

In-vitro Evaluation of Botanicals, Fungi-toxic Chemicals and Bio-control Agent for Efficacy against *Turcicum* Leaf Blight of Maize

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ABSTRACT

Maize is the second most important cereal crop of Nepal. Its growth and production is severely affected by *Turcicum* leaf blight caused by *Exserohilum turcicum* at pre-harvest stage. A total of 6 botanicals, 4 chemical fungicides and 1 bio-control agent were evaluated for efficacy against *Exserohilum turcicum* under *in vitro* conditions following poisoned food technique at National Maize Research Program, Rampur, Nepal. The experiment was carried out in a completely randomized design with 5 replications. All the tested botanicals, fungicides and bio-control agent exhibited fungicidal action and significantly inhibited mycelial growth of the test pathogen over untreated control. Among botanicals, extract of *Acorus calamus* L. at 1% W/V checked the pathogen growth completely *in-vitro*. The mycelial growth inhibition percent of *Artimisia indica* Willd, *Lantana camera* L., *Allium sativum* L., *Xanthoxylum armatum* DC., and *Azadirachta indica* A. Juss. at the concentration of 2.5% W/V on PDA was 75.18%, 74.00%, 44.68%, 44.21% and 37.59% respectively. Among fungicides, the mycelial growth inhibition percent of *E. turcicum* due to ACME-COP (Copper oxychloride 50% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), Dithane M-45 (Mancozeb 75% WP) and Bavistin (Carbendazim 50% WP) at the concentration of 1000 ppm on PDA was 70.69%, 68.44%, 61.23%, and 60.52% respectively. Antagonist *Trichoderma viride* developed more rapidly than *E. turcicum* in single as well as in dual cultures. *T. viride* caused significantly inhibition of 35% of the pathogen *E. turcicum* on the 5th day of incubation. These results have important implications for the management of *turcicum* leaf blight disease in maize.

Keywords: Biological control, *Exserohilum turcicum*, fungicides, mycelial inhibition, plant extracts.

सारांश

मकै वालीमा लागने उत्तरी पात डडुवा रोगको कारक दुस्री इक्जेरोहीलम टरसीकम विरुद्ध स्थानिय तहमा नै उपलब्ध छ प्रकारका वानस्पतिक जडिबुटीहरु, ४ प्रकारका रसायनिक विषादी र १ प्रकारको जैविक दुसिको प्रयोग गरि राष्ट्रिय मकैवाली अनुसन्धान कार्यक्रम रामपुर, चितवनको केन्द्रिय प्रयोगशालामा सजिव वस्तु वाहिर (इनभिट्रो) अर्न्तगत विशाक्त खाद्य प्रविधि परिक्षण मार्फत गरिएको थियो । उक्त परिक्षणहरु पूर्ण रेन्डमाइज्ड ढाँचा (सि.आर.डि) मा पाँचपटक दोहोऱ्याइ व्यवस्थित गरिएको थियो । वानस्पतिक जडिबुटी तर्फबाट बोभोको पानी मिश्रित सार तत्वको न्यूनतम मात्रा (१% डव्लु./भी.) ले रोग कारक दुसिको वृद्धि पूर्ण रुपमा रोकी दुसिलाइ मारेको पाइयो साथै तीतेपाती, वनफाडाकांडा, लसुन, टीमुर र नीमको छुट्टा-छुट्टै पानी मिश्रित सार तत्वको मात्रा (२.५% डव्लु./भी.) ले रोग कारक दुसिको माइसेलियम वृद्धि निसेध प्रतिशत क्रमशः ७५.१८%, ७४%, ४४.६८%, ४४.२१% र ३७.५९% गरेको पाइयो । रसायनिक विषादी (मात्रा १००० पी.पी.एम) को तर्फबाट एक्मीकप (कपर अक्सीक्लोराइड ५०% डव्लु.पी.) ले दुसिको माइसेलियम वृद्धि निसेध प्रतिशत ७०.६९% गरेको पाइयो भने साफ (कारवेन्डाजीम १२% + म्यान्कोजेव ६३% डव्लु.पी) को हकमा ६८.४४%, डाइथेन एम ४५ (म्यान्कोजेव ७५% डव्लु.पी) को हकमा ६१.२३% र वेभिष्टिन (कारवेन्डाजीम ५०% डव्लु.पी) को हकमा ६०.५२% पाइयो । जैविक दुसि र रोगकारक दुसि एकैसाथ वृद्धि गर्ने प्रविधि (ड्यूलकल्चर टेक्निक) को नातिजा अनुसार उष्मायन अवधिको पाँचौ दिनमा नै जैविक दुसि ट्राइकोडर्मा भिरिडी पर्स. ले रोग कारक दुसि इक्जेरोहीलम टरसीकमको माइसेलियमलाई पूर्ण रुपमा छोपी दुसिको वृद्धि रोक्न सफल भएको पाइयो जसमा दुसिको माइसेलियम वृद्धि निसेध प्रतिशत ३५ % रहेको थियो ।

INTRODUCTION

There are various biotic and abiotic stresses which are responsible for the feeble yield of maize (*Zea mays* L.) in Nepal; however, different diseases and poor crop management practices are most important among them (Subedi 2015). Maize is invaded by several fungal pathogens, but ubiquitous incidence of turcicum leaf blight caused by *Exserohilum turcicum* at pre-harvest stage is the most important bottleneck in satisfactory maize production (NMRP 2015). Heavy dews, cool temperature and frequent rains are environmental conditions conducive for disease development (Jordan et al 1983). The pathogen first identified in Nepal in 1964 from Patan, Lalitpur (Khadka and Shah 1967, Manandhar 1980). The disease is widely distributed throughout the country; however, it is more prevalent in the cool and humid conditions of the foothills and also common in the winter maize of the terai region. It was not considered as the major disease of maize crop in Nepal until 1985 (Dahal et al 1992). The disease usually appears before tasseling cause yield loss upto 40 to 70% and has become the most important disease at present in Nepal (NMRP 2016). Late sown maize in the summer season is most affected by this disease in hills (Manandhar et al 2007). The objective of this work was to evaluate the plant extracts, chemical fungicides and bio-control agents for their efficacy against *Exserohilum turcicum* causing northern leaf blight of maize under *in vitro* condition.

MATERIALS AND METHODS

Experimental site and isolation of pathogen

The samples of northern leaf blight disease specimens were collected from maize (*Zea mays* L. 2n = 2x = 20) growing maize fields of National Maize Research Program (NMRP), Rampur and brought in to the Plant pathology laboratory during winter season of 2017. The infected leaves were cut in to several small sections 5-10 mm-square from the margin of the infected lesion to contain both diseased and healthy tissue. Then they were placed in surface sterilizing agents' solutions (1% NaOCl) for about 15-30 seconds and the sections were taken out aseptically and blotted dry on clean, sterile paper towels or washed in three changes of sterile water and placed on the nutrient medium, usually three to five sections per dish. The pathogen, *E. turcicum*, grew from the sections and the colonies of the pathogen were sub cultured aseptically for further study.

Botanicals and preparation of extracts

The botanicals selected for the experiments were Sweet flag (*Acorus calamus* L. 2n = 4x = 48), Prickly ash (*Xanthoxylum armatum* DC. 2n = 2x = 66), Mugwort (*Artemisia indica* Willd. 2n = 2x = 18), Neem (*Azadirachta indica* A. Juss. 2n = 2x = 28), Lantana (*Lantana camera* L. 2n = 2x = 22, 44) and Garlic (*Allium sativum* L. 2n = 2x = 16). The dried fruits of prickly ash, stolos of sweet flag, garlic and fresh leaves of neem, mugwort and *Lantana camera*, were used to prepare the extract. These materials were dried in the shade for two days. While preparing the extracts, the unwanted debris was removed, surface sterilized for 2 minutes in 70% ethanol, and washed in three changes of sterile distilled water. The paste was obtained by grinding 1, 2 and 2.5g each of prickly ash, sweet flag, neem, garlic, mugwort and lantana plants separately in 100 ml of sterile distilled water using a mortar and pestle. The paste was squeezed and filtered through 4 folds of sterile cotton wool into a 150 ml conical flask to give concentrations of 1, 2 and 2.5% W/V (Wokocha and Okereke 2005). The volume of the filtrate collected was 100 ml and each of the extract concentration was kept aseptically in 150 ml conical flasks. The content in the flasks was exposed to UV light for further sterilization.

Fungicides and bio-control agents

The chemical fungicides used for the experiment were Dithane M-45 (Mancozeb 75% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), ACME-COP (Copper oxychloride 50% WP) and Bavistin (Carbendazim 50% WP). The concentration level maintained was 1000 ppm. The bio-control agent used for the experiment was laboratory isolated culture of *Trichoderma viride*.

In-vitro test

The in-vitro test of plant extracts and fungicides against *Exserohilum turcicum* was done at the laboratory of NMRP, Rampur, Chitwan during 2017 winter. The experiment was carried out by poisoned food technique with completely randomized design (CRD). The linear growth was measured after 15 days of incubation. Different concentrations of water extracts of plants and fungicides (1000

ppm) were individually incorporated into different conical flasks containing 100 ml of sterile molten potato dextrose agar medium prepared from the dehydrated PDA (HIMEDIA, REF MO96-500G) as per the instructions of the manufacturer in the laboratory condition. The plant extracts and fungicides were added to the PDA separately when it was cooled to about 50°C with the help of micro-pipette. The flasks were gently agitated for 2 min to allow for a proper mixing of extract with the nutritive medium then 20 ml medium was poured in the petri-plates (9cm diameter). Strepto-penicillin (Bistrepen-V) (250 mg per litre) was added to the medium at the time of pouring to prevent bacterial contamination. In case of plant extracts, there were a total of seven different treatments consisting of prickly ash, sweet flag, mugwort, neem, lantana, garlic and control. The concentration of botanicals was maintained to 1, 2 and 2.5% W/V. There were 6 experimental setting consisting of prickly ash, sweet flag, mug-wort, neem, lantana and garlic extract. Each experiment included 4 treatments; plant extract at 1% W/V, 2% W/V and 2.5% W/V including control (without any extract). In case of fungicides, there were 5 treatments consisting of Dithane M-45 (Mancozeb 75% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), ACME-COP (Copper oxychloride 50% WP), Bavistin (Carbendazim 50% WP) and control. The concentration level maintained for the fungicides was 1000 ppm. Four mm diameter of *E. turcicum* of one week old culture was cut by cork borer and picked up with the help of inoculating needle and placed onto the center of the plate. The plate contained PDA amended plant extract and fungicides where the cut piece was kept upside down for better contact of pathogen to the media. The plates were incubated in incubator at 25°C for up to 15 days.

Observation

The colony diameter (mm) of the pathogen was determined by measuring the average radial growth on different incubation dates. Average radial growth was recorded by using a measuring scale from the lower view of the petri-plates.

Mycelial growth inhibition percent was calculated by following formula.

$$\text{Mycelial growth inhibition (\%)} = [(dc-dt) / dc] \times 100$$

Where dc = average diameter of fungal colony in the control and dt = average diameter of fungal colony in the treatment group

The antagonist effect of bio-control agent was evaluated against *E. turcicum* in dual culture technique. Four mm diameter of tested pathogen of one week old culture and above stated bio control agent was cut by cork borer and picked up with the help of inoculating needle and placed apart on solidified PDA medium in an equal distance comprising 5 replications and incubated as stated earlier. Observations were recorded by measuring colony diameter of both the test fungus and bio agent on different days of incubation up to 5 days and per cent inhibition was calculated.

Statistical analysis

The recorded observations were analyzed statistically using MSTAT-C computer package program and treatment means were compared using Duncan's Multiple Range Test (DMRT) at 1% levels of significance

RESULTS

All tested botanicals inhibited radial mycelial growth of *Exserohilum turcicum* significantly over the control at different concentrations (**Table 1**). The sweet flag (*Acorus calamus* L.) even at lower dose (1% W/V on PDA) was able to check completely the growth of pathogen (4.00 mm). With the increase in concentration (2.5% W/V), the mean colony diameter of the *E. turcicum* in the plate amended with mugwort (*Artemisia indica*) and lantana (*Lantana camera* L.) was significantly lower i.e. 21.0 mm and 22.0 mm respectively than the diameter recorded at control plate. However, the mean colony diameter of the pathogen did not differ significantly at 2.5% W/V dose when the plates were amended with prickly ash (*Xanthoxylum armatum* DC.) and garlic (*Allium sativum*) separately up to 15th day of incubation period (**Figure 1**). The mycelial growth inhibition percent of *Artemisia indica* Willd, *Lantana camera* L., *Allium sativum* L., *Xanthoxylum armatum* DC., and *Azadirachta indica* A. Juss. at the concentration of 2.5% W/V on PDA was 75.18%, 74.00%, 44.68%, 44.21% and 37.59% respectively (**Table 2**).

Table 1. Effect of plant extracts incorporated PDA on the growth of *E. turcicum* in different concentration levels at 25°C temperature during 2017 at NMRP, Rampur, Chitwan, Nepal

Treatment	Mean colony diameter (mm)		
	1% W/V	2% W/V	2.5% W/V
<i>Acorus calamus</i>	†4.00 ^g	4.00 ^f	4.00 ^e
<i>Xanthoxylum armatum</i>	73.00 ^c	57.00 ^c	47.20 ^c
<i>Artimisia indica</i>	43.60 ^e	23.80 ^e	21.00 ^d
<i>Azadirachta indica</i>	56.00 ^d	55.40 ^c	52.80 ^b
<i>Lantana camera</i>	57.60 ^d	26.80 ^d	22.00 ^d
<i>Allium sativum</i>	79.00 ^b	72.80 ^b	46.80 ^c
PDA only	84.60 ^a	84.60 ^a	84.60 ^a
F test	**	**	**
LSD (0.01)	2.42	2.66	3.13
CV%	2.43	3.28	4.50

†Means of 5 replications. Means in column with same superscript are not significantly differed by DMRT ($P \leq 0.01$). mm- millimeter, W/V- Weight by volume, PDA- Potato Dextrose Agar, **: highly significant

Table 2. Effect of plant extracts incorporated PDA on mycelial growth inhibition of *E. turcicum* in different concentration levels at 25°C temperature during 2017 at NMRP, Rampur, Chitwan, Nepal

Treatment	MGI %		
	1% W/V	2% W/V	2.5% W/V
<i>Acorus calamus</i>	95.27	95.27	95.27
<i>Xanthoxylum armatum</i>	13.71	32.62	44.21
<i>Artimisia indica</i>	48.46	71.87	75.18
<i>Azadirachta indica</i>	33.81	34.52	37.59
<i>Lantana camera</i>	31.91	68.32	74.00
<i>Allium sativum</i>	6.62	13.95	44.68
PDA only			

MGI- Mycelial Growth Inhibition, W/V- Weight by volume, PDA- Potato Dextrose Agar

All the tested fungicides incorporated PDA had significant ($p < 0.01$) effect on the growth of the *Exserohilum turcicum* at 25°C as compared to control at 1000 ppm concentrations up to 15th day of incubation (Table 3). There was no significant difference between Bavistin (Carbendazim 50% WP) and Dithane M-45 (Mancozeb 75% WP) to reduce the growth of *Exserohilum turcicum* in all the dates of incubation. The fungicides SAAF (Carbendazim 12% + Mancozeb 63% WP) and ACME-COP (Copper oxychloride 50% WP) had also the same effect to check the growth of pathogen (Figure 2). The mean colony diameter of the pathogen in the plate amended with ACME-COP (Copper oxychloride 50% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), Dithane M-45 (Mancozeb 75% WP) and Bavistin (Carbendazim 50% WP) was significantly lower ie 24.80 mm, 26.70 mm, 32.80 mm and 33.40 mm respectively than the control plate (84.60 mm) on 15th day of incubation. The mycelial growth inhibition percent of *E. turcicum* due to ACME-COP (Copper oxychloride 50% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), Dithane M-45 (Mancozeb 75% WP) and Bavistin (Carbendazim 50% WP) at the concentration of 1000 ppm on PDA was 70.69%, 68.44%, 61.23%, and 60.52% respectively.

Table 3. Effect of fungicides incorporated PDA on the growth of *E. turcicum* in concentration level (1000 ppm) at 25°C during 2017 at NMRP, Rampur, Chitwan, Nepal

Treatments (Concentration: 1000 ppm)	Mean colony diameter (mm)	MGI%
Dithane M-45 (Mancozeb 75% WP)	†32.80 ^b	61.23
SAAF (Carbendazim 12% + Mancozeb 63% WP)	26.70 ^c	68.44
ACME-COP (Copper oxychloride 50% WP)	24.80 ^c	70.69
Bavistin (Carbendazim 50% WP)	33.40 ^b	60.52
Control (only PDA)	84.60 ^a	
F test	**	
LSD (0.01)	3.15	
CV%	4.33	

†Means of 5 replications. Means in column with same superscript are not significantly different by LSD ($P < 0.01$) mm-millimeter, MGI- Mycelial Growth Inhibition, ppm- parts per million, WP- wettable powder, PDA = Potato Dextrose Agar, **: highly significant

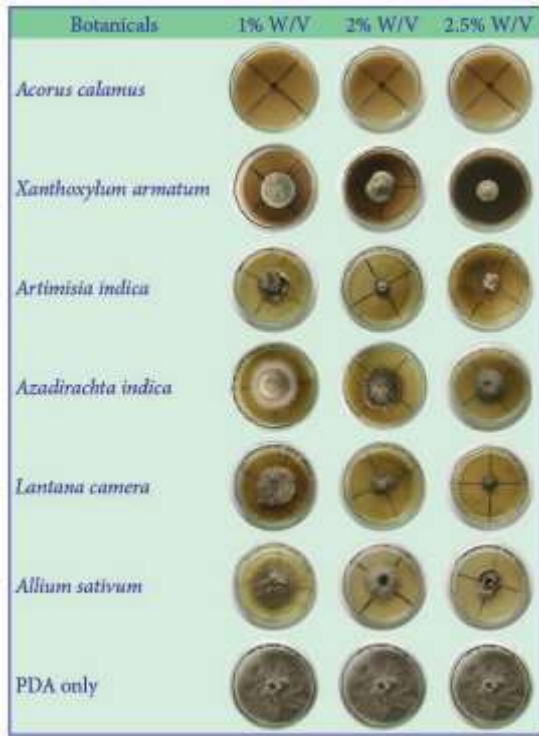


Figure 1. Effect of plant extracts on the growth of *E. turcicum* in different concentration levels at 25°C during 2017 at NMRP, Rampur, Chitwan

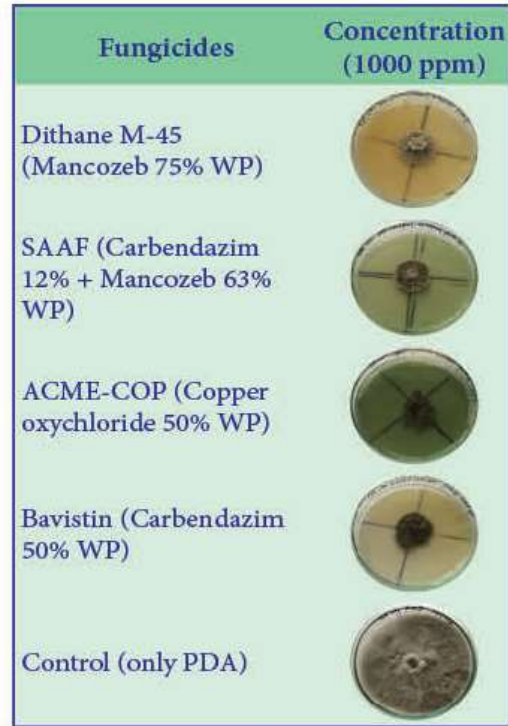


Figure 2. Effect of fungicides on the growth of *E. turcicum* in different concentration levels at 25°C during 2017 at NMRP, Rampur, Chitwan

The mean colony diameter of the antagonist was significantly higher than the pathogen, *E. turcicum*, on 5th day of incubation at 25°C (Table 4). Less (18.60 mm) mean colony diameter of the pathogen was measured when dual culture was done with *Trichoderma viride* Pers. (PPD isolate). The diameter of the pathogen alone was 28.60 mm on 5th day of incubation at room temperature. The mean colony diameter of the antagonist *T. viride* was noticed higher in dual culture with *E. turcicum* in all days of incubation compared to the diameter of *T. viride* grown alone in PDA (Figure 3). It was observed that the growth of the antagonist was enhanced in dual culture with pathogen than the antagonist alone grown in PDA while the growth of the pathogen was less in dual culture with antagonist compared to the pathogen grown alone in PDA in all days of incubation.

Table 4. Effect of *Trichoderma viride* on the growth of *E. turcicum* in different incubation periods at 25°C during 2017 at NMRP, Rampur, Chitwan

Treatment	Mean colony diameter (mm)				
	1st day	2nd day	3rdday	4th day	5th day
<i>Trichoderma viride</i> only	†20.80 ^b	50.20 ^b	68.00 ^b	83.20 ^a	88.80 ^a
<i>T. viride</i> in dual culture with <i>E. turcicum</i>	28.80 ^a	61.80 ^a	73.90 ^a	85.10 ^a	88.30 ^a
<i>Excerohilum turcicum</i> only	11.00 ^c	14.40 ^c	21.80 ^c	24.40 ^b	28.60 ^b
<i>E. turcicum</i> in dual culture with <i>T. viride</i>	7.40 ^d	13.60 ^c	15.40 ^d	17.80 ^c	18.60 ^c
F test	**	**	**	**	**
LSD (0.01)	2.00	2.63	3.67	2.92	2.69
CV%	6.38	4.07	4.44	3.00	2.60

†Means of 5 replications. Means in column with same superscript are not significantly different by LSD ($P < 0.01$), mm-millimeter, **- highly significant

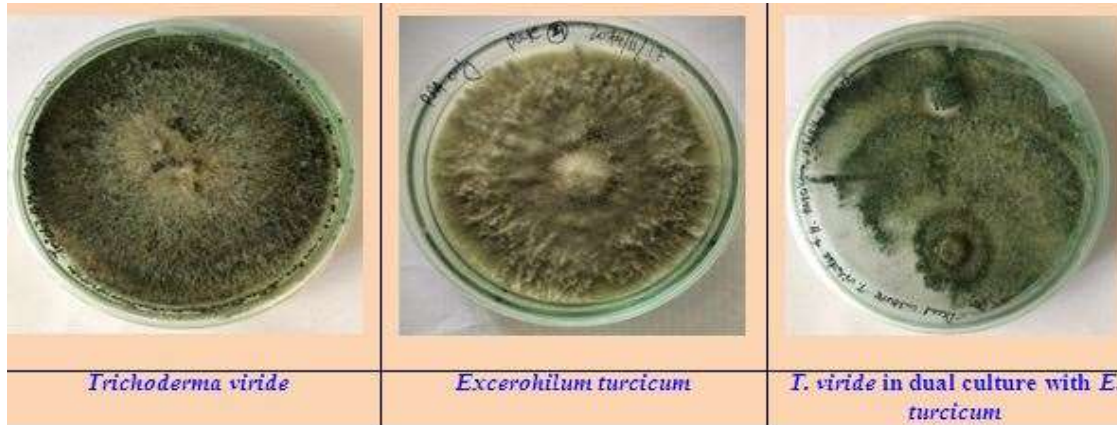


Figure 3. Effect of *Trichoderma viride* on the growth of *E. turcicum* at 25°C during 2017 at NMRP, Rampur, Chitwan.

DISCUSSION

As the plant pathogens develop resistance to many fungicides it creates immense problem to control various crop diseases which pushes to search for new and alternative fungi-toxic substance from various sources including plant extracts. The results indicated that the various phyto-extracts tested inhibited the mycelia growth of *E. turcicum* as compared to control. However, there was no further growth of the pathogen on the PDA incorporated with *Acorus calamus* extract at 1% W/V in all the dates of incubation. Even at the lower dose 0.5 % W/V on PDA, *Acorus calamus* extract significantly check the growth of the pathogen followed by *Artimisia indica* and *Lantana camera* at higher concentration level (2.5% W/V). Similar results on antifungal activity of aqueous extracts of different plants has been reported by various workers (Shivapuri et al 1997, Meena et al 2003). From the present study, it is concluded that *Acorus calamus* L., *Artimisia indica* and *Lantana camera* L. were effective botanicals to suppress the growth of *E. turcicum*. A perusal of literature showed similar experiments regarding the control of *E. turcicum* using plant extracts has not yet been done in Nepal and very few literatures were available. Phongpaichit et al(2005) investigated that an antimicrobial guided one of methanol extracts of *A. calamus* containing β -asarone as a major component showing high antifungal activity against *Microsporium gypseum* Bodin, *Trichophyton rubrum* Castell. and *Penicillium marneffeii* Segretain and had moderate activity against *Candida albicans* Berkhout and *Cryptococcus neoformans* Vuill. The findings of Kungtha (1999) also indicated that alcoholic extract of *A. calamus* could inhibit many fungi including *Alternaria brassicae* Sacc, *Fusarium oxysporum* f.sp. lycopersici, *Rhizoctonia solani* Kuhn, *Sclerotiana sclerotiorum* de Bary at the concentration of 0.10% upward. The major antifungal compound found in *A. calamus* is β -asarone which is considered to be the most biological active compound in the rhizome of *A. calamus* and quantities of β -asarone vary 10-20% in the rhizome of European origin while in Asia, it was found in the range of 70-90% (Karwowska et al 1997). Findings of current study are in agreement with the report of Deena and Thoppil (2000) who described the essential oil of *Lantana camera* containing β -caryophyllene, geranyl acetate, terpinyl acetate, bornyl acetate and limonene remarkably inhibited the growth of many tested bacteria and fungi. *Aspergillus niger* Tieghem, *Fusarium solani* Schlecht and *Candida albicans* Berkhout appeared as the most sensitive ones. Khedkar et al (2012) studied the efficacy of phyto extract on *E. turcicum* by poisoned food technique under in vitro conditions and found that Nimbicidin @ 5% concentration was superior to all other treatments with the maximum mycelial inhibition of 68.85% followed by negunda (38.88%) and neem seed kernel extract (37.01%). Subedi et al (2015) also reported that *Acorus calamus* L. was the most effective botanical followed by *Xanthoxylum armatum* when tested against *Stemphylium botryosum* Walr. both at *in vitro* and *in vivo* condition.

The finding of this experiment is also in agreement with the Wani et al (2017) who found that among fungicides, Mancozeb was found best (95.23% mean inhibition) and among plant extracts, neem oil inhibited maximum mycelial growth of *E. turcicum* (62.33%). Investigations also indicated that

mancozeb was most effective in controlling Turcicum leaf blight of maize (Pandurangegowda 1987, Begum et al 1993). Reddy et al (2013) evaluated seven fungicides in vitro against *E. turcicum* causing leaf blight of maize and found treatment mancozeb @ 0.25% and combination treatment of mancozeb and carbendazim, i.e, Saff @ 0.25% recorded the lowest per cent disease index (PDI) reducing the disease by 73.0 and 72.1%, respectively.

Antagonist *Trichoderma viride* Pers. showed the potentials to suppress the radial colony growth of *E. turcicum* under laboratory condition. *T. viride* developed more rapidly than *E. turcicum* in single as well as in dual cultures. The intensive development of *Trichoderma* gives it a significant advantage in competition with pathogens for nutrients and space, besides the production of mycotoxins. *In vitro* evaluation of bio-control agents revealed that *Trichoderma viride* caused significantly inhibition (35%) of the pathogen *E. turcicum* on 5th day of incubation. The inhibitory effect of these bio-agents was probably due to competition and / or antibiosis. The antagonism of *T. viride* observed in the present studies is in tune with the findings of various workers (Biles and Hill 1988, Mahamood et al 1995, Ramachandra 2000). The mechanism of *Trichoderma* to control pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Cal et al 2004). *Trichoderma sp.* grown over the pathogenic fungal hyphae, coils around them and degrades the cell walls. This action of parasitism restricts the development and activity of pathogenic fungi. Additionally, or together with mycoparasitism, some *Trichoderma* species release antibiotics (Harman 2006). According to Rosado et al (2007), the main factor for ecological success of this genus is a combination of very active mycoparasitism mechanisms and an effective defensive strategy, induced in the plants.

All the tested botanicals, fungicides and bio-control agent exhibit fungicidal action and inhibit mycelial growth of the *Exserohilum turcicum*. Among botanicals, extract of *Acorus calamus* L. at lower dose also completely check the pathogen growth *in-vitro*. At higher dose, plant extracts of *Artimisia indica* Willd, *Lantana camera* L., *Xanthoxylum armatum* DC., *Allium sativum* L. and *Azadirachta indica* A. Juss. are also effective to reduce the mycelial growth of *E. turcicum*. Among fungicides, ACME-COP (Copper oxychloride 50% WP), SAAF (Carbendazim 12% + Mancozeb 63% WP), Dithane M-45 (Mancozeb 75% WP) and Bavistin (Carbendazim 50% WP) at the concentration of 1000 ppm on PDA are effective to check the growth of the pathogen. Antagonist *Trichoderma viride* Pers. suppresses the radial colony growth of *E. turcicum* and develops more rapidly than *E. turcicum* in single as well as in dual cultures.

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REFERENCES

- Begum H, RB Raj and E Satyanarayana. 1993. Field evaluation of five fungicides to control Turcicum leaf blight in maize. Indian Journal of Plant Protection. **21**:110-116.
- Biles CL and JPHILL. 1988. Effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on excised wheat seedling leaves. Phytopathology. **78**:656-659.
- Cal A, I Larena, P Sabuquillo and P Melgarejo. 2004. Biological control of tomato wilts. Recent Research Development and Crop Science. **1**:97-115.
- Dahal G, P Amatya and HK Manadhar. 1992. Plant diseases in Nepal-country profile. Review of Plant Pathology. **71**(11):797-807.
- Deena MJ and JE Thoppil. 2000. Antimicrobial activity of the essential oil of *L. camara*. Fitoter. **71**:453-457.
- Harman GE. 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology. **96**:190-194.
- Jordan EG, JM Perkins, RA Schall and WL Pederson. 1983. Occurrence of race 2 of *Exserohilum turcicum* on corn in central United States. Plant Disease. **67**:1163-1165.
- Karwowska K, J Stegman, W Duszkiwicz-reinhard and A Dobrzeniecka. 1997. Studies on isolation and chemical composition of biologically active compounds of calamus (*Acorus calamus*). Horticulture **18**:109-113.

- Khadka BB and SM Shah. 1967. Preliminary list of plant diseases recorded in Nepal. Nepalese Journal of Agriculture.2:47-76.
- Khedekar SA, SI Harlapur, S Kulkarni and VI Benagi. 2012. Evaluation of fungicides, botanicals and bioagents against *Turcicum* leaf blight of maize caused by *Exserohilum turcicum*(Pass.) Leonard and Suggs. International Journal of Plant Protection 5(1):58-62.
- Kungha T. 1999. Effect of clove, sweet flag, star anise and Chinese cassia extracts on some plant pathogenic molds. Master Thesis. Chiang Mai University, Chiang Mai. 80p.
- Mahamood A, N Javed, R Ahmad and ZI Raheel. 1995. Biological control of maize leaf blight caused by *Helminthosporium turcicum* Pass in vitro. Pakistan Journal of Phytopathology 7:62-64.
- Manandhar G, S Baidya, DC Paudel and RR Burlakoti. 2007. Evaluation of maize genotypes for resistant to northern leaf blight disease in Nepal. In: Proceedings of the 25th National Summer Crops Research Workshop on Maize Research and Production in Nepal (DB Gurung, DC Paudel, G KC, SR Upadhyay and BB Pokhrel, eds). June 21-23, 2007, Nepal Agriculture Research Institute, NARC, Khumaltar, Lalitpur, Nepal. pp.273-275.
- Manandhar KL. 1980. Review on maize diseases investigation from 1975-1980. In: Proceedings of the 8th Summer Crops Workshop, 25-30 January 1980, National Maize Development Program, Rampur, Chitwan, Nepal. pp.255-278.
- Meena RL, RS Rathore and K Mathur. 2003. Evaluation of fungicides and plant extracts against banded leaf and sheath blight of maize. Indian Journal of Plant Protection. 31:94-97.
- NMRP. 2015. Annual Report 2071/72 (2014/15) (S Subedi, TB Karki, J Shrestha and G Bhandari, eds). National Maize Research Program, NARC, Rampur, Chitwan, Nepal. 198p.
- NMRP. 2016. Annual Report 2072/73 (2015/16) (S Neupnae, R Sharma, S Sharma and J Shrestha, eds). National Maize Research Program, NARC, Rampur, Chitwan, Nepal. 157p.
- Pandurangegowda KT. 1987. Status of breeding and management of *Turcicum* leaf blight of maize. In: Progressive Report of 30th Annual Maize Workshop, 17-20 April, 1987, AICMIP, IARI, New Delhi, India. pp.21-28.
- Phongpaichit S, N Pujenjob, V Rukachaisirikul and M Ongsakul. 2005. Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn. Journal of Science and Technology. 27(2):517- 523.
- Ramachandra CG. 2000. Studies on leaf blight of *Dicoccum* wheat caused by *Exserohilum hawaiiensis* (Bugnicourt). Master Thesis. Subram. and Jain, Ex. Ellis, M.B. University of Agricultural Sciences, Dharwad. 120p.
- Reddy TR, NP Reddy, RR Reddy and SS Reddy. 2013. Management of *Turcicum* leaf blight of maize caused by *Exserohilum turcicum* in maize. International Journal of Scientific and Research publications 3(10):1-4.
- Rosado I, M Rey, A Codon, J Gonavites, MA Moreno-mateos and T Benitez. 2007. QID74 Cell wall protein of *Trichoderma harzianum* is involved in cell protection and adherence to hydrophobic surfaces. Fungal Genetics and Biology 44(10):950-964.
- Shivapuri A. OP Sharma and SL Jhamaria. 1997. Fungitoxic properties of plant extracts against pathogenic fungi. Journal of Mycology and Plant Pathology. 27:29-31.
- Subedi S. 2015. A review on important maize diseases and their management in Nepal. Journal of Maize Research and Development 1(1):28-52. DOI: <https://doi.org/10.3126/jmrd.v1i1.14242>
- Subedi S, SM Shrestha, GB KC, RB Thapa, SK Ghimire, DB Gharti and S Neupane. 2015. Evaluation of plant extracts against *Stemphylium* blight of lentil. Nepal Journal of Science and Technology. 16 (1):11-16.
- Wani TA, A Mushta and A Anwar. 2017. Evaluation of Fungicides, Bioagents and Plant Extracts against *Exserohilum turcicum* causing *Turcicum* Leaf Blight of Maize. International Journal of Current Microbiological Applied Science 6(8):2754-2762.
- Wokocha RC and VC Okereke. 2005. Fungitoxic activity of extracts of some medicinal plants on *Sclerotium rolfsii* causal organism of the basal stem rot diseases of tomato. Nigerian Journal of Plant Protection. 22:106-110.

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