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Efficacy of different fungicides against *Alternaria tusi* cause leaf blight of peas (*Alternaria tusi*) was first time discovered from Kharmang Olding and Skardu Baltistan

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Abstract

Leaf blight disease of peas caused by *Alternaria alternata* and *Alternaria tusi* which is one of the major and important foliage diseases of peas. Leaf blight of peas was first reported occasionally in visconsin a commercial production fields in 1968 and 1976 but recently it has been seen in all peas growing areas of skardu and Kharmang olding. The losses occurs 20- 80% due to the attack of this disease depending upon the management practices. *Alternaria tusi* was fist reported from kwardu in 2018 at Tusi scientific research center kwardu. Keeping in view the losses caused by *Alternaria alternata* and *Alternaria tusi* in peas. The present study was carried out at Tusi scientific research center kwardu during the year 2018. The key objectives of this study were (a) Survey and sampling of leaf blight disease of peas in the surrounding of District Kharmang and different localities of Skardu (b) Isolation and identification of the leaf blight disease causing agent, (c) evaluation of *in-vitro* chemical control measures against the *Alternaria* sp. causing leaf blight of peas. The fungicides were tested at (50, 100, 200 ppm) concentrations whereas, All treatments were tested with three replications. The result of this study showed that the leaf blight disease of peas occurs in all fields with different intensities during the surveyed area. The maximum disease incidence (22%) was recorded at Muntazir abad followed by Bindiaq (18%) Maqpon abad (17%) whereas, the minimum disease incidence (11%) and 12% was recorded at Gulshan abad and hasnain abad respectively. Different fungi such as *Alternaria alternata*, *Alternaria tusi Aspergillus* sp and *Penicillium* sp with different number of colony frequencies (%) were being isolated from the leaves of leaf blight disease samples. The fungus *Alternaria alternata* and *Alternaria tusi* was isolated with maximum (88%) from muntazir abad followed by maqpon abad. Among all the tested fungicides the minimum colony mycelial growth 22.33mm and 32.33mm was recorded when Nativo fungicide used at highest doses 200 and 100 ppm whereas, at lowest dose 50 ppm, the growth of the tested fungus was recorded 39.33 mm. The Cabriotop resulted 33.66mm, 42.66mm and 52.00mm at 200, 100 and 50ppm doses respectively. The Topsin-M and Topas Fungicides did not proved better as compare to Nativo and Cabriotop fungicides. The overall results showed that all tested fungicites retarded the colony mycelail growth of the *Alternaria tusi* as compare to control.

Keywords: fungicides, *Alternaria tusi*, leaf blight, discovered, Skardu Baltistan

Introduction

Pea (*Pisum sativum* L.) member of Fabaceae family is considered as one of the major vegetable grown all over the world. It is generally nutritious vegetable and is mostly cultivated as winter crop throughout the world. The crop is very much precious in crop rotation (Duke, 1981) [9]. It is considered as an important cultivated legume next to soybean, groundnut and beans. Economically pea is predominant export and cash crop in world trade and represents about 40% of the total trading in pulses (Oram & Agcaoili, 1994) [19]. Legumes are supposed to be the richest plant families in the world that are extending with more than hundreds of genera and thousands of species over all continents. Several members of this family have cost-effective importance and are being used for various purposes, including animal feeding, human consumption, and green manure for the betterment of soil (Mikic *et al.* 2006) [18]

The peas were first cultivated in western Asia, from where it spread to other different countries such as Europe, China and India. In conventional times, Greek and Roman authors mentioned its cultivation as a pulse and fodder crop. Pea was already recognized in the mountain regions of Central and East Africa before the arrival of the Europeans and was a well-established and important food crop in Rwanda and southwestern Uganda by 1860. The use of the cooked pods was first described in the Netherlands and France during the 16th century, whereas the use of immature seeds as a vegetable began in Europe a century later. At present, *Pisum sativum* is originate in all temperate countries and in most tropical elevated- lands. In Africa there are two types of peas such as garden pea and sugar pea and are considered as exotic crop of the regions. They are formerly having very much importance, sugar pea is found in Francophone countries, whereas, garden pea found in Anglophone countries. The other imported canned garden pea seeds which are mostly available everywhere especially in food shops (Agriculture, Forestry and Fisheries. 2011) [2].

Many countries of the tropics and subtropics regions of the world are under the cultivation of peas including Burma, India, Ethiopia, Morocco, Columbia, Ecuador, Peru and Pakistan (Javaid *et al.*, 2002) [13]. In Pakistan peas are grown as winter crop in plains of all four provinces and also as summer crop in high lands. It is grown as rain-fed and irrigated crop. The leading province in terms of cultivation of pea in Pakistan is Punjab, which contributes about 71% of the total production (Anon., 1999) [5]. The pea crop also occupies the third position among the major grain legume in Pakistan (Aslam *et al.*, 2000; Kazmi *et al.*, 2002) [7, 15]. Where as it is grown only in an area of 784 hectares in Baluchistan province, with total production of 8611 tons or 10983 kg ha-1 (Anon., 2002-2003) [4].

Peas are cultivated an area of 528.71 thousand hectares worldwide and it ranks fourth in production (441.53 thousand tons) among grains legume after soybean, groundnut and beans respectively (Ashraf *et al.*, 2011) [6]. Canada is growing and exporting more than More than 70 percent of the peas. The Spain is the largest buyers of Alberta peas (52,332 tonnes), Belgium (5 1, 2 1 1 tonnes), Cuba (25,220 tonnes), India (22,682 tonnes), France (1 1,922 tonnes) and the Peoples Republic of China (1 1, 82 1 tonnes) (Pekalski 1997) [20]. Few fungal diseases of peas such as Powdery mildew, Root rot and Blight are very important for the vegetable growers in term of yield losses (Iqbal *et al.*, 2000) [12]. Epidemics of viruslike diseases are widespread in winter-grown peas in Pakistan, but the causal viruses have not been identified. Thirty-five viruses have been reported to transmit a disease to peas naturally or experimentally (Hagedorn, 1984) [11].

Among the others fungal diseases in peas the *Alternaria* blight is caused by a fungus, *Alternaria tusi*. It infects chickpea, lentil, and field pea. The disease causing agent is seed borne and it can be reach by planting infected seeds. *A. alternata* produces the foliar lesions which are oval with a concentric ring pattern inside, tannish-brown in the centre and lighter towards the margins. Up to half or more of the leaves and stipules can be covered by lesions when an outbreak occurs. Pod lesions are smaller, raised and brownish in appearance. Small stunting of pea plants can also occur. Long-standing high humidity (3 or more days) and warm temperatures (16 O - 24°C) favour progress of *Alternaria* blight.

Objectives of the study

1. Survey and sampling of leaf blight disease of peas in the surrounding of District Kharmang and Skardu.
2. Isolation and identification of the leaf blight disease causing agent.
3. Evaluation of *in-vitro* chemical control measures against the *Alternaria* sp. causing leaf blight of peas.

Material and Methods

Survey and sampling of leaf blight disease of peas

The survey of the peas crop was being carried out randomly from the different vicinities of district kharmang and kwardu, such as Muntazir abad, maqpon, Gulshan abad hassan abad and kharmang olding. The diseases incidence Percentage was calculated in each surveyed field in zigzag method. The infected leaf samples showing the leaf blight symptoms of peas were collected in paper envelope, brought in Tusi Phytological research center kuardu, for the fungal detection and diagnosis purpose. The diseases incidence% from all surveyed localities was recorded by using the formula given bellow.

$$\text{Disease incidence \%} = \frac{\text{Number of infected plants}}{\text{Total number of Plants observed}} \times 100$$

Isolation and identification of the leaf blight disease causing agent

The collected diseased leaf samples showing leaf blight of peas symptoms were processed through isolation and identification in Tusi scientific research center kuardu. The infected leaves were washed with tape water by removing the dust and mud. These samples were air dried and cut in to small pieces with sterilized scissor. The cut samples were surface sterilized with 0.05% sodium hypchloride NaHCl for one minutes followed by tape water twice for two minutes. These sterilized samples were dried on sterilized filter paper for removing moisture. 5 tissue /samples per Petriplate were inoculated in sterilized Petriplate containing sterilized PDA. The inoculated plates were incubated at 25+1 c for seven days in incubators for the growth and multiplication of the disease causing fungus. After the inoculation the Isolated fungus was identified on basis of morphological characteristics as described by Simmons (2007) and with the help of the characteristics of the fungus mentioned in book "isolation and identification of fungi" by Frank. M. Dugan (2005). The measurement of the fungus colonies were calculated by using the following formula.

$$\text{Colonization \%} = \frac{\text{Number of pieces colonized by the fungus} * 100}{\text{Total number of colonies}}$$

Identification of the *Alternaria alternata* and *A. tusi*

The fungus *Alternaria alternata* and *A. tusi* was isolated from the infected leaves of pea showing the Blight symptoms. The fungus was identified on the basis of their color of colony growth, characteristics and conidial morphology as described by Bessadat *et al.*, (2014) [8] and with the help of a book "isolation and identification of fungi" by Frank. M. Dugan (2005). *Alternaria tusi* have one septate in center but *Alternaria alternata* have amorphous shape and 3 to 4 iregular deptate seen under microcope.

Evaluation of *in-vitro* chemical control measures against the *Alternaria* sp. causing leaf blight of peas.

The available four different fungicides such as Topsin-M, Topas, Nativo and Cabriotop were tested against the colony mycelial growth of the test fungus *Alternaria tusi* causing leaf blight of peas *in-vitro* condition at Tusi scientific research center kuardu. The solutions of the fungicides were made with standard scientific method (*Aqueous*) in percentage of their active ingredients. The trial was laid down in complete randomized design (CRD) with four treatment and three replications. The food poisoning techniques was used *in-vitro*

conditions as described by Aftab *et al.*, (2012) [1]. Different doses of the fungicides such as 50, 100 and 200 ppm were made. The sterilized media PDA was poured in sterilized petriplates. The 5 mm disc of the 5-7 days pure culture (*Alternaria tusi*) fungus was placed in the centre of 90 mm Petriplates with the help of sterilized cork borer. Three PDA petriplates without fungicides were served as control. These Petriplates were incubated in the incubator at 25 °C for seven days. The data was recorded after 24 hrs of each petriplates in (mm) till the full growth in control Petri plate.

Table 1: List of the fungicides used for the experiment

Serial number	Name of fungicides	Active ingredients	Name of company	Doses used for the trial (ppm)
1	Topsin-M	Thiophanate –methyl	Arysta Life Science	50, 100 and 200 ppm
2	Topas	Propiconazole	Syngenta	50, 100 and 200ppm
3	Nativo	Trifloxystrobin 50% + tobuconazole 25%	Bayer Crop Science	50, 100 and 200ppm
4	Cabriotop	Pyraclostrobin 50% + metiram 55%),	Arysta Life Science	50, 100 and 200 ppm

Results

1. Intensity of the leaf blight disease of peas at different localities

The results of the each surveyed area were showed that the leaf blight disease occurs in all fields with different intensities. The maximum disease incidence (22%) was recorded at Muntazir abad followed by maqpon (18%) and gulshan abad (17% whereas the minimum disease incidence (11%) and 12% was recorded at hasain abad and Kharmang olding respectively. (Table-1)

2. Isolation and identification of the *Alternaria tusi*

Different fungi such as *Alternaria alternata*, *Alternaria tusi*, *Aspergillus* sp and *penicillium* sp with different number of colony frequencies (%) were being isolated from the leaves of peas showing the leaf blight symptoms of disease. The disease causing fungus *Alternaria tusi* was isolated with maximum (88%) from muntazir abad followed by maqpon 85%, gulshan abad 83% and hassan abad 80% frequencies whereas 78% frequency was isolated from the Kharmang olding locality. The minimum frequency 4% and 5% was isolated from kharmang olding and Hasnain abad kwardu of the *penicillium* sp. The overall maximum 82.8% fungal frequency of *A. tusi* was recorded followed by 10.8% and 6.6% of *Aspergillus* sp and *Penicillium* sp from all surveyed localities respectively. (Table-2).

3. Effect of different fungicides on the linear mycelial growth of *Alternaria tusi*

Four different fungicides such as Topsin-M, Topass, Nativo and cabriotop with different doses 50, 100 and 200 ppm were evaluated *in-vitro* condition against the colony mycelial growth of the *Alternaria tusi* causing leaf blight disease of peas. The results showed that the all tested fungicides checked the mycelial growth of the *Alternaria tusi* at all applied doses. The increase in doses of the fungicides reduces the mycelial growth of the target fungus *in-vitro* conditions. Among all the tested fungicides the Nativo was found most effective at all applied doses. The minimum colony mycelia growth 22.33mm and 32.33mm was recorded when native fungicide used at highest doses 100 and 200 ppm. Whereas at lowest dose 50 ppm, the growth of the tested fungus recorded 39.33mm after seven days of the inoculation. The Cabriotop fungicide was also found effective at their highest dose as compare to Topas and Topsin-M fungicides. The result of the

Cabriotop fungicide was found 33.66mm, 42.66mm and 52.00mm at 200,100 and 50ppm doses respectively. The Topsin-m and Topas Fungicides did not proved better as compare to Nativo and Cabriotop fungicides.

The Topsin-M gave the maximum 72.00 mm colony mycelia growth at the lowest dose whereas, at moderate dose resulted 54.66 mm but found effective 39.33mm at their highest dose. The Topasin-M Fungicide was found the moderate effective against the test fungus. It resulted 35.33mm colony growth at the highest dose applied, followed by 43.33mm and 65.66 mm at moderate and lowest doses respectively. All the tested fungicides slow down the colony mycelia growth of the *Alternaria tusi* as compare to control 83.33mm. Table-3

Table 2: disease incidence% of leaf blight disease of pea in different locations

S. No	Locality	Disease Incidence%
1	Muntazir abad	22%
2	Maqpon	18%
3	Gulshan	17%
4	Hasnain abad	12%
5	Kharmang olding	11%

Disease incidence%= Number of disease plants/Total number plants*100

Table 3: Efficacy of different fungicides against the colony mycelia growth of *Alternaria tusi*

S. No	Fungicides	Doses		Radial colony mycelia growth (mm)	
		a	b	c	d
1	Topsin-M	50 ppm	a	72.00	a
		100 ppm	b	54.66	d
		200 ppm	c	44.66	e
2	Nativo	50 ppm	a	39.33	f g
		100 ppm	b	32.33	h
		200 ppm	c	22.33	i
3	Topas	50 ppm	a	65.66	c
		100 ppm	b	43.00	e f
		200 ppm	c	35.33	g h
4	Cabriotop	50 ppm	a	52.00	d
		100 ppm	b	42.66	e f
		200 ppm	c	33.66	h
5	Control	-----		83.33	
	LSD(P<0.05)	-----		0.0000	

Efficacy of different fungicides against the mycelial colony growth of *Alternaria tusi*

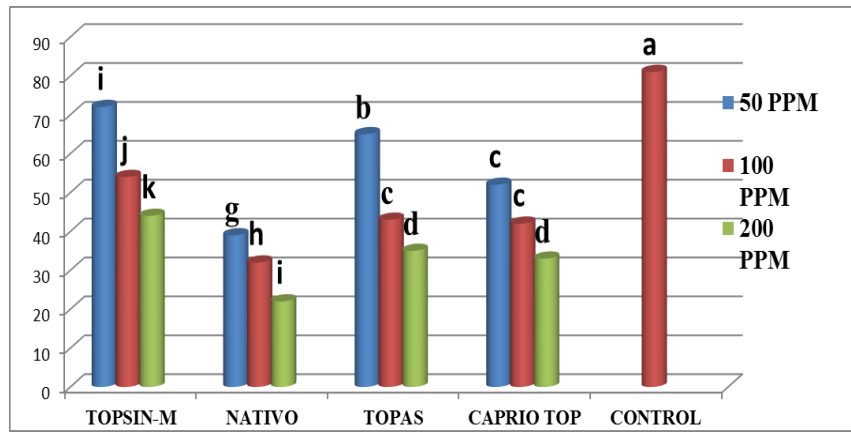
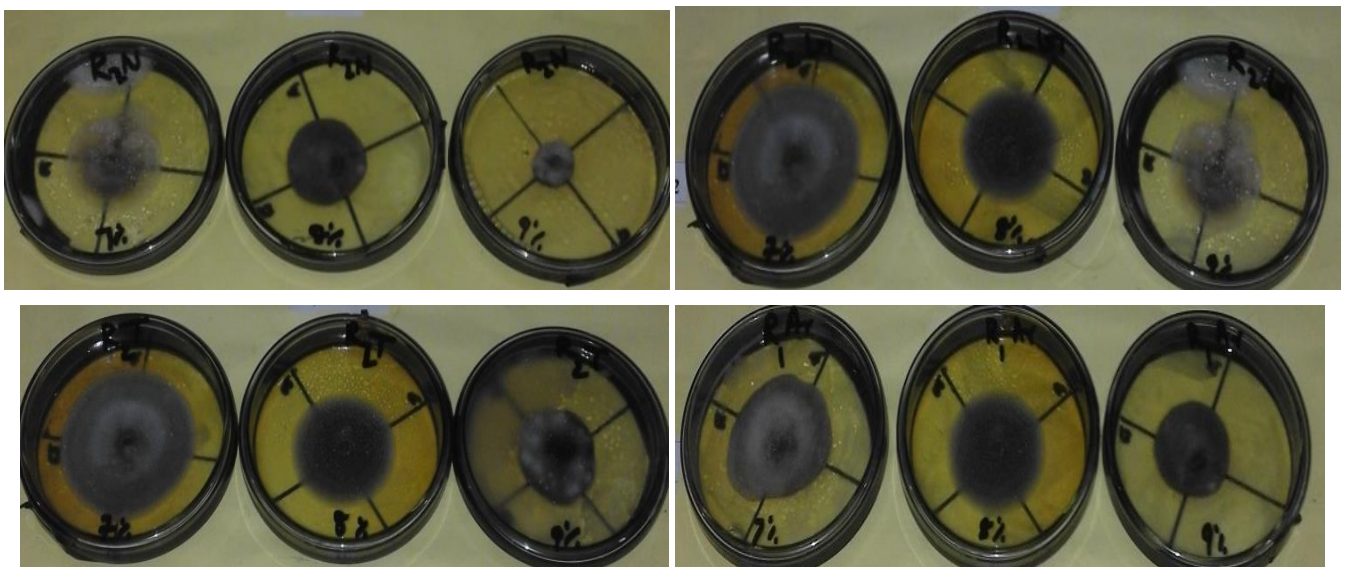


Fig 1: Pea plant showing the leaf blight symptoms in field infected by *A. tusi* with spores.



Concentration of chemicals 50ppm 100ppm and 200ppm

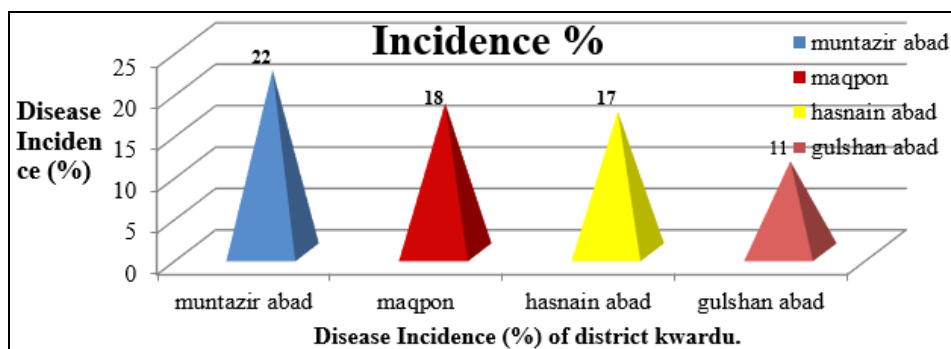


Fig 4: Pictures showing effect of fungicides against the colony mycelial growth of *Alternaria tusi* Disease Incidence (%) of district kwardu.

Discussion

In Pakistan peas are grown as winter crop in plains of all four provinces and also as summer crop in kharmang olding and kwardu. The Average pea yield per hectare is 678 kg which is quite low as compared to pea yield in developed countries. The yield of the pea crop is reduced due to many diseases. Among the diseases, the losses occur due to the Blight are important in reducing the yield (Anonymous, 2013) [3]. Keeping in view the losses occurs due to blight diseases a surveyed was conducted of the major pea producing areas of District kharmang and kwardu was being carried out to find out the disease incidence of leaf blight disease. Different intensities were observed in all surveyed fields. The maximum disease incidence (22%) was recorded at muntazir abad followed by maqpon (18%) Gulshan abad (17% whereas the minimum disease incidence (11%) and 12% was recorded at hasnain abad and kharmang olding respectively.

The *Alternaria tusi* is a major and dominant fungus responsible to cause the leaf blight of pea. The fungi which are frequently associated with leaf blight, stem blight and apical fruit rot of *Solanaceae* family are *Alternaria arborescens*, *A. alternata* and *A. tenuissima* (Bessadat *et al.*, 2014) [8]. During our research different fungi such as *A. tusi*, *Alternaria alternata*, *Aspergillus* sp and *Penicillium* sp with different frequencies (%) were being isolated from the leaves of peas showing the leaf blight symptoms of disease. The disease causing fungus *Alternaria tusi* was isolated dominantly with (88%) fungal frequency from muntazir abad followed by maqpon 85%, Gulshan abad 83% and hasnain abad 80% frequencies whereas 78% frequency was isolated from the kharmang olding. The minimum frequency 4% and 5% was isolated from hasnain abad and kharmang olding of the *Penicillium* sp. The overall maximum 82.8% fungal frequency of *Alternaria tusi* was recorded followed by 10.8% and 6.6% of *Aspergillus* sp and *Penicillium* sp from all surveyed localities respectively.

Many scientists have studied on different type of fungicides against the blight diseases of various crops. Khan *et al.*, (2007) [17] tested Three systemic fungicides: Topsin-M (Thiophanate methyl, 70% WP), Ridomil MZ (Mancozeb, 64% +Metalaxyl, 8% WP), and Bavistin (Carbendazim, 50% WP) alone and in combination with four non-systemic fungicides Captan (Captan, 50% WP), Indofil M-45 (Mancozeb, 75% WP), Indofil Z-78 (Zineb, 75% WP), and Thiram (Thiram, 75% WP) both *in-vitro* and *in-vivo* for their effectiveness to manage *Alternaria* blight of rape seed mustard caused by *Alternaria brassicae*. All the fungicides were evaluated for their efficacy at various concentrations, 50, 100, 150, 200 and 500 ppm. For our experiment we selected four different fungicides such as Topsin-M, Topass, Nativo and cabriotop with different doses 50, 100 and 150 ppm *in-vitro* condition against the colony

mycelial growth of the *Alternaria alternata* causing leaf blight disease of peas. The results showed that the all tested fungicides checked the mycelial growth of the *Alternaria tusi* at all applied doses. The increase in doses of the fungicides reduces the mycelia growth of the target fungus *in-vitro* conditions. Among all the tested fungicides the Nativo was found most effective at all applied doses. The minimum colony mycelia growth 22.33mm and 32.33mm was recorded when Native fungicide used at highest doses 200 and 100ppm. Whereas at lowest dose 50 ppm, the growth of the tested fungus recorded 39.33mm after seven days of the inoculation. The Cabriotop fungicide was also found effective at their highest dose as compare to Topas and Topsin-m fungicides. The result of the Cabriotop fungicide was found 33.66mm, 42.66mm and 52.00mm at 200, 100 and 50ppm doses respectively. The Topsin-M and Topas Fungicides did not proved better as compare to Nativo and Cabriotop fungicides. The Topsin-M gave the maximum 72.00 mm colony mycelia growth at the lowest dose whereas, at moderate dose resulted 54.66 mm but found effective 39.33mm at their highest dose. The Topas Fungicide was found the moderate effective against the test fungus. It resulted 35.33mm colony growth at the highest dose applied, followed by 43.33mm and 65.66 mm at moderate and lowest doses respectively. All the tested fungicides slow down the colony linear growth of the *Alternaria tusi* as compare to control 83.33mm.

Conclusions

The study was design to estimate the present status and losses occurs due to the leaf blight disease of pea in the vicinities of major vegetable growing District kharmang and Skardu baltistan. The disease was found in all vicinities with different intensities. The maximum disease incidence was recorded at Muntazir abad followed by Maqpon and gulshan abad, whereas the lowest disease incidence was recorded at hasnain abad and Kharmang olding. The fungicides were most effective. Nativo was found to be the best fungicide followed by Cabriotop, Topas and Topsin-M at all applied. *Alternaria tusi* was first time discovered from different localities of Skardu and kharmang olding. Fungi *Alternaria tusi* was observed first time in baltistan.

Suggestions

In our research there are clear variations in result between Chemicals. The chemicals are most effective and easily available in the markets and also looking effective can be used against the leaf blight disease of pea at different concentrations. Few more chemicals should be searched and study could be continuing for the further management of this disease. The entire studies were being carried out *in-vitro* conditions the same trial should be repeated in the field

conditions for the evaluation of best control measure against the Leaf Blight Disease of peas.

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