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Research Article

COMPARATIVE APPRAISEMENT OF SYNTHETIC CHEMICALS, PHYTOCHEMICALS AND HOST RESISTANCE TOWARDS *FUSARIUM MONILIFORME* CAUSING STALK ROT OF MAIZE

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Abstract

Stalk rot of maize is one of the most important emerging threat to the successful production of Pakistan. It causes 10-40% yield losses which may reach up to 100% due to conducive conditions in some areas of country. Current research effort was made to cope with stalk rot of maize caused by *Fusarium moniliforme* (*Fm*) through synthetic chemicals, botanical extracts and source of resistance. Disease samples of maize were collected from Faisalabad regions to isolate pathogenic fungus. Screening of ten varieties (Gohar-19, Sahiwal Gold, Malka-2016, FH-1046, YH-5427, Pearl, DK-6317 MMRI, AS-5101, and DK-9108) was done. Among these varieties, YH-5427 expressed highly resistance response. By using poisoned food technique, five synthetic chemicals (Tilt, Belanty, Forum-Top, Cabrio-Top, and Enervin Duo) and eight phytochemicals (*Azadirachta indica*, *Allium sativum*, *Eucalyptus globulus*, *Zingiber officinale*, *Ficus benjamina*, *Cinnamomum tamala*, *Mentha piperita* and *Moringa olerifas*) were evaluated against *Fm* at three different concentrations (50, 100, 150ppm) and (3, 5 and 7%) respectively under lab conditions. Among all tested treatments, tilt and moringa showed significant results under lab conditions and further investigated under field conditions alone and in combination against stalk rot of maize. Minimum disease incidence was expressed by Tilt + Moringa (9.69%) in integration followed by Tilt (16.78%) and Moringa (30.89%) as compared to control. Lab and field experiments were conducted under Complete Randomized Design (CRD) and Randomized Complete Block Design (RCBD) respectively. To observe the difference among the treatments towards maize stalk rot, least significant difference (LSD) was used at 0.05 % probability level.

Keywords: *Zea mays* L, Phytochemicals, Synthetic chemicals, *Fusarium moniliforme*, Genetic resistance
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1. INTRODUCTION

Maize (Zea mays L.) is the world's second most imperative cereal crop that is widely cultivated all over the world and has a high economic value. (Syam'un and Nasruddin, 2022). It belongs to the family Poaceae. The prime origin of maize is Central America and Mexico (Tabassum et al. 2020). It is highly rich in carbohydrates, protein, oil, fiber, sugar and ash as well as it is used as cocking oil for human and fodder for animals (Wang et al. 2018). Flour, meals, flakes, porridge, poultry,fish meals, bran feed, gluten meal, and stalk fodder are its byproducts. Worldwide, it is cultivated on an area of 202 million hectares with an annual production of 1162.35 million tons, while in Pakistan it is grown on an area of 1.42 million hectares with an annual production of 8.46 million tons (FAOSTAT, 2020) with 0.6% GDP share (GOP, 2021).

Maize production is influenced by different biotic (Brown spot, Head smut, Maize lethal necrosis, Stewart's wilt, Northern blight, Southern blight) and abiotic (temperature, water, humidity, light, rain fall, and cultural practices) factors (Wang et al., 2020). One of the most important biotic threats to the successful

production of maize in Pakistan is stalk rot of maize caused by *Fusarium moniliforme (Fm)*. Fusarium is a soil born fungi that penetrates through the roots and causes internal blackening or browning of the xylem vessels from the roots to the stem (Lima et al., 2019). It has whitish or pale salmon-colored colony, and it produces three types of spores (macrospores, microspores, and chlamydospores). Macroconidia are 4-8 celled and encased in a sickle-shaped sac-like structure, while kidney-shaped microconidia are 1-2 celled (Ngure, 2020). Chlamydospores are the resting spores of Fm (Cotten and Munkvold, 1998). Characteristic symptoms are reddish discoloration of the xylem vessel, yellowing and wilting of leaves. White to pink, or orange fungal growth appeared on stem and root surface of infected plant (Pfordt et al., 2020). Severe outbreak of disease occurred at 31℃ temperature and 70% relative humidity (Shekhar and Singh, 2021). Fm is severe in warm and dry areas and spread all over the world just before tasseling stage. It causes 10-42% yield losses which may reach up to 100% due to favorable conditions in some areas of the country (Khokhar et al., 2021). Different management strategies such as cultural, biological, chemical, and resistant varieties are used for stalk rot management (Trueman et al., 2019). Among all these strategies, use of resistant source is the most fruitful tool to combat this disease. For this purpose, screening of available germplasm of maize is necessary to find the resistant source. Due to this in current study the available germplasm of maize was evaluated against stalk rot of maize in field conditions. But in field, under conducive environmental conditions the disease appeared in epidemic form, then farmer have the only choice to use chemicals because they are quick in action, easily available, easy to handle, and easy to apply. That's why in current study 5 different chemicals were evaluated at 3 concentrations. Korate et al. (2021) evaluated different chemicals and concluded that Carbendazim (0.1%) and carbendazim + mancozeb (0.1%) were found most effective. Jing and Suga (2021) conducted an experiment and concluded that tebuconazole and thiabendazole were found most efficient against Fm. Jiskani et al. (2021) evaluated six fungicides at 3 concentrations and concluded that two were found most effective against *Fm.*

Of course, chemicals have quick action towards stalk rot of maize and are easily available but poses an acute threat to human health. Due to their residual effects, they are causing environmental pollution. Continuous use of chemicals caused the resistance in pathogen against these chemicals due to which more virulent strains were developed. Therefore, it is pivotal to move towards alternative approaches i.e., phytoextracts because they are plant based, eco-friendly and easily available. Plant extracts obtained from many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties (Sowley et al., 2017). That's why in present study 8 phytoextracts were evaluated at 3 concentrations against Fm. Bhavya et al. (2019) evaluated six phytoextracts against *Fm* and concluded that *C. impressinervium* had the highest antifungal activity at 1000 ppm. Subedi and Neupane (2021) evaluated five aqueous extracts for antifungal activity at three concentrations (1, 2 and 3 %) against *Fm* and concluded that Acorus calamus (1 %) completely inhibited the growth of Fm. It was revealed that phytoextracts are a suitable substitute for controlling fungal pathogens rather than using environmentally hazardous commercial fungicides.

2. Materials and Methods

2.1. Isolation, purification, and identification of pathogen

Disease samples showing typical symptoms of stalk rot of maize were collected in brown bags (12cm) from the Research Area of Department of Plant Pathology University of Agriculture Faisalabad (UAF) and brought to the Plant Pathology

laboratory. Diseased samples along with some healthy portions were cut with the help of sterilized scissor into small pieces (2-3 mm). These samples were sterilized with 5% NaOCl2 followed by 2-3 rinsing of distilled water and dried by putting on sterilized tissue pepper. Potato Dextrose Agar (PDA) media was prepared and autoclaved (RTA-85, Robus United Kingdom) by using standard conditions (15 Psi, 121°C for 15-20 minutes) to isolate the pathogen. The PDA media was poured into the Petri plates (90 mm) and allowed to solidify. By using sterilized forceps, the diseased samples were placed in a Petri plate having PDA media and wrapped with paraffin tape. All the work was done in the laminar flow chamber (RTVL-1312, Robus United Kingdom) to avoid contamination. The petri plates were placed in an incubator at 28 ℃. The fungal growth was observed after 2-3 days of inoculation.

Purification of fungus was done by single hyphal tip method (Liu et al. 2022). By using a sterile needle, small fungal hyphae were picked from the culture and placed into another petri plate having PDA media and incubate at 28oC in an incubator (LFGI-101, Labocon, United Kingdom).

2.2. Pathogenicity test

Pathogenicity test was done by following the Koch's postulates for the confirmation of the pathogen. For this purpose, inoculum was prepared, and plants were grown in the pots size (25cm). The soil was sterilized with 40% formalin solution and filled in the pots. After this, the inoculum was mixed with soil, and the seeds were sown in the infected soil. When plants attained 10 leaf stage the inoculum was also injected in the stem with the help of syringe needle size (25 gauge) for the confirmation of infection. After 15 days of inoculation, symptoms were developed. Pathogen was re-isolated on PDA and re-identified on the base of morphological characteristics (colony shape, colony color, spore shape, and spore color) (Nelson, 1992).

2.3. Screening of maize varieties against stalk rot of maize

For screening of germplasm sick field was prepared. For this purpose, an isolated plot (100m2) was selected in the Research Area of Department of Plant Pathology at UAF to avoid the spread of fungus in another field. The soil was sterilized with a 40% formalin solution to make sure that the soil is free of pathogen (Afolabi et al., 2008). In the lab, spore suspension was prepared by using a hemocytometer (HBG AND MARIENFELD, HBG Germany) at a concentration 106 per mL concentration of distilled water. After the preparation of suspension, it was sprayed on the sterilized soil. The most susceptible variety was sown on this soil when the plants reached at tasseling stage rotavate in the soil and mix through plough, irrigate the field, and allow for multiplication of pathogen in soil for at least 7 days (Pandey et al., 2012). The field was prepared, and blocking was done perpendicular to the treatments. Ten varieties (Gohar-19, Sahiwal Gold, Malka-2016, FH-1046, YH-5427, Pearl, DK-6317 MMRI, AS-5101, and DK-9108) were collected from Maize and Millet Research Institute, Sahiwal (MMRI). Each variety with 10 seeds were sown by maintaining R×R distance (45cm) and P×P distance (15-20cm) (Babu et al., 2020) in already prepared sick field at Research Area of Department of Plant Pathology at UAF. All the cultural practices like irrigation, fertilizer, and hoeing were followed to keep the plants in good health. Data recording disease incidence was collected after 1 week of interval according to the disease ration scale of Campus (2012). According to this scale 1.0-2.0=Highly Resistant, 2.1-4.0=Resistant, 4.1-5.0=Moderate Resistant, 5.1-6.0=Moderate Susceptible, 6.1-7.0=Susceptible, 7.1-9.0=Highly Susceptible. Data regarding disease incidence was recorded by using the following formula:

Disease incidence (%) = (Number of infected plants)/(Number of total plants)x 100 (James, 1974)

2.4. *In-vitro* evaluation of synthetic chemicals against *F. moniliforme*

By using poisoned food technique, five synthetic chemical [Tilt (25%), Belanty (400g/L), Forum-Top (9%+55%), Cabrio-Top (5% + 55%), and Enervin Duo (300g+225g/L)] were used at 3 different concentrations (50, 100, and 150ppm). Stock solution was prepared for each chemical. To prepare 50ppm concentration, from the stock solution 0.5mL was taken and mixed in 100mL of PDA media. Then 1mL for 100ppm and 1.5mL from the stock solution for 150ppm. Poisoned PDA media were poured into 90 mm (millimeter) Patri plates. After solidification of media, a disc (5mm) of 7 days old culture was placed in the center of petri plates with 3 replications and one control (Distilled water). These plates were incubated at 28°C. The data regarding fungal colony growth was taken after 1, 2 and 3 days of incubation.

2.5. *In-vitro evaluation of phytochemicals against F. moniliforme*

Eight phytochemicals (neem, garlic, sufaida, ginger, ficus, taiz pata, and moringa) were evaluated against *F. moniliforme.* For the evaluation of phytochemicals, the experiment was carried out by using poisoned food technique at three concentrations (3, 5, and 7%). To prepare 3, 5, and 7% concentrations, 3, 5 and 7mL of plant leaves extract were added to 100 mL of PDA media respectively. PDA media containing phytochemical was poured into the Petri-plates (90mm). Antibiotic was added to the medium at the time of pouring to prevent bacterial contamination. After solidification 5 mm disc of 7 days old fungal culture was placed at centre of the plate with three replications and one control (distilled water). The plates were incubated at 28°C. The growth of fungal colony was measured after 24, 48, and 72 hours of incubation.

2.6. *In-vivo evaluation of synthetic and phytochemicals against stalk rot of maize*

In vitro Tilt (25%) and Moringa showed significant results and further evaluated under field conditions alone and with their combinations. For field management, the most susceptible variety (Malkh-2016) was selected, and 40 plants were sown by maintaining R×R distance (45cm) and P×P distance (15-20cm) (Babu et al., 2020) in the Research Area of Department of Plant Pathology at UAF. When plants are at 6 leaves stage the pathogen was inoculated by the pin prick method (Di Ming et al., 1991) and soil drenching method. In the laminar flow chamber, fungal spore suspension was prepared by using a hemocytometer at a concentration of 106 per mL of distilled water. After preparation of suspension, the spores were applied by flooding, soil drenching, and pinprick method to ensure that pathogen caused the disease. Three concentrations (2, 2.5, and 3%) with three replications were applied by hand sprayer in the morning time. To prepare 2, 2.5, and 3 % solution for spray 2, 2.5, and 3mL of the synthetic and phytochemical were added to 100 mL of distilled water respectively. 10 plants were sprayed with Tilt, 10 with moringa and 10 with the combination of both (Tilt + moringa) to check the combination effect of the treatments and 10 plants were left for control. Data regarding disease incidence was recorded by using the following formula:

Disease incidence (%) = (Number of infected plants)/(Number of total plants)x 100 (James, 1974)

2.7. *Data analysis*

Lab experiments were conducted under Complete Randomized Design (CRD) to evaluate synthetic and phytochemicals against Fm. Field experiments were conducted under Randomized Complete Block Design (RCBD) to evaluate maize varieties, Phyto-extracts, and chemicals against stalk rot of maize. To observe the difference among the treatments towards maize stalk rot, least significant difference (LSD) was used at 0.05 % probability level (Steel et al., 1997). Data was analyzed by using Statistix 8.1(St and Wold, 1989).

3. Results:

3.1. Screening of maize varieties to find the resistance source against stalk rot of maize

Ten maize varieties were collected and screened against stalk rot of maize under field conditions by artificial inoculation using a syringe and soil drenching method during Kharif 2021 and results are presented in (Table 1 and). Maize varieties expressed different responses such as YH-5427 (highly resistant), DK-6317, AS5101 and DK-1908 (resistant), MMRI and Gohar-19 (moderate resistance), Pearl, FH-1046 (moderate susceptible), Sahiwal gold (susceptible), and Malkha-2016 (highly susceptible) response.

Table 1 Response of maize varieties against stalk rot of maize under field conditions

Varieties	Rating scale	Disease incidence (%)	Reaction (Response)
YH-5427	2	14.36i	HR
AS-5101	4	26.33g	R
DK-6317	4	28.39f	R
DK-9108	3	23.57h	R
MMRI	5	44.00d	MR
Gohar-19	5	34.77e	MR
Pearl	5.5	56.88c	MS
FH-1046	6	58.33c	MS
Sahiwal Gold	7	67.86b	S
Malkha-2016	9	82.96a	HS
LSD		**0.0198**	

3.2. Evaluation of synthetic chemicals against *F. moniliforme in-vitro* conditions

Among all the treatments, Tilt expressed minimum fungal growth (2.459) followed by Belanty (6.11), Cabrio Top (9.80), Forum Top (14.03), and Enervin Duo (18.25) as compared to the control (Table 2 & Fig. 1). Interaction between treatments and concentrations (T×C) showed that minimum fungal growth was exhibited by Tilt (0.323,2.016,5.038) followed by Enervin Duo (21.82,18.17,14.76) Belanty (9.44,6.33,2.57), Cabrio Top (13.27,9.76,6.38) Forum Top (17.52,13.87,10.70) at 150ppm, 100ppm and 50ppm respectively as compared to control (Table 3). Interaction between treatments and days (T×D) showed that Tilt expressed maximum fungal growth (3.45,2.45,1.46) followed by Belanty (4.44,6.24, 7.64), Cabrio Top (8.38,10.01,11.01), Forum Top (12.45,14.28,15.36), and Enervin Duo (16.75,18.28, 19.72) after 24, 48, and 72 hours respectively as compared to control (Fig.2)

3.3. In vitro evaluation of phytochemicals for the management of *F. moniliforme*

Among all the treatments, *Moringa olerifas* exhibited minimum fungal growth (7.92mm) followed by *Cinnamomum tamala* (9.30mm), *Zingiber officinale* (10.44mm), *Azadirachta indica* (13.80mm), *Allium sativum* (16.28mm), *Eucalyptus globulus* (18.03mm), *Mentha piperita* (21.21), and *Ficus benjamina* (24.85) as compared to control (Table 4 & Fig. 3). Interaction between treatments and concentrations (T×C) showed that minimum fungal growth was observed by *Moringa olerifas* (6.84, 7.92, and 8.99mm) followed by *C. tamala* (10.48,9.38, 8.03mm), *Z. officinale* (11.43, 10.47, 9.42mm), *A. indica* (15.75, 13.79, 11.88mm), *A. sativum* (17.98, 15.91, 14.97mm), *E. globu* (20.05, 18.00, 16.03mm), *M. piperita* (23.21, 21.22, 19.20mm), and *F. benjamina* (27.26, 24.76, 22.52mm) at 7%, 5%, 3% respectively as compared to control (Table 5). Interaction between treatments and days (T×D) showed that *Moringa olerifas* exhibited maximum fungal growth (10.6,7.50,6.00) followed by *C. tamala* (6.91,9.31,11.69), *Z. officinale* (8.70,10.23,12.41), *A. indica*

(11.79,13.38,16.26), *A. sativum* (14.61,15.8018.46), *E. globu* (16.17,17.64,20.28), *M. piperita* (19.29,20.49,23.87), and *F. benjamina* (23.06,24.53,26.96) after 24, 48, and 72 hours respectively as compared to control (Fig. 4).

3.4. *In vitro evaluation of phytochemicals for the management of F. moniliforme*

Among all the treatments, *Moringa olerifas* exhibited minimum fungal growth (7.92mm) followed by *Cinnamomum tamala* (9.30mm), *Zingiber officinale*

Table 2 Impact of different synthetic chemicals against *F. moniliforme* under lab conditions

Sr. #	**Treatments**	**Active ingredient**	**Fungal growth** (mm)
T_1	Tilt	Propiconazole	2.459f
T_2	Belanty	Mefentrifluconazole	6.115e
T_3	Cabrio Top	Pyraclostrobin +metiram	9.806d
T_4	Forum Top	Diamatamorph	14.033c
T_5	Enervin Duo	Mefentrifluconazole	18.255b
T_6	Control	Distilled water	30.00a
	LSD	**0.3359**	

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05)

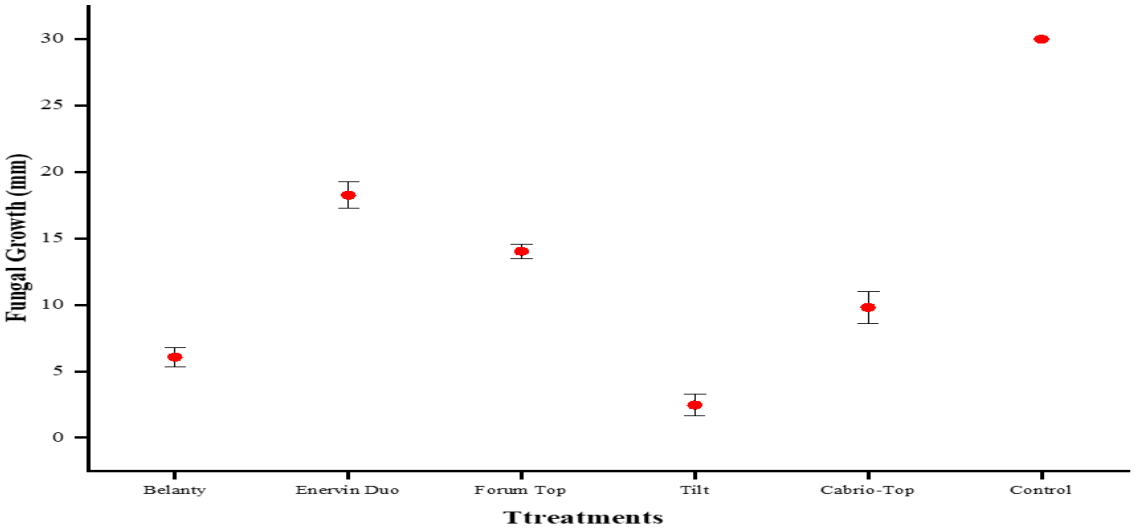

Fig. 1 Impact of different synthetic chemicals against *F. moniliforme* under lab conditions

Table 3 Impact of interaction between treatments and their concentrations (T×C) against *F. moniliforme* under lab conditions

Sr. #	**Treatments**	**Fungal growth** (mm)		
		Concentrations		
		50ppm	**100ppm**	**150ppm**
T_1	Tilt	5.038i	2.016j	0.323k
T_2	Belanty	9.444g	6.330h	2.571j
T_3	Cabrio Top	13.273e	9.762fg	6.383h
T_4	Forum Top	17.524c	13.870de	10.704f
T_5	Enervin Duo	21.826b	18.177c	14.763d
T_6	Control	30.000a	30.000a	30.000a
	LSD	**0.5818**		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05)

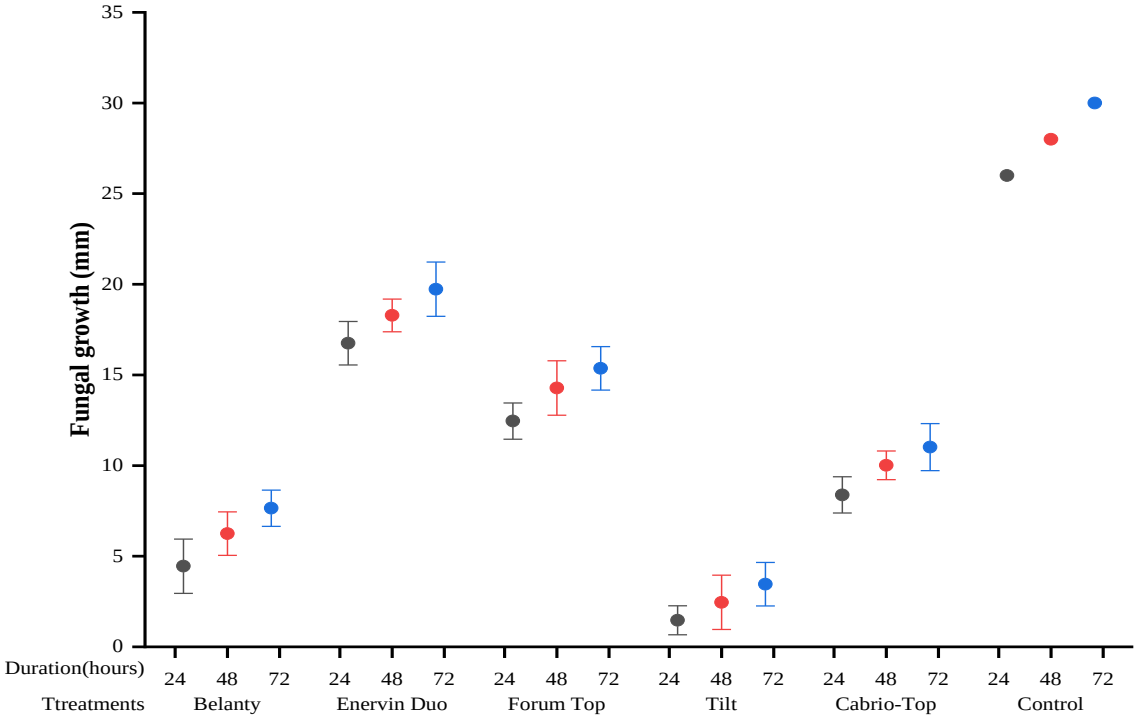

Fig. 2 Impact of interaction between treatments and days (T×D) against *F. moniliforme* under lab conditions

(10.44mm), *Azadirachta indica* (13.80mm), *Allium sativum* (16.28mm), *Eucalyptus globulus* (18.03mm), *Mentha piperita* (21.21), and *Ficus benjamina* (24.85) as compared to control (Table 4 & Fig. 3). Interaction between treatments and concentrations (T×C) showed that minimum fungal growth was observed by *Moringa olerifas* (6.84, 7.92, and 8.99mm) followed by *C. tamala* (10.48,9.38, 8.03mm), *Z. officinale* (11.43, 10.47, 9.42mm), *A. indica* (15.75, 13.79, 11.88mm), *A. sativum* (17.98, 15.91, 14.97mm), *E. globu* (20.05, 18.00, 16.03mm), *M. piperita* (23.21, 21.22, 19.20mm), and *F. benjamina* (27.26, 24.76, 22.52mm) at 7%, 5%, 3% respectively as compared to control (Table 5). Interaction between treatments and days (T×D) showed that *Moringa olerifas* exhibited maximum fungal growth (10.6,7.50,6.00) followed by *C. tamala* (6.91,9.31,11.69), *Z. officinale* (8.70,10.23,12.41), *A. indica* (11.79,13.38,16.26), *A. sativum* (14.61,15.8018.46), *E. globu* (16.17,17.64,20.28), *M. piperita* (19.29,20.49,23.87), and *F. benjamina* (23.06,24.53,26.96) after 24, 48, and 72 hours respectively as compared to control (Fig. 4).

3.5. *Evaluation of synthetic and phytochemicals against stalk rot of maize in-vivo conditions*

Among all the treatments, maximum disease reduction was observed by the combination Tilt + Moringa (9.69%) followed by Tilt (16.78%) and Moringa (30.89%) as compared to control (Table 6 & Fig. 5). Interaction between treatments and concentrations (T×C) showed that minimum disease incidence was observed by the combination of Tilt + Moringa (5.53, 9.99, 13.54%) followed by Tilt (21.73, 16.49, 12.12%) and Moringa (34.98, 31.08, 26.62%) at 3, 2.5 and 2% respectively as compared to control (Table 7). Interaction between treatments and days (T×D) showed that minimum disease incidence was observed by combination of Tilt + Moringa (5.62, 10.12, 13.31%) followed by Tilt (21.700, 17.77, 10.86 %) and Moringa (35.72, 31.66, 25.30%) after 7, 14 and 21days respectively as compared to control (Table 8 & Fig. 6).

Table 4 Impact of different phytochemicals against *F. moniliforme* under lab conditions

Sr. #	Treatments	Common Name	Fungal growth (mm)
T_1	*Moringa olerifas*	Moringa	7.92i
T_2	*Cinnamomum tamala*	Taiz pata	9.303h
T_3	*Zingiber officinale*	Ginger	10.446g
T_4	*Azadirachta indica*	Neem	13.809f
T_5	*Allium sativum*	Garlic	16.289e
T_6	*Eucalyptus globulus*	Eucalyptus	18.031d
T_7	*Mentha piperita*	Mint	21.216c
T_8	*Ficus benjamina*	Ficus	24.851b
T_9	Control	Distilled water	30.00a
	LSD	**0.4251**	

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05)

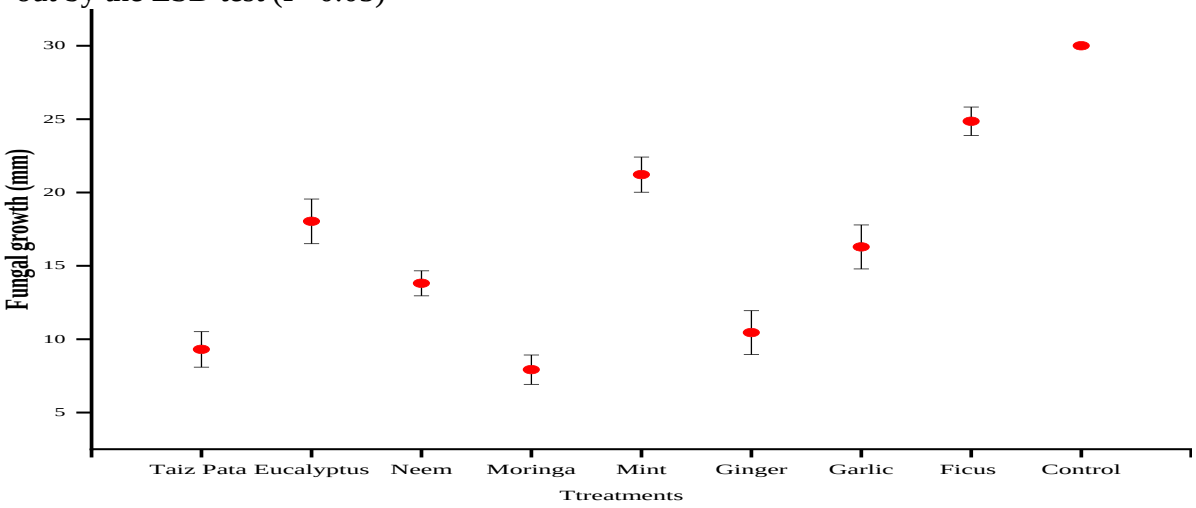

Fig. 3 Impact of different phytochemicals against *F. moniliforme* under lab conditions

Table 5 Impact of interaction between treatments and concentrations (T×C) against *F. moniliforme* under lab conditions

Sr. #	Treatments	**Fungal growth** (mm)		
		Concentrations		
		3%	**5%**	**7%**
T_1	*Moringa olerifas*	8.99o	7.92no	6.84o
T_2	*Cinnamomum tamala*	10.48kl	9.38lm	8.03mno
T_3	*Zingiber officinale*	11.43k	10.47kl	9.42lm
T_4	*Azadirachta indica*	15.75i	13.79j	11.88k
T_5	*Allium sativum*	17.98h	15.91i	14.97ij
T_6	*Eucalyptus globulus*	20.05fg	18.00h	16.03i
T_7	*Mentha piperita*	23.21d	21.22ef	19.20gh
T_8	*Ficus benjamina*	27.26b	24.76c	22.52de
T_9	Control	30.00a	30.00a	30.00a
	LSD	**0.7362**		

Mean value in a column sharing similar letters do not differ significantly as figured out by the LSD test (P<0.05)

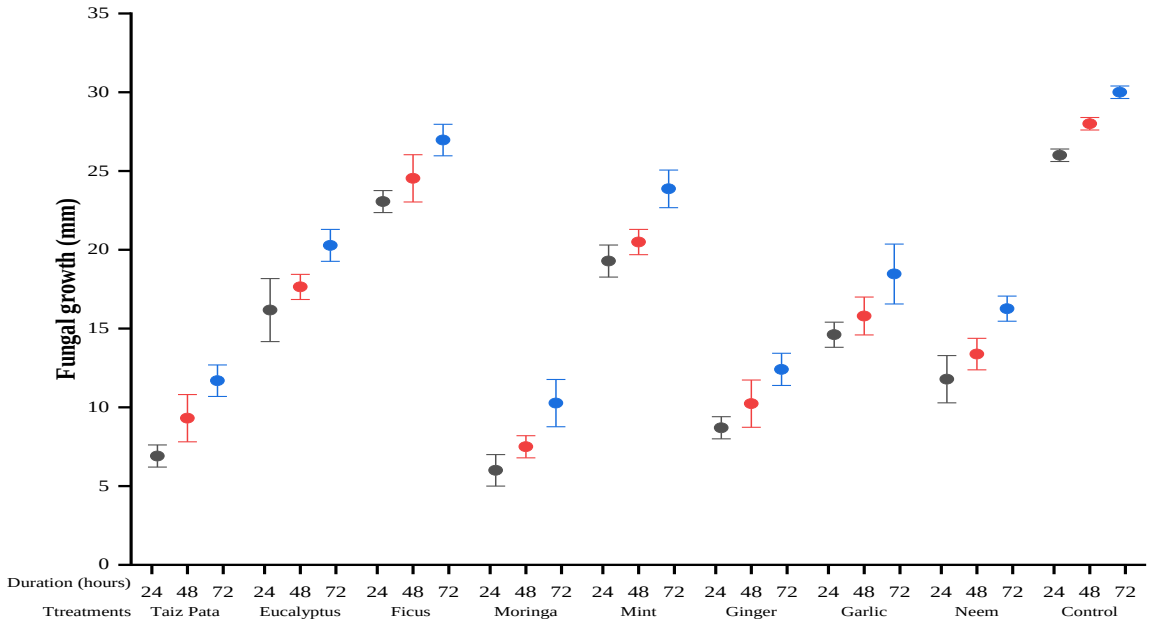

Fig. 4 Impact of interaction between treatments and days (T×D) against *F. moniliforme* under lab. conditions

Table 6 Impact of different synthetic chemicals alone and in combination against stalk rot of maize under field conditions

Sr. #	**Treatments**	**Disease incidence** (%)
T_1	Tilt + Moringa	9.69d
T_2	Tilt	16.781c
T_3	Moringa	30.898b
T_4	Control	38.667a
	LSD	**1.3415**

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05)

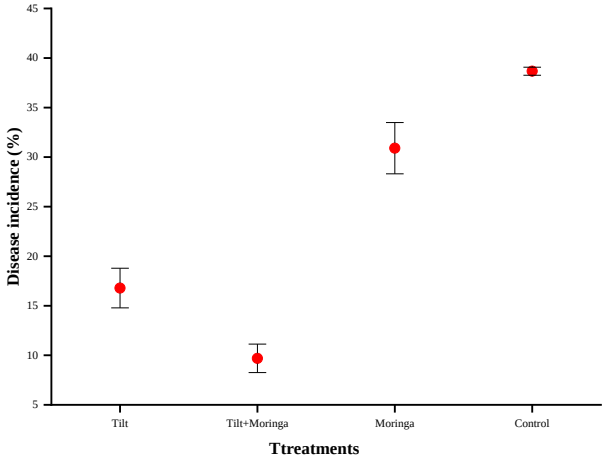

Fig. 5 Impact of different synthetic and phytochemicals alone and in combination against stalk

Table7 Impact of interaction between treatments and concentrations (T×C) against stalk rot of maize under field conditions

Sr. #	**Treatments**	**Disease incidence** (%)		
		Concentrations		
		2%	**2.5%**	**3%**
T_1	Tilt + Moringa	13.54fg	9.99gh	5.53h
T_2	Tilt	21.73e	16.49f	12.12fg
T_3	Moringa	34.98bc	31.08cd	26.62d
T_4	Control	41a	39ab	36b
	LSD	**2.3236**		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05)

Table 8 Impact of interaction between treatments and days (T×D) against stalk rot of maize under field conditions

Sr. #	Treatments	**Disease incidence** (%)		
		Days		
		7	**14**	**21**
T_1	Tilt + Moringa	13.31ef	10.12fg	5.62g
T_2	Tilt	21.700cd	17.77de	10.86f
T_3	Moringa	35.72ab	31.66b	25.30c
T_4	Control	38.67a	38.67a	38.67a
	LSD	**2.3236**		

The mean value in a column sharing similar letters does not differ significantly as figured out by the LSD test (P<0.05)

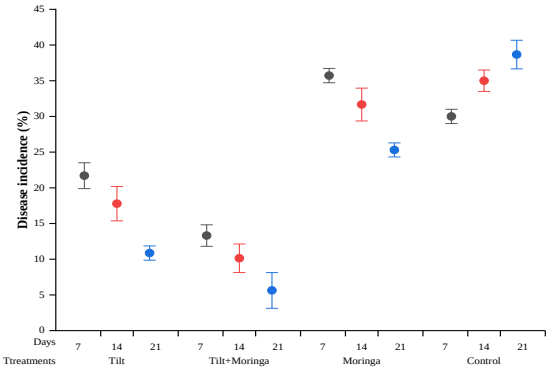

Fig. 6 Impact of interaction between treatments and days (T×D) against stalk rot of maize

4. Discussion

Maize (Zea mays L) is a parsimoniously important cereal crop after wheat and rice because it is high in carbohydrates, protein, oil, fiber, sugar, and ash, use as cooking oil for human and fodder for animals (Wang et al., 2018). Maize production is influenced by different biotic (Brown spot, Head smut, Maize lethal necrosis, Stewart's wilt, Northern blight, Southern blight) and abiotic (temperature, water, humidity, light, rain fall, and cultural practices) factors (Wang et al. 2020). The stalk rot of maize (caused by *Fusarium moniliforme*) is one of the most important emerging threats to the successful production of maize in Pakistan. To avoid yield losses different management approaches such as use of cultural practices, biological, chemical, and resistant variety were used against stalk rot of maize. Among all these strategies, resistant variety is the most effective to control this disease. For this purpose, screening of available germplasm was done and concluded that YH-5427 expressed highly resistant response against stalk rot of maize. Inbred line YH-5427 has dominant Fm resistance gene, Rfg1 (Yang et al., 2004). Both resistant and susceptible maize genotypes had different biochemical makeups, resistant genotypes had higher crude protein, fiber, ash, carbohydrate, fat, and total energy than susceptible genotypes. Biochemical studies help to explain the resistance of maize against Fm that shows the strong relationship between antioxidant efficiency, total phenolic content, and total flavonoid content. As the phenolic and flavonoid content contributes more to antioxidant activity, its increasing value raises the percentage of free radicals trapped (Rashmi et al., 2017). The high phenolic and flavonoid content of resistant genotypes may be attributable to the presence of more sugar, which serves as a precursor for the synthesis of antioxidant agents during pathogen infection. It has been reported that lignin accumulation and cell wall-bound phenolic compounds fortify the cell walls against many plant pathogens (Niemann et al., 1991). Results of current study were supported by Qureshi et al. (2015) who conducted a trial at Maize and Millet Research Institute, Yousaf Wala, Pakistan. Results showed that two maize genotypes, EL7 and All, exhibited a highly resistant response to Fusarium stalk rot in both growing seasons. Results of current study was also supported by Ghani et al. (2020) who conducted an experiment on single-cross maize hybrid and concluded

that FH-5427 is high yielding and resistant to stalk rot of maize.

But in field, under conducive environmental conditions the disease appeared in epidemic form, then farmer has the only choice to use chemicals because they are quick in action, easily available, easy to handle, and easy to apply. In the contemporary study, five synthetic chemicals (Table 11) [Tilt (25%), Belanty (400g/L), Forum-Top (9%+55%), Cabrio-Top (5% + 55%), and Enervin Duo (300g+225g/L)] were evaluated against Fm under lab conditions. Among these synthetic chemicals, Tilt was the most effective in inhibiting the fungal growth at 150ppm concentration. Tilt contains propiconazole that stop the growth of fungi by interfering the biosynthesis of cell membrane. It also inhibited sporulation and spore germination of Fm. Triazole fungicides act by binding to hemoproteins involved in the biosynthesis of sterols and other functions, such as cytochrome P-450. When ergosterol biosynthesis takes place, propiconazole demethylates C-14, causing a formation of C-14 methyl sterols.

The biosynthesis of these ergosterols is essential for the development of fungal cell walls (Ginoya and Gohel, 2015). This lack of normal sterol production effectively prevents further infection and invasion of host tissues by slowing or stopping the growth of the fungus. Pectinolytic and cellulase production was also reduced by these fungicides (Meon, 1982). These enzymes directly contribute to pathogenesis by inducing electrolyte loss, tissue maceration, and cell death. Fm's enzyme production was lower in the fungicide-incorporated medium than in the control group in the present study. Decreased enzyme production linked to the decreased mycelial biomass observed in treatment plates. The results of the current study were supported by the findings of Naz et al., (2013) who studied and described the fungicidal effect of Tilt against *F. moniliforme*. Outcomes of the present study were also supported by Khokhar et al., (2014) who evaluated five synthetic chemicals and concluded that Tilt showed maximum growth inhibition against *F. moniliforme.*

The use of synthetic chemicals is efficient but harmful to the environment. Continuous use of chemicals causes resistance to the pathogen (Waqas et al., 2018). That is why in the current study eight plant extracts (Table 12) (*Moringa olerifera, Cinnamomum tamala, Zingiber officinale, Azadirachta indica, Allium sativum, Eucalyptus globulus, Mentha piperita,* and *Ficus benjamina)* were evaluated against Fm causing stalk rot of maize under lab conditions. As they have antifungal compounds which are less toxic, ecofriendly, safe, and easily biodegradable (Shuping and Eloff, 2017).

Among these phytoextracts *M. olerifera* showed significant results against *Fm* under lab conditions. *M. olerifera* has various antimicrobial compounds like zeatin, quercetin, β-sitsterol, caffeoylquinic acid, and kaempferol (Anjorin et al., 2010). Which damages the external membrane of the fungal cell wall by disturbing its structure at specific binding sites and affecting multiple targeted sites and causing a reduction in cytoplasmic pH which results in cell wall disruption. Antimicrobial compounds of *M. olerifera* disrupt the structure of fungal spores and inhibit enzyme synthesis. They also change the shape and size of the cell which leads to the death of fungi (Prasad et al., 2019). The results of the current study were supported by the findings of El-Mohamedy and Abdalla, (2014) who investigated the antifungal activity of plant extracts against *F. moniliforme* and concluded that phytoextracts are eco-friendly, less toxic, and effective. M. olerifas was found to be the most effective plant extract against stalk rot of maize in the field as well as in lab conditions. Same results were reported by Subedi and Neupane, (2021) who studies six different plant extracts (*Acorus calamus* L., *Xanthoxylum armatum* DC., *Azadirachta indica* A. Juss., *Lantana*

Table 11 Assessment of synthetic chemicals along with their mode of action

Chemical	Active ingredient	Mode of action	C	Reference
TiTilt	Propiconazole 2 5%	Stop the growth of fungi by interfering the biosynthesis of cell membrane		Biehl, (2019)
CCabrio Top	Pyraclostrobi n +Metiaram 5 5+55 %	Interfere with the ATP pproduction in the mitochondria o of the fungi by blocking eelectron transport at the site of q quinol oxidation (Qo site) in the cytochrome bc1		Shridhar *et al*., (2018)
E Enervin Duo	Ametoctradin +Dimethomo h 3 300+ 225g/L	Inhibit mitochondrial r respiration by interacting with complex III at the quinone ininner site (QiI).		Cherrad *et al*., (2018)
F Forum Top	Diamatamorh +Metiram 9 44%	Inhibition of sterol (ergosterol) synthesis		Lamberth *et al*., (2021)
BBelanty	Mefentrifluco nazole 4 400g/L	Strongest inhibiting effects on conidium germination, cell membrane integrity		Liu *et al*., (2022)

Table 12 Phytochemicals used for experiment work

Common name	Technical name	Plant part	Anti-fungal compound
Neem	*Azadirachta indica*	Leaves	Triterpenoids (Chen *et al*., 2011)
Sufaida	*Eucalyptus globulus*	Leaves	Polyphenolic compound (Jafari *et al*., (2021)
Garlic	*Allium sativum*	Bulb	Allicin (Wang *et al*., 2014)
Ginger	*Zingiber officinale*	Roots	C.albicans (Maekawa *et al*., 2013)
Moringa	*Moringa olerifera*	Leaves	Methanolic and ethyl acetate (Jayasree, 2012)
Ficus	*Ficus benjamina*	Leaves	Alkaloid and phenolic compounds (Salehi *et al*., 2021)
Taiz pata	*C, tamala*	Leaves	Phenylpropanoids Pandey *et al*., (2012)
Mint	*Mentha piperita*	Leaves	Alkaloid compounds (Wenji *et al*., 2019)

camera L. and *Artemisia indica*) under lab conditions and concluded that A. calamus significantly reduced the growth. Similarly, Gwa and Nwankiti, (2017) evaluated ten plant extracts (*Allium sativum* L., *Allium cepa* L., *Azadirachta indica, Capsicum Annum, Calotropisgi gantea, Dalbrgia* sissoo, *Eucalyptus camelduensis, Gardenia florida, Melia azedarach, Zingiber officinalis*) against *F. moniliforme* through poisoned food technique and are noteworthy results. Various antimicrobial compounds are present in plants therefore it is the most pressing need to use and

evaluate the maximum potential of these plant extracts for making them a part of different integrated disease management strategies. This strategy will also help in reducing the environmental hazards and their toxic effects on human beings.

5. Conclusion

In current comparative investigation of synthetic chemicals and phytochemicals, minimum disease incidence was expressed by Tilt and Moringa in integration followed by Tilt and Moringa. Among all host genotypes, YH-5427 expressed highly resistance response.

6. Conflict of Interest

There is no conflict of interest regarding submission of this research paper.

7. Authors' Contribution Statements

MA conceive idea of research, MK conduct lab and field experiments, NAR supervised field experiments, FY wrote methodology, AA draw graphs, LM correct references, AS analyzed data, MA correct the references, AW collect references and help in research, ZA collect field and lab data and complied them.

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