Research Article

INVITRO EVALUATION OF FUNGICIDES AND BOTANICALS AGAINST *FUSARIUM OXYSPORUM* F.SP. *CUBENSE* OF BANANA

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ABSTRACT

Fusarium wilt caused due to *Fusarium oxysporum* f.sp *cubense* is one of the most destructive diseases of banana worldwide. Three fungicides namely Bavistin (Carbendazim 50% WP), Sectin (Fenamidone 10%+Mancozeb 50%), Dhanucop (Copper oxychloride 50% WP) in two concentrations (100 and 200ppm) and three botanicals (*Azadirachta indica*, *Justicia adhatoda* and *Eucalyptus globules*) at (5 and 10%) concentration were evaluated against *F. oxysporum* under laboratory condition following poison food technique. The design of the experiment was completely randomized design with four replications and seven treatments including control. Observations were taken at 24 hours interval upto 144 hours for the assessment of their inhibitory effect. Isolates of pathogen used in this study were collected from Chitwan district of Nepal. All the fungicide showed effectiveness in decreasing the fungal growth at increased concentrations. Total inhibition of the fungal growth at both concentrations was found in case of bavistin whereas sectin showed 21.94 and 34.48% inhibition at 100 and 200 ppm concentration, respectively. In case of botanicals, Eucalyptus showed highest percent inhibition (36.67%) followed by Asuro (9.10%) and Neem (5.32%) at 10% concentration. Fungicide Bavistin and Masala among botanicals were proved to be the best which inhibited the fungal growth in all concentrations.

Key words: *Botanicals, fungicides, Fusarium oxysporum f.sp. cubense*

INTRODUCTION

Banana is considered one of the major fruits in social and economic respect in Nepal and stands fourth position after citrus, mango and apple in term of fruit growing area in the country (K.C. *et al*., 2009).

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Malbhog, an indigenous banana variety of Nepal valued for its flavor and delicacy suffers production losses due to *Fusarium oxysporum* f.sp. *cubense*. Fusarium wilt of several agricultural and horticultural crops is caused by *F. oxysporum,* a pathogenic fungus common in soil around the world (Mongkutkarn and Kasem, 2016). Pathogen enters the plants through the roots and colonizes the xylem vessels thereby blocking the flow of water and nutrients (Ploetz and Churchill, 2011).Yellowing of oldest leaves or a longitudinal splitting of the lower portion of the outer leaf sheaths on the pseudostem is the first external symptoms of panama wilt of banana. Evidence suggests that *F. oxysporum* f. sp.*cubense* originated in Southeast Asia (Ploetz and Pegg, 1997) and from there was disseminated rapidly throughout the world with infected rhizomes (Stover, 1962). Infected rhizomes or suckers and adhering soil are the means of transmission of panama wilt from one country or growing region to another region. These rhizomes and suckers often did not exhibit symptoms (Moore *et al.*, 1995). *F. oxysporum* is spread from infested to non-infested areas between fields or within production areas in contaminated water, and with farm equipment, shoes and vehicles (Ploetz, 1994).

Chemical control of Panama wilt of banana has received little attention in the past 40 years although fungicides remain an important part of disease control strategies. At present, botanical fungicides are gaining importance as they are considered as an alternative source for chemicals in the management of soil borne pathogens. The active ingredient present in botanicals may directly act on the pathogen or induce systemic resistance in the host plants resulting in the reduction of disease development (Yulier and Toyata, 2015). For the management of various plant diseases including panama wilt of banana, botanicals are considered eco-friendly, less expensive and easily available (Akila *et al*., 2011). Hence, in the study different chemical fungicides and botanical extractions were included for testing the efficacy level to control the panama disease of banana.

MATERIALS AND METHODS

An in-vitro experiment was conducted at Plant Pathology Division of Nepal Agricultural Research Council to find out the effective fungicides and botanicals against *Fusarium oxysporum* f. sp. *cubense*. The experiment was conducted in Complete Randomized design with four replications and seven treatments including control. Three fungicides (Bavistin: Carbendazim 50% WP, Sectin: Fenamidone 10%+Mancozeb 50%WP) and (Dhanucop: Copper oxychloride 50% WP) with two concentrations viz., 100 and 200 ppm and three botanical extracts such as Neem (*Azadirachta indica*), Masala (*Eucalyptus globulus*) and Asuro (*Adhatoda vasica*) with 5 and 10% concentrations were incorporated to Potato Dextrose Agar medium for inoculation of the *Fusarium* in sterilized petridishes. For botanical extract, leaves were grinded in mixture grinder with equal amount of sterilized water and heated in hot water bath at 100 degree centigrade for 15 minutes. The grinded material was filtered through muslin cloth to get stock solution considered as 100% concentration (1:1 w/v). Five and ten ml of stock solution was mixed with 95 and 90 ml of

sterilized PDA medium respectively, for making 5 and 10 percent concentration solution. The isolated pathogen was grown on potato dextrose agar medium prior to the setting of the experiment. Twenty ml of poisoned medium was poured in each sterilized petriplate and suitable checks were maintained without addition of fungicides and botanicals. An isolate of *Fusarium oxysporum* f.sp *cubense* (Foc) used in this study were isolated from banana plants of Chitwan district of Nepal. Four millimeter of seven days old fungal disc was taken from the periphery of the culture and was placed in the centre of the poisoned medium aseptically and incubated at 25°C. The diameter of the colony was measured in two directions and the average was recorded after incubation for five to six days.

Percent inhibition of the fungus was calculated by using the following formula (Vincent, 1947)

$I = (C\text{-}T/C) \times 100$

Where,
I= Percent inhibition.
C= Growth of the pathogen in control plate.
T= Growth of the pathogen in treated plate.
Statistical package IRRI STAR was used for the analysis of variance to test the significance of treatment effect on mycelia growth and sporulation of *Fusarium oxysporum*. Least significant difference test was used to compare the values of significant treatment means at 1% level of significance.

RESULTS AND DISCUSSION

Different chemicals and botanicals were tested against *F. oxysporum* f.sp. *cubense* following poisoned food technique. Fungicides were tested at 100 and 200 ppm and botanicals at 5 and 10% concentrations each. The observations on colony diameter and percent inhibition of colony growth over control are presented in Table 1, 2 and Fig. 1. A gradual increase of fungal mycelia growth was observed with increasing periods of incubations in control as well as all other treatments. The maximum radial growth of fungus at 120 hours was observed in control (7.9 cm) followed by those treated with Copper oxychloride (COC) 100 ppm (7.72 cm), COC 200 ppm (7.53), Fenamidone 10%+Mancozeb 50% 100 ppm (6.22cm), Fenamidone 10%+Mancozeb 50% WP 200 ppm (5.22cm). In case of botanicals, maximum radial growth of fungus after same hour of incubation was observed in Neem 10% (7.55 cm) followed by Neem 5% (7.42 cm), Asuro 10% (7.25 cm), Asuro 5% (7.15 cm), Eucalyptus 5% (5.78 cm) and Eucalyptus 10 % (5.07 cm). Carbendazim 50% WP, completely inhibited the mycelia growth of the fungus (100%) followed by Fenamidone 10%+Mancozeb 50% WP (21.94%), COC (3.13%) at 100 ppm. Whereas at 200 ppm, Fenamidone 10%+Mancozeb 50% WP showed 34.48% inhibition followed by COC (5.64%).

The results showed that, effect of plant extracts on the fungal growth inhibition was also significant than control. In eucalyptus, 36.67% inhibition was found which was most effective and significantly superior in inhibiting mycelia growth of *F. oxysporum* and this was followed by Asuro (9.10%) and Neem (5.32%) inhibition at 10% concentration.

In this study, two concentrations of Carbendazim 50% WP completely suppressed mycelia growth of *F. oxysporum* f.sp. *cubense*. This result is in agreement with Soma *et al*., 2008 who found that Bavistin and Vitavax showed complete inhibition of pathogen *F. oxysporum* f.sp. *cubense* at 100 and 200 ppm. There was complete inhibition of mycelia growth of *Fusarium* sp. when treated with fungicide Bavistin. Singh and Jha, 2003 also found Carbendazim as the most effective fungicide against *F. oxysporum* f.sp. *ciceris* under in vitro conditions. Sectin (Fenamidone 10%+Mancozeb 50% WP) was found to be less effective than Bavistin (Carbendazim 50% WP) but possesses more inhibition capacity than Copper oxychloride. The efficacy of Copper oxychloride and neem was not found significantly different. Similarly, efficacy of Sectin (Fenamidone 10%+Mancozeb 50% WP) and Eucalyptus was also not significant with each other (Table 1 & 2). Hence, these botanical products can be used as alternative for Copper oxychloride and Sectin (Fenamidone 10%+Mancozeb 50% WP) to reduce the radial growth of pathogen. Similarly, Asuro was found more effective than COC in term of inhibition of the pathogen.

There was no significant difference on inhibition of mycelium growth of pathogen in application of 5 and 10% of Asuro and Neem whereas there was significant difference on inhibition of colony growth of pathogen when the concentration of eucalyptus was increased to 10% (Table 2). The present results of this study exhibit the radial growth of *Fusarium* sp. was inhibited in vitro by leaf extracts of Eucalyptus, Neem and Asuro, suggesting the presence of antifungal substances in the plant tissue, which agrees with the results reported by other workers on different pathogens and plants ((Tewari and Nayek, 1991; Alabed *et al.*, 1993; Qasem *et al*., 1996; Amadioha, 1998; Amadioha, 2003). Results also showed that Eucalyptus leaf extract, at 10% concentration was significantly superior over 5% concentration but the similar trend was not found with increasing the concentration of leaves extract of Neem and Asuro to inhibit the growth of pathogen (Table 2). Results obtained with Eucalyptus (Masala) leaf extract on radial growth of *Fusarium oxysporum* f.sp. *cubense* indicated that it was effective in reducing growth of fungus at both 5 and 10% concentrations as compared to Asuro and Neem leaf extracts. The inhibitory effects of Eucalyptus on *Fusarium oxysporum* f.sp *cubense* has also been previously reported in an in vitro assay (Mengane *et al*. 2014).

CONCLUSION

From this study, it is concluded that chemical fungicide Bavistin (Carbendazim 50% WP) and botanical product, Masala (*Eucalyptus globulus*) are found effective to suppress the growth of pathogen *F. oxysporum* f.sp. *cubense* of banana. The level of inhibition of

pathogen is increased with increasing the level of concentration of leaf extract of Eucalyptus. Further green-house and field trials are necessary for evaluating these chemicals and botanicals against the pathogen for additional confirmation. The botanical extraction can also be used as alternative application for the chemical fungicides.

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Table 1. Effect of Fungicides and botanical extract on radial growth (cm) of *Fusarium oxysporum* during 2019

Treatments	24hrs	48hrs	72hrs	96hrs	120hrs
Control	1.65^{a}	3.33^{a}	4.55^{a}	6.10^{a}	7.97^{a}
Bavistin 100ppm	0.00^{h}	0.00^{g}	0.00^{h}	0.00^{g}	0.00^{i}
Bavistin 200 ppm	0.00^{h}	0.00^{g}	0.00^{h}	0.00^{g}	0.00^{i}
Sectin 100ppm	1.15^{def}	2.2^{e}	3.17^{f}	4.20^{e}	6.22^{f}
Sectin 200ppm	1^{g}	1.70^{f}	2.73^{g}	3.58^{f}	5.22^{h}
Copper oxychloride 100ppm	1.27^{c}	2.60^{c}	4.09^{c}	5.50^{b}	7.72^{b}
Copper oxychloride 200ppm	1.48^{b}	2.85^{b}	4.30^{b}	5.62^{b}	7.53^{bc}
Neem 5%	1.18^{cdef}	2.45^{cd}	3.85^{d}	5.25^{c}	7.42^{cd}
Neem 10%	1^{g}	1.15^{e}	3.60^{e}	5.05^{d}	7.55^{bc}
Asuro 5%	1.23^{cd}	2.65^{bc}	3.88^{d}	5.15^{cd}	7.15^{e}
Asuro 10%	1.10^{efg}	2.42^{cd}	3.67^{e}	5.03^{d}	7.25^{de}
Eucalyptus 5%	1.20^{cde}	2.25^{de}	3.10^{f}	4.10^{e}	5.78^{g}
Eucalyptus 10%	1.07^{fg}	1.88^{f}	3.73^{g}	3.52^{f}	5.07^{f}
CV	7.61	7.96	3.19	3.33	2.77
LSD	0.11	0.23	0.13	0.19	0.22
P<0.01	**	**	**	**	**

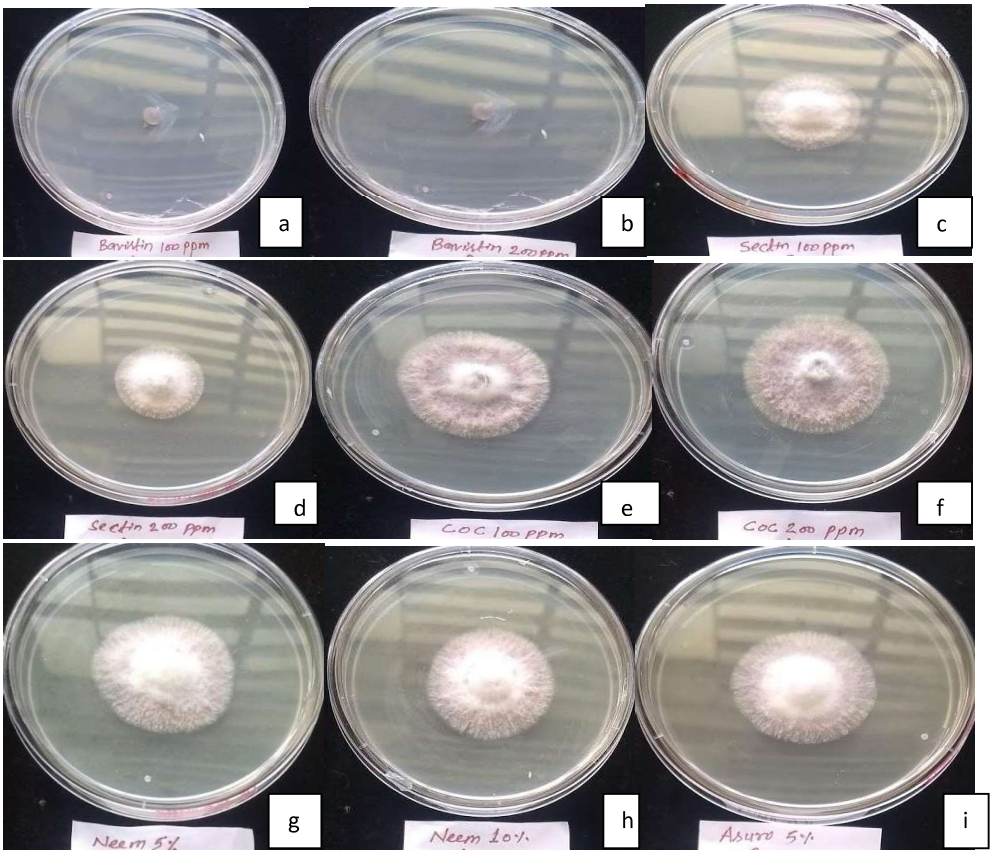
Note: Values in the same column followed by similar letter are not significantly different.

Table 2. Effect of Fungicides and botanical extract on percent inhibition (cm) of *Fusarium oxysporum* during 2019

Treatments	24hrs	48hrs	72hrs	96hrs	120hrs
Control	0.00^{g}	0.00^{h}	0.00^{h}	0.00^{g}	0.00^{i}
Bavistin 100ppm	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Bavistin 200ppm	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}
Sectin 100ppm	30.33^{cd}	36.13^{cd}	30.22^{c}	31.13^{c}	21.94^{d}
Sectin 200ppm	39.34^{b}	48.75^{b}	40.09^{b}	41.37^{b}	34.48^{b}
Copper oxychloride 100ppm	22.61^{e}	21.64f^{g}	10.15^{f}	9.81^{f}	3.13^{h}
Copper oxycloride 200ppm	10.57^{f}	14.22^{g}	5.47^{g}	7.75^{f}	5.64^{gf}
Neem 5%	28.68^{cde}	26.26^{ef}	15.36^{ef}	13.87^{e}	6.89^{fg}

Treatments	24hrs	48hrs	72hrs	96hrs	120hrs
Neem 10%	39.34^{b}	35.29^{d}	20.88^{d}	17.15^{de}	5.32^{g}h
Asuro 5%	33.18^{bc}	20.31^{fg}	14.82^{e}	15.58^{de}	10.34^{e}
Asuro 10%	25.55^{de}	26.97^{ef}	19.21^{d}	17.63^{d}	9.10^{ef}
Eucalyptus 5%	27.30^{cde}	32.23^{de}	31.87^{c}	32.75^{c}	27.57^{c}
Eucalyptus 10%	34.74^{bc}	43.60^{bc}	40.11^{b}	42.20^{b}	36.37^{b}
CV	14	13.78	7.17	7.72	7.73
LSD	7.57	7.66	3.37	3.64	3.06
P<0.01	**	**	**	**	**

Note: Values in the same column followed by similar letter are not significantly different.



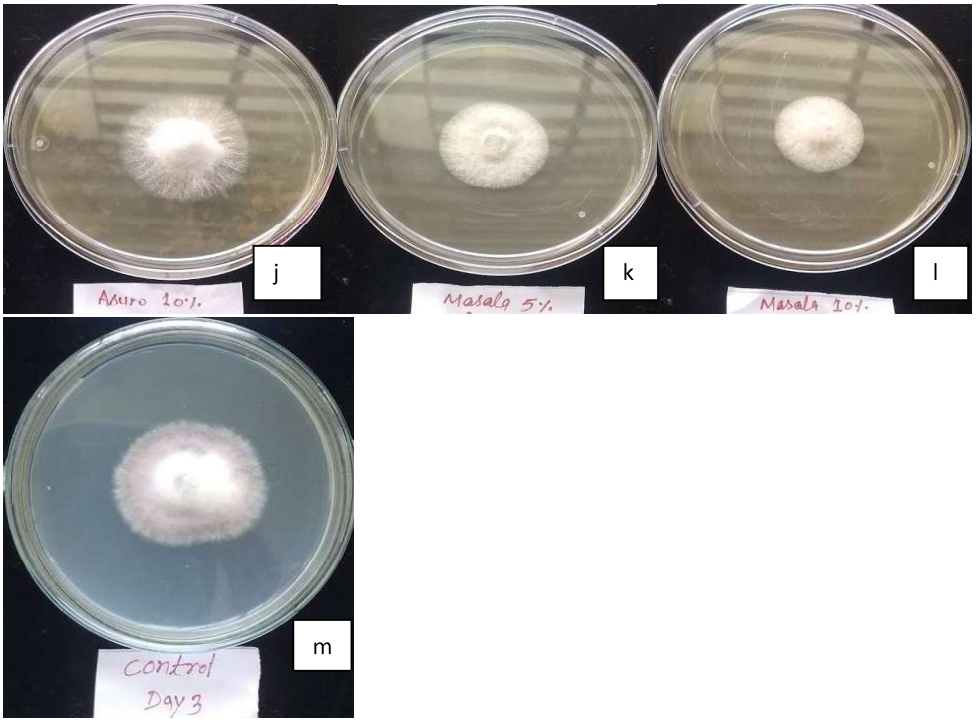


Fig. 1: Inhibitory effect of fungicides and botanical extracts on radial growth of *Fusarium oxysporum*

a) Bavistin 100 ppm, b) Bavistin 200 ppm, c) Sectin100 ppm,
d) Sectin 200 ppm, e) Copper oxychloride 100 ppm, f) Copper oxychloride 200 ppm,
g) Neem 5%, h) Neem 10%, i) Asuro 5%,
j) Asuro 10%, k) Masala 5%, l) Masala 10% and
m) Control