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Research Article

CHEMOTHERAPEUTIC MANAGEMENT OF *RALSTONIA SOLANACEARUM* CAUSING BACTERIAL WILT OF TOMATO

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Abstract

Bacterial wilt is an important disease of tomato caused by *Ralstonia solanacearum*, which causes 90% yield losses. The present research was done to evaluate the effect of different chemicals against bacterial wilt of tomato. By using inhibition zone technique, five chemicals (Oxyrich, Forum top, Electus Super, Cabrio-Top and Kocide) at three different concentrations (250, 300 and 350 ppm) along with control were evaluated against *R. solanacearum* under lab conditions with Complete Randomized Design (CRD). Among all the treatments, maximum inhibition zone was expressed by Oxyrich (20.687mm). Under field conditions using Randomized Complete Block Design (RCBD), Oxyrich and Kocide were evaluated alone and in combination at three different concentrations (2, 2.5 and 3%) along with control. Among these treatments, maximum reduction in disease incidence was expressed by combination of Oxyrich + Kocide (18.38%). Difference among treatments was observed using least significant difference (LSD) at probability level of 0.05%. It is concluded that identification of different chemicals will be helpful in future studies for the management of bacterial wilt disease of tomato.

Keywords: *Solanum lycopersicum* chemicals, Inhibition zone technique, Disease incidence. Forum top

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1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is the most consumed vegetable in the world and comprises of important nutrients such as vitamin A, vitamin C, potassium, carbohydrates and folates (Wu *et al.*, 2022). It also contains carotenoids, antioxidants, ascorbic acid, phenolic compounds, lycopene and tocopherols which helps to prevent cancer, diabetes and cardiovascular diseases (Capobianco-Uriarte *et al.*, 2021). It is cultivated on an area of 5 million hectares worldwide with production of 186 million tonnes while in Pakistan, it is cultivated on an area of about 57 thousand hectares with annual production of 594 thousand tonnes (FAOSTAT, 2020).

Tomato crop is prone to attack by a number of diseases that lower the production and damage quality of the produce, but the most important and the devastating one is tomato bacterial wilt which is caused by soilborne pathogen, *Ralstonia solanacearum* and is responsible for yield reduction up to 90% (Campos *et al.*, 2022; Kemboi *et al.*, 2022). Drooping of upper leaves, yellowing of lower leaves and wilting of the entire plant are the characteristic symptoms of tomato bacterial wilt (Chattopadhyay *et al.*, 2022). The presence of excessive bacterial colonies blocks the xylem vessels and prevents the sufficient amount of water to reach the leaves which results in wilting (Choudhary *et al.*, 2018). When the infected stem or root is cut, bacterial ooze and sometimes brown discoloration is also observed (Manda *et al.*, 2020). *Ralstonia solanacearum* is a gram-negative, tiny straight rod like bacterium with 1.5–3.12µ×0.25–2.5µ size and exhibits smooth, round, elevated and filthy white colonies (García *et al.*, 2019). It can survive and persists in the soil for a longer time period of up to 8 years (Zhang *et al.*, 2020). It enters the plants through roots, wounds, organic openings and colonized the plants (Lowe-Power *et al.*, 2018). It can spread through irrigation, farm machinery, crop debris and host weeds (Hasnain *et al.*, 2020).

Scientists, researchers, experts and growers have utilized a number of management approaches such as cultural control, chemical control, biological control and other field management strategies (Ahmed *et al.*, 2022). Among these approaches, the use of resistant varieties is the most effective and environmentally sustainable method for managing this disease but under favourable environmental conditions, these resistant cultivars may become susceptible and the disease appears in an epidemic form (Aslam *et al.*, 2017). In this situation, farmers have no other option except the use of chemicals. Chemicals are easy to use, give a quick response against the disease (Mihajlović *et al.*, 2017). A study was conducted in which different chemicals such as copper hydroxide, copper oxychloride, kasugamycin and streptocycline were evaluated against *R. solanacearum.* The results revealed that copper hydroxide followed by copper oxychloride was effective (Revathi *et al.*, 2017). Another study was conducted in which thymol and acibenzolar-S-methyl were used and the results showed that the disease incidence and severity was significantly reduced (Ganiyu *et al.*, 2018). Keeping in view the importance of chemicals, the present study was designed to evaluate the efficacy of five different chemicals against bacterial wilt of tomato.

2. MATERIALS AND METHODS

2.1. Collection of infected Samples

Tomato samples infected with bacterial wilt were collected from different areas of district Faisalabad. The samples were collected in the zipper bags and brought to the Phyto bacteriology lab, Department of Plant Pathology, University of Agriculture, Faisalabad for further process. Precautionary measures were followed during the whole procedure.

2.2. Isolation, Purification and Identification of Pathogen

For isolation of pathogen, Nutrient Agar media was prepared. To prepare 1 litre NA media, 29 g of synthetic NA was mixed thoroughly in 1000 mL of distilled water. The media was autoclaved at 121^{o}C temperature at 15 Psi pressure for 15 minutes. The media was then poured in the Petri plates and allowed to solidify. The samples were washed with tap water to remove the dust. The diseased samples along with some healthy portion were cut into small pieces of 2-3 mm in size. Surface sterilization of the samples was done through 1% Sodium hypochlorite solution followed by two consecutive washings with distilled water in order to remove the residual effects of NaOCl solution. The samples were dried on the blotter paper and transferred to Petri plates containing NA media with the help of sterilized forceps in the laminar air flow chamber. After that, plates were wrapped, labelled and incubated for 24 hours at 25-28^{o}C. After 24 hours, filthy white bacterial colonies in the Petri plates were observed (Ranjan *et al.*, 2021).

Purification of the pathogen was done through streaking method. Using a sterilized loop, bacterial colony was picked and streaked on another fresh media plate in a zig zag manner. After 24 hours, single colony was picked and again purified using streaking method on another media plate (Ranjan *et al.*, 2021). Identification of the pathogen was done on the basis of morphological characteristics such as colony colour, spore size and growth pattern under microscopic observation (Sharma and Singh, 2019).

2.3. Pathogenicity Test

Pathogenicity test was done to fulfil the Koch's postulates. Healthy tomato plants were grown in pots containing sterilized soil. The bacterial suspension was adjusted to 10^6 CFU/mL with the help of spectrophotometer. After that, inoculation of bacteria was done in the early morning and the suspension was injected into the stems of one-month old tomato plants with the help of sterile injecting needle (30G × 1/2″). Some plants were injected with sterile distilled water and taken as control. After 7 days, these plants were observed for symptoms development. Re-isolated bacteria showed the same colony pattern as original culture (Wang *et al.*, 2021).

2.4. *In* vitro Assessment of Chemicals against *R. solanacearum*

Five different chemicals namely Oxyrich, Cabrio Top, Forum Top, Kocide and Electus Super were evaluated against *Ralstonia solanacearum* under lab conditions through inhibition zone technique (Atiq *et al*., 2021). Stock solution of each chemical was prepared. Three concentrations of each chemical i.e. 250, 300 and 350 ppm were used. The concentrations were prepared by adding 2.5, 3 and 3.5 mL of stock solutions in 100 mL of distilled water. Streaking was done on nutrient agar media plates. Blotter paper discs of 1 Cm size were cut, dipped in concentrations and then placed at the centre of the plates. Three replications were used and one plate was treated as control and blotter paper dipped in distilled water was placed in the centre of that plate. The plates were wrapped, labelled and placed in incubator at 28^{o}C. Complete Randomized Design (CRD) was used and data regarding inhibition zone was recorded after 24, 48 and 72 hours.

Table 1 Chemicals, their Active Ingredients, Company name and Mode of Action

Chemicals	Active ingredients	Company Name	Mode of Action	References
Oxyrich	Copper Oxychloride (30%) +Cymoxanil (10%)	Agrow	Inhibits synthesis of nuclei acids and amino acids	(Yanju *et al.*, 2019)
Forum Top	Dimetamorph (9%) + Metiram (44%)	BASF	Inhibition of sterol synthesis	(Gogoi *et al.*, 2022)
Electus Super	Azoxystrobin (22%) + Difenaconazole (8%)	Evyol Group	Inhibit mitochondrial respiration by blocking electron transport.	(Flampouri, 2021)
Cabrio Top	Pyraclostrobin (5%) + Metiram (55%)	FMC	It provides good resistance by blocking electron transport	
Kocide	Copper Hydroxide (52.4%)	FMC	It disrupts the cellular proteins	(Valdes *et al.*, 2020)
Control	Distilled Water			

2.5. *In vivo* Assessment of Chemicals against *R. solanacearum*

For field experiment, susceptible varieties of tomato plants were grown under Randomized Complete Block Design (RCBD) in the Research Area of Plant Pathology, University of Agriculture, Faisalabad. The plants were grown by maintaining P×P= 1ft and R×R= 2.5 ft distances. All the horticultural practices were maintained for healthy growth of tomato. Then, these plants were inoculated with bacterial suspension while control plants with distilled water. After this the chemicals which showed the most effective results in the lab were assessed under field experiment and sprayed at three concentrations i.e. 2%, 2.5% and 3%. Three replications of each treatment were used. The symptoms were observed after 7 days interval and disease incidence was recorded by following formula:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

2.6. Data Analysis

Under field conditions, Randomized Complete Block Design while in lab conditions, Complete Randomized Design was used. Analysis of variance (ANOVA) was applied on the recorded data to find out the significant results among treatment means. The least significant difference (LSD) with the probability level of 0.05% was used to separate the means of all the treatments.

3. RESULTS

3.1. Evaluation of different chemicals against *Ralstonia solanacearum* under *in vitro* conditions

Among all the treatments used, maximum inhibition zone was expressed by Oxyrich (20.68mm) followed by Kocide (17.85 mm), Electus Super (15.79 mm), Cabrio Top (13.79 mm) and Forum Top (10.83 mm) respectively as compared to control (Table 2 and Fig. 1). Interaction between Treatments and Concentrations (T×C) showed that Oxyrich expressed maximum inhibition zone (17.96, 20.53, 23.55 mm) at 250 ppm, 300 ppm and 350 ppm concentrations followed by Kocide (15.24, 17.74, 20.58 mm), Electus Super (13.06, 15.63, 18.69 mm), Cabrio Top (11.19, 13.66, 16.52 mm) and Forum Top (8.06, 10.81, 13.62 mm) respectively as compared to control (Table 3 and Fig. 2). Among all the treatments used, the interaction between Treatments and Days (T×D) showed that Oxyrich expressed maximum inhibition zone (18.56, 20.51, 22.98 mm) with three days interval followed by Kocide (15.82, 17.70, 20.04 mm), Electus Super (13.72, 15.62, 18.03 mm), Cabrio Top (11.69, 13.63, 16.05 mm) and Forum Top (8.78, 10.67, 13.05 mm) respectively, as compared to control (Table 4 and Fig. 3).

Table 2 Evaluation of different treatments that exhibited inhibition zone against *R. solanacearum* under *in vitro* conditions

Treatments	**Active Ingredients**	**Inhibition zone (mm)**
Oxyrich	Copper Oxychloride (30%) +Cymoxanil (10%)	20.68a
Forum Top	Dimetamorph (9%) + Metiram (44%)	10.83e
Electus Super	Azoxystrobin (22%) + Difenaconazole (8%)	15.79c
Cabrio Top	Pyraclostrobin (5%) + Metiram (55%)	13.79d
Kocide	Copper Hydroxide (52.4%)	17.85b
Control	Distilled Water	0.000f
LSD		**0.325**

*Mean values in the column sharing similar letter does not differ significantly as determined by the LSD test (P<0.05)

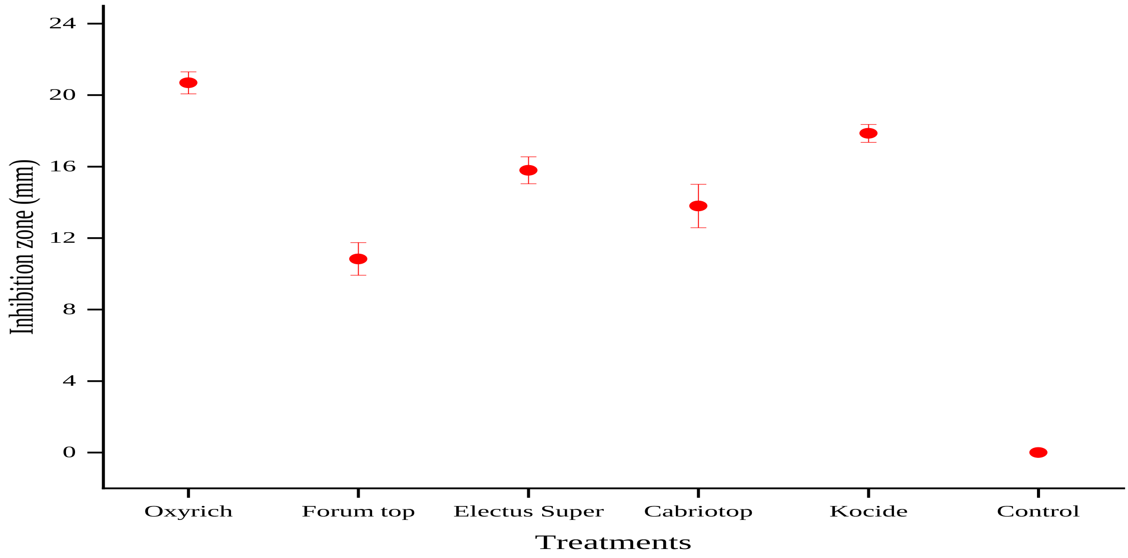

Fig. 1 In-vitro assessment of different treatments against *R. solanacearum* through inhibition zone technique

Table 3: Evaluation of interaction between treatments and concentrations that showed inhibition zone against *R. solanacearum* under *in vitro* conditions

Treatments	**Inhibition zone** (mm)		
	Concentrations (ppm)		
	250 ppm	**300** ppm	**350** ppm
Oxyrich	17.96c	20.53b	23.55a
Forum Top	8.06h	10.81g	13.62f
Electus Super	13.20ij	16.82f	19.80c
Cabrio Top	13.06f	15.63de	18.69c
Kocide	11.19g	13.66f	16.53d
Control	0i	0i	0i
LSD	**0.564**		

*Mean values in the column sharing similar letter does not differ significantly as determined by the LSD test (P<0.05)

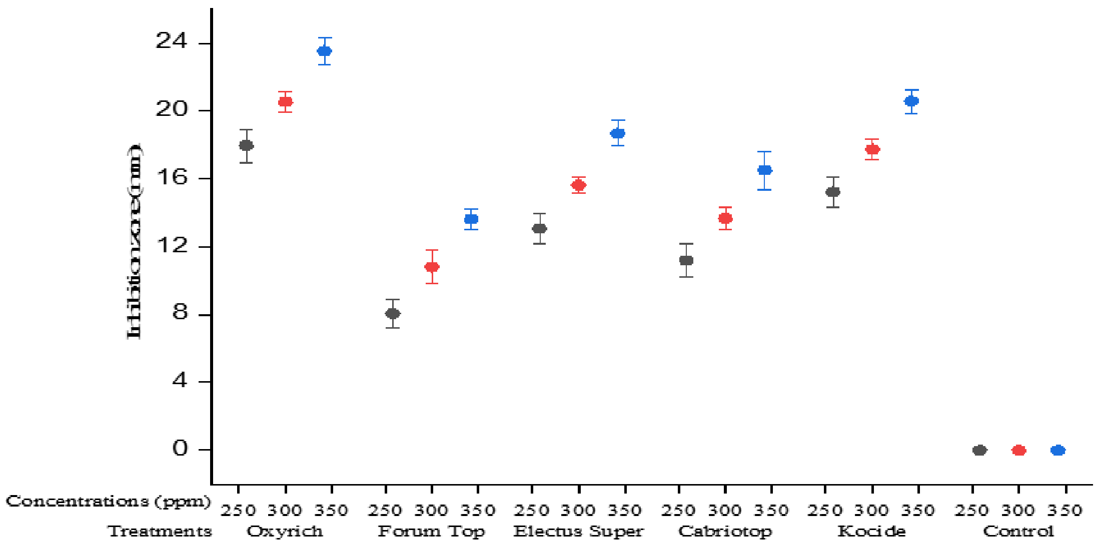

Fig. 2. Impact of interaction between treatments and concentrations against *R. solanacearum* under *in vitro* conditions

Table 4 Evaluation of interaction between treatments and days that showed inhibition zone against *R. solanacearum* under *in vitro* conditions

Treatments	Inhibition zone (mm)		
	Day1	Day2	Day3
Oxyrich	18.56c	20.51b	22.98a
Forum Top	8.78g	10.67f	13.05e
Electus Super	13.72e	15.62d	18.03c
Cabrio Top	11.69f	13.63e	16.05d
Kocide	15.82d	17.70c	20.04b
Control	0h	0h	0h
LSD	**0.564**		

*Mean values in the column sharing similar letter does not differ significantly as determined by the LSD test (P<0.05)

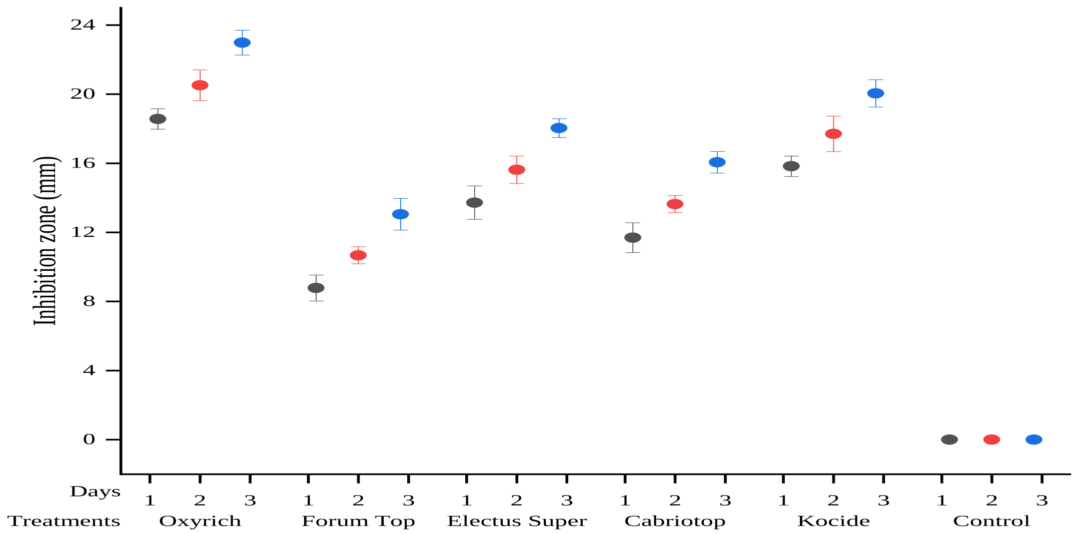

Fig. 3. Impact of interaction between treatments and days against *R. solanacearum* under *in vitro* conditions

Table 5 Evaluation of different treatments against bacterial wilt of tomato under *in vivo* conditions

Treatments	Disease incidence (%)
Oxyrich	26.58c
Kocide	35.37b
Oxyrich + Kocide	18.38d
Control	72.69a
LSD	**2.103**

*Mean values in the column sharing similar letter does not differ significantly as determined by the LSD test (P<0.05)

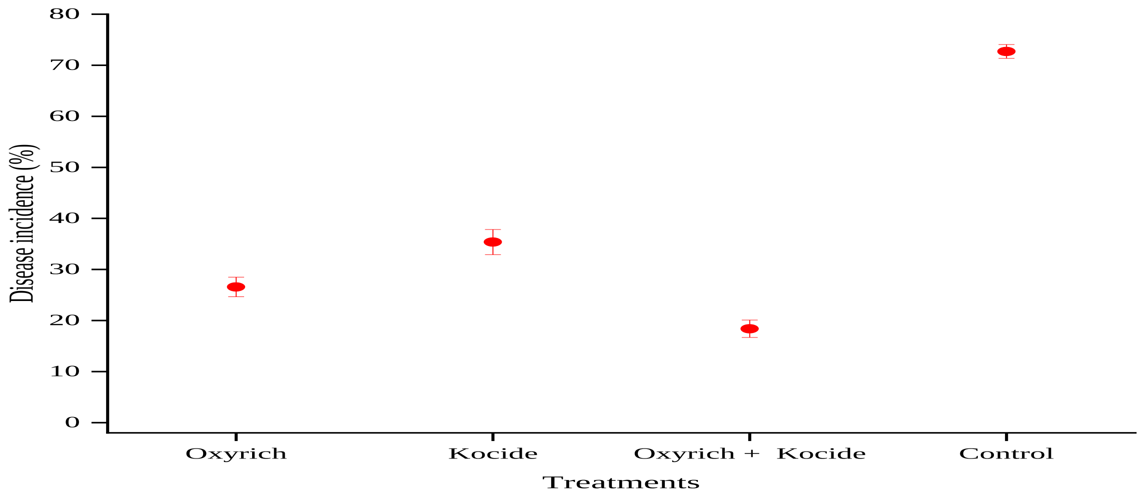

Fig. 4 In-vitro assessment of different treatments against bacterial wilt of tomato

Table 6 Evaluation of interaction between treatments and concentrations against bacterial wilt of tomato under *in vivo* conditions

Treatments	Disease incidence (%)		
	Concentrations (%)		
	2%	2.5%	3%
Oxyrich	31.61de	26.33ef	21.79fg
Kocide	40.41c	35.26cd	30.45de
Oxyrich + Kocide	22.66fg	18.59gh	13.87h
Control	68.63b	72.73ab	76.70a
LSD	**3.643**		

*Mean values in the column sharing similar letter does not differ significantly as determined by the LSD test (P<0.05)

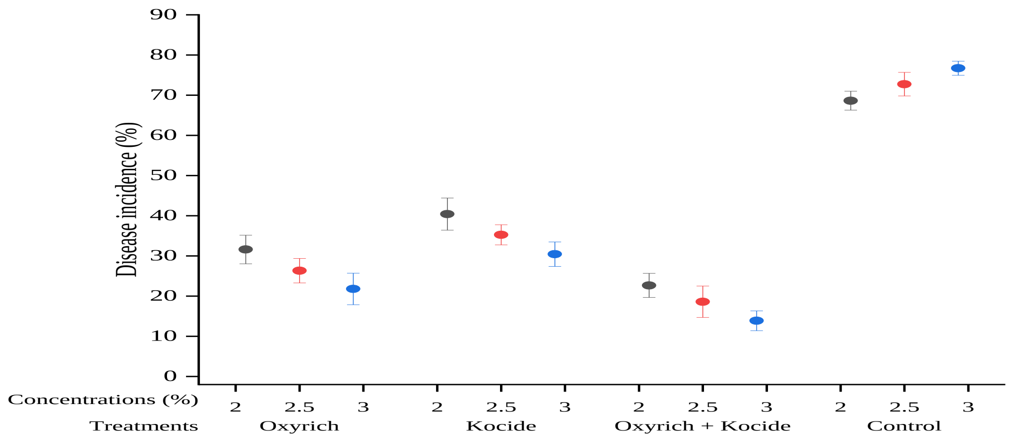

Fig. 5. Impact of interaction between treatments and concentrations against bacterial wilt of tomato under *in vivo* conditions

Table 7 Evaluation of interaction between treatments and weeks against bacterial wilt of tomato under *in vivo* conditions

Treatments	Disease incidence (%)		
	Week1	Week2	Week3
Oxyrich	31.45de	26.44ef	21.84fg
Kocide	40.44c	35.40cd	30.29de
Oxyrich+ Kocide	22.69fg	18.55gh	13.89h
Control	68.65b	72.75ab	76.67a
LSD	**3.643**		

*Mean values in the column sharing similar letter does not differ significantly as determined by the LSD test (P<0.05)

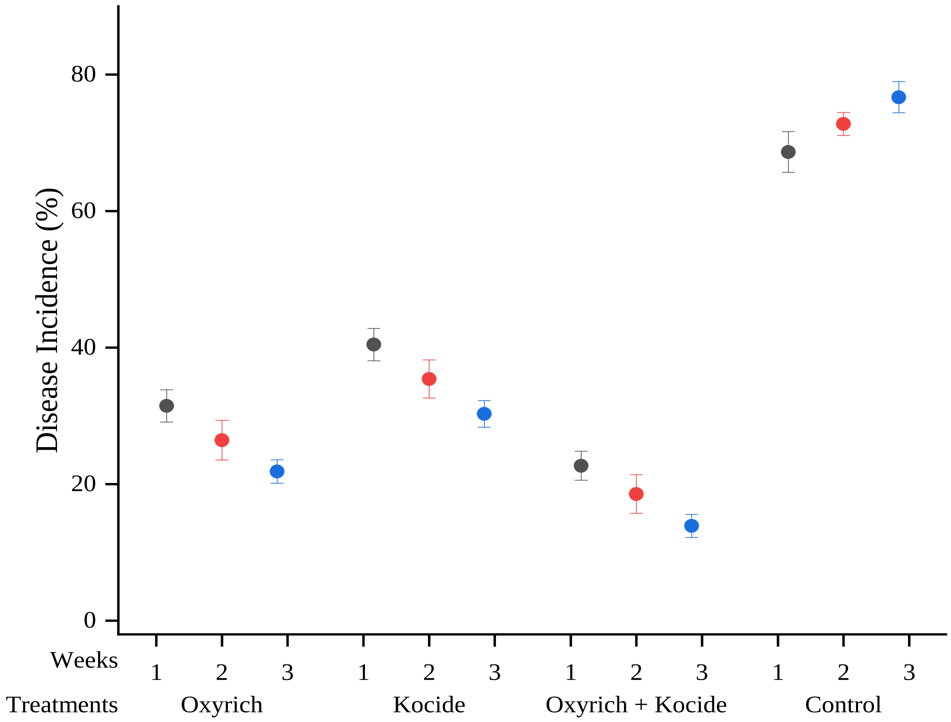

Fig. 6. Impact of interaction between treatments and weeks against bacterial wilt of tomato under *in vivo* conditions

3.2. Evaluation of different chemicals against bacterial wilt of tomato under *in vivo* conditions

Among all the treatments used, maximum reduction in disease incidence was expressed by combination of Oxyrich + Kocide (18.38%) followed by Oxyrich (26.58%) and Kocide (35.37%) respectively, as compared to control. The highest disease incidence was recorded in control with mean of 72.69% (Table 5 and Fig. 4). Interaction between Treatments and Concentrations (T×C) showed that combination of Oxyrich + Kocide expressed maximum reduction in disease incidence (22.66, 18.59, 13.87%) at 2%, 2.5% and 3% concentrations followed by Oxyrich (31.61, 26.33, 21.79 %) and Kocide (40.41, 35.26, 30.45%) respectively, as compared to control. The control showed maximum disease incidence (Table 6 and Fig. 5). Among all the treatments used, the interaction between Treatments and Weeks (T×W) showed that combination of Oxyrich + Kocide expressed maximum reduction in disease incidence (22.69, 18.55, 13.89%) with three weeks interval followed by Oxyrich (31.45, 26.44, 21.84%) and Kocide (40.44, 35.40, 30.29%) respectively, as compared to control. The control showed maximum disease incidence against bacterial wilt of tomato (Table 7 and Fig. 6).

4. DISCUSSION

Nowadays, abiotic and biotic stresses are causing a threat to the tomato yield around the globe. Due to diverse climatic environment, tomato is infected by numerous diseases, but the most important one is Bacterial Wilt, resulting in significant crop production losses (Manda *et al.*, 2020).

The most efficient management strategy to manage bacterial wilt is the use of resistant varieties (Huet, 2014; Nion and Toyota, 2015). Due to unavailability of resistant varieties, there is only choice to use chemicals, when the disease emerges in an epidemic form. Chemicals are affordable, convenient to use and provide immediate control against the disease and also increase soil microorganism density, produce systemic resistance, and increase tolerance to *R. solanacearum* (Kurabachew and Wydra, 2014). In the current study, five chemicals (Oxyrich, Forum Top, Electus Super, Cabrio Top and Kocide) were evaluated in the lab conditions against *R. solanacearum* causing bacterial wilt in tomato. Among these chemicals, oxyrich and kocide expressed maximum inhibition zone under *in vitro* conditions. However, Oxyrich, Kocide and combination of Oxyrich + Kocide were used against bacterial wilt of tomato under *in vivo* conditions. The combination of Oxyrich + Kocide results in minimum disease incidence.

The active ingredients of Oxyrich and Kocide are Copper oxychloride + Cymoxanil and Copper hydroxide, respectively. The most effective chemicals for treating bacterial diseases are those based on copper (Kan *et al.*, 2019). Copper is a structural component of various regulatory proteins. It plays an important

Tomato (*Solanum lycopersicum*) is a very important fruit or vegetable in the world next to potato crop (Quinet *et al.*, 2019). This fruit or vegetable is high in antioxidants and low in cholesterol, saturated fatty acids and salts and is extensively consumed because of frequent health benefits (Cammarano *et al.*, 2020).

role in physiological and biochemical mechanisms within plants like ethylene sensing, protein synthesis, oxidative stress responses, photosynthesis, electron transport, metabolism in cell wall and mitochondrial respiration which results in increase in growth and development of plants. Copper has a redox nature due to which it readily produced hydrogen peroxide, which is a signalling molecule and helps in activating resistance in plants (Nazir *et al.*, 2019). Through root absorption, xylem loading, and root-shoot transport, copper is carried into and within cells of plants (Ghazaryan *et al.*, 2019). Copper-based chemicals have been widely utilized for plant protection over time, such as copper hydroxide and copper oxychloride causing copper deposition in agricultural soils (El-Hak and Mobarak, 2019). Copper is a crucial element that degrades protein function by affecting nucleic acids, thus inhibiting microbial activity (Zhang *et al.*, 2018). Copper ions could activate defence signalling mechanisms such as ethylene biosynthesis pathway to successfully protect the plants from bacterial infection (Liu *et al.*, 2015). Cymoxanil suppress the seed germination and prevents transferring of electrons in the pathogenic bacteria's mitochondria which results in killing of cells of bacteria (Tellier *et al.*, 2002). Copper oxychloride has been used for ages to prevent cellular growth by bringing toxic amounts of copper ions into the phylloplane, which inhibit the growth of bacterial pathogens (Ottesen *et al.*, 2015).

Findings of the present study were supported by (Revathi *et al.*, 2017). In this study, an investigation on *in vitro* research was conducted to determine the efficacy of antibacterial chemicals in preventing the growth of *R. solanacearum.* Antibacterial chemicals such as copper oxychloride, copper hydroxide, kasugamycin, streptocycline and combinations were used. The results of the study showed that copper hydroxide gives the maximum inhibition zone followed by copper oxychloride (Revathi *et al.*, 2017). Another study reported the use of antibacterial chemicals against *R. solanacearum.* The results of that study revealed that copper oxychloride in combination with streptocycline was most effective followed by copper oxychloride, azoxystrobin and kocide (Singh and Jagtap, 2017). In another research work, bactericides were evaluated against *R. solanacearum* and the results expressed that azoxystrobin gives the maximum inhibition zone followed by Carbendazim + Mancozeb and Copper oxychloride (Kumari *et al.*, 2021). Chemicals are cost-effective, easy to use and give a quick response against plant diseases, therefore it is need of hour to utilize and determine the effectiveness of chemicals.

5. Conclusion

The current study showed that the chemicals have a potential effect against the growth of *Ralstonia solanacearum*. Oxyrich can be used to control bacterial wilt because it results in maximum inhibition zone under lab conditions while oxyrich in combination with kocide expressed maximum reduction in disease incidence under field conditions. The findings of the study concluded that chemicals can be used to manage bacterial wilt disease of tomato.

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