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Effect of Different Media, pH and temperature on Growth and Sporulation of Fusarium Wilt of Soybean cause by *Fusarium oxysporum* f. sp. *glycines*

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ABSTRACT: The pathogen *Fusarium oxysporum* f.sp. *glycines* was tested for variation in growth and cultural characters an seven different soiled media, Potato dextrose agar and Potato dextrose broth medium were found best for growth and sporulation. The optimum growth and sporulation of *Fusarium oxysporum* f. sp. *glycines* was found on pH 6.0 to 7.0. The highest growth of pathogen was recorded at 30ºC with higher sporulation.

Keywords: Soybean, pH, media, temperature, wilt, *Fusarium oxysporum.*

INTRODUCTION

Soybean (*Glycine max* L.) is one of oilseeds with a high nutritional value (Hesseltine, 1985), native to Asia. It was domesticated in China (1550-1027 B.C.) and introduced to Romania in 1911 (Hartman *et al*., 1999; Dencescu and Popa 1973). Soybean has great potential as an exceptionally nutritive and very rich protein food. It can supply much needed protein to human diets, because it contains more than forty per cent protein of superior quality and all the essential amino acids particularly glycine, tryptophan and lysine, similar to cow's milk and animal proteins. Soybean also contains about twenty per cent oil with an important fatty acid, lecithin and Vitamin A and D. Its edible oil contain about 1.6 – 3.1 per cent lecithin which is essential for building up nerve tissues. The four percent mineral salts of soybeans are fairly rich in phosphorous and calcium. Oil is also used as raw material in manufacturing antibiotics, paints, varnishes, adhesives and lubricants.

In India, annual yield losses due to various diseases are estimated as 12% of total production. Over hundred pathogens are known to affect soybean, of which 66 fungi, six bacteria and eight viruses have reported to be associated with soybean seeds (Sinclair, 1978). The diseases include rust, wilts, leaf spot, rots, powdery mildew, bacterial and viral diseases. Among these, soil borne diseases like root rot or collar rot caused by *Sclerotium rolfsii* (Saccardo, 1911), *Rhizoctonia* sp. and *Fusarium* sp. are gaining more importance as they reduce plant populations in the field resulting in heavy yield losses. *Fusarium* species can infect plants at any stage of soybean development but infection is particularly favored when plants are weakened. *Fusarium* species are commonly isolated from soybean roots (Arias *et al*., 2013). *F. oxysporum* f. sp. *glycines* reduced seed germination and seedling survivability by 40% and caused pre-emergence damping off of seedlings (Begum *et al*., 2007). *Fusarium* species are widespread soilborne organisms capable of surviving for long periods of time as chlamydospores and as mycelium in plant residues and in soil (Gheorghies and Cristea 2001; Cristea, 2005). *F. oxysporum* (Schlecht. Snyder and Hansen 1940) f. sp. *glycines* belongs to Kingdom Fungi, Phylum *Ascomycota*; Class *Sordariomycetes*; Order *Hypocreales*; Family *Nectriaceae*; Genus *Fusarium* (Kirk *et al*., 2008). The pathogens are soil inhabitants and polyphagous facultative parasites. These are having wide hosts.

Sangeetha and Jahagirdar (2013) studied the effect of different solid media. *viz.,* potato dextrose agar, czapek's-dox agar, oat meal agar, sabouraud's agar and host extract agar on *Fusarium* in soybean. The results indicated that best mycelial growth was made on potato dextrose agar (90 mm) followed by czapek's agar (78.03 mm). Least growth was recorded in host extract (56.44 mm) and sabouraud's agar (50.20 mm). Gheorghe *et al*. (2015) reported that energy sources are important for *Fusarium oxysporum* f. sp. *glycines* colonies development. The fungus metabolizes carbon very efficiently from monosaccharide's: arabinose, dextrose, glucose, levulose, maltose, mannose, mannitose, ribose, and trehalose. On the substrate containing polysaccharides, such as cellulose, fungus colony development was weak and carbon metabolism of starch was relatively good. The lack of a carbon

source stopped the development of colonies. It is observed that the fungus easily metabolized inorganic nitrogen compounds such as sodium nitrate, ammonium nitrate and ammonium sulphate. Asparagine and peptone had instead of have the same effect on the growth of the fungus. Gheorghe *et al*. (2015) studied the influence of pH value on the development of the fungus *Fusarium oxysporum* f. sp. *glycines.* He observed that there is a wide range of pH values substrates development of strong acid up to alkaline, colonies forming a good vegetative mass with conidia appearance at pH=3; the optimal values are between pH=4 and pH=7. With alkalinization culture medium, the fungus grew less vegetatively, but sporulated well. The optimum temperature for colony development for *Fusarium oxysporum* f. sp. *glycines* was between 22-28°C; the minimum temperature for colony development was 8°C and the highest at 36°C; the lethal temperature level was identified at 38°C

MATERIALS AND METHODS

Isolation and purification of the pathogen. Small pieces of infected tissues 1–2 mm dimension from the advancing margin of the spot, adjacent to healthy portions were cut with blade, washed well in distilled water to remove dust adhered to the infected pieces. Pieces were dipped in 0.1 percent mercuric chloride solution for 30 seconds and finally washed well in three changes of sterilized distilled water.

The bits were then transferred to PDA slants with the help of inoculating needle under aseptic condition and incubated at 28 ± 1ºC. After 72 hrs, fragments of hyphal growth from the growing tips were transferred to fresh PDA slants. Pure culture was made, following repeated hyphal tip transfer method.

Effect of culture media. Following seven culture media were used to find out the most suitable one for the mycelial growth and sporulation. Each culture medium was prepared in 1 liter of water and autoclaved at 121.6ºC at 15 psi for 20 min. These were cooled to 45ºC and then poured in 90 mm Petri dishes for solidification.

1. Potato Dextrose agar (PDA) medium (Peeled and sliced potato 200g, Dextrose 20g, Agar-agar 20g).
2. Richards's agar (RA) medium (Potassium nitrate 10g, Potassium monobasic phosphate 5g, Ferric chloride 0.02g, Magnesium sulphate 2.5g, Sucrose 50g, Agar-agar 20g).
3. Ashby's agar medium (Mannitol 20g, Di potassium phosphate 0.2g, Sodium chloride 0.2g, Potassium sulphate 0.1g, Magnesium sulphate 0.2g, Calcium carbonate 5g, Agar-agar 15g, final pH (at 25°C) 7.4±0.2).
4. Asthana and Hawker's medium (D-Glucose 5g, Potassium nitrate 3.50g, Magnesium sulphate 0.75g, Potassium dihydrogen Phosphate 1.75g, Agar- agar 20g).
5. Czapeks Dox agar (CDA) medium (Sodium nitrate 2g, Di potassium hydrogen phosphate 1g, Potassium chloride 0.5g, Sucrose 30g, Magnesium sulphate 0.5g, Ferrous sulphate 0.01g, Agar-agar 20g).
6. Coon's agar (CA) medium (Sucrose 7.2 g, Dextrose 3.60g, Potassium nitrate 2.02g, Potassium di- phosphate 2.72g, Magnesium sulphate 1.23g, Agar- agar 15g).
7. Browns agar (BA) medium (Dextrose 2g, Magnesium sulphate 0.75g, Tri basic potassium phosphate 1.25g, Agar-agar 20g).

Estimation of dry weight of mycelial growth. The target pathogen was inoculated in liquid media contained in Erlenmeyer flask. These inoculated flasks were incubated for 21 days at 28 ± 1ºC in order to determine the dry weight of mycelial mat. The mycelial mats were filtered through previously dried and weighed whatman's filter paper no. 42 and washed thoroughly with hot distilled water to remove the traces of suspended sugars. Mycelial mats along with filter papers were dried at 60ºC for 24 hrs. They were cooled in desiccators. The mycelial mats were weighed and again dried in oven until the constant weights were obtained. Weight of mycelial mat was calculated with help of the following formulae:

DW = (W2 – W1)

Where,

DW = Dry weight of mycelial mat
W2 = Weight of test pathogen along with filter paper
W1 = Weight of filter paper

Effect of pH

There were eight different pH level ranging from 5.0 to 8.5 with a difference of 0.5 were prepared by using pH meter and by using either N/10 HCl or NaOH before autoclaving the PDA medium. For each pH value, three replications were maintained. The Petriplates containing sterilized medium was inoculated with 5mm mycelium disc and incubated at 28 + 1ºC. At the interval of 24hrs, the linear growth was measured till 7 days. The range of sporulation test ranges on various pH was recorded after 7 days. Sporulation was calculated with the help of haemocytometer.

Effect of temperature. The experiments were conducted to find out, the most suitable temperature for mycelial growth and sporulation of *F. oxysporum* f. sp. *glycines*. The sterilized poured petriplates with PDA were inoculated with 5 mm disc of the test pathogen of seven days old culture. The petriplates were incubated at 10, 15, 20, 25, 30, 35 and 40ºC temperature. Three replications were maintained for each treatment and observation for mycelial growth was recorded after seven days. Sporulation was recorded at seven days after inoculation with the help of haemocytometer.

RESULTS AND DISCUSSION

The radial growth was maximum on Potato dextrose agar (87.17 mm) which was significantly superior over all other medium. This was followed by Richards's agar (85.50 mm), Coon's agar (63.83 mm) and Asthana and Hawker's agar (59.33 mm) and Czapek's Dox agar (55.00 mm). No radial growth was obtained in Asbhy's agar and Browns agar media, respectively. Growth characters of *Fusarium oxysporum* f. sp. *glycines* studied on different solid media indicated that Potato dextrose agar supported maximum growth of fungal colony. Margin was regular in Potato dextrose agar, Richards's agar and Czapek's dox agar medium. In case

of Asthana and Hawker's agar and Coon's agar medium the margin were irregular. Mycelium was whitish in all the media.

The test fungus supported excellent sporulation in Potato dextrose agar and Richards's agar medium where as fair sporulation was recorded in Coon's medium and Asthana and Hawker's agar medium. Poor sporulation was observed in case of Czapek's Dox agar medium, No sporulation was recorded in Ashby's agar and Browns agar medium.

Table 1: Effect of solid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines*.

T. No.	Name of the medium	Radial growth (mm)		Growth characters	Sporulation
		After 120 hrs*	After 168 hrs*		
T1	Potato dextrose agar	64.83	87.17	White cottony growth with regular margin	++++
T2	Richard's agar	61.67	85.50	White cottony growth with regular margin	++++
T3	Coon's agar	44.67	63.83	White sparsh growth with irregular margin	++
T4	Asthana and Hawker's agar	40.50	59.33	White sparsh growth with irregular margin	++
T5	Czapek's Dox agar	38.83	55.00	Dull white appressed growth with regular margin	+
T6	Ashby's agar	0.00	0.00	No growth	-
T7	Browns agar	0.00	0.00	No growth	-
	SE(m)	**0.33**	**0.32**		
	CD (0.05)	**1.02**	**1.00**		

*Average of 3 replications

Table 2: Effect of liquid media on dry mycelial weight and sporulation of *Fusarium oxysporum* f. sp. *glycines*.

Treatment Number	Name of the medium	Dry mycelial weight (mg) after 21 days *	Sporulation
T1	Potato dextrose broth	374.90	+++
T2	Richard's broth	359.83	+++
T3	Asthana and Hawker's broth	284.73	++
T4	Coon's broth	294.73	++
T5	Czapek's Dox broth	255.17	++
T6	Ashby's broth	0.00	-
T7	Browns broth	0.00	-
	SE(m)	**0.76**	
	CD (0.05)	**2.34**	

*Average of 3 replications

Effect of seven different liquid media maximum dry mycelial weight of *Fusarium oxysporum* f. sp. *glycines* was recorded in Potato dextrose broth medium (374.90 mg) which was significantly superior to the dry mycelial weight recorded in rest of the medium. Next best medium supporting the growth of *Fusarium oxysporum* f. sp. *glycines* was Richard's broth medium, which yielded 359.83 mg dry mycelial weight. Dry mycelial weight of 294.73, 284.73 and 255.17 mg were recorded in Coon's, Asthana and Hawker's and Czapek's Dox medium respectively. Brown's and Ashby's broth medium supported no mycelial growth. Gheorghe *et al* (2015) reported that the most favorable carbon sources for the development of the *Fusarium oxysporum* f. sp. *glycines* are: arabinose, dextrose, glucose, levulose, maltose, mannose, mannitose, ribose, and trehalose. The reason for poor growth in Asbhy's agar medium and Browns medium may be due to the presence very low amount of sucrose in the medium. The present results were also in confirmation with Singh *et al*. (2016) also reported that Potato dextrose agar and Richard's agar were the best medium for radial growth and sporutation of *Fusarium oxysporum* f. sp. *lentis*. which also support the present study. The maximum suitable media for growth of *Fusarium oxysporum* f. sp. *pisi* was Potato dextrose agar (81.15 mm) followed by Coons agar and Czapex dox agar. The maximum mycelial growth of *Fusarium oxysporum* f.sp. *lini* was recorded in the Potato dextrose agar (39.50 mm) which was superior, followed by Oat seed extract dextrose agar (36.33 mm).

Effect of pH. Effect of different pH *viz*., 5, 5.5, 6, 6.5, 7, 7.5, 8 and 8.5 on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines* were studied and observations have been presented in growth of the test pathogen was obtained at all the pH levels tested but it was maximum at pH 6.0 (89.83mm) after 168 hrs of inoculation. pH 6.5 (85.00mm) and pH 7 (82.83mm) were also found favorable. It was observed that there was a wide range of pH values substrates development of strong acid up to alkaline, colonies forming a good vegetative mass. The optimal values were between pH 6.0 and pH 7.0. With alkalinization culture medium, the fungus grow slowly. The foremost acidic and alkaline pH is not suitable for the growth of test pathogen. Excellent sporulation was observed at pH 6.0. Good sporulation was recorded at pH 6.5. pH 5.5, 7.0, 7.5 and 8 which supported fair sporulation while, poor

sporulation was observed at pH 5.0 and 8.5. Data presented in Table 3, clearly indicate that pH 6.0 to 7.0 is optimum for growth and sporulation of *Fusarium oxysporum* f. sp. *glycines*. The present results are in agreement with the result of Gheorghe *et al*. (2015) reported that the pH values of the culture medium influenced the fungus *Fusarium oxysporum* f. sp. *glycines* growth. The pH reaction substrate was optimal for values between 4.0 and 7.0. The results of the present study are also in agreement with those achieved by Somesh *et al.* (2019) reported that pathogen grows over a wide range of pH i.e., from 3.0 to 8.0, but the most suitable pH for *Fusarium oxysporum* f. sp *lini* growth was observed to be 7.0 at which the maximum growth of the fungus was recorded which was closely followed by pH 6.5. However, with the increase of acidity or alkalinity, the growth of the fungus was hampered and lowest growth was recorded at pH 3.0. The maximum growth of *Fusarium oxysporum* f.sp. *lini* (88.16 mm) was recorded at pH 6 with highest growth rate (12.59 mm per day) and maximum sporulation after seven days of incubation.

Table 3: Effect of pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines*.

Treatment Number	pH	Radial growth (mm)		Sporulation
		After 120 hrs*	After 168 hrs*	
T1	5.0	58.17	76.67	++
T2	5.5	59.83	82.33	+++
T3	6.0	70.50	89.83	++++
T4	6.5	61.67	85.00	+++
T5	7.0	60.17	82.83	+++
T6	7.5	59.50	80.83	+++
T7	8.0	57.83	79.17	+++
T8	8.5	54.83	75.67	++
SE(m)		**0.47**	**0.54**	
CD (0.05)		**1.44**	**1.64**	

*Average of 3 replications

Effect of temperature. Growth of *F. oxysporum* f. sp. *glycines* was studied from 10 to 40ºC temperature and result is presented in Table 4. It was seen that there was quite a large variation in the growth of these isolate at different temperature after 7 days. The maximum mycelial growth was recorded at 30ºC (85.00mm) followed by 25ºC (71.33mm), 20ºC (47.67mm), 15ºC (26.33mm), 35ºC (21.00mm) and 10ºC (13.00mm). While no mycelial growth was recorded at 40ºC. Temperatures from 25 to 35ºC were most favorable for the growth of target pathogen. The highest growth of pathogen was recorded at 30ºC with higher sporulation. Gheorghe *et al*. (2015) observed that the optimum temperature for colony development for *Fusarium oxysporum* f. sp. *glycines* was between 22-28°C; the minimum temperature for colony development was 8°C and the highest at 36°C; the lethal temperature level was identified at 38°C. Cruz *et al.* (2019) observed that There was a significant interaction between temperature and pH ($P < 0.05$) for *in vitro* radial growth and root rot severity in soybean caused by *F. oxysporum*. Isolates showed the most *in vitro* radial growth after incubation at pH 6 and 25°C. For the rolled-towel assay, the pathogenic isolate caused the most severe root rot at pH 6 and 30°C. Gaussian regression analysis estimates for optimal conditions were pH 6.3 at 27.1°C for maximal fungal growth and pH 5.9 at 30°C for maximal root rot severity. Somesh *et al.,* (2019) observed that optimum temperature range for growth of *Fusarium oxysporum* f.sp. *lini*, was found to be 25°C to 30°C. However, the minimum growth was recorded at 45°C and 10°C. No growth and sporulation were observed at 50°C temperature. Chakrapani *et al.* (2023) showed that all isolates of *Fusarium oxysporum* f. sp. *pisi,* exhibited the highest growth at a temperature of 25°C and the optimal temperature range for growth of *Fusarium oxysporum* was 23–27°C. All isolates showed the highest growth at pH5.

Table 4: Effect of deferent temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines*.

Treatment Number	Temprature ^{o}C	Radial growth (mm) After 168 hrs*	Sporulation
T1	10	13.00	+
T2	15	26.33	++
T3	20	47.67	++
T4	25	71.33	+++
T5	30	85.00	++++
T6	35	24.00	++
T7	40	0.00	-
SE(m)		**0.44**	
CD (0.05)		**1.34**	

*Average of 3 replications

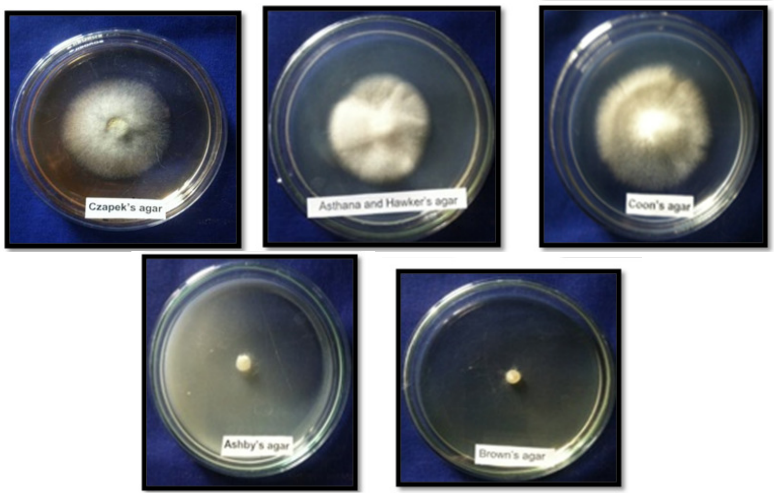

Plate 1. Effect of solid media on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines.*

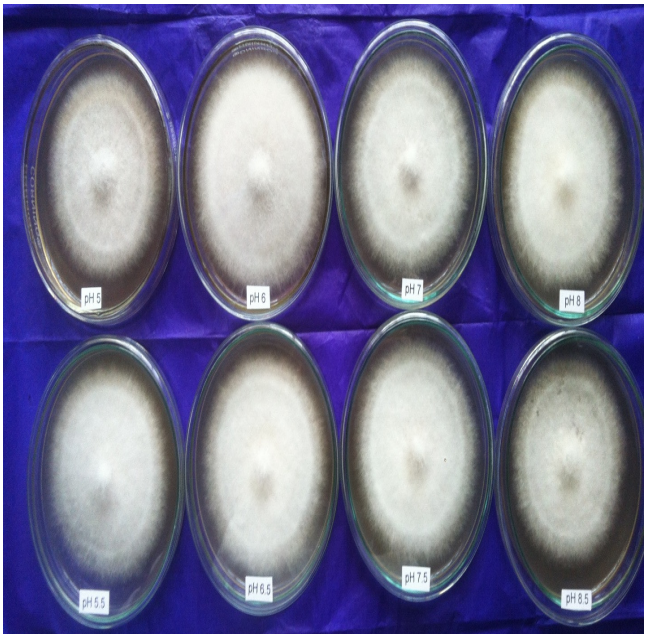

Plate 2. Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *Glycines.*

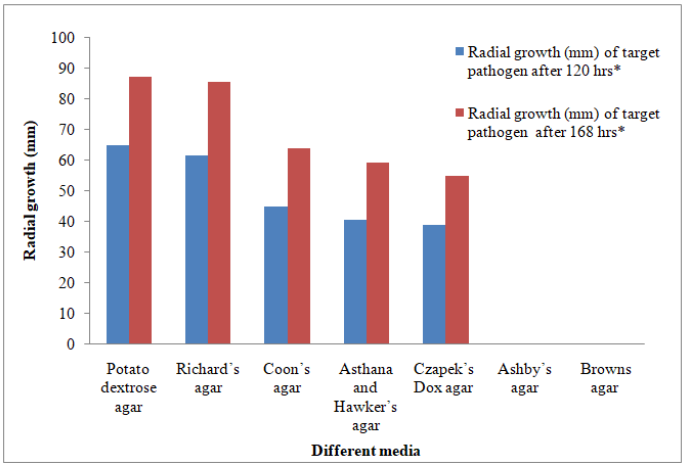

Fig. 1. Effect of solid media on radial growth of *Fusarium oxysporum* f. sp. *glycines.*

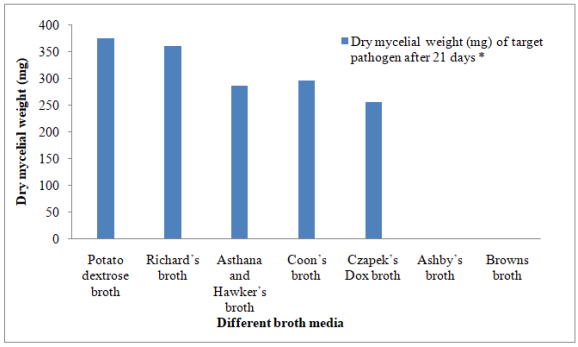

Fig. 2. Effect of liquid media on dry mycelial weight of *Fusarium oxysporum* f. sp. *Glycines.*

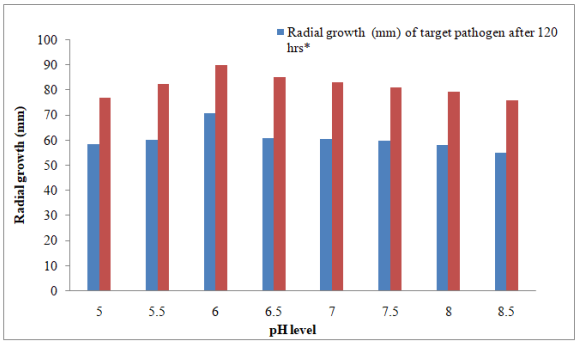

Fig. 3. Effect of various pH on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines.*

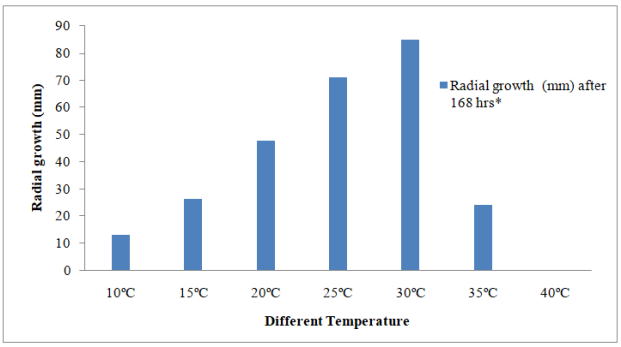

Fig. 4. Effect of deferent temperature on radial growth and sporulation of *Fusarium oxysporum* f. sp. *glycines.*

CONCLUSIONS

Following the observations carried out on different media and pH on growth and sporulation of the fungus *Fusarium oxysporum* f. sp. *glycines* can be concluded that: Potato dextrose agar medium was found best for growth and sporulation. Potato dextrose broth medium was found best for dry mycelia weight and sporulation. The optimum growth and sporulation of *Fusarium oxysporum* f. sp. *glycines* was found on pH 6.0 to 7.0. The highest growth of pathogen was recorded at 30ºC with higher sporulation.

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