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**Arshad Husain**

Department of Plant Pathology,  
C.S.A. University of Agriculture  
and Technology, Kanpur, Uttar  
Pradesh, India

**Md Mahtab Rashid**

Department of Mycology and  
Plant Pathology, Institute of  
Agricultural Sciences, Banaras  
Hindu University, Varanasi,  
Uttar Pradesh, India

**Nishar Akhtar**

Department of Plant Pathology,  
Birsra Agricultural University,  
Ranchi, Jharkhand, India

**Abdul Muin**

Department of Plant Pathology,  
C.S.A. University of Agriculture  
and Technology, Kanpur, Uttar  
Pradesh, India

**Gufran Ahmad**

Krishi Vigyan Kendra, Mahoba,  
Banda University of Agriculture  
and Technology, Banda, Uttar  
Pradesh, India

**Corresponding Author:****Gufran Ahmad**

Krishi Vigyan Kendra, Mahoba,  
Banda University of Agriculture  
and Technology, Banda, Uttar  
Pradesh, India

## *In-vitro* evaluation of fungicides at different concentrations against *Alternaria solani* causing early blight of potato

**Arshad Husain, Md Mahtab Rashid, Nishar Akhtar, Abdul Muin and Gufran Ahmad**

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**Abstract**

Potato (*Solanum tuberosum* L.) is an eminent cash crop and also an important part human diet in India. It forms a major source of carbohydrate that is taken in the form of vegetables and almost the cooked in meals of every Indian household. The potential production of potato is mainly constricted by Early blight disease caused by the fungal phytopathogen *Alternaria solani*. This disease is almost prevalent in all parts of our country, and the farmers indiscriminately use the fungicides for its management. The injudicious use of fungicides brings down the profit of farmers along with causing environmental pollution. An experiment was carried out to find the potency of five fungicides of varied chemistry at different concentrations against the mycelial growth of *Alternaria solani* under *in-vitro* condition. It was found that Propiconazole 25EC and Carbendazim 50WP completely inhibit the mycelial growth at 250ppm. Thus, it was concluded that these two fungicides could be used in field conditions for the management of early blight at the same concentration in a sequential manner to avoid environmental pollution and resistance development in the phytopathogen along with increasing the profit of the farmers.

**Keywords:** Potato, early blight disease, pathogenicity, poisoned food technique, conventional identification, pure culture, single-spore isolation

**Introduction**

Potato (*Solanum tuberosum* L.) is the third most important food crop in the world and is grown almost all around the world (FAOSTAT, 2020) [6]. It belongs to the Solanaceae family of plants. It is cultivated as a cash crop in many states of India like Uttar Pradesh, Bihar, Orissa, Karnataka, Gujarat, Maharashtra, Andhra Pradesh, West Bengal, and Madhya Pradesh. In India, a total of 48,529 Mt of potato was produced from an area of 2.151 Mha (FAOSTAT, 2020) [6]. It is also known as the “king of vegetables,” and a vegetable market in India is incomplete without it. A high dry matter and edible protein content imparts nutritional superiority to potato over other vegetables and is thus one of the world's staple food crops. Potato crop has more potential to give more production than the present in our country, but it is attacked by several phytopathogens which brings down its potential production. Early blight disease caused by *Alternaria solani* is one of the major diseases which causes heavy losses in the cultivation of potato crop. It is estimated that it can cause up to a 40 percent yield loss in India (Bansode *et al.*, 2018) [2]. It is among the most common diseases of the potato crops in places where it is cultivated under irrigated conditions, especially when there are alternating dry and humid conditions with high temperature (Van der Waals *et al.*, 2003; Leiminger and Hausladen, 2012) [31, 14].

*Alternaria solani* is a phytopathogen that causes early blight disease in a wide variety of solanaceous and vegetable crops leading to a huge loss in their production. Among the solanaceous host group, it is a regular pathogen of potato, tomato, and tobacco crops (Zhang *et al.*, 2020) [34]. It is a necrotrophic fungal phytopathogen of the ascomycetous group which causes up to 80 percent of yield losses in potato crop on an annual basis in some parts of the world (Morgan *et al.*, 2002; Pasche *et al.*, 2004; Peters *et al.*, 2008) [16, 20, 21]. The phytopathogen can survive for extensive durations of time in the soil in form of conidia and/or mycelia on leftover plant parts (Runno-Paurson *et al.*, 2015) [24]. It can take an epidemic form depending on the predisposing factors constituting of temperature, relative humidity in the atmosphere, and duration of leaf wetness (Harrison *et al.*, 1965; Adams and Stevenson, 1990; Vlutoglou and Kalogerakis, 2000; Olanya *et al.*, 2009) [12, 1, 32, 19]. It is majorly a foliar pathogen but can negatively impact the quality of tubers as well under severe infections (Shahbazi *et al.*, 2010) [28].

The primary infection starts from the base of potato plant and thus the symptoms occur from lower to upper leaves leading to senescence of leaves (Franc and Christ, 2001; Secor and Gudmestad, 2009) [7, 27]. The foliar symptoms are in the form of small and dark concentric lesions which restrict the photosynthetic area of the plant (Franc and Christ, 2001, Horsfield *et al.*, 2010; Gudmestad *et al.*, 2013) [7, 13, 11].

The disease can be managed through various cultural practices such as crop rotation, field sanitization, growing of the non-host crop, and site selection but all these management practices consume time and also take away the economic benefit of growing the potato crop. The phytopathogen can be managed by various chemical fungicides but an injudicious use of these fungicides also has various negative impacts. The over-use of fungicides in the context of concentration, as well as the frequency of application, has led to the development of resistance in the phytopathogen against various fungicidal groups and even mixture of them (Pasche *et al.*, 2004; Rosenweig *et al.*, 2008) [20, 23]. Additionally, the excessive runoff of these fungicides in the soil have a negative impact on the non-target fungal species and also degrades the soil by accumulating as pesticidal residues over time. This indiscriminate use of fungicide also has an adverse effect on human health by the mechanism of bio-accumulation. Thus, given all these facts, an in-vitro experimental trial was conducted to find out the efficacy of different diverse fungicides in controlling the phytopathogen, *Alternaria solani* at different concentrations so that the results can be forwarded to the field trials.

## Materials and Methods

**Isolation and pure-culture of the pathogen:** The potato plants were grown at Vegetable Research Farm, C.S.A. University of Agriculture and Technology, Kanpur; with all the recommended package of practices for cultivation of crop in the region. The leaves of infected plants showing the symptoms of early blight were collected from the field and kept in butter paper bags. The leaf samples were surface sterilized with 0.5% of sodium hypochlorite solution and then washed thoroughly with distilled sterilized water for several times. Some small pieces of leaves were cut containing nearly half an infected portion and half healthy portion. These small pieces of leaves were placed on potato dextrose agar (PDA) medium containing streptomycin to avoid bacterial contamination. The plates were incubated at  $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for one week at 12 hours dark/light photoperiod (Meena *et al.*, 2017) [15]. The isolated pathogen culture was purified through the single spore isolation technique and maintained in PDA slants for further investigations.

**Identification of the pathogen culture isolate:** The isolated fungal culture was identified to be potential *Alternaria* spp. on the basis of conventional taxonomic methods, which included characteristics of the colony and the spore. The conidia were harvested from the border of isolated culture and placed on a microscopic slide having a drop lactophenol solution to study the spore characteristics. The fungus was also regularly checked at 24 hours interval for their colony characteristics. The identification was done on the basis of description given of the phytopathogen in different manuals and books (Ellis, 1971; Subramaniam, 1971; Barnett and Hunter, 1998; Gilman and Joseph, 1998) [5, 30, 3, 9].

**Pathogenicity test:** The identified *Alternaria* spp. isolate was tested for its pathogenicity on healthy potato plants to establish its pathogenic nature as per Koch's postulates. The susceptible cultivar of potato "Kufri Chandramukhi" (Dey and Chakraborty, 2012) [4] propagules were taken and surface sterilized with 0.5% sodium hypochlorite solution followed by thorough washing with distilled sterilized water for several times. The surface-sterilized plant propagules were air-dried, sown in pots containing sterilized soil, and allowed to grow for one month. A spore suspension of the *Alternaria* spp. isolate was prepared @  $2 \times 10^6$  CFU ml<sup>-1</sup> and sprayed on the one-month-old whole potato plants. The inoculated plants were covered with plastic bags for maintaining the humidity. The control plants were sprayed with sterilized distilled water. The symptoms development was recorded at 2, 4, and 8 days after inoculation (DAI). The phytopathogen was re-isolated from the leaves of inoculated plants and compared with the original culture.

**In-vitro evaluation of the fungicides against the fungal pathogen:** The evaluation of five fungicides, namely Propiconazole 25EC, Carbendazim 50WP, Curzate 50WP, Mancozeb 75WP, and Copper oxychloride was performed for their respective ability to control the pathogen by poisoned food technique (Schmitz, 1930) [26]. The PDA medium was amended with 50, 100, 250, and 500ppm of all the fungicides along with one without any fungicides as the control were poured into sterilized Petri-plates and kept for solidification. A seven days old pure culture of the *Alternaria solani* isolate was used for inoculation and 5mm discs were placed in the center of each plate containing the fungicide along with the control one. All the treatments were in three replications and kept at  $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$  sealed with paraffin wax strips. The growth of the colony in each of the treatments was recorded at 24 hours interval until the plates got completely covered with the fungus. The inhibition percent in the fungicide treatments were calculated over the control by using the following formula:

$$\text{Inhibition percent} = \frac{\text{Growth of the colony in control (mm)} - \text{Growth of the colony in fungicide treatment (mm)} \times 100}{\text{Growth of the colony in control (mm)}}$$

**Statistical analysis:** The experiment was conducted in a completely randomized design (CRD). The data recorded in percent were transformed by arcsine square root transformation before the statistical analysis. The statistical analysis was carried out by using analysis of variance (ANOVA) method and the critical difference was measured at a 5% level of significance (Gomez and Gomez, 1984) [10].

## Results and Discussion

**Isolation and identification of the phytopathogen:** The isolated fungal phytopathogen formed a white mycelial

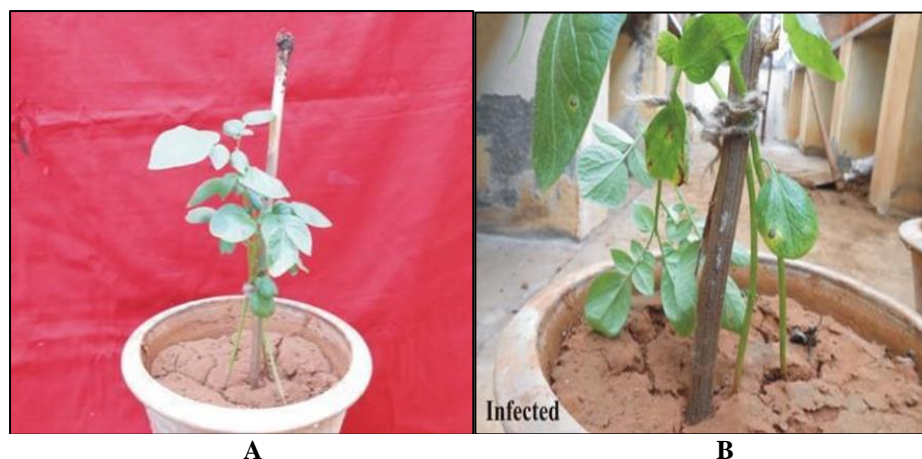
colony initially, which turned into greyish-green in appearance due to pigmentation. The colony was smooth, circular in shape with concentric zonation (**Figure 1B**). The isolated fungal produced dark-coloured septate mycelium with short, erect, and flexuous conidiophores. The conidia were single, dark, muriform, with several transverse and 1-2 longitudinal septa in matured ones. The beak was ellipsoidal and relatively long. The morphological descriptions of the colony and conidia the phytopathogen substantially validate the descriptions given by Neergard (1945) [18] and Ellis (1971) [5] about *Alternaria solani*.

**Pathogenicity test:** The initial symptoms of inoculated susceptible potato cultivar “Kufri Chandramukhi” were observed at 3 DAI. The symptoms appeared on lower leaves of inoculated plants leaves as pale to dark green spots at the tips and margins, which gradually turned into black/brown lesions. The lesions grew in size and enlarged rapidly when given favourable conditions. The spots were small, dark brown, irregular to circular, and measuring to about 0.5 mm in diameter. They grew bigger and formed concentric rings in irregular shape forming a lesion giving the characteristics

“target-board” or “bull’s eye” appearance. These symptoms correspond to the symptoms of early blight disease of potato (Weber and Halterman, 2012; Ganie *et al.*, 2013; Strammler *et al.*, 2014) [33, 8, 29] (Figure 2). The fungus was re-isolated using the symptomatic leaves of the inoculated plants also showed almost the same characteristics to the original culture, thus, completing all the criteria of Koch’s postulates and confirming the pathogenicity of isolated *Alternaria solani* culture to be pathogenic on potato plants.



**Fig 1:** A - The symptomatic leaves of potato plant in the field, B – The isolated culture of *Alternaria solani*.



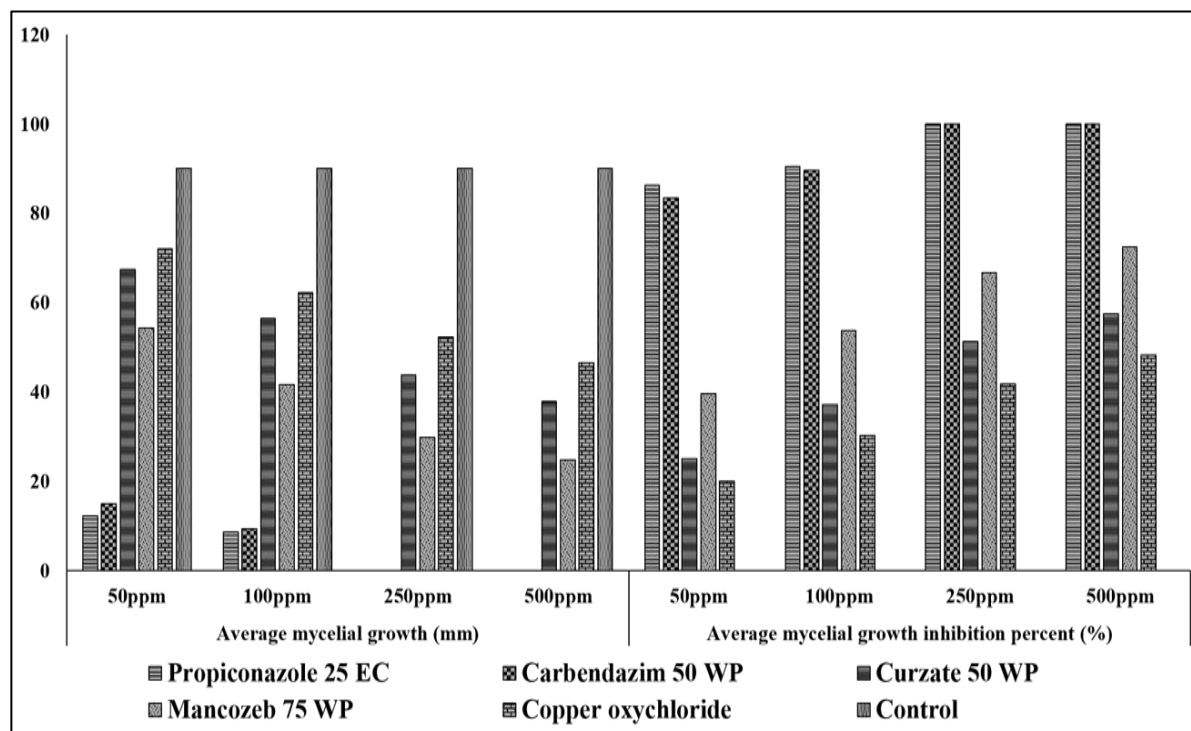
**Fig 2:** A- Healthy potato plant sprayed with sterilized distilled water, B- Infected plant sprayed with  $2 \times 10^6$  CFU ml<sup>-1</sup> of isolated *Alternaria solani* spore suspension.

**In-vitro evaluation of the fungicides against the fungal pathogen:** The *in-vitro* evaluation of Propiconazole 25EC, Carbendazim 50WP, Curzate 50WP, Mancozeb 75WP, and Copper oxychloride at 50, 100, 250, and 500ppm showed that they all could significantly inhibit the growth of *Alternaria solani* at all the concentrations (Table 1). At 50 and 100ppm concentration, Propiconazole 25EC showed maximum inhibition of mycelial growth while the minimum inhibition was showed by Copper oxychloride. At 250ppm of the fungicide concentration, total inhibition of the mycelial growth was shown by Propiconazole 25EC and Carbendazim 50WP, followed by a Mancozeb 75WP (66.77% inhibition). Again, at 500ppm of the fungicidal concentrations showed similar results in that at 250ppm (Figure 3). This proves the

inhibitory action of all the fungicide against *Alternaria solani* as all the results are significantly superior. Further, Propiconazole 25EC and Carbendazim 50WP are the most effective against the phytopathogen that can completely inhibit the pathogen at 250ppm. A similar inhibitory action of Propiconazole 25EC and Carbendazim 50WP was demonstrated by Naik and Jayalakshmi (2017) [17], Kumar *et al.* (2017), Saha *et al.* (2018) [25], and Rajeswari and Balasupramani (2020) [22] on *Alternaria* leaf blight of bhendi, tomato, cabbage, and pigeon-pea, respectively. Thus, the results suggest that Propiconazole 25EC and Carbendazim 50WP at 250ppm have a promising potential in reducing the growth of *Alternaria solani*.

**Table 1:** *In-vitro* evaluation of the fungicides against the fungal pathogen *Alternaria solani*.

Treatment	Average mycelial growth (mm)				Average mycelial growth inhibition percent (%)			
	50ppm	100ppm	250ppm	500ppm	50ppm	100ppm	250ppm	500ppm
Propiconazole 25 EC	12.25	8.60	0.00	0.00	86.38	90.44	100	100
Carbendazim 50 WP	14.96	9.31	0.00	0.00	83.38	89.65	100	100
Curzate 50 WP	67.38	56.50	43.85	37.88	25.13	37.22	51.28	57.51
Mancozeb 75 WP	54.30	41.66	29.90	24.77	39.67	53.71	66.77	72.48
Copper oxychloride	72.00	62.27	52.33	46.55	20.00	30.31	41.85	48.27
Control	90.00	90.00	90.00	90.00	00.00	-	-	-
S.E.	0.54	1.12	1.29	1.09	-	-	-	-
C.D. 5%	1.69	3.50	2.85	3.41	-	-	-	-

**Fig 3:** Average mycelial growth (mm) and inhibition percent (%) of *Alternaria solani* against five fungicides.

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