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In-vitro Efficacy of Thiram against *Alternaria tenuis* causing Seed Rot of Green Gram (*Vigna radiata*)

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Abstract

Chemical control is one of the measures to prevent the disease and evade losses. The evaluation study was therefore conducted in vitro. Six fungicides were tested against the pathogen, i.e., *Alternaria tenuis* Ness *in vitro*. The effect of Thiram on *Alternaria tenuis* Ness was studied using the food poisoning technique. The sensitivity of Thiram was found to be more inhibitory as a compound to other fungicides. As a result, investigation will be helpful in the control of seed-borne fungi of green gram seeds. The different concentrations of Thiram were used from 100μg/mL to 800μg/mL. The lowest PCE was 100 g/mL, and the highest was 1000μg/mL. The MIC was found to be 700μg/mL. Mung bean seed samples were collected from pulse research in Badnapur before the experiment. The laboratory experiment was conducted in the Department of Botany Mahatma Phule Arts, Science and Commerce College Panvel district Raigad University of Mumbai (India).

Key words: Green gram, *Alternaria tenuis* Ness, Fungicides, Percent control efficacy (PCE)

Green gram, also known as *Vigna radiata*, is one of the most important legume crops that are grown in the Marathwada region. The agroecological conditions of this country are favorable for the cultivation of green gram. It has been discovered that the seeds of a green gram are highly infested with a group of different types of fungi [1]. Evidence shows that the seeds and contents are deteriorating due to these related fungi. In India, it is commonly planted in the states of Maharashtra, Uttar Pradesh, Gujarat, Tamil Nadu, Andhra Pradesh, and Bihar. It is grown on an area of around 2.4 million hectares and produces one million tonnes of seeds. The Kharif season is the primary time for its cultivation. According to Zahran *et al*. [2], it has the ability to improve soil fertility and fix atmospheric nitrogen through a symbiotic connection with soil microorganisms. Certain plants, particularly legumes, have the unique ability to improve soil fertility by forming a symbiotic relationship with nitrogen-fixing soil microorganisms such as *Rhizobium* bacteria. This mutually beneficial relationship not only supports the growth of the host plant but also enriches the surrounding soil with biologically available nitrogen, which remains in the soil even after the plant has completed its life cycle. These bacteria inhabit root nodules of the host plant, where they convert atmospheric nitrogen (N_2), which is unusable by plants, into ammonia (NH_3), a form readily absorbed and utilized by plants for growth. This natural process not only provides essential nitrogen to the host plant but also enriches the surrounding soil, benefiting subsequent crops and reducing the need for synthetic fertilizers. As the plant matures and eventually decomposes, the fixed nitrogen is released into the soil, further enhancing its fertility. This symbiotic nitrogen fixation is a key component of sustainable agriculture, promoting soil health, reducing chemical input, and supporting long-term productivity. In our country, illnesses are considered the most significant constraint, which is one of the many factors contributing to the reduced yield of mungbean [3].

The Thirum was discovered to have inhibitory effects on the growth of the test fungus. Fungicides have been extensively employed to manage fungal infections and enhance agricultural yield. Fungal infections are a major concern in agriculture as they can lead to reduced seed viability, poor germination rates, and ultimately lower crop yields. To combat this, fungicides are widely used to prevent and control the spread of fungal diseases, thereby improving plant health and enhancing overall agricultural productivity. In this context, the present research was designed with two main objectives: first, to identify seed-borne fungal pathogens affecting green gram (Vigna radiata), a vital legume crop; and second, to evaluate the effectiveness of different chemical treatments, including Thirum, under controlled laboratory conditions. By understanding the types of fungi associated with green gram seeds and testing various fungicides for their antifungal activity, the study contributes to the development of more targeted and efficient plant disease management strategies, ultimately supporting sustainable crop production. In 2004, Amaresh and Nargund [4] conducted a laboratory study to assess the effectiveness of fungicides against *Alternaria helianthi*, a fungus that causes leaf blight in sunflowers. Fungicides are utilized for the chemical management of seed-borne diseases. The fungicides utilized were categorized as either systemic or non-systemic. The fungicides employed included Thiram, Carbendazim, Mancozeb, Copper oxychloride, Captan, and Captafol. These fungicides can be applied as seed dressers to manage seed-

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borne diseases effectively. The effectiveness of captafol was evaluated against the growth of the fungi obtained from the seeds of green gram

MATERIALS AND METHODS

The procedure involved preparing various quantities of fungicides, ranging from 100 to 1200 µg/mL, based on their active components. Following sterilization, a beaker that had been sterilized was used to add 10 mL of the recommended concentration of fungicide to 10 mL of Potato Dextrose Agar (PDA) medium. Once thoroughly mixed, the solution was transferred into a sterile Petri dish and left undisturbed until it solidified. Following the solidification process, a 5mm disc of an 8-day-old culture of the test fungus was introduced into the Centre of the PDA plate. The plates were placed in an incubator at a temperature of 28±1°C. All treatments, including the control, were made by adding 10mL of sterilized distilled water to 10mL of medium. Each treatment was made in triplicate, with all plates treated this way.

The data was documented as the linear progression of the fungal pathogen in millimeters (mm) daily for 8 days. The linear growth was measured until it reached the maximum growth observed in the control plate when it was entirely filled.

The recorded value was the minimum inhibitory concentration (MIC) of the fungicide. The diameter of the fungal pathogens' colonies on the medium was measured, and the percentage of inhibition was estimated using the formula provided by Vincent [5].

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where;
C = growth of the test fungus in the untreated control plate
T = growth of the test fungus in treated plate

With the individual fungicide treatment, minimum inhibitory concentration (MIC) was determined. Thiram minimum inhibitory concentration (MIC) to *Alternaria tennis* was recorded.

RESULTS AND DISCUSSION

The effect of Thiram on percent control efficacy on *Alternaria tenuis* was noted as shown in (Table 1, Fig 1). From the results, it is clear that when thiram was used against *Alternaria tenuis* at 100g/mL, the PCE recorded were found to be 80%, 75%, 70%, 55%, 40%, 38%, 20%, and 8% from 1st day to 8th day. When the concentration of thiram increased up to 700g/mL, the PCE recorded was 100% for seven days and 90% on the 8th day. This means the MIC for *Alternaria tenuis* was 700µg/mL.

Table 1 Effect of Thiram on percent control efficacy (PCE) of *Alternaria tenuis* Ness

Conc. µg/mL	Percent control efficacy (PCE)							
	Incubation period (Days)							
	1	2	3	4	5	6	7	8
100	80	75	70	55	40	38	20	8
200	82	80	74	65	67	40	33	15
300	83	81	75	67	65	55	50	35
400	85	82	78	72	66	61	58	40
500	87	85	80	75	77	62	60	45
600	90	87	85	80	81	75	70	67
700	100	100	100	100	100	100	100	90
800	100	100	100	100	100	100	100	100
S.E ±	2.58	3.04	3.81	5.35	6.58	7.93	9.47	11.02
C.D. at 5%	6.11	7.20	9.02	18.72	15.59	18.79	22.44	26.11

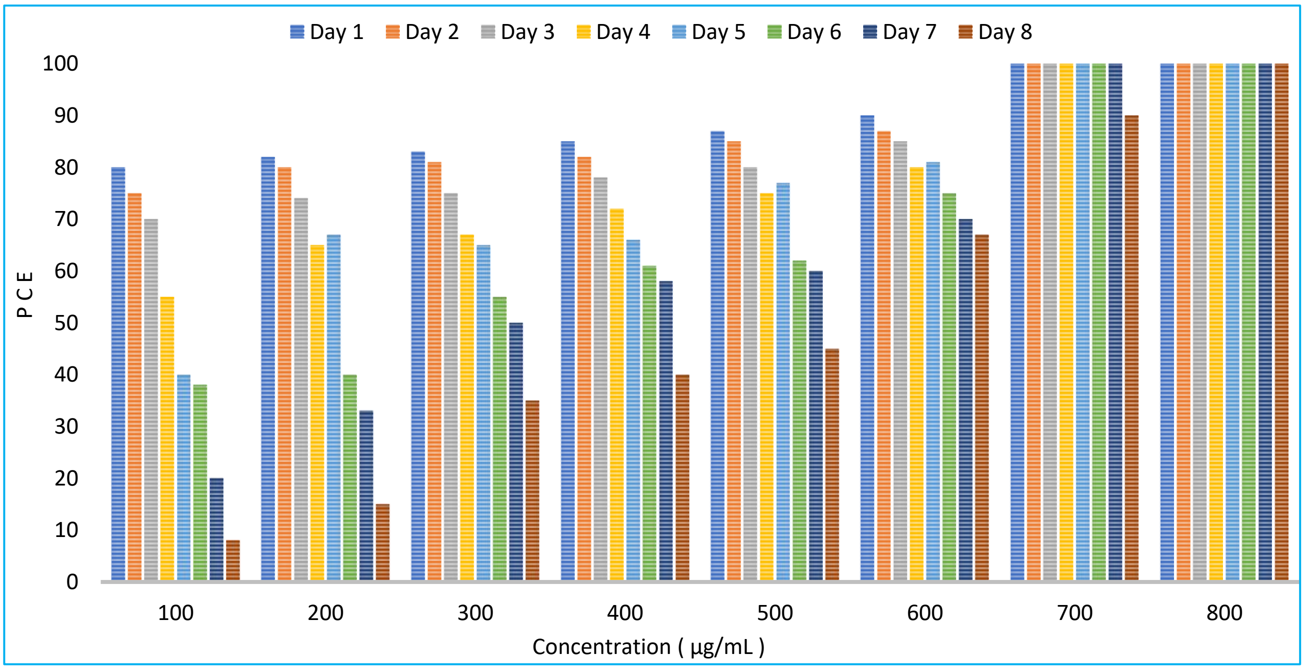

Fig 1 Effect of Thiram on percent control efficacy (PCE) of *Alternaria tenuis* Ness.

Vincent [5] observed the deformation of fungal hyphae when certain inhibitors were present. Nene and Thapliyal [6] released the third version of their book, which focuses on the use of fungicides for controlling plant diseases. In their 1994 study, Zahran *et al*. [2] examined the management of pulse crops in sequential cropping. In 1998, Bakr and Rahman [3] presented a study paper on the current state of research on diseases affecting mungbeans and black grams, as well as the future requirements in this field. The authors of the publication titled Madhuri and Rangaswamy [7]. Impact of certain fungicides on microbial population in groundnut (*Arachis hypogaea L.*) soils. Amaresh and Nargund [4] conducted a study in 2004. An in vitro assessment was conducted to evaluate the efficacy of fungicides against *Alternaria helianthi*, the causal agent of leaf blight in sunflower. Kharakwal *et al*. [8] investigated the effects of several fungicides on the in vitro spore germination and growth of *Altemaria solanine*. Akbari and Parakhia [9] conducted a study on the management of *Alternaria alternata*, which causes blight in sesame, using fungicides. In their study, Rajani and Rakholia [10] examined the use of fungicides to manage fruit rot caused by *Alternaria alternata* in chilli plants. Chaudhary *et al*. [11] conducted a study to evaluate the effectiveness of several fungicides against dry root rot (*Macrophomina phaseolina*) of Soybean using in vitro methods. Devamani *et al*. [12] investigated the effectiveness of fungicides and bioagents on the fungal population found on mung bean seeds (*Vigna radiata* L.). Nene and Thapliyal [13] authored a book titled "Fungicides in Plant Disease Control" in its 4th edition. Balram *et al*. [14] conducted a study to assess the effectiveness of fungicides against *Alternaria alternata,* the pathogen responsible for causing blight disease in tomato plants (*Solanum lycopersicum* L.), using in-vitro methods.

CONCLUSION

Different fungicides were used to study sensitivity against *Alternaria tenuis*: Thiram, Carbendazim, Mancozeb, Copper Oxychloride, Captan, and Captafol. The MIC of these fungicides varied against *Alternaria tenuis. Alternaria tenuis* was more sensitive to Thiram. *Alternaria tenuis* cause seed rot of green gram.

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