

ANTIFUNGAL EXPLOITATION OF FUNGICIDES AND PLANT EXTRACTS AGAINST *FUSARIUM* OXYSPORUM F.SP. MELONGENAE CAUSING FUSARIUM WILT OF EGGPLANT

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ABSTRACT

Eggplant/Brinjal (*Solanum melongena* L.) is the most significant and widely consumed as nutritious vegetable. *Fusarium* wilt is the major destructive disease in most of the vegetable growing regions of Pakistan. The research was conducted to assess fungicides against *Fusarium oxysporum* f. sp. melongenae from eggplant in field and invitro efficacy of numerous plant extracts. In current research, three treatments (T) i.e; shincar, flumax, Topsin-M, along with control were used at recommended dose with three consecutive sprays, on comparison of disease incidence with control, the Shincar showed 21.03% disease incidence, Topsin-M showed 31.94% disease incidence and the Flumax was close to control as it showed the least effect against the *Fusarium* wilt by showing 35.85% disease incidence. Furthermore, for plant extracts, poisoned food technique was used in the lab. The results were significant for all treatments (T), concentrations of (C), days (D) and their interactions (T x D), (T x C) and (C x D). Zingiber officinale showed less growth of colony (1.28cm) following *Allium sativum* (1.62cm), *Datura stramonium* (2.17cm), *Eucalyptus globulus* (2.50cm), *Azadirachta indica* (2.76cm) compared to control, respectively. Results of the present study showed a significant decrease in fungal growth of tested fungicides such as Shincar (Carbendazim) and *Zingiber officinale* (Ginger).

Keywords: Eggplant, Fusarium f.sp. melongenae, In-vitro, fungal exploitation, Poison-food technique and fungicides spray.

INTRODUCTION

Brinjal/Eggplant (Solanum melongena L.) is the most significant and widely consumed as nutritious vegetable. It has great status/importance as vegetable in diet of human because of its worth in vitamins, proteins and fundamental minerals. On facing malnutrition problems, vegetables are important producers in developing nations (Rahman et al., 2011, Perrone et al., 2012). In Pakistan, brinjal is used as a common vegetable in cooking due to its availability at reasonable price round the year, area under cultivation is 8,427 hectares with a total annual production of 84,255 tons (Anonymous, 2019). Pakistan stood at 22nd position in world ranking for Eggplant cultivation (FAO, 2021). Successful eggplant production is subject to numerous fungal, viral and bacterial disorders due to the inability to obtain disease resistant seeds (Abdel-Monaim, 2012). Among those constraints, Fusarium wilt caused by Fusarium oxysporum has major

borne and systemic pathogen, which leads to the deterioration of the vascular system, nutrient inhibition and water transfer, yield and quality by disrupt physiological procedures (Morid *et al.*, 2012). The *Fusarium is* a pathogen which invades into the roots of the host eggplant through the feeding of nematode pinholes, natural and other wounds. Afterward its invasion, it penetrates in the xylem vessels of plant which transfer water and nutrients from roots towards crown and fungal mycelium makes toxins, as it proliferate through plant. Suddenly, death of plant occurs owing to blockage of the xylem vessel (Pietro *et al.*, 2003). *Fusarium* wilt characterized by yellowing of leaves and drooping of shoots which results in expiry of entire plant. Soil inhabiting fungi which colonized

destructive impact. F. oxysporum decreases level of

quality and quantity of eggplant's fruit in vegetable

growing areas (Fayzan, 2012). Thermophilic, soil-

in senescing tissues of infected plant and could persist in soil for decades (Joseph et al., 2008). A sequence of wilt and death may occur in mature leaves. Xylem vessel of infected tissue show brown discoloration. The most pronounced symptom is epinasty of leaves and partial stunting of eggplant by Fusarium wilt (Altinok, H.H 2005). Numerous management strategies are widely adopted to reduce disease incidences such as cultural operations, biological controls, resistant varities, soil solarization, crop rotation & less availability of soil moisture (Kamal et al., 2009), but for all these techniques it is necessary to demonstrate their activities against new prolonged virulently pathogen races as well as highly skilled precise measurements (Amini and Sidovich 2010). Production can be increased by cultivating diseased and pest free crops. The healthy fruit with the good appearance takes hold of high prices at market level. In the emerging countries, there are some vegetables and fruits are banned to grow because of their defenselessness to sever disease and insect/pest attacks (Sain, 2018). Researchers are finding low-priced and more ecofriendly compounds to control plant infectious diseases due to environmental and economic issues. Some analysis has shown that extracts of some plants and bacteria could be applied as biocontrol agents for managing soil-inhabiting pathogens like Fusarium oxysporum (Pegg and Brady, 2002). Conversely, using systemic fungicides in order to prevent the occurrence, gravity and growth of Fusarium wilt has been easily handled, alternatives are developed and drained into the soil, directly and quickly (Shah et al., 2014). Pakistani farmers are aware rather than adverse effects of these fungicides that are increasing their output, vield and financial return (Sitara and Hasan, 2011). A wide range of fungicide classes have also achieved a reputation in Pakistan because of irrelatively low costs and efficiency (Diaz et al., 2005) and are widely used worldwide. The protection of plants and curative interventions depend on the use of suitable systemic fungicides gradually. In addition, successfully managing wilting problems is crucial to ensuring that the brinjal farming is economical. However, it is an essential component of modern research to prevent the incidence of this thermophilic soil-borne disease through substances. Furthermore, structural and ecological fungicide recognition is the requirement of the present era. Therefore, it is very necessary to implement such fungicides that are quick to act and less phytotoxicity effect against Fusarium oxysporum. Therefore, in the current studies, the most efficient fungicides have been used to avoid disease for brinjal crop. Current study was performed with the aim of identifying best fungicide against Fusarium wilt commercially suited for treatment in Pakistan induced in field and in laboratory conditions for Fusarium f. sp.

melongenae by plants extracts. In the eggplant world, this disease causes huge losses.

MATERIALS AND METHODS:

Collection, isolation, purification and identification of F. oxysporum f. sp. Melongenae: Brinjal plants which were already infected with Fusarium wilt were collected from different fields of Faisalabad, the collected diseased samples were processed in lab for isolation of pathogen Fusarium oxysporum f.sp. melongenae. Thoroughly cleaned and sliced the infected roots into tiny parts and sterilized with 1% of HgCl2. Roots were dried on sterilized filter paper and placed on watch glass. Atleast, two portions of diseased roots were placed in petri-dish which consists of potato dextrose agar (PDA) medium. The plates were incubated for fungal growth at 25 $^{\circ}C \pm 2 ^{\circ}C$ (Sarwar et al., 2005) for 48 to 72 hours. Then the pathogen colonies have been purified. Fusarium oxysporum f.sp. melongenae were identified by morphological features under stereomicroscope such as white and violet mycelium and brief microconidia on short conidiophores (Soesanto et al., 2011).

Pathogenicity test through sick field: The pathogenicity test was conducted through a nursery in which the pure *Fusarium oxysporum* f. sp. *melongenae* cultures were mixed into the soils before transplant.

Establishment of sick field: Two consecutive pure culture sprays F. oxysporum f.sp. melongenae were performed in experimental areas at an interval of 10 days before the most susceptible eggplant varieties were replanted like desi on the ground. Susceptible varieties with a fungal suspension of $1 \ge 10^6$ spores per ml of H₂O were again inoculated. This suspension was done by inserting 3-4 ml of fluid in petri-plates containing F. oxysporum 7-10 days old, and after shaking water was poured into 250 ml beaker. 1ml of this suspension in a haemocytometer was retained and no. of spores was evaluated three times, stereo microscopically. Spores in the beaker were adjusted by incorporating sterilized water in the beaker to 1 x 10⁶ spores/ml of H₂O (Sarwar et al., 2005). Inoculation of this spore suspension was done close to plant's root area for the peak production of inoculum in field. The plants were brought into the laboratory for an evaluation on presence of F. oxysporum f. sp. *melongenae* after symptoms observation. After testing, the affected crops were ploughed in soil and irrigated to improve decomposition of crop waste and to create circumstances for peak fungal development (Naik et al., 2008). Artificially diseased crops have been reisolated to fulfill postulates of Koch (Ignjatov et al., 2012).

Exploitation of commercial fungicides against *Fusarium* wilts of Eggplant: Most effective concentrations of fungicides Topsin-M, Flumax and Shincar with control (water) were evaluated against *Fusarium* wilt of eggplant in field environments. In diseased fields, 15 day old seedlings of susceptible varieties were planted and row-to-row and plant-to-plant distance was maintained under RCBD. Every treatment was applied with three replicates. Spray was done with 2 weeks interval and influence of spray on disease was observed. Data related to disease incidence (%) has been collected after one week interval up to crop maturity. T1=Topsin-M (Thiophanate-Methyl) @ 3gm/L, T2= Flumax (Fluazinam Metalaxal-M) @ 2ml/L, T3= Shincar (Carbendazim) @ 3ml/L, T4= Control (water).

In-vitro evaluation and their potential against Fusarium oxysporum f.sp. Melongena of several plant extracts: Extracts of 5 plants namely Neem (Azadirachta indica), Garlic (Allium sativum), Eucalyptus (Eucalyptus globulus), Datura (Datura stramonium) and (Zingiber officinale) with one control (water) were examined against Fusarium oxysporum f.sp. melongenae by using poisoned food-technique. Several parts of these plants like leaves and roots @30g/plant were collected and brought to lab for processing and extraction. These were suspended in 100 ml sterilized water and grand in blender apparatus (Yelmame et al., 2010) and then 1 gram washing powder was added to the solution and then filtered with muslin cloth in beaker and then left for 24 hours. Then, 50 ml distilled water was added and mixed in a beaker. 10% and 15% concentration were made by adding prepared plant extract in sterilized water, and the extract was added in semicooled PDA media in vessel and mixed well by using rotatory shaker and 15ml medium was poured into 9 cm petri dish in laminar

flow (Yelmame *et al.*, 2010). In cooled medium PDA center and a pure medium of culture of the previously incubated pathogen, a sterilized cork borer was used for making of the 6 mm hole with one diameter in hole. Every treatment with control (water) has been replicated 3 times and then incubation was done at \pm 25C for 2 to 3 days under CRD. Data collection was carried out after 4, 7 and 10 days based upon colony growth. T1= Neem, T2= Ginger, T3= Garlic, T4= Eucalyptus, T5= Datura, T6= Control (water).

Data Analysis: SAS/STAT statistical softwares were used to perform all statistical tests (SAS Institure, 1990). Means of all treatments were separated via Fisher's protected least significant difference (ANOVA) and SAS/STAT software packages were used to develop interaction among different treatments and their combinations.

RESULTS

ANOVA indicated that all the treatments (T), sprays, varieties and their interactions showed significant results as shown in Table 1. The minimum disease incidence has been recorded as 21.03% by fungicide Shincar (Carbendazim) then Topsin-M showed 31.94% and then Flumax showed the 35.85% disease incidence. In (Table 5) the ANOVA indicated that all the treatments (T), concentrations (C), Days (D) and their interactions expressed significant results. *Zingiber officinale* showed less growth of colony (1.28cm) following *Allium sativum* (1.62cm), *Datura stramonium* (2.17cm), *Eucalyptus globulus* (2.50cm), *Azadirachta indica* (2.76cm) compared to control

SOV	DF	SS	MS	F	Р
REP	2	0.1	04399		
SPRAY	2	21120.6	10560.3	81183.3	0.0000*
TR	3	2385.8	795.261	6113.63	0.0000*
V	1	956.7	956.667	7354.45	0.0000*
SPRAY*TR	6	2212.2	368.70	2834.41	0.0000*
SPRAY*V	2	342.9	171.467	1318.16	0.0000*
TR*V	3	314.1	104.704	804.92	0.0000*
SPRAY*TR*V	6	251.5	41.9176	322.24	0.0000*
ERROR	46	6.0	0.13008		

 Table1. ANOVA for field evaluation of fungicides against Fusarium oxysporum f. sp. Melongenae

Where P > 0.05=Non-Significant ; P < 0.05=Significant * ; CV = 1.17

Sr.#	Treatment	Disease Incidence (%)
T1	Topsin-M	31.94c
T2	Flumax	34.85b
T3	Shincar	21.03b
T4	Control	35.31a
LSD	2.013	

Mean values in rows and column sharing similar small and capital letters does not differ significantly P>0.05

The above (Table. 2) describe the results of the field evaluation of fungicides, on comparison of disease incidence with control the Shincar fungicide was the best against the *Fusarium* wilt disease as it showed 21.03% disease incidence and then the Topsin-M

showed 31.94% disease incidence and the Flumax was close to control as it showed the least effect against the *Fusarium* wilt disease by showing 35.85% disease incidence.

Treatments	Spray 1	Spray 2	Spray 3	
T1 Topsin-M	8.70i	30.01e	57.11b	
T ₂ Flumax	8.90hi	35.51d	57.36a	
T ₃ Shincar	9.15j	25.26g	28.83f	
T ₄ Control	9.31h	39.25c	60.1b	
LSD	2.0	13		

Table.3: Impact on growth of *Fusarium* wilt disease by Interaction between treatments and sprays.

Mean values in rows and column sharing similar small and capital letters do not differ significantly P>0.05.

The comparison of the three sprays with the fungicidal effects is discussed in the (Table 3). It shows that disease incidence recorded after first sprays of Topsin-M was 8.70% and that time control was at 9.31%, therefore, Flumax (8.90%) and Shincar (9.15%) respectively. After 2nd spray, the Shincar showed (25.26%), Topsin-M showed 30.01% and Flumax showed 35.51%. After the third spray, there was significant difference between Shincar effect and control as it showed 28.83% incidence and then Topsin-M showed 57.11% following Flumax (57.36%).

Treatments	Spray 1		Spray 2	Spray 2		Spray 3	
	WER-2	DESI	WER-2	DESI	WER-2	DESI	
T1 Topsin-M	8.20u	9.20rs	28.70n	31.33m	52.76f	58.56b	
T ₂ Flumax	8.31Tv	9.50qr	32.761	38.26i	54.86e	61.46a	
T ₃ Shincar	8.50Tu	9.80q	14.80p	35.73j	18.260	39.40h	
T ₄ Control	8.80St	9.83q	33.70k	44.80g	56.16d	65.43c	
LSD	•	2.013					

Table: 4 Impact of Interaction between treatments spray and varieties

Mean values in rows and column sharing similar small and capital letters do not differ significantly P>0.05.

The above (Table 4) shows the relation/comparison of Fungicides (Shincar, Flumax, Topsin-M) with the disease incidence showed by two susceptible varieties (WER-2, DESI/Local) along-with three sprays. The disease incidence shown by variety WER-2 after first spray of Topsin-M was 8.20% and 9.20% was of Desi. The first spray of Flumax showed 8.31% disease incidence on WER-2 and 9.50% was on Desi variety. The Shincar showed the disease incidence on both WER-2 and Desi (8.50 and 9.80%) respectively. On comparison, control was at 8.80% on WER-2 and 9.83% on Desi. After the second spray the Topsin-M showed 28.70% on WER-3 and 31.33% disease incidence on Desi variety, Flumax showed 32.76% on the WER-2 and 38.26% on Desi, Shincar showed 14.80%. And at that time the control had 33.70% on WER-2 and 44.80% on Desi variety. After the last spray the Shincar showed the best results by showing 18.26% disease incidence on WER-2 and 39.40% on Desi variety. Then Topsin-M showed the somehow best result after the Shincar by showing 52.76% disease incidence on WE\$-2 and 58.56% on Desi variety. Then Flumax showed 54.86% on WER-2 and 61.46% disease incidence on Desi variety as compared to control (56.16% on WER-2 and 65.43% on desi Variety).

In-vitro evaluation of plant extracts against melongenae: Fusarium oxysporum f.sp. All treatments (T), concentrations (C), days (D) and their interactions (T \times D), (T \times C), and (C \times D) were showed significant results as shown in (Table 5). Zingiber officinale showed less growth of colony (1.28cm) Allium sativum (1.62cm), following Datura stramonium (2.17cm), Eucalyptus globulus (2.50cm), Azadirachta indica (2.76cm) compared to control, respectively (Table 6). Interaction among treatments and concentrations $(T \times C)$ revealed that 10% concentration of Zingiber officinale shows lesser colony growth (1.56cm) as compared to Allium sativum (1.96cm), Datura stramonium (2.37cm), Eucalyptus globulus (2.65cm), Azadirachta indica (2.90cm) while at 15% concentration of these treatments showed 0.99,1.28, 1.98, 2.31, 2.60 and 3.37 cm colony growth of fungus respectively (Table 5 and Table 6). The interaction of treatment and days showed Zingiber officinale (0.85,1.30) followed by Allium sativum (1.22,1.60,2.04), Datura stramonium (0.95, 2.15, 3.40), Eucalyptus globulus (1.15,2.99,3.38), Azadirachta indica (1.85,2.42,4.01) and control (2.16,3.17,4.84) respectively (Table 6 and Table 6).

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SOV	DF	SS	MS	F	Р
С	1	1.2920	1.2920	82.93	0.0000*
DAYS	2	20.7986	10.3993	667.50	0.0000*
TR	5	17.7986	3.5597	228.49	0.0000*
C*DAYS	2	0.0852	0.0426	2.73	0.0130*
C*TR	5	0.3936	0.0787	5.05	0.0144*
DAYS*TR	10	4.5521	0.4552	29.22	0.0000*
Error	10	0.1558	0.0156		
Total	35	45.0758			

Table: 5 ANOVA for In-vitro testing of plant extracts against Fusarium oxysporum f.sp. melongenae

P<0.05=Significant CV 5.45

Sr#	Treatment Name	Fungal growth (cm)
1	Azadirachta indica	2.76 b
2	Zingiber officinale	1.28 f
3	Eucalyptus globulus	2.50 c
4	Datura stramonium	2.17 d
5	Allium sativum	1.62 e
6	Control	3.39 a
LSD		2.28

Mean values in rows and column sharing similar small and capital letters do not differ significantly P>0.05

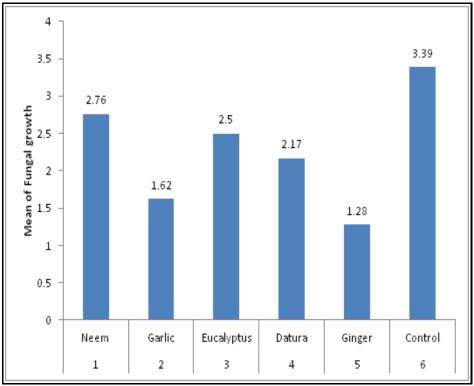


Fig:1 Evaluation of different plants extracts against Fusarium oxysporum f.sp. melongenae

The above graph showed the response of five plant extracts (neem, garlic, datura, ginger and garlic against the *Fusarium oxysporum*. As comparison with the control the plant extract of Ginger shows the best

response (by showing minimum growth of 1.28 cm) against *Fusarium oxysporum* and followed by Garlic (1.62 cm), Datura (2.17 cm), Eucalyptus (2.5 cm) and neem (2.76 cm) respectively (Fig 1).

Treatments	Colony Growth (cm)	Colony Growth (cm)			
	Concentration (10%)	Concentration (15%)			
Azadirachta indica	2.90b	2.60d			
Zingiber officinale	1.56i	0.99k			
Eucalyptus globulus	2.65c	2.31f			
Datura stramonium	2.37e	1.98g			
Allium sativum	1.96h	1.28j			
Control	3.41a	3.37a			
LSD	2.28				

Table: 7 Effect of various plant extracts and their concentrations on Fusarium oxysporum f.sp. melongenae colony growth

Mean values in rows and column sharing similar small and capital letters do not differ significantly P>0.05

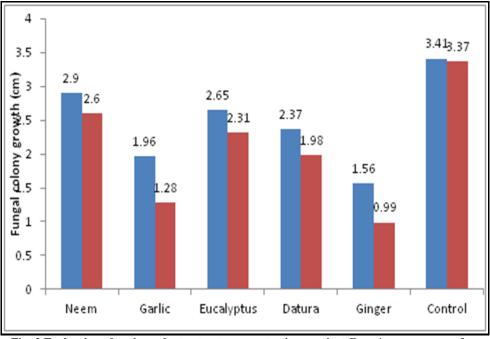


Fig: 2 Evaluation of various plant extracts concentrations against Fusarium oxysporum f.sp.

The above graph showed the response of five plants extracts conc. @ 10% blue bars and 15% red bars (neem, garlic, datura, ginger and garlic) against the *Fusarium oxysporum*. As comparison with the control the plant extract of Ginger shows the best response at 15% (by showing reduced growth of 0.99)

cm against *Fusarium oxysporum* and followed by Garlic (1.28 cm), Datura (1.98 cm), Eucalyptus (2.31 cm) and neem (2.6 cm) respectively. And at 10% concentration the fungus growth was ginger (1.56cm), Garlic (1.96 cm), Datura (2.37 cm), Eucalyptus (2.65 cm) and neem (2.9 cm) respectively (Fig 2).

Table:8 Effect of different plant extracts and days on colonial growth of Fusarium oxysporum f.sp. melongenae

Treatments	Fungal colony growth			
	After 3 days	After 7 days	After 10 days	
Azadirachta indica	1.850GH	2.425E	4.010B	
Zingiber officinale	0.850K	1.300I	1.690H	
Eucalyptus globulus	1.155L	2.990D	3.3800	
Datura stramonium	0.950JK	2.155N	3.405C	
Allium sativum	1.225IJ	1.605M	2.040FG	
Control	2.165EF	3.175CD	4.840A	
LSD	2.28			

Mean values in rows and column sharing similar small and capital letters do not differ significantly P > 0.05

DISCUSSION

Fusarium wilt produced by Fusarium oxysporum f.sp *melongenae* is major issue in cropping regions, in Pakistan; under favorable environment it can cause almost 70% vield losses (Ashfaq et al., 2014). Fusarium wilt indicates signs of a yellowing of the leaves and the fall of apical bud, which kills the whole crop. Soil inhabiting fungi which colonized the senescing tissues of the infected plant and could persist in the soil for many years (Joseph et al., 2008). A subsequent wilt and death of older leaves may occur. Xylem vessel of infected tissue show brown discoloration (Rani et al., 2008). The most pronounced symptom is epinasty of leaves and partial stunting of eggplant by Fusarium wilt (Altinok, 2005). For soil inhabiting diseases namely Fusarium wilt, using resistant varieties was good and appropriate method (Naik et al., 2007). Use or resistant host minimizes the disease incidences and avoid application of fungicides on soil due to its toxic effects (Russo and Howard, 2002).

Current study indicated that the disease-uptake caused soil and air temperature rise of 19% to 70%, soil-humidity and wind-speed while the comparative moisture rise causes diseases to decrease, with other soil and environmental conditions such as minimum land and aerial-temperature. These results were matched with Karimi *et al.*,(2012) and Mehmood *et al.*,(2013) results, respectively, who evaluated that temperature of soil and air alongwith soil moisture leads towards significant disease development because of thermopolic nature of pathogen (Land *et al.*, 2006; Chand and Khirbat, 2009).

Different plant species were identified since year's having antifungal characteristics (Sridhar *et al.*, 2003; Singh *et al.*, 2004). Plants have naturally occurring substances with appropriate amount; they were less toxic, safe to use and eco-friendly, easily biodegradable and translocated (Duru *et al.*, 2003; Saxena *et al.*, 2005; Lee *et al.*, 2007 and Sitara *et al.*, 2008).

Plant extracts are being used because they are alternative to the fungicidal chemicals, plants extracts are names botanical pesticides as they are not detrimental to plants, human beings and animals (Asthana et al., 2001). These bio-pesticides are utilized in various formulations namely sprays, dip solutions, fumigants, cakes and water solutions (Siripornivisal and Ngamchawee, 2011). Current study shows five different plants were used against Fusarium wilt disease as botanical pesticides (Azadirachta indica, Datura stramonium, Zingiber officinales, Eucalyptus globulus, and Allium sativum). From all there extracts the Z. officinales exhibited outstanding results compared to other extracts of plants. These extracts have lectins, trypsin and proteinase inhibitors and antifungal properties (Goyal et al., 2007; Bijina et al.,

2011). They inhibit germination of Fusarium oxysporum spores. Results of this research also matched with observations of Jamil et al., 2007, Neela et al., (2014) who demonstrated anti-fungal properties of many extracts of plants against pathogen Fusarium oxysporum casual agent of Fusarium wilt disease. If resistant varieties are unavailable and application of fungicides is an essential method to control soil inhabiting pathogens to prevent yield losses during epidemically widespread of diseases. Evaluation of fungicides has been a good way to manage infectious diseases for protection of large amount of money and environment (Song et al., 2004). During contemporary studies, three fungicides (Flumax, Shincar and Topsin-M) with recommended dose have been checked against Fusarium wilt. Among these, the Shincar showed significant results. Shincar (Carbendazim) disturbs metabolism and delays growth and development of Fusarium wilt pathogen (Iqbal et al., 2010). It forms strong bonds with chemical force to pathogens microtublules and change its ionic concentrations (Magnucka et al., 2007). Results of current studies were also favored by results of Naik et al., 2007 who tested carbendazim, benomyl, Thiophanate methyl (Topsin-M) and triadimefon against Fusarium oxysporum and observed carbendazim revealed significant results when compared to other fungicides. Conclusion

Five plant extracts and three fungicides are used in current research. It is concluded that *Zingiber officinale* (Ginger) and Shincar (carbendazim) are statistically significant antifungal agents to minimize losses and disease incidence caused by *Fusarium* wilt of eggplant.

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