ASSESSMENT OF PHYTOEXTRACTS AND CHEMICALS AGAINST *Colletotrichum Falcatum* CAUSING RED ROT OF SUGARCANE

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ABSTRACT

Red rot is one of the most alarming threat to successful production of sugarcane crop. It is caused by *Colletotrichum falcatum* which is responsible for significant yield losses along with 29-83% reduction in cane weight and reduces 31-75% sugar contents. Different management strategies are used to manage red rot of sugarcane. In current study, five chemicals (Benomyl, Captan, Score, Cabriotop and Ridomil), five plant extracts (*Curcuma longa, Piper longum, Jatropha curcas, Citrullus colocynthis* and *Trigonella foenum-graecum*) and different combinations of these (*C.colocynthis* + Ridomil, *C.colocynthis* + Benomyl, Benomyl + Ridomil, *C.colocynthis* + *Jatropha curcas, C.colocynthis*+ Benomyl and *Jatropha curcas* + Ridomil) were evaluated at three different concentrations. Completely randomized design (CRD) was used by maintaining three replications. Results showed that most effective fungicide was Benomyl while Ridomil showed minimum growth inhibition. From plant extracts, *Curcuma longa* expressed minimum fungal growth followed by *Piper longum*, *Jatropha curcas*, *Citrullus colocynthis* and *Trigonella foenum-graecum*. Combination of *C.colocynthis* +Ridomil exhibit maximum fungal growth and showed best results. In future, Benomyl and *Curcuma longa* alone and in combinations can be used for the management of red rot disease of sugarcane.

Keywords: *C.colocynthis* , Ridomil, *Trigonella foenum-graecum, Jatropha curcas,* Benomyl

INTRODUCTION

Sugarcane is a perennial crop belongs to family *Poaceae* and genus *Saccharum.* It is considered as an old source of energy for mankind and fossil fuel for motor automobiles. It has high nutritional value and contains high levels of sugar calculating major (80%) world sugar production (Menossi *et al.,* 2008). Its juice contains magnesium, sodium, potassium, calcium and iron, as well as saccharose and organic acids (Kaavya *et al*., 2019). The total cultivated area of sugarcane worldwide is about 26.9 million hectares with production of 1.91 billion tones (Dotaniya *et al.,* 2016). In Pakistan it is cultivated on 1341.8 hectors with total production 0.083 million tons (FAO, 2017).

Sugarcane crop is attacked by a number of fungal, bacterial and viral pathogens causing different diseases including red rot, Wilt, Grassy shoot, red rot, smut, leaf scald disease, red striped disease, eye spot, rust, mosaic disease and Pokkah boeng. Among them, the most important is red rot (Cancer) of sugarcane which is caused by *Colletotrichum falcatum* (Costa *et al.,* 2021). It belongs to *Glomerallaceae* family (Alexander *et al.,*2002). It produces asexual fruiting bodies, conidiophores are hyaline, linear or club-shaped, forming single, thin walled, colorless conidia with single cells (Kumar *et al.,* 2016). Red rot reduces about 29-83% cane weight and 31-75% sugar contents (Ahmad *et al.,* 1986). Losses

worldwide due to this disease is recorded by 5-10% (Viswanathan and Samiyappan, 2003).

Different management strategies are used to manage red rot disease of sugarcane. Use of resistant varieties are most effective towards disease management. Due to sudden climatic changes and mutation in pathogen, out resistant genomes are becoming susceptible against disease. Moreover, there is very less number of resistant varieties available towards red rot disease. When disease appears in epidemic form, farmers have no option except the use of fungicides. Because fungicides give quick or sudden response towards disease management. Moreover, these are easily available to the farmers. That's why in current study, five different fungicides were used against *C. falcatum.*

Synthetic chemicals have toxic effects on environment and living society. These are persistent in nature as their residues remain for long term. These fungicides put heavy costs to farmer community. Moreover, the pathogen is becoming resistant against red rot due to injudicious use of fungicides. That's why, there is a need to find out an alternate approach for the management of red rot disease of sugarcane. Phytoextracts are the bi-products of plant parts which have active chemical in them that is helpful for the inhibition of fungal growth. These are ecofriendly in nature and have no toxic effects on humans and environment. That's why in current study, five different plant extracts were used against red rot of sugarcane.

MATERIALS AND METHODS

Isolation, purification and identification of pathogen

For isolation, samples were collected from different sugarcane growing areas of district Faisalabad and experiment was conducted in Citrus Pathology Lab, Department of Plant Pathology, University of Agriculture, Faisalabad. Isolation of pathogen was done on potato dextrose agar medium. PDA medium was prepared by dissolving 39g of synthetic potato dextrose agar in 1000ml of distilled water. This media was sterilized at 121ºC for 15 minutes at 15 psi. For isolation, samples showing typical symptoms of the red rot disease were bring into laboratory. These samples were washed with tap water to remove the dust and other unwanted materiel. After washing samples were cut and disinfected in 1% sodium hypochlorite. After giving two washings with distilled water, the small infected pieces were kept on blotter paper and left for dry. Petri plates of size 9cm were poured with potato dextrose agar media. When media in the Petri plates was solidified than, four to five disinfected samples pieces were placed on Petri plates with the help of forceps and wrapped with paraffin tape. Plates were placed in the incubator at 28-30^{o}C for the growth of fungus. When the fungus starts to grow, purification was done by single hyphal technique. With the help of needle single mycelium of the fungus was picked and placed on the Petri plates heaving PDA. Plates were wrapped, labeled and again placed in the incubator for next 48 hours. After incubation purified growth of the fungus was observed and again examined under microscope to confirm the pure culture.

In-vitro evaluation of Phytoextracts against *Colletotrichum falcatum* causing red rot disease in Sugarcane

Five plants (*Citrullus colocynthis, Curcuma longa, Piper longum, Jatropha curcas* and *Trigonella foenum-graecum*) were used for the preparation of plant extracts. These plants were collected from nursery and dried under shade and then oven dried at 28^{o}C to remove moisture. Fine powder of these plants was prepared and passed through muslin cloth. Three concentrations (3, 5, 7%) for each plant extract 3g, 5g and 7g were prepared and added in 100 ml of distilled water separately to make solution. Three Petri plates for each concentration of phytoextracts and three plates for control were used. Through poisoned food technique antifungal potential

of phytoextracts was evaluated. Each concentration of plant extract was mixed well with PDA and poured into Petri plates. Purified culture of the *C. falcatum* was placed in the center of petri plate. Each concentration of plant extract was mixed well with PDA and poured into Petri plates. Petri plates were incubated at 28-30^{o}C. Fungus growth was recorded after 3, 7, and 9 days.

***In-vitro* evaluation of chemicals against *Colletotrichum falcatum* causing red rot disease in Sugarcane**

Five chemicals (Benomyl, Captan, Score, Cabriotop and Ridomil) were evaluated against *Colletotrichum falcatum*. For each chemical stock solution was prepared. Three Concentrations (200, 500, 700 ppm) of each chemical were prepared. Through poisoned food technique each concentration was evaluated. In autoclaved media each concentration was mixed well and poured into Petri plates, culture was placed at the center of the plate and incubated at 28-30^{o}C for 2-3 days for fungal growth and data was recorded.

***In-vitro* evaluation of most effective Chemicals and Phyto-extracts combinations against *Colletotrichum falcatum* causing red rot disease in Sugarcane**

Combination of the chemicals and phyto-extracts which expressed significant results were evaluated against *Colletotrichum falcatum* under lab conditions through poisoned food technique.

Data Analysis

The experiment was performed under Completely Randomized Design (CRD) with three replications for each Chemicals and Phytoextract concentration**.** the data was analyzed by using a statistical software (Steel et al., 1997).

RESULTS

In vitro evaluation of chemicals against red rot of Sugarcane caused by *Colletotrichum falcatum*

Benomyl 50 WP exhibited minimum fungal growth (18.185) followed by Captan (22.074), Score (22.926), Cabriotop (23), Ridomil 75wp (26.741) and control expressed (58.481) mm. Interaction between treatment and concentration showed that Benomyl 50 WP expressed minimum fungal growth (13.778) at 0.3% followed by Cabriotop (15.889) at 0.3%, Score (16.444) at 0.3%, Cabriotop (16.778) at 0.3%, Benomyl 50 WP (17.889) at 0.2%, Cabriotop (20.333) at 0.2%, Ridomil 75wp (20.333)) at 0.3%, Score (22) at 0.2%, Benomyl 50 WP (22.889) at 0.1%, Cabriotop (24.889) at 0.2%, Ridomil 75wp (27.778) at 0.2%, Cabriotop (28.222) at 0.1%, Captan (29.111) at 0.1%, Score (30.333) at 0.1%, Ridomil 75wp (31.889) at 0.1% and control expressed (63) mm at 0.3%.D

In vitro* evaluation of phyto-extracts against *Colletotrichum falcatum

Curcuma longa expressed minimum fungal growth (18.741), followed by *Piper longum* (19.444), *Jatropha curcas* (24.231), *Citrullus colocynthis* (24.639), (33.370), *Trigonella foenum-graecum* (29.648), control expressed (65.333) mm. Interaction between treatment and concentration exhibited that 3% concentration of *Piper longum* showed minimum growth of fungus (23.444) followed by *Curcuma longa* (24.056),*Citrullus colocynthis* (31.111), *Jatropha curcas* (31.611), *Trigonella foenum-graecum* (34.556) and control expressed (60.778) mm. At 5% concentration *Curcuma longa* gives minimum growth of fungus (18.75), *Piper longum* followed by (21.25), *Jatropha curcas* (21.667), *Citrullus colocynthis* (25.417), *Trigonella foenum-graecum* (30.75). Control expressed (65.111) mm fungal growth. 7% concentration of *Curcuma longa* showed minimum fungal growth (13.417), followed by *Piper longum* (13.639), *Citrullus colocynthis*, (17.389), *Jatropha curcas* (19.417), *Trigonella foenum-graecum* (23.639), Control expressed (70.111) mm fungal growth

In vitro* evaluation of chemicals and phytoextracts combination against *Colletotrichum falcatum

Combination of one chemical and one phytoextract (*C.colocynthis* +Ridomil

75WP) expressed minimum fugal growth (16.519) followed by *J.curcas*+ Benomyl50 WP (17.407), Benomyl 50WP+ Ridomi 75WP (21.074), *C. colocynthis* + *Jatropha curcas* (21.407), *C.colocynthis* + Benomyl 50WP (23.259), *J.curcas* + Ridomil75WP (27.519) and control expressed (64.778). Interaction of treatment and concentration showed that combination of *C.colocynthis*+ Ridomil 75WP at 0.7% exhibited (13) followed by *C.colocynthis* + Ridomil 75WP at 0.7% (13.667), Benomyl 50WP+ Ridomil 75WP (14.556), *C.colocynthis* +*J.curcas* (16.333), *C.colocynthis* + Benomy l50WP (19.556), *J.curcas*+ Ridomil 75WP (21.222)and control expressed (70). Combination *C.colocynthi s*+Ridomil 75WP at 0.5% showed (15.333) followed by *J.curcas*+Benomyl (17.778), *C.colocynthis*+*J.curcas* (21.444), Benomyl 50WP+ Ridomil 75WP (22), *C.colocynthis* + Benomyl 50WP (23.222), *J.curcas* + Ridomil75WP (28) and control gives (64). At 0.3% concentration *J.curcas*+ Benomyl 50WP (20.778) followed by *C.colocynthis*+ Ridomil 75WP (21.222), *C.colocynthis* + *J.curcas* (26.444), Benomyl 50+ Ridomil 75WP (26.667), *C.colocynthis*+ Benomyl 50WP (27), *J.curcas* + Ridomil75WP (33.333) and control expressed (60.333).

DISCUSSION

Red rot of sugarcane is caused by *Colletotrichum falcatum*, which is the most destructive disease of sugarcane (Chandra *et al.,* 2015). It is mainly appeared in tropical and subtropical regions of the world. Disease losses are recorded in all phases from seedling to maturity during crop life cycle. With the passage of time *Colletotrichum falcatum*, evolving itself through genetic mutations, which results in the susceptibility of the previously resistant sugarcane verities. Suitable management practices against this disease are still a big task to be done. In field, if the disease appears in an epidemic form and there is no availability of the resistant variety then the farmer has only one option to use of chemicals. Chemicals application is the most efficient way to manage the disease, because they inhibit the growth and development of the pathogen. To develop resistance against pathogen the use of chemicals is best strategy (Heydari and Pessarakli, 2010). Recently, it was found that chemicals such as Difenoconazole 11.4%, Azoxystrobin 23%, Vitavax, Bavistin, Benomyl 50 WP and Captan can significantly reduce the disease by inhibit the fungal growth (Kumar, 2016). Hence, an experiment was conducted under lab conditions to evaluate different chemicals, phytoextracts and their combinations. Benomyl 50 WP found to be most effective against pathogen under lab conditions by inhibiting minimum fungal growth (18.185) followed by Captan (22.074). It was noticed that Benomyl 50 WP at 0.3% showed minimum fungal growth (13.778) followed by Cabriotop (15.889) mm at 0.3%. Toxicological and environmental problems are arising due to the over use of chemicals to manage different plant diseases. Extracts of plants are mainly used to control the diseases, because this method is ecofriendly and cheap. (Thakur *et al.,* 2020) Plants are also being used as chemotherapeutant to protect plants. Commercially different bio-pesticides such as neem oil and pyrethroid products are available. Oils of some plants are being used as antimicrobial agent against pests of storage. (Dalavayi et al., 2021) Volatile oil is used as botanical pesticides and also to reduce food spoilage (Gurjar *et al,* 2012). In recent study five phytoextract (*C. longa*, *Piper longum*, *J. curcas*, *Citrullus colocynthis* and *T.graecum*) at different concentrations were used to manage red rot disease of sugarcane. According to the results, *Curcuma longa* extract showed minimum fungal growth followed by *Piper longum*. Treatments with interaction to concentration showed that *Piper longum*at 3% effectively limited the growth of fungus *Curcuma longa* at 5%.

Combination of chemicals (Ridomil75WP and Benomyl 50WP) and phytoextracts (*J.curcas* and *C.colocynthis*) against *Colletotrichum falcatum*, is another approach

to control the disease at significant level. Combination of one chemical and one phytoextract (*C.colocynthis* + Ridomil 75WP) is also the most effective approach against pathogen control, followed by *J.curcas* + Benomyl50 WP. Antibiological components are found in *C.colocynthis* due to which it has ability to inhibit the fungal growth. With interaction to treatment *C.colocynthis*+Ridomil 75WP at 0.7% found most effective followed by *C.colocynthis* + Ridomil 75WP at 0.7%. Combination of *J.curcas* + Ridomil75WP at 3% found least effective.

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Table-1 Impact of chemicals on growth of *Colletotrichum falcatum* under lab conditions

Fungicides	Active ingredients	Fungal growth (mm)
Benomyl	Benzimidazole	18.185
Captan	N-Trichloromethylthio-4-cyclohexene dicarboximide	22.074
Score	Difenoconazole	22.926
Cabriotop	Pyraclostrobin + Metiram	23
Ridomil	Metalyxal + Mencozeb	26.741
Control	Distilled water	58.481
LSD	**1.982**	

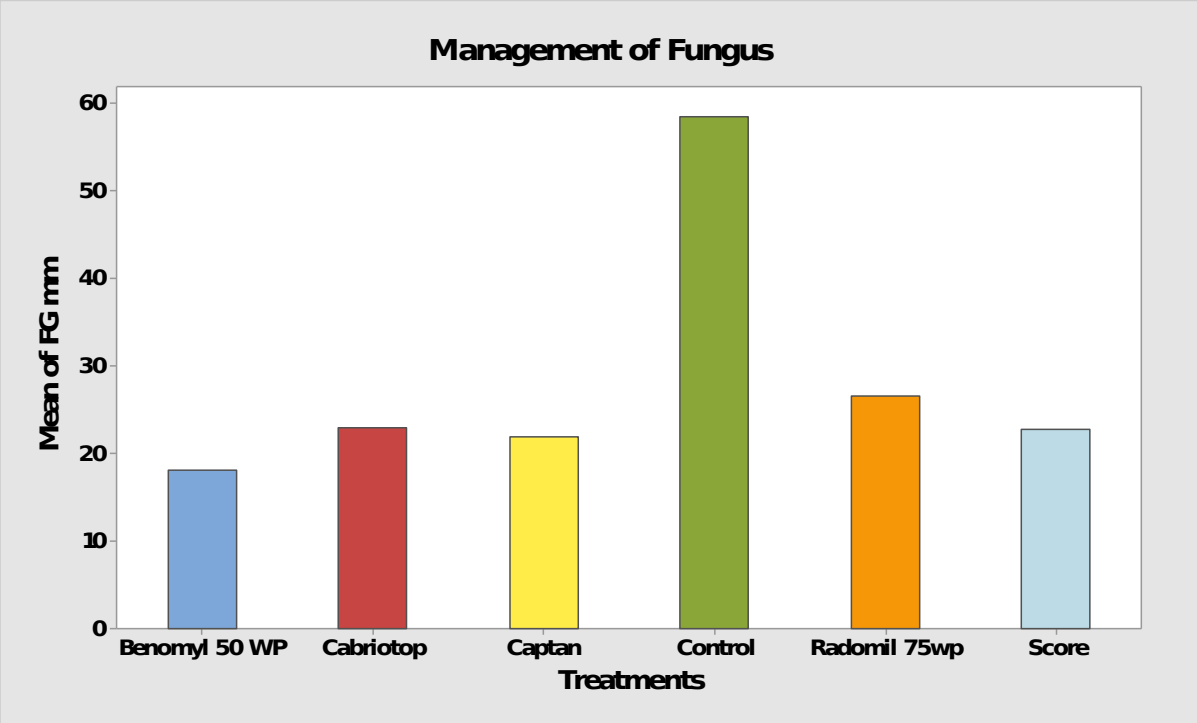

Fig.1: Impact of chemicals on growth of *Colletotrichum falcatum*

Table-2 Impact of chemicals in relation to concentration on growth of *Colletotrichum falcatum*

Treatments	Fungal growth (mm)		
	Concentration (200ppm)	**Concentration (500ppm)**	**Concentration (700ppm)**
Benomyl	22.889gh	17.889jk	13.778l
Ridomil	31.889d	27.778b	20.556hi
Cabriotop	28.222ef	24.889gt	15.889kl
Captan	29.111ef	20.333ij	16.778k
Score 250EC	30.333df	22hi	16.444k
Control	54c	58.444b	63c
LSD			**1.2782**

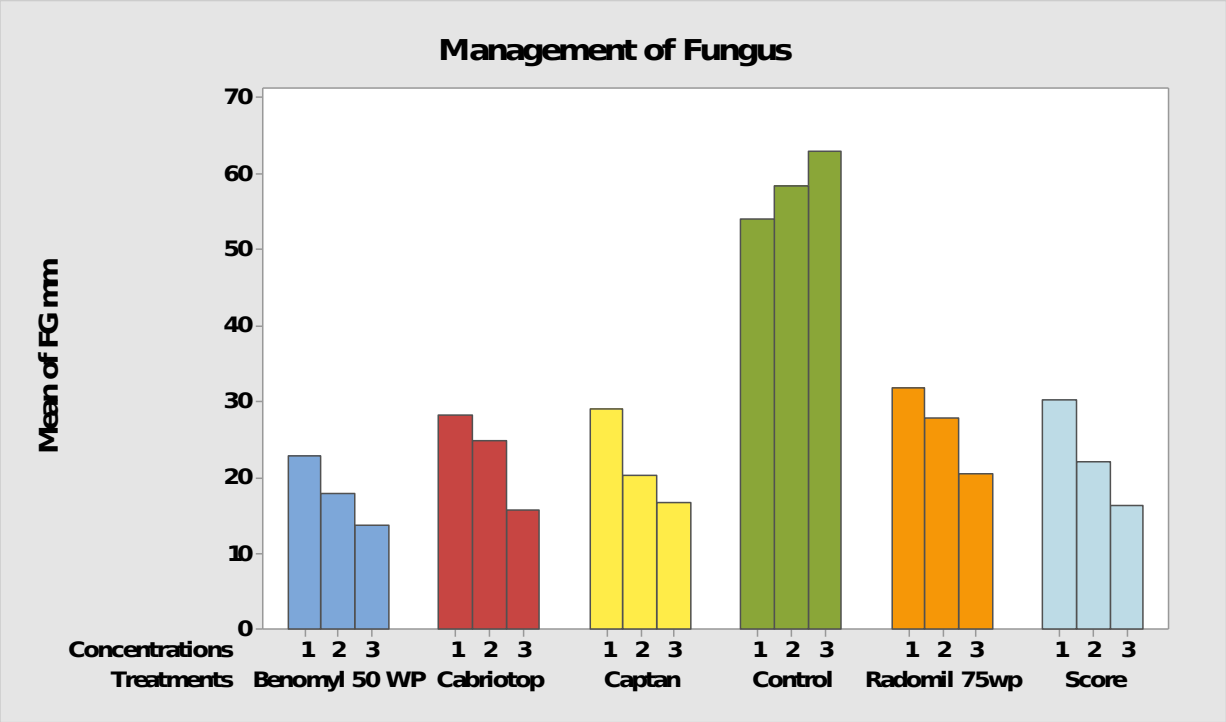

Fig.2 Impact of chemicals in relation to concentration on growth of *Colletotrichum falcatum*

Table-3 Impact of Phytoextracts on growth of under lab conditions *Colletotrichum falcatum*

Treatment	Technical Name	Fungal growth (mm)
Turmeric	*Curcuma longa*	18.741d
Long piper	*Piper longum*	19.444d
Jatropha	*Jatropha curcas*	24.231c
Kor tumma	*Citrullus colocynthis*	24.639c
Fenugreek	*Trigonella foenum-graecum*	29.648b
Control	Distilled water	65.333a
LSD	**1.982**	

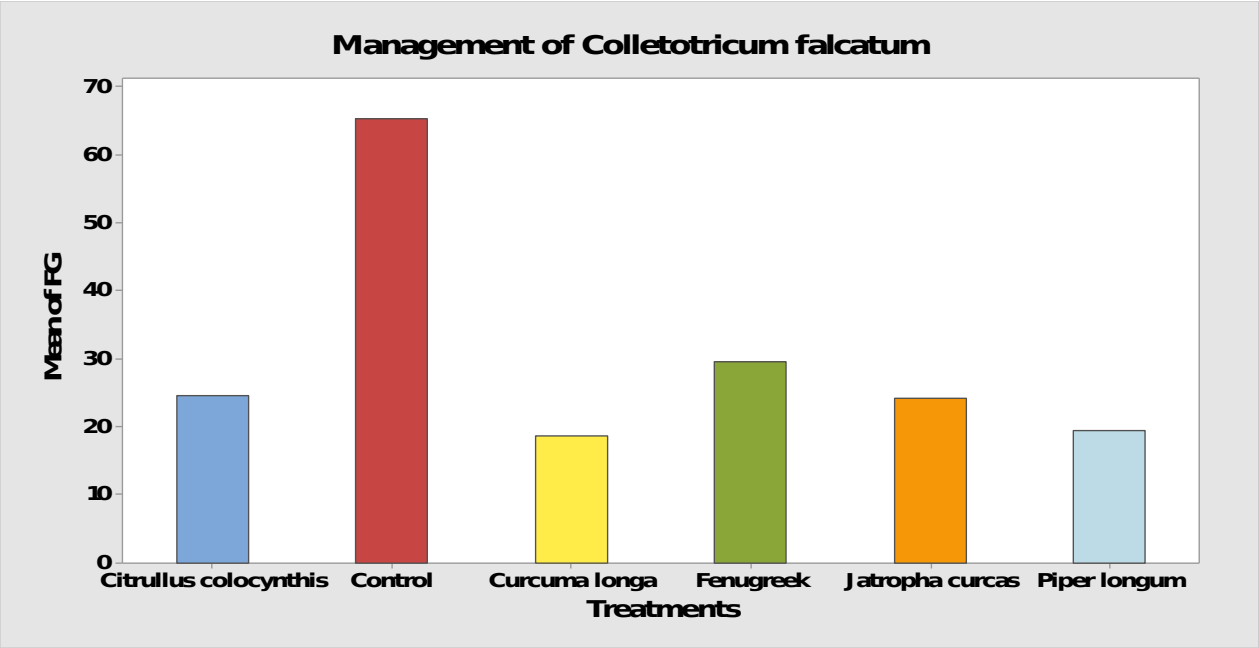

Fig.3 Impact of Phytoextracts on growth of under lab conditions *Colletotrichum falcatum*

Table-4 Impact of phytoextracts in relation to concentration on growth of *Colletotrichum falcatum* under lab conditions

Treatments	Fungal growth (mm)		
	Conc.I (3%)	Conc,II (5%)	Conc.III (7%)
Curcuma longa	24.056fg	18.75ij	13.417k
Citrullus colocynthis	31.111e	25.417f	17.389j
Piper longum	23.444fg	21.25ghi	13.639k
Jatropha curcas	31.611e	21.667gh	19.417hij
Trigonella foenum-graecum	34.556d	30.75e	23.639fg
Control	60.778c	65.111b	70.111a
LSD	**1.4478**		

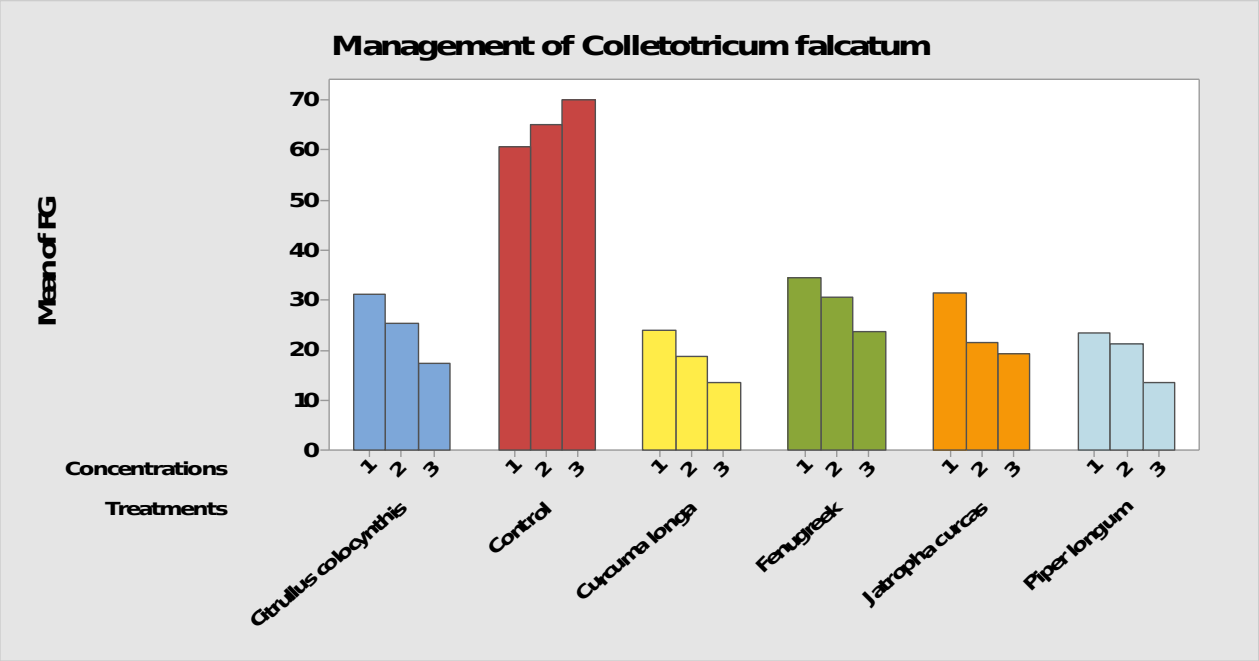

Fig.4 Impact of phytoextracts in relation to concentration on growth of *Colletotrichum falcatum* under lab conditions

Table-5 Impact of combination of chemicals and phytoextracts combination against *Colletotrichum falcatum* under lab conditions

Treatments	Technical Names and Active Ingredients	Fungal growth (mm)
Kor tumma+Ridomil	*C.colocynthis* + (Metalyxal + Mencozeb)	16.519e
Kor tumma+Benomyl	*C.colocynthis* + Benzimidazole	17.407e
Benomyl+ Ridomil	Benomyl50 WP + (Metalyxal + Mencozeb)	21.074d
Kor tumma+ Jatropha	*C.colocynthis* + *Jatropha curcas*	21.407d
Kor tumma+ Benomyl	*C.colocynthis* + Benzimidazole	23.259c
Jatropha + Radomil	*Jatropha curcas* + (Metalyxal + Mencozeb)	27.519b
Control	Distilled water	64.778a

Table-6 Impact of combinations of chemicals and phytoextracts combination in relation to concentration on growth of *Colletotrichum falcatum* under lab conditions

Treatments	**Fungal growth** (mm)		
	Concentrations		
	Conc,III (3%)	Conc.II (5%)	Conc. I (7%)
C.colocynthis + *Jatropha curcas*	26.444e	21.444fg	16.333ij
C.colocynthis+ Benomyl50 WP	27.000e	23.222f	19.556gh
C.colocynthis + Ridomil 75WP	21.222fg	15.333ijk	13k
Jatropha curcas + Benomyl50WP	20.778fg	17.778hi	13.667k
Jatropha curcas + Ridomil 75WP	33.333d	28e	21.222fg
Benomyl50 WP + Ridomil 75WP	26.667e	22fg	14.556jk
Control	60.333c	64b	70a
LSD	**1.2524**		

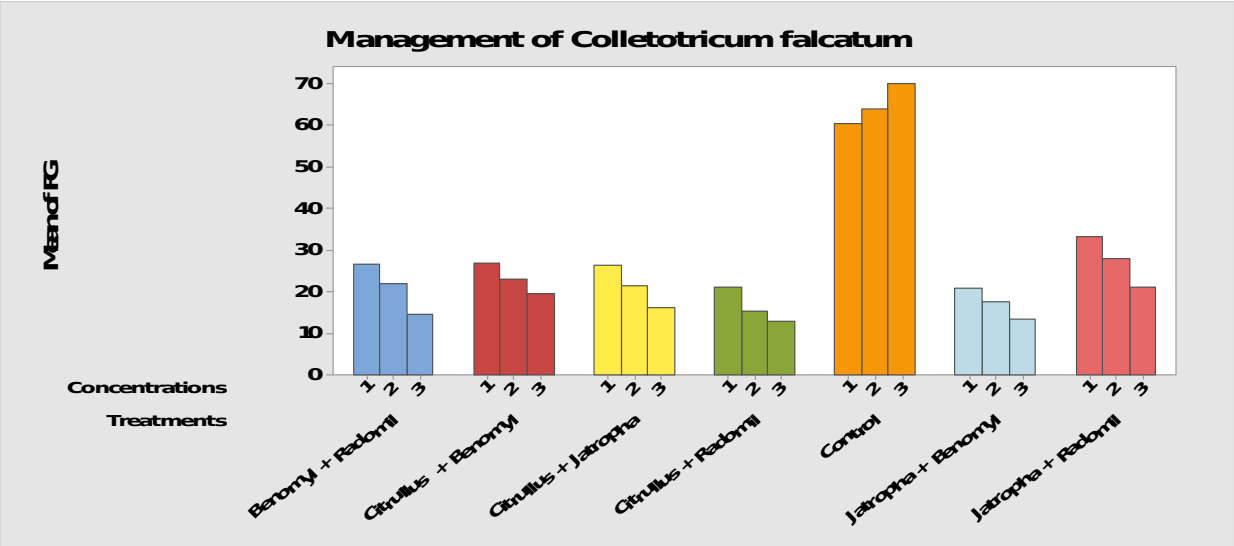

Fig.6 Impact of combinations of chemicals and phytoextracts combination in relation to concentration on growth of *Colletotrichum falcatum* under lab conditions