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Bio-efficacy of Kresoxim Methyl 44.3 % SC Against Late Blight of Tomato under *In vitro* Condition

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Authors' contributions

This work was carried out in collaboration among all authors. Author RKS conducted the laboratory experiment and analyzed data. Author DKJ design the experiment. Authors LC and SC prepared the manuscript. Author JPS guided in every step of the experiment. All authors read and approved the final manuscript.

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ABSTRACT

Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable crops grown throughout the globe for its nutritional and antioxidant properties. In recent years, the production of this crop is hindered by various pests and diseases. Late blight is one of the most common and devastating disease of tomato crop which is caused by the fungus *Phytophthora infestans.* In order to manage this disease, an experiment was conducted in the laboratory of IPFT, Gurgaon. For this, different concentration of Kresoxim methyl 44.3 % SC at 80 ppm, 40 ppm, 20 ppm, 10 ppm, 5 ppm

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and 2.5 ppm were evaluated against late blight of tomato under *in vitro* using food poison technique. Observations on radial growth of the pathogen recorded after 3, 7 and 10 days of transferring culture in media showed that the Potato Dextrose Agar (PDA) plates with 80 ppm Kresoxim methyl 44.3 % SC recorded minimum radial growth of pathogen (1.53, 3.13 and 2.73 cm) followed by 40 ppm (1.69, 3.50 and 3.10 cm) after 3, 7 and 10 days of treatment application. Moreover, Kresoxim methyl 44.3 % SC at 80, 40, 20 and 10 ppm were effective over control as they exhibit more than 50 % reduction in radial growth over control. The percent inhibition of mycelial growth over control was highest (67.82 %) in PDA plates consisting of 80 ppm Kresoxim methyl 44.3 % SC followed by 40 ppm (62.96 %) after 10 days of transferring culture in media. The treatment with 5 ppm and 2 ppm recorded less than 50 % reduction of pathogen growth.

Keywords: Kresoxim methyl; phytophthora infestans; tomato; late blight; In vitro.

1. INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) belonging to the family Solanaceae is one of the most important vegetable crops of the world and also widely grown in India. This crop has socioeconomic importance to families, gardeners, farmers, laborers, marketers, retailers, chefs and other workers and services in the food and restaurant industries (Ravikumar et al., 2019). Despite of its importance and health benefits, the production of tomato has been hindered by several factors. The reduction in yield levels could be due to attack of insect pests and diseases or due to biotic and abiotic stress like drought, weeds, excessive heat, declining soil fertility, and use of poor agronomic practices (Biswas and Das, 2024)**.** There are several causal agents of diseases like fungus, bacteria, virus, nematodes etc. Among all the causal agents, fungus is causing most of the disease in agricultural crops. In tomato, diseases like early blight, late blight, damping off, powdery mildew, anthracnose and fusarium wilt are most common, reducing the crop yield. Among them, late blight is the most important disease of tomato, as its infestation incurred a yield loss of 79.47 in NS 501 variety (Kumar et al., 2022) and even up to 100 % (Nowicki et al., 2012) if not controlled at the right time. It is also notoriously known for the Irish famine in the 1840's causing a death of 1 million people and mass migrations from Ireland to the USA and other European countries (Zadoks, 2008). It is caused by *Phytophthora infestans* which produces asexual propagating structures -sporangia and zoospores -which are dispersed by wind and rain for further infection while sexual reproduction results in yielding oospores through which the pathogen survived for longer periods (Brylinska et al., 2016). *Phytophthora infestans* (Mont.) de Bary which is regarded as a fungus-like organism is classified as an Oomycete that belongs to the order Peronosporales. This order contains Phytophthora species and a number of other very important plant-pathogenic genera, including the genus Pythium (Nelson, 2008**).** The pathogen affects all parts of plants including leaves; stem and can destroyed the whole field within a few days. Symptoms typically manifest as dark, water-soaked lesions on the leaves, initially appearing as small, olive-green spots which eventually enlarge and turn brown or black. Under humid conditions, a white, fuzzy growth may develop on the undersides of the leaves, particularly where lesions are present. As the disease progresses, lesions can also appear on stems, petioles, and fruit, leading to the collapse of entire plants. In advanced stages, affected fruits exhibit dark, sunken areas with a leathery texture.

Kresoxim methyl is a type of fungicide used in agriculture to manage fungal diseases in crops. It belongs to the class of chemicals known as oxime ethers. Mode of action of kresoxim-methyl specifically inhibits respiration by binding to the mitochondrial bc1 complex, subsequently blocking electron transfer and ATP synthesis (Ypema and Gold, 1999). Kresoxim methyl is available in various formulations, including sprays and granules. Most studies on fungicides for late blight disease in tomatoes have focused on their effectiveness, but not on the efficiency of different concentrations. This experiment aimed to determine the lowest effective concentration of Kresoxim methyl 44.3 % SC fungicide against late blight caused by *Phytophthora infestans*.

2. MATERIALS AND METHODS

The experiment was conducted *In vitro* at the Bioscience Division of the Institute of Pesticide Formulation Technology in Gurugram, Haryana. The Kresoxim methyl 44.3 % SC fungicide used in this research was purchased from the local

market in Haryana. This selection was made according to the list of major pesticide uses registered under the Insecticides Act of 1968, as of June 1, 2023. The present investigation was carried out under laboratory conditions, using a Completely Randomized Design (CRD) for the experimental setup. The field was closely monitored for the occurrence of late blight diseases. Once the disease symptoms appear, they were collected, stored in a sterilized polythene bag in refrigerator for future use.

2.1 Preparation of Potato Dextrose Agar (PDA)

The media for culturing the pathogen was prepared by taking 40 g of PDA in 1000 ml distilled water in a conical flask, stirred and autoclaved at 121^{0}C at 15 psi. Twenty ml of sterilized media was then poured in a petridish in an aseptic manner and kept it for solidification for future use.

2.2 Isolation, Identification and Purification of Pathogen

The disease sample was washed with running tap water and dry padded with tissue paper, cut in to small pieces with help sterilized blade. It was then treated with sodium hypochlorite 2 % solution and wash with sterile water. Small pieces of the disease sample were transferred to the sterilized PDA petri-plates and incubated in BOD at 25 ^{0}C for 5 to 7 days. For identification of the pathogen, single hypha from the PDA plates was isolated and morphological observation was carried out using microscopic image examination whereas for purification, single spore technique was used. Pure cultures were then stored at 4 ^{0}C on PDA slants.

2.3 Antifungal Activity Assay

Antifungal activity of Kresoxim methyl 44.3 % SC was performed by the food poison technique (Grover and Moore, 1962). Potato Dextrose Agar (PDA) medium with different concentrations of the fungicide i.e. T_1 (80 ppm), T_2 (40 ppm), T_3 (20 ppm), T_4 (10 ppm), T_5 (5 ppm), T_6 (2.5 ppm), with T_7 serving as the control was undertaken. About 20 ml of supplemented medium with each treatment of Kresoxim methyl 44.3 % SC was poured in to the petridish and replicated thrice. To each plate, the pathogen from the pure culture slant was inoculated at the centre and incubated in Biochemical Oxygen Demand (BOD) at 25 ^{0}C and observed for their growth inhibition at different day's interval. Percent inhibition of mycelial growth of the fungus was calculated by using the formula given by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

I -Per cent inhibition
C -Radial growth in control
T -Radial growth in treatment (fungicide)

3. RESULTS AND DISCUSSION

Chemical control has been regarded as the most successful strategy for management of diseases of many economically important crops against various pathogens. This study was conducted under *in vitro* condition to evaluate the bio-efficacy of Kresoxim methyl 44.3% SC against *Phytophthora infestans.* Results from the microscopic examination showed distinctively semi-papillate and lemon shaped sporangia with compound and sympodial sporangiophores which was similar to the identification made by Khalid et al. (2018) in late blight infected leaves of tomato. The morphological characters of the mycelium pattern of *P. infestans* isolates were profusely branching, fluffy, and white which confirms the isolated pathogen as *Phytophthora infestans* in the present study.

3.1 Antifungal Activity of Kresoxim Methyl 44.3 %

The data on the antifungal activity of the Kresoxim methyl 44.3 % SC evaluated by applying Food Poison Technique is presented in Table 1. Results from the table showed that the mycelial growth of the pathogen significantly reduces in all the treatment and ranged from 1.53 to 7.10 cm. Pathogen inoculated in PDA with 80 ppm (T_1) of Kresoxim methyl 44.3 % SC recorded significantly low radial growth (1.53, 3.13 and 2.73 cm) and closely followed by T_2 (1.69, 3.50 and 3.10 cm) after 3, 7 and 10 days of treatment respectively. The radial growth of the fungal mycelium was observed to be in ascending order of T_1, T_2, T_3, T_4, T_5, T_6 and T_7 at all observations time. The maximum radial growth of the fungus was observed in the treatment T_1, T_2, T_3 and T_4, (3.13, 3.50, 3.73 and 4.13 cm) after 7 days, which reduced to 2.73, 3.10, 3.13 and 3.40 cm after 10 days of treatment. However, the fungal growth in T_5 and T_6 continues to increase even after 10 days of

treatment application. This may due to susceptibility of the pathogen at higher concentration of fungicides in the former four treatments where the growth of the pathogen ceases with time. This study was in close conformity with the findings of Gupta and Jarial (2010) who reported *in vitro* inhibition of *Phytophthora nicotianae* up to 65.06 % when Kresoxim methyl 44.3 % SC at 583 ppm was treated with PDA plates. This fungicide showed a high level of persistence period under laboratory (7 days) and field condition (9 days) (Kumar et al., 2022). Similarly, Yadav et al. (2021) reported 4.17 mm radial growth and 94.16 % growth inhibition of *Rhizoctonia solani* at 0.2 % Kresoxim methyl under *in vitro* tested against sheath blight of rice. Complete inhibition of downy mildew with zero sporulation was also achieved by Sudisha et al. (2010) when Kresoxim methyl was used at 5 µg/ml as seed treatment and foliar spray in sunflower. However, Pithiya et al. (2024) reported the same chemical to be least effective in controlling the radial growth of *Fusarium pallidoroseum* in coriander compared to other systemic fungicides.

3.2 Percent Growth Inhibition

The percent radial growth inhibition of *P. infestans* by Kresoxim methyl 44.3 % was calculated over control for all the treatments and presented in Fig. 1. High percent inhibition of radial growth (67.82 %) over control was recorded at 80 ppm (T_1) followed by T_2 (40 ppm) which recorded up to 62.96 % (Figs. 1 & 2). It was observed that treatment T_1 (80 ppm), T_2 (40 ppm), T_3 (20 ppm) and T_4 (10 ppm) showed more than 50 % percent inhibition of radial growth over control after 10 days of culture. However, at 5 ppm (T_5) and 2.5 ppm (T_6), a very high fungal growth was observed and percent inhibition of growth over control was below 50 % indicating

Table 1. Efficacy of Kresoxim methyl 44.3 % against *Phytopthora infestans* based on radial growth at 3, 7 and 10 days

Treatment	Concentration (ppm)	Radial growth (cm)(days after treatment)		
		3 days	7 days	10 days
T_1	80	1.53 (1.24)	3.13 (1.77)	2.73 (1.65)
T_2	40	1.69 (1.30)	3.50 (1.87)	3.10 (1.76)
T_3	20	2.29 (1.51)	3.73 (1.93)	3.13 (1.77)
T_4	10	2.53 (1.59)	4.13 (2.03)	3.40 (1.84)
T_5	5	2.85 (1.69)	4.70 (2.17)	6.33 (2.52)
T_6	2.5	3.94 (1.98)	5.30 (2.30)	7.10 (2.66)
T_7	Control	4.27 (2.06)	6.23 (2.50)	8.37 (2.89)
S.Em ±		0.020	0.017	0.010
C.D (p=0.05)		0.06	0.05	0.03

(Figures in parentheses are square root transformed values)

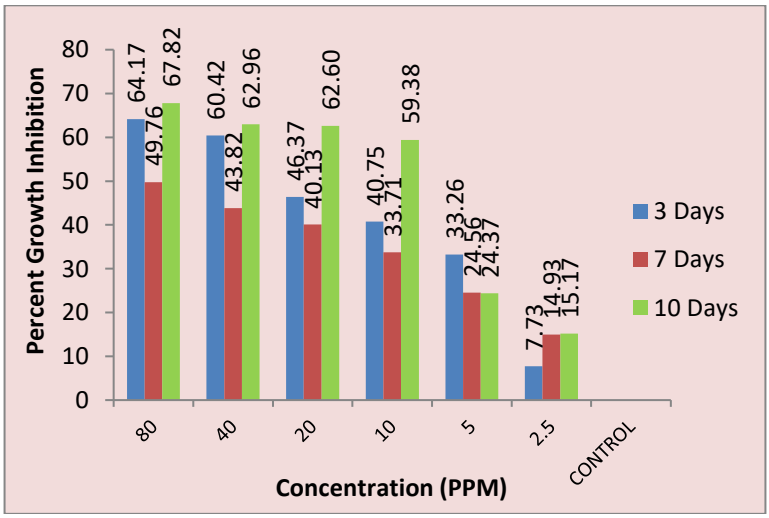

Fig. 1. Efficacy of Kresoxim methyl 44.3% based on percent inhibition of pathogen growth

Fig. 2. Radial growth of *Phytophthora infestans* on PDA with different concentrations of Kresoxim methyl 44.3%

ineffectiveness at these two concentrations. Similar results was reported by Rini et al. (2022) who recorded 92.37 % and 55.77 % reduction of disease over control in capsicum field during prophylactic spray and post infection spray respectively when Kresoxim methyl 44.3 % SC was used at 0.1 % concentration. Thus, Kesoxim methyl 44.3 % SC at a concentration of 80 ppm was highly effective in inhibiting the growth of *Phytophthora infestans* under lab condition.

In this study, Kresoxim methyl 44.3 % SC demonstrated effective control of *Phytophthora infestans* across a range of concentrations, particularly exhibiting maximum efficacy at 80 ppm under *in vitro* conditions. These findings suggest that further research is required to evaluate the fungicide's performance against late blight in field conditions at various dosages.

4. CONCLUSION

In this study, Kresoxim methyl 44.3 % SC demonstrated effective control of *Phytophthora infestans* across a range of concentrations, particularly exhibiting maximum efficacy at 80 ppm under *in vitro* conditions. These findings suggest that further research is required to evaluate the fungicide's performance against late blight in field conditions at various dosages.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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