *et al*., 2023).

Nerium oleander, a member of the Apocyanaceae family, is valued not only for its ornamental qualities but also for its potent antibacterial properties, making it a viable candidate in integrated disease management (IDM) strategies. Known for its perennial nature and resilience, *N. oleander* produces biologically active compounds that effectively target bacterial wilt pathogens, especially *Ralstonia solanacearum*. The plant's phytochemicals, particularly concentrated in powdered formulations, exhibit prolonged antibacterial action, positioning it as a promising, long-lasting tool in agricultural disease control (Ayouaz *et al*., 2023; Chandramohan *et al*., 2019). By leveraging these medicinal properties, N. oleander stands out as a sustainable choice for mitigating soil-borne diseases in crops.

The objective of the study was to check different amounts of *Nerium oleander* powder formulations with both fine and coarse particle sizes which were applied to potted soil that was infected with *Ralstonia solanacearum* in order to control tomato bacterial wilt.

Materials and Methods

Collection of medicinal plant

In District Peshawar (KP) green tops, also known as succulent shoots, of the *Nerium oleander* plant were collected. These shoots consisted of leaves, flower buds, soft wooded branches, etc. After collection, the plant was authenticated by a professional botanist.

Plant extract formation

The selected plant parts were air-dried until they became brittle, washed with tap water, and then finely chopped. Subsequently, the chopped fragments were ground into a coarse powder using an electric grinder with a mesh size of approximately 1000 μm. Pot tests were conducted using this coarse powder (both in vivo and in vitro). To obtain a finer powder with a mesh size of 1000 μm, the coarse powder underwent a second grinding process. The fine powder obtained was used to prepare water extracts. Quantities of 5, 10, and 15 grams of fine powder were mixed with 95, 90, and 85 milliliters of sterilized distilled water, respectively, to get concentrations of 5% (weight/volume), 10% (weight/volume), and 15% (weight/volume). The suspensions were placed at room temperature and stirred continuously with a magnetic stirrer for a duration of 48 hours. Filtration was performed to remove solid particles from the suspensions.

Isolation of bacterial culture and production of mass-inoculum

The UAP's Department of Plant Pathology provided a pure culture of *Ralstonia solanacearum* bacteria acquired from preserved plant pathogenic bacterial cultures. In order to verify the pathogenicity of the culture after storage, it was cultivated on a medium called tetrazolium chloride nutritional agar (TZCNA). A pathogenic colony with a liquid appearance and a pink center on TZCNA was chosen and grown in large quantities on nutritional agar media (NA) by incubating it for 48 hours at a temperature of 27°C. Abundant bacterial proliferation was seen on the nutrient agar plates. To eliminate bacterial growth, a small amount of sterile distilled water (SDW) was poured onto each plate and then meticulously removed along with the bacteria using sterile cotton balls. A 0.85% brine solution was added to the bacterial and water mixture, and the mixture was gently stirred using a magnetic stirrer to create a consistent suspension. The bacterial concentration per milliliter was measured using a spectrophotometer set to an optical density of 600 (OD 600). Following the adjustment of the concentration to OD600 = 0.3, which is equivalent to 10^8 colony forming units per milliliter (cfu/ml), this standardized concentration was utilized for all future *in vitro* and *in vivo* experiments (Chandramohan *et al*., 2019).

In-vitro antibacterial activity

The antibacterial potential of the aqueous extract of *Nerium oleander* was evaluated by generating concentrations of 5%, 10%, and 15% fine powder and examining their antibacterial activity in a controlled laboratory setting. A culture medium called nutrient agar (NA) was produced and then placed into Petri dishes. After solidification, each plate was injected with 50 μl of the prepared bacterial suspension, ensuring uniform distribution across the surface. Using a sterile cork borer, five wells were meticulously created on the surface of each inoculation plate. Each well had a diameter of 9 mm. Afterwards, 100 μl of the aqueous extract concentrations (5%, 10%, and 15%) were applied to the first three wells. To serve as a positive control, 100 μl of streptomycin sulfate (100 ppm) was introduced into the fourth well. Conversely, 100 μl of sterile distilled water (SDW) was added to the fifth well as a negative control. The diameter (in millimeters) of the zones of bacterial growth inhibition was measured after a 24-hour incubation period at 27°C. These zone diameters were used to assess the effectiveness of the extract concentrations as antibacterial agents. The data collected were arranged using five replications of a completely randomized design (CRD) before statistical analysis (Khan *et al*., 2015; Lin *et al*., 2014).

Greenhouse experiment

Role of powder doses and particle size in controlling bacterial wilt (BW) of tomato: The reduction of bacterial wilt (BW) in tomatoes was the subject of an investigation that was carried out in order to determine the effect that various doses of *Nerium oleander* powder formulation per kilogram of potted soil (0, 14, 28, and 42g) had on the growth of tomatoes. In addition, we examined two different particle sizes, specifically 250 µm and 1000 µm, to assess how they affect BW control. The soil was treated with powder 20 days before transplantation to promote sufficient breakdown of organic matter. A total of eight treatments were created, which involved the combination of two different particle sizes and four different powder dosages. This was done using a completely randomized design (CRD) with 10 replications for each treatment.

The experiment employed plastic pots of fifteen centimeters in diameter, each containing one kilogram of field dirt. One group of pots received the fine powder doses, while the other group received the coarse powder doses. This was done to ensure that the powder was thoroughly mixed with the soil. Prior to being inoculated with a 30 cc bacterial suspension, the soil in each pot was moistened. The bacterial suspension was uniformly distributed in the center of each pot, and then one-month-old tomato seedlings of consistent size and vitality were transplanted into their separate containers. The plants were thereafter fertilized and irrigated in accordance with horticultural guidelines.

Following measurements encompassed the evaluation of plant fresh biomass, root and shoot lengths, and disease progression, which was observed at intervals of fifteen days. Moreover, fluctuations in the number of colony-forming units (cfu) per gram of soil were noted during the duration of the experiment.

Role of powder doses and application methods in controlling BW of tomato

Two application methods were employed to investigate possible impacts on bacterial wilt management: thorough mixing and surface mulching. The powder preparation was meticulously mixed with the dirt in the pot. Afterwards, the powder was given in four distinct quantities (0, 14, 28 g, and 42 g per kilogram of potted soil). The trial consisted of eight treatment groups, achieved by utilizing a mix of these two application modalities and varied doses. The experiment consisted of ten replicates for each treatment, using a completely randomized design (CRD). Out of the total of eighty pots used in the experiment, forty pots were subjected to top-mulching with the powder, while the other forty pots had the dried powder evenly incorporated into the soil. The experiment ensured consistent parameters, such as the uniform size of plastic pots, standardized soil filling techniques, controlled artificial inoculation, transplantation of tomato seedlings, regular watering, fertilizer, and systematic collecting of plant characteristic data.

Effect of treatments on changes in CFU/g soil

The experiment involved measuring bacterial counts per gram of soil on two occasions: Once after the initial inoculation and again at the end of the experiment. The difference between the final and initial counts was calculated, and the values were then transformed into a logarithmic (log10) scale. The objective of the study was to investigate the potential of different treatments, which varied in the quantity of powder preparation applied and the application methods used, to decrease the population of bacterial pathogens in the soil.

Final colonies-initial colonies than converted to (log10)

Soil population of *R. solanacearum*: Soil samples were collected using a 10 mm diameter cork borer, which was inserted to a depth of 10 cm. The objective was to ascertain the initial and final numbers of bacteria per gramme of soil. Each plant container yielded three soil cores. The soil cores were later merged and thoroughly blended to create a composite sample, following the procedure outlined by (Chin *et al*., 2001). Subsequently, three subsets were derived from every composite sample. The subsets underwent a ten-fold series dilution, and 100 µl of the 10^-10 dilution was applied to the TZCNA (tetrazolium chloride nutritional agar) selective medium, following the procedure described by (Grüter *et al*., 2006). Following a 24-hour incubation period at a temperature of 27°C, colonies exhibiting an off-white hue with pink centres were identified as the causative agents of bacterial wilt. The colony counts obtained from these colonies were used to calculate the number of colony-forming units (cfu) per gram of soil.

Data analysis

The trials were conducted for a duration of 60 days following the transplanting before reaching a conclusion. The measurements consisted of the disease severity for each treatment, the lengths of shoots and roots (in centimeters) per plant, and the fresh weight (in grams) per plant. The statistical analysis was conducted using IBM Corp.'s Statistical Package for the Social Sciences (SPSS) version 8.1, located in Armonk, NY. The Fisher's Least Significant Difference (LSD) test was employed to ascertain the disparities among treatment means.

Results and Discussion

In vitro effect of N. oleander extract on inhibition of BW

This study aimed to assess the inhibitory potential of aqueous extracts obtained from shed dried leaves and twigs on the growth of a bacterial pathogen in a controlled laboratory environment. The extracts were evaluated at three separate concentrations (5%, 10%, and 15%), demonstrating statistically significant differences across the concentrations (P≤0.05). The water extract, comprising 15% of the solution, had notable inhibitory effects, demonstrating potent antibacterial properties against the bacterial pathogen. Furthermore, the 10% water extract shown significant effectiveness in suppressing bacterial proliferation. Furthermore, significant effects were seen when comparing the 5% aqueous extract to both the control and sterilised distilled water (SDW) samples, as depicted in Table 1.

Table 1: *Effect of different doses on roots length (cm) of different particle sizes (fine and coarse) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 1).*

P. size	Doses (g)				
	0	14	28	42	Mean
Fine	5.97	8.97	11.76	14.83	10.383A (98.54)%□
Coarse	5.60	8.57	11.40	14.36	9.982 B
Mean	5.785 D	8.77 C	11.580 B	14.595 A	

LSD of doses = 0.45; LSD of particle size =0.32; □[8.97+11.76+14.83/3-5.97/5.97x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage Increase over Unamended Control Shown in Parenthesis.

Optimum particle sizes of powder preparation and its safe doses

In the study, it was discovered that there were significant differences (P≤0.05) in the impacts of two distinct plant powder sizes (250 µm and 1000 µm) on stem length, fresh biomass, and root length. These were the variables that were being investigated. By increasing the quantity of the powder preparation and extending the duration of time during which the powder was allowed to degrade prior to transplantation, a positive influence was achieved on all of the parameters that are connected with plant production. In the first experiment, plants that were treated with a dosage of 42 grams per kilogram of soil twenty days before to being transplanted exhibited significant gains in root length (98.54% cm), stem length (23.11% cm), and fresh biomass (18.97%) in comparison to the group that served as the control. The findings of Experiment II revealed that the majority of plant growth indicators continued to show an upward trend that was consistent. Additionally, the root length grows.

Optimum application methods and safe doses

The study conducts a comparative analysis on the effects of two application procedures, mulching and mixing, together with varying amounts of powder preparation (0, 14, 28, and 42g/kg potted soil) administered 20 days prior to transplantation. The statistical analysis results indicated that there were significant differences (P ≤ 0.05) seen among the treatments. According to the results, the powdered combination proved to be more efficient than surface mulching in reducing bacterial wilt and enhancing plant growth indices. The initial experiment yielded substantial improvements in the mean length of the roots (98.54 percent), the stems (23.11 percent), and the fresh biomass (18.97 percent). Experiment II demonstrated significant increases in root length (50% cm), stem length (33.04% cm), and new biomass (31.26% g). If you require additional information, please consult Tables 2–7.

Table 2: *Effect of different doses on Roots length (cm) of different application methods (Mixing and mulching) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 2).*

Application method	Doses (g)				
	0	14	28	42	Mean
Fine mixing	6.80	8.40	10.30	11.90	9.35 A (50)%□
Fine mulching	6.40	7.90	9.80	11.60	8.925 B
Mean	6.600D	8.150C	10.050B	11.75A	

LSD of Doses =0.56; LSD of Application method = 0.39 □[8.40+10.30+11.90/3-6.80/6.80x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage Increase over Unamended Control Shown in Parenthesis.

CFU/g soil as affected by powder size (Fine and coarse) and different doses

It was proved by the findings that increasing the period of powder decomposition and adjusting the dosage were both effective techniques for reducing

Table 3: *Effect of different doses on stem length (cm) of different particle sizes (fine and coarse) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 1).*

P. Size	Doses (g)				
	0	**14**	**28**	**42**	**Mean**
Fine	15.00	16.7	18.5	20.2	17.6 A (23.11)%□
Coarse	14.6	16.7	18.0	19.7	17.1 B
Mean	14.8 D	16.7 C	18.25 B	19.95 A	

LSD of doses =0.58; LSD of application methods = 0.41; □[16.7+18.5+20.2/3-15.00/15.00x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage Increase over Unamended Control Shown in Parenthesis.

Table 4: *Effect Of different doses on Stem length (cm) of different application methods (Mixing and mulching) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 2).*

Application method	Doses (g)				
	0	**14**	**28**	**42**	**Mean**
Fine mixing	11.40	13.40	15.20	16.90	14.225 A (33.04)%□
Fine mulching	10.80	12.80	14.50	16.10	13.55 B
Mean	11.10D	13.10C	14.85B	16.50A	

LSD of Doses =0.45; LSD of application times =0.32; □[13.40+15.20+16.90/3-11.40/11.40x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage Increase over Unamend Control Shown in Parenthesis.

Table 5: *Effect of different doses on Fresh biomass (g) of different particle sizes (fine and coarse) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 1).*

P. Size	Doses (g)				
	0	**14**	**28**	**42**	**Mean**
Fine	12.4	13.66	14.8	15.8	14.165 A (18.97)%□
Coarse	11.8	13.1	14.3	15.3	13.625 B
Mean	12.1 D	13.38 C	14.55 B	15.55 A	

LSD of doses =0.52; LSD of application method =0.38; □[13.66+14.8+15.8/3-12.4/12.4x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage Increase over Unamended Control Shown in Parenthesis.

the number of bacterial counts per gram of soil. This was demonstrated by the fact that the findings demonstrated a significant influence on the reduction of bacterial counts. In the first trial, it was proven that the treatment with a dosage of 42 grams resulted in the most substantial reduction in population when compared to the other treatments. A decrease of 1.99 units occurred as a consequence of the logarithm of base 10 being dropped from 11.99 to 10, which resulted in the value being reduced. The application of 28 grams of treatment resulted in a reduction in the number of bacteria that were present in each gram of soil, which is comparable to the example that was presented before. It may be deduced from the fact that the initial log10 value was lower than the end log10 value that the quantity of units has decreased. The second experiment demonstrated that the treatment with a mass of 42 grams was the most efficient in reducing the number of bacteria in the population. This was demonstrated by the fact that the effect was observed. Viewing Table 7 makes it abundantly evident that the log10 value decreased from its initial value to its final value over the course of the study.

Table 6: *Effect of different doses on Fresh biomass (g) of different application methods (Mixing and mulching) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 2).*

Application method	Doses (g)				
	0	**14**	**28**	**42**	**Mean**
Fine mixing	11.30	12.90	14.90	16.70	13.95 A (31.26)%□
Fine mulching	10.60	12.50	14.30	16.20	13.40 B
Mean	10.95D	12.70C	14.60B	16.45A	of BW

LSD of Doses =0.50; LSD of application method=0.35; □[12.90+14.90+16.70/3-11.30/11.30x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage Increase over Unamended Control Shown in Parenthesis.

Table 7: *Effect of powder application and different particle sizes (Fine and Coarse) of (Nerium oleander) on decrease in soil population density pathogen (Experiment 1).*

Application method	Doses				
	0	**14**	**28**	**42**	**Mean**
Fine mixing	1.64d	1.77 c	1.84b	1.99a (21.34)%□	1.81
Coarse	0.70h	1.03g	1.23f	1.42 e	0.93
Mean	0.85	1.40	1.53	1.70	

LSD of doses= 0.02; LSD of particle Size= 0.01; LSD of interaction= 0.030; □[1.99-1.64/1.64x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05). Percentage increase over unamended control shown in parenthesis.

CFU/g of soil as affected by application methods and doses

The data that are provided depict the results that were obtained from two different application processes, namely mixing and top mulching, with varying amounts of powder preparation. These procedures were used during the application process. It displays a higher level of efficacy in reducing the number of bacterial counts per gram of soil when the powder preparation is fully blended with the soil in the pot, particularly at larger dosages per kilogram. This is in comparison to top mulching and lesser doses, which are less effective in this regard. The statistics demonstrate that the mixing method is more effective than other approaches when it comes to the management of bacterial populations in the soil, particularly when it is applied in larger dosages.

Table 8: *Effect of powder and different particle sizes (Fine and Coarse) of (Nerium oleander) on the decrease in soil population density of BW pathogen (Experiment 2).*

Application	Doses (g)				
Fine mixing	0.88 h	2.04 c	2.26 b	2.67 a (203.40)%□	1.9650
Fine mulching	1.50 g	1.77 f	1.85 e	1.98 d	1.7767
Mean	1.1933	1.9083	2.0550	2.3267	

LSD of doses= 0.02; LSD of application methods= 0.01; LSD of interaction= 0.037; □[2.67-0.88/0.88x100]. The values in each table represent the average of four replicates. Values with the same letter are not statistically different (P≤0.05).

It was discovered in the first experiment that the number of bacterial counts fell from the beginning log10 value to the end log10 value. This was the case with the first experiment. This was shown by the fact that the number of bacteria that were present was reduced. The number of bacterial counts decreased from the initial log10 value to the end log10 value as a result of the application of top mulching treatment at a rate of 42 grams per kilogram of soil. This resulted in a reduction in the number of bacterial counts. This situation is related to the one that was discussed earlier in the presentation. Experiment II demonstrated that top mulching with the same quantity of powder preparation gave better results than fully mixing 42 grams of powder preparation per kilogram of soil. This was discovered through the process of fully mixing the powder preparation. When comparing the two approaches, this was the situation that arose. The application of 14 grams of powdered preparation as a top mulch on one kilogram of soil for potted plants resulted in the least amount of bacterial population reduction that was conceivable.

Disease severity values in % as affected by powder application methods and doses

The following tables depict the relationship between varying lengths of deterioration and different doses of *N. Oleander* powder formulation, and how they impact the severity of the sickness. The statistical analysis revealed significant variations among the treatments (P ≤ 0.05). In Experiment I, the therapy known as DBT was given 20 days before transplantation. It was supplied at a greater dosage of 42 grams per kilogram of soil and had a small particle size. This treatment resulted in the lowest level of disease severity. There was a reduction of 34.25 percent compared to the control treatment of 20 grams per kilogram of soil. Experiment II revealed that the use of this therapy resulted in a reduction of 25.25 percent. The therapy with the highest sickness severity percentage, including 44.25% of cases, will be used as the standard for future comparisons. Refer to Figures 1 and 2 for further data.

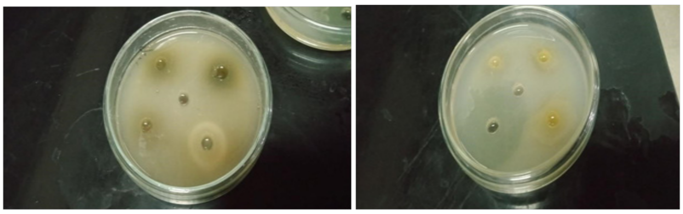

Figure 1: *Effect on disease severity of different Particle sizes (Fine and coarse) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 1).*

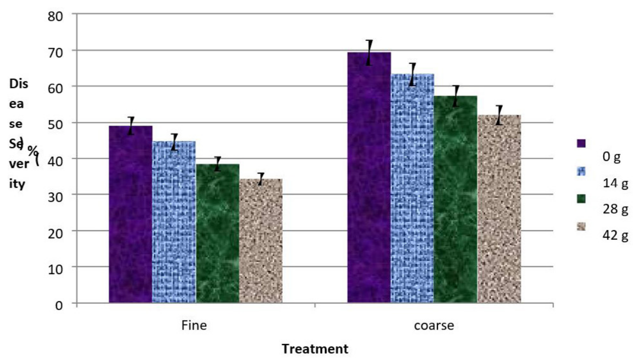

Figure 2: *Effect on disease severity of different application methods (Mix and Mulch) of (Nerium oleander) on Ralstonia solanacearum inoculated tomato plants (Experiment 2).*

Bacterial wilt, caused by a bacterium present in the soil, poses a substantial risk to tomato cultivation and other crops worldwide. *Ralstonia solanacearum* is the main causative agent of this disease, and it is most commonly found in tropical, subtropical,

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and warm temperature zones (Goszczynska *et al.*, 2000; Hayward, 1991). Bacterial wilt, caused by *Ralstonia solanacearum*, poses a significant threat to vegetable crops in Pakistan, particularly tomatoes. After a plant becomes infected, the bacteria has the ability to propagate to nearby plants via the vascular system. Controlling this disease is difficult because of its complex biology, extensive distribution, and its capacity to impact a diverse array of host plants (Pradhanang *et al.*, 2005).

Various methods, including as sanitation practices, chemical treatments, the utilization of resistant cultivars, and soil management techniques, are included in the contemporary management measures that are being implemented. Although it is possible to use chemical methods to manage *Ralstonia solanacearum*, their effectiveness is still restricted. Interventions in this context refer to actions taken to address a problem. Some examples of interventions are soil fumigation, which is the process of applying chemicals to the soil to control pests or diseases, and the use of antibiotics such as streptomycin, ampicillin, tetracycline, and penicillin, which are used to combat bacterial infections (Murakoshi *et al.*, 1984). Introducing crop rotation using crops that are not easily affected by diseases or pests is a possible method of control (Smith *et al.*, 2011). Nevertheless, it is crucial to acknowledge that no single treatment can ensure the entire elimination of the disease or provide absolute protection for host plants. Studies conducted both in laboratory settings (*in vitro*) and in living organisms (*in vivo*) have investigated the antibacterial characteristics of several plant species, demonstrating their potential effectiveness (Vashist and Jindal, 2012). Furthermore, the utilization of natural plant-based products has been helpful in the creation of novel agrochemicals designed to tackle a wide range of plant diseases (Loiseleur, 2017).

It is becoming more recognized that the technique of incorporating organic matter that possesses antibacterial properties into the soil is an efficient method for controlling illnesses that are transmitted through the soil. study has demonstrated that this strategy is effective in managing *Ralstonia solanacearum* in both controlled laboratory (*in vitro*) and living creature (*in vivo*) environments. This has been established through study. The usage of dried powder or green manure made from medicinal herbs or weeds such as *Withania somnifera, Withania coagulans,* and *Xanthium strumarium*, for example, has been reported to lower the incidence of bacterial wilt disease in tomato plants. This is one example of what has been observed (Loiseleur, 2017). Numerous plants possess secondary metabolites that have antimicrobial effects, especially those with therapeutic attributes. These metabolites show potential for being used in the creation of natural pesticides (Ahmad *et al.*, 2022). Several plant species have shown effectiveness against a range of diseases, such as bacteria, fungus, nematodes, protozoa, and viruses (Mann and Kaufman, 2012; Kareem *et al.*, 2008; Al-Shaibani *et al.*, 2008). It is possible to introduce dried powders or green manures into the soil as organic amendments by making use of the environmentally friendly and productive qualities of these plants (Pires *et al.*, 2022). Thus, effective disease management hinges on the implementation of integrated strategies that encompass several components (Rashid *et al.*, 2020). The aforementioned attributes render amendments made from cannabis highly appealing for the purpose of integrated disease management, especially for farmers in developing nations such as Pakistan who have little resources.

For our *in vitro* investigations, we utilized fresh leaf extracts obtained from dried powder of *Nerium oleander*. Peshawar boasts a substantial population of easily accessible *Nerium oleander*s. We used the powdered formulations of this plant in our in vivo experiments, as opposed to the liquid formulations. The in vitro investigation revealed that the efficacy of *N. oleander* extracts was linked to the dosage, with higher doses leading to a more pronounced suppression of bacterial growth. The aqueous extract with a concentration of 15% exhibited the greatest amount of zone of inhibition (ZI), followed by the aqueous extract with a concentration of 10%. This discovery is noteworthy because it significantly reduces the population of *Ralstonia solanacearum*, leading to improved development of disease-free transplant seedlings. Previous studies have documented similar results (Kumar *et al.*, 2023; Malik *et al.*, 2015; Jamal *et al.*, 2012). The results illuminate the substantial impact that the timing and amount of dried plant powder application have on different plant growth factors and the prevention of *R. solanacearum* infection.

When employed as an organic amendment, the efficiency of a plant-based product in managing plant diseases is contingent upon a number of factors, including the dosage and the mode of delivery. For

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example, when dried powder from *Nerium oleander* was used in studies, it was found that incorporating the powder into the soil at various rates led to a considerable reduction in bacterial wilt (BW), a drop in the number of pathogens, and an improvement in plant growth. It was observed that administering higher dosages of the dehydrated powder (42 g/kg soil) produced better outcomes in comparison to lesser doses provided in proximity to transplanting. Similarly, research on *Adhatoda vasica* and *Calotropis procera* has documented similar advantages, as well as the use of mulching strategies including flowering-stage Brassica species.

Scientists are investigating chemical substitutes in response to the increasing environmental contamination caused by agricultural chemicals and the growing prevalence of pathogen resistance. Multiple investigations (Jasim and Sultan, 2023; Lo Cantore *et al*., 2004) have discovered a wide range of plant species that possess strong antibacterial capabilities. In addition, Iacobellis *et al.* (2005) proposes that natural plant products pose lower threats to the environment and public health in comparison to industrial agricultural chemicals. The use of these plants in different ways, such as green manure, finely powdered supplements for soil, or extracts for foliar sprays, holds potential in reducing bacterial plant diseases such as bacterial wilt (BW).

The study emphasizes the increased efficacy of larger doses of powdered formulations, which have important practical consequences. *Nerium oleander*, due to its ready availability and perennial nature, offers a feasible choice for efficiently mitigating bacterial wilt (BW) in tomato crops when administered in higher quantities. Our research highlights the effectiveness of powdered preparations as important elements in integrated disease management (IDM) methods against bacterial wilt disease. When tomato plants were subjected to doses of up to 42 grammes, they did not exhibit any negative effects. In spite of this, it is essential to exercise caution when assessing the potential levels of plant toxicity prior to making considerable use of it in any crop. As a result of our experiments, we discovered that the powder formulations retained a powerful antibacterial action for more than two years when they were stored at room temperature. This makes it a long-lasting instrument for the management of bacterial wilt. Powder may be easily applied to each tomato plant, allowing Pakistani labour to be utilized in a manner that is both efficient and cost-effective. In addition, the incorporation of the dehydrated powder into plastic mulch on hot and sunny days has shown promise in effectively preventing soil-borne pathogens such as *R. solanacearum*. This is particularly true when the soil temperatures climb to 45 degrees Celsius for extended periods of time.

Conclusions and Recommendations

The results of our study emphasize the increased efficacy of larger doses of powdered formulations, which have important practical consequences. *Nerium oleander*, due to its ready availability and perennial nature, offers a feasible choice for efficiently mitigating bacterial wilt (BW) in tomato crops when administered in higher quantities. This highlights the effectiveness of powdered preparations as important elements in integrated disease management (IDM) methods against bacterial wilt disease. When tomato plants were subjected to doses of up to 42 g, they did not exhibit any negative effects. In spite of this, it is essential to exercise caution when assessing the potential levels of plant toxicity prior to making considerable use of it in any crop. As a result of our experiments, we discovered that the powder formulations retained a powerful antibacterial action for more than two years when they were stored at room temperature. This makes it a long-lasting instrument for the management of bacterial wilt. Powder may be easily applied to each tomato plant, allowing Pakistani labor to be utilized in a manner that is both efficient and cost-effective. In addition, the incorporation of the dehydrated powder into plastic mulch on hot and sunny days has shown promise in effectively preventing soil-borne pathogens such as R. solanacearum. This is particularly true when the soil temperatures climb to 45 degrees Celsius for extended periods of time. Future research should investigate the long-term impacts of *Nerium oleander* powder on soil health and crop yield while assessing optimal application methods to maximize its antibacterial efficacy and minimize potential toxicity in various environmental conditions.

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Novelty Statement

The novelty lies in the exploration of Nerium oleander, a plant typically overlooked in agricultural contexts, as a sustainable organic soil amendment. This study investigates its unique biochemical properties and effects on soil health, nutrient availability, and plant growth, providing insights into an innovative use of a commonly available resource.

Author's Contribution

Aamir Sohail, Sidra Ahmad and Mehran Gul: Conceived and wrote the manuscript.
Irshad Ali Khan, Ahmed Shamkhi Jabbar, Nayab Ahmad, Farzana, Abdur Rehman, Arooba Bashir, Syed Muhammad Owais Shah and Ayesha Alam: Revised the manuscript.
All authors have read and agreed to the published version of the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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