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Study of factors affecting mycelial growth and sporulation of *Gibbago trainthemae in vitro*

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Abstract
Thirteen different natural, semi-synthetic and synthetic media were evaluated for its growth and sporulation. Richard's Agar, Asthana and Hawker's Agar and Sabouraud's agar were supported good growth and sporulation. Of the eight different natural products evaluated for growth and sporulation of *G. trainthemae*, wheat bran Agar supported the maximum mycelial while chickpea flour agar supported maximum sporulation. Of the seven different micronutrient evaluated at lower and higher doses, the maximum mycelial growth (76.20 mm) occurred with Ferric Nitrate at 500 mg/lit , while the maximum sporulation was found with 25 mg/lit of ammonium molybdate. The maximum mycelial growth and sporulation occurred at 35°C and 30°C respectively. The maximum dry mycelial weight of *G. trainthemae* were observed at the pH 5.5, while in liquid medium sporulation was low.

Keywords: *Gibbago trainthemae, Trianthema portulacastrum,* media, pH, temperature.

Introduction
In India, little work has been done on mycoherbicides. Though some pathogens exlusively infecting most problematic weeds have been reported as new records. On *Parthenium hysterophorus* (*Congress grass*) *Alternaria zinniae* and *Phoma sorghina* (Kumar and Kumar, 2000a) [7]; *Alternaria alternata*, *Sclerotium* sp. and *Aspergillus niger* (Joshi *et al.* 2001) [6] have been reported while on *Lantana camara Alternaria* spp., *Phoma* spp., *Fusarium* sp. and *Curvularia* sp. have been reported to cause moderate to severe disease (Saxena and Pandey, 2000) [6].

Trianthema partulacastrum L., commonly known as horse purslane, black pigweed, carpet weed, gudbur, hogweed, itcit or santha is an important terrestrial weed of the family Aizoaceae. It is indigenuous to South Africa, but now has spread into tropical and sub-tropical countries such as West Asia, India, Sri Lanka, Africa and Tropical America. *T. portulacastrum* has been reported to be very aggressive weed in mustard, maize, pegion pea, soybean, potato and onion crops in the states of Haryana and Punjab. Upto 60-70 per cent infestation of this weed has been reported in pegion pea and soybean fields. Because of its infestation in various agricultural crops especially during the rainy season, horse purslane has been referred to as number one problematic weed of agricultural crops in Northern India (Aneja and Kaushal, 1999) [1].

The pathogens reported on *T. porulacastrum* are *Cercospora trianthemae* (Chiddarwar, 1962) *Gibbago Trianthemae* (Simmons, 1986) [7]; *Drechslera* indica (=*Bipolaris indica)* (Rao and Rao, 1987); Taber *et al.* 1998 [9] and soil born pathogen (*Fusarium oxysporum*); Darshika and Daniel, 1992). Babu *et al.* (2004b) [2] reported a leaf blight of *T. portulacastrum* caused by *Paecilomyces varioti* as a new host record. Bohra *et al.* (2005) [3] evaluated a strain of *Alternaria alternata* as mycoherbicies of *T. protulacastrum*, A strain of *Myrothecium verrucaria* applied of 2×10^7 conidia ml^{-1} also killed Horse purslane seedlings in addition to spotted spurge and prostrate spurge in tomato field (Boyette *et al*., 2007) [4].

Materials and Methods
Effects of different culture media on growth and sporulation of *G. trianthemae*
The fungus was grown on different natural, semi synthetic, synthetic media. All the ingredients of media were dissolved in 500 ml of distilled water. In another lot of 500 ml distilled water, 20 g of agar was melted and mixed with above solution. The volume of mixture was adjusted finally to one litre. The media were sterilized in auto clave at 1.045 kg/cm^2 pressure for 20 minutes. Twenty (20) ml of sterilized medium was poured in 90 mm sterilized petri plates and allowed to solidify. The plates were inoculated with 5 mm disc of 7 – days- old culture of *G. trianthemae*.

Each treatment had five replications. Inoculated petri plates were incubated at 25±2 °C in an incubator or BOD. The observations of colony diameter and sporulation were recorded after 15 days of incubation.

Effect of different substrates on growth and sporulation of *G. trianthemae.*

Eight different substrates were evaluated for increased growth and sorulation of *G. trianthemae*. These were- Rice flour, wheat bran, maize flour, corn starch, shelled corn cob (powder), molasses, chickpea flour and soybean flour. The materials were used sieved through a 425 mesh sieve to obtain fine powder. The media were sterilized in autoclave at 1.045 kg/cm^2 pressure for 20 minutes, and allowed to cool. Twenty (20) ml of sterilized medium was poured in 90 mm sterilized petri plates and allowed to solidify. The plates were inoculated with 5 mm disc of 7 –days- old culture of *G. trianthemae*. Each treatment had five replications. Inoculated petri plates were incubated at 25±2 °C in BOD incubator. The observation of colony diameter and sporulation were recorded after 15 days of incubation.

Effect of different micronutrient on growth and sporulation of *Gibbago triantheame*

Seven different micronutrient, namely, copper sulphate at 25 mg and 50 mg, cobalt chloride at 50 mg and 100 mg, Ferric nitrate at 250 mg and 500 mg, Ferric sulphate 5 mg and 10 mg, manganese chloride 25 mg and 50 mg, zinc sulphate 10 mg and 50 mg, Ammonium molybdate at 25 and 50 mg (Dhingra and Sinclare 1993) [5].

Sabouraud' agar medium was used for this study as it had shown consistency in supporting both, growth and sporulation of *G. trianthemae*. The selected micronutrient at lower and higher dose were individually added in Sabouraud's Agar medium, while the medium without micronutrient served as control. The variously amended media were sterilized in autoclave at 1.045 kg/cm^2 pressure for 20 minutes and allowed to cool. Twenty (20) ml of sterilized medium was poured in 90 mm sterilized petri plates and allowed to solidify. The plates were inoculated with 5 mm disc of 7 –days- old culture of *G. trianthemae*. Each treatment had five replications, inoculated petri plates were incubated at 25 ±2°C in BOD incubator. The observation of colony diameter and sporulation were recorded after 15 days of incubation.

Growth and sporulation of *G. trianthemae* at different temperatures

Studies were conducted to determine the optimum range of temperature for growth and sporulation of *G. trianthemae*. *G. trianthemae* were inoculated in plates containing potato dextrose agar by placing 5mm diameter mycelial discs cut from periphery of 7–days- old culture of *G. trianthemae.* The plates were incubated at 15, 20, 25, 30 and 35 °C. Five plates as five replications were maintained for each temperature. The diameter of the colonies were measured after 15 days of incubation and rate of sporulation were also recorded by haemocytometer.

Growth and sporulation of *G. trianthemae* on different pH levels

G. trianthemae was grown on potato dextrose broth media of 5 different pH levels, which ranged from 4.5 to 7.0 with to standardize the most suitable pH level for its growth and sporulation. Different pH levels were adjusted by adding the citrate phosphate buffer solution given by Dhingra and Sinclair (1993) [5]. Stock solution were prepared for citrate phosphate buffer viz., 0.1 M citric acid (19.21 g litre^{-1}) and 0.2 M Na_2HPO_4 (53.69 g litre^{-1}) and then mixed x ml of citric acid stock solution with y ml of phosphate stock solution and the volume was made one litre for different pH levels.

Twenty (20) ml potato dextrose broth medium of different pH levels were dispensed in 100 ml flask, keeping five replication for each level, and the flasks were autoclaved at 1.045 kg cm^{-2} pressure for 20 minutes. After cooling to room temperature, the flasks were inoculated with 5 mm discs of 7 –days- old culture of *G. trianthemae.* Inoculated flasks were incubated at 25±2 ºC in an incubator. The observations of dry mycelial weight and rate of sporulation were recorded after 15 days of incubation.

Results

Effect of thirteen different natural, semi-synthetic and synthetic media on growth and sporulation of *G. trianthemae*

Solid media: Thirteen different natural, semi-synthetic and synthetic media viz., *Trianthema* extract Dextrose Agar, Potato Dextrose Agar, Potato-Carrot Agar, Potaot-Sucrose Agar, Yeast extract Dextrose Agar, Peptone Glucose Agar, Malt and Yeast extract Agar, Richard's Agar, Sabouraud's Agar, Czapek Dox Agar, Asthana & Hawker's Agar, WFP Agar, Leonian Agar were prepared and autoclaved at 1.045 Kg/cm^2 pressure for 20 minute. Sterilized solid media were poured aseptically in sterilized petri plates which were inoculated with 5 mm diameter disc of 7- days- old culture of *G. trianthemae.* The plates were incubated at 25±2° C. Each treatment had five replications. The linear growth of *G. trianthemae* was measured along two diameters at right angles to each other in each replication after 15 day of inoculation and rate of sporulation was also recorded after 15 day of inoculation. The results are presented in Table-1.

Table 1: Effect of different natural, semi-synthetic and synthetic media on growth and sporulation of *Gibbago trianthemae*

S. No	Media	Linear growth in diameter (mm)* after 15 days of incubation	Sporulation after15 days of incubation (× 10^4 mm^{-2})*
A.	**Natural media**		
1.	*Trianthema* extract Dextrose Agar	37.5	80.2
2.	Potato Dextrose Agar	35.5	10.2
3.	Potato-Carrot Agar	34.5	6.6
4.	Potato Sucrose Agar	22.2	6.2
B.	**Semi-synthetic media**		
5.	Yeast extract Dextrose Agar	34.5	29.6
6.	Peptone Glucose Agar	29.6	51.0
7.	Malt and Yeast extract Agar	21.7	58.0
C.	**Synthetic media**		
8.	Richard's Agar	78.8	12.0

9.	Sabouraud's Agar	70.2	73.0
10.	Czapek Dox Agar	62.0	14.2
11.	Asthana and Hawaker's Agar	55.9	88.0
12.	WFP Agar	52.6	14.2
13.	Leonian Agar	49.2	8.0
	SEm±	1.33	2.18
	CD at 5%	3.77	6.19
	CD at 1%	5.03	8.24

* Average of five replications.

Maximum mycelial growth (78.8 mm) was observed on Richard's Agar medium followed by Sabouraud's Agar (70.2 mm), Czapek Dox Agar (62.00 mm) and Asthana and Hawker's Agar (55.9 mm). On WFP Agar and Leonian Agar 52.6 mm and 49.2 mm were recorded. Poor mycelial growth was recorded on *Trianthema* extract Dextrose Agar (37.5 mm) followed by Potato Dextrose Agar (35.5 mm) and Potato carrot Agar (34.5 mm) and Yeast extract Dextrose Agar (34.5). Peptone glucose Agar (29.6 mm) Potato Sucrose Agar (22.2 mm) and Malt and Yeast extract Agar (21.7 mm) also supported low growth (Table 1). Overall, Richard's Agar medium (78.8 mm) gave the best mycelial growth and Malt and Yeast extract Agar gave minimum mycelial growth (21.7 mm) of *G. trainthemae* (Table-1).

Maximum sporulation (88.0×10^4 mm^{-2}) was also observed on Asthana and Hawker's Agar medium followed by *Trianthema* extract Dextrose Agar (80.2×10^4 mm^{-2}), Sabouraud's Agar (73.0×10^4 mm^{-2}), Malt and Yeast extract Agar (58.0×10^4 mm^{-2}), Peptone Glucose Agar (51.0×10^4 mm^{-2}) and Yeast extract Dextrose Agar (29.6×10^4 mm^{-2}). WFP Agar and Czapex Dox Agar (14.2×10^4 mm^{-2}) Richard'Agar ($12x10^4$ mm^{-2}) Potato Dextrose Agar ($10.2x10^4$ mm^{-2}) Leonian Agar ($8x10^4$ mm^{-2}), Potato carrot Agar (6.6×10^4 mm^{-2}) and The least sporulation was observed on potato sucrose Agar (6.2×10^4 mm^{-2}), (Table-1)

Effect of different agar media amended with natural products on growth and sporulation of *G. trianthemae*

To find out the most suitable medium for mycelial growth and sporulation of *Gibbago trianthemae* eight different agar media amended with natural products viz., Corn starch agar, Wheat bran agar, Soybean agar, Corn cob agar, Molasses agar, Chickpea flour agar, Maize agar and Rice agar, were evaluated for growth and sporulation of *G. trianthemae*. The media were autoclaved at 1.045 kg/cm^2 pressure for 20 min. sterilized agar media were poured aseptically in sterilized petri plates which were inoculated with 5 mm diameter disc of 7- days- old culture of *G. trianthemae.* These plates were incubated at 25±2 °C. Each treatment had five replications. The liner growth of *G. trianthemae* and rate of sporulation were recorded after 15 days of incubation. The results are presented in Table-2

The maximum mycelial growth (74.00 mm) was observed on wheat bran agar medium followed by corn starch agar (70.10 mm), Molasses agar (67.80 mm) and corn cob agar (62.40 mm). Mycelial growth (48.10 mm) occurred on soybean agar followed by maize agar (45.90 mm) chick pea flour agar (42.60 mm) and rice agar (38.70 mm). The lowest growth was on rice agar. Maximum sporulation (155.2×10^4 mm^2) was observed on chickpea flour agar followed by maize agar (42.40×10^4 mm^2). Minimum sporulation was observed on rice agar ($7.2x10^4$ mm^{-2}. No sporulation occurred on corn starch agar, wheat bran agar, corn cob agar, soybean agar and molasses agar upto 15 days of incubation (Table-2).

Table 2: Effect of eight different Agar media amended with natural products on growth and sporulation of *Gibbogo trianthemae*

S. No.	Agar Media	Linear growth in diameter (mm)* after 15 days of incubation	Sporulation after 15 days of incubation ($\times 10^4$ mm^{-2})*
1.	Wheat bran Agar	74.00	0.00
2.	Corn starch Agar	70.10	0.00
3.	Molasses Agar	67.80	0.00
4.	Corn cob Agar	62.40	0.00
5.	Soybean Agar	48.10	0.00
6.	Maize Agar	45.90	42.40
7.	Chickpea flour Agar	42.60	155.20
8.	Rice Agar	38.70	7.20
	SEm±	3.65	1.96
	CD at 5%	10.50	5.66
	CD at 1%	14.13	7.60

* Average of five replications.

Effect of different micronutrient on growth and sporulation of *G. trianthemae*

Seven different micronutrient namely Copper sulphate at 25 mg and 50 mg, Cobalt chloride at 50 mg and 100 mg, Ferric nitrate at 250 mg and 500 mg, Ferric sulphate at 5 mg and 10 mg, Mangenese chloride at 25 mg and 50 mg, Zinc sulphate at 10 mg and 50 mg, and Ammonium molybdate at 25 mg and 50 mg L^{-1} were evaluated for enhancing the growth and sporulation of *G. trianthemae*. The micronutrients were added separately in Sabouraud's agar at lower and higher dose. Sabouraud' agar without micronutrient served as control. The variously amended media were autoclaved at 1.045 kg/cm^2 pressure for 20 min., allowed to cool and were dispensed aseptically in sterilized petri plates, keeping five plates as five replications of each treatment. After solidification of the media, the plates were inoculated with 5 mm diameter disc of 7- days- old culture of *G. trianthemae.* These plates were incubated at 25±2 °C for fifteen (15) days and then observations for colony diameter and sporulation were recorded. The results are presented in Table-3

Table 3: Effect of seven different micronutrient at lower and higher dose on growth and sporulation of *Gibbago trianthemae*

S. No.	Micronutrient	Liner growth in diameter (mm)* after 15 days of incubation	Sporulation after 15 days of incubation ($\times 10^4$ mm^{-2})*
	Copper Sulphate		
1.	$CUSO_4$ 25 mg	64.10	67.00
2.	$CUSO_4$ 50 mg	36.80	0.00
	Cobalt Chloride		
3.	$CoCl_2$ 50 mg	61.10	13.40
4.	$CoCl_2$ 100 mg	52.20	0.00
	Ferric Nitrate		
5.	$Fe_2 (No_3)_3$ 250 mg	54.90	0.00
6.	$Fe_2 (No_3)_3$ 500 mg	76.20	0.00
	Ferric sulphate		
7.	$Fe_2 (So_4)_3$ 5 mg	66.90	1.80
8.	$Fe (SO_4)_3$ 10 mg	41.00	0.00
	Mangenese Chloride		
9.	$Mncl_2$ 25 mg	68.70	0.00
10.	$Mncl_2$ 50 mg	62.60	0.00
	Zinc Sulphate		
11.	$ZnSO_4$ 10 mg	67.80	23.00
12.	$ZnSO_4$ 50 mg	59.70	87.20
	Ammonium Molybdate		
13.	$NH_4mo_7O_4$ 25 mg	64.6	151.80
14.	$NH_4mo_7O_4$ 50 mg	39.50	0.00
15.	Control	69.10	79.80
	SEm±	2.81	1.79
	CD at 5%	7.95	5.05
	CD at 1%	10.58	6.72

*Average of five replications

The linear growth of *G. trianthemae* and rate of sporulation was also recorded after 15 days of inoculation the results are presented in Table-4. The maximum (76.20 mm) and significantly high growth occurred at higher dose of Ferric Nitrate 500 mg, as compared to 69.10 mm colony diameter in unammended Sabouraud's agar (control). The medium amended with Copper sulphate recorded 64.10 mm and 36.80 mm colony diameter, that with cobalt chloride showed 61.10 mm and 52.20 mm colony diameter, with ferric sulphate growth was 66.90 mm and 41.0 mm, with manganese chloride the growth was 68.70 mm and 62.60 mm, with zinc sulphate it was 67.80 mm and 59.70 mm, and with ammonium molybdate it was 64.6 mm and 39.50 mm, at lower and higher doses, respectively Table-3

Although the mycelial growth at lower and higher dose of these micronutrients was significantly less than control Sabouraud's agar medium, but some of these showed significantly increased sporulation over it (Table-3). The maximum sporulation (151.8×10^4 mm^{-2}) was recorded at lower dose (25 mg) of ammonium molybdate , followed by (87.20×10^4 mm^{-2}) in higher dose (50 mg) of zinc sulphate, while on Sabouraud's agar (79.80×10^4 conidia mm^{-2}) were recorded. Medium amended with Copper sulphate at 25 mg/lit yielded (67.0 x10^4conidia mm^{-2}), the sporulation was (23.0×10^4 mm^{-2}) in Zinc sulphate at 10mg/lit;, (13.4×10^4 mm^{-2}) with Cobalt chloride and 1.80×10^4 mm^{-2} with Ferric sulphate at 5mg/lit. The remaining treatments did not support sporulation. No sporulation occurred till fifteen (15) days in medium amended with both the doses of Ferric nitrate, Mangenese chloride, and at higher doses of Ammonium molybdate, Cobalt chloride and Copper sulphate and Ferric sulphate (Table-3)

Effect of different temperatures on growth and sporulation of *G. trianthemae*

In order to find out optimum temperature for growth and sporulation of *G. trianthemae* it was grown on chickpea flour agar (besan agar medium) at different temperature ranging between 15 to 35 °C the results are presented in Table-4

G. trianthemae could grow over a wide range of temperature ranging from 15 to 35° C,. The maximum growth (62.10 mm) was observed at 35° C followed by (30° C, 25° C and 20° C) 57.40 mm, 54.20 mm and 52.60 mm respectively. The least growth (30.60) mm was observed at 15° C (Table-4).

Maximum sporulation (269 x10^4mm^{-2}) was observed at 30° C followed by (25° C, 20° C, and 15°C) 189x10^4mm^{-2}, 164x10^4mm^{-2} and 153x10^4mm^{-2} respectively. The least sporation (138.60x10^4 mm^{-2}) was observed at 35° C (Table-4).

Table 4: Effect of different temperature on growth and sporulation of *Gibbago trianthemae*

S. No	Temperature	Liner growth in diameter (mm)* after 15 days of incubation	Sporulation after 15 days of incubation ($\times 10^4$ mm^{-2})*
1.	15° C	30.60	153.00
2.	20° C	52.60	164.00
3.	25° C	54.20	189.00
4.	30° C	57.40	269.00
5.	35° C	62.10	138.60
	SEm±	2.63	10.13
	CD at 5%	7.76	29.88
	CD at 1%	10.59	40.76

*Average of five replications.

Effect of different pH on growth and sporulation of *G. trianthemae*

The effect of variation in hydrogen ion activity on growth and

sporulation of *G. trianthemae* was studied on six pH levels ranging from 4.5 to 7.0. The potato dextrose broth medium was used as a basal medium. Dry mycelial weight and sporulation were recorded after 15 days of incubation. The results are presented in Table-5.

G. trianthemae was capable of growing and sporulating over a wide range of pH, ranging from 4.5 to 7.0. The maximum dry mycelial weight (394 mg) was observed at 5.5 pH, followed by pH 4.5, 5.0, 6.0, 7.0 and 6.5 (376 mg, 370 mg, 352 mg, 348 mg, 340 mg respectively (Table-5).

Poor sporulation was observed on potato dextrose broth media of different pH. Poor sporulation ($1x10^4 mm^{-2}$) was observed at 4.5 pH. At pH 5.0 and 5.5 sporulation was recorded ($2x10^4$ mm^{-2}). ($3x10^4$ mm^{-2}) sporulation was recorded at pH 6.0. At pH 6.5 and 7.0 sporulation was recorded ($4x10^4$ mm^{-2}), Table-5.

Table 5: Effect of different pH on growth and sporulation of *Gibbago trianthemae*

S.No.	pH	Liner growth in diameter (mm)* after 15 day of incubation	Sporulation after 15 day of incubation ($\times 10^4$ mm^{-2}))*
1.	4.5	376	1
2.	5.0	370	2
3.	5.5	394	2
4.	6.0	352	3
5.	6.5	340	4
6.	7.0	348	4
	SEm±	18.01	
	CD at 5%	52.57	
	CD at 1%	71.25	

*Average of five replications.

Discussion

Of the thirteen different cultural media including natural, semi-synthetic and synthetic media evaluated, maximum mycelial growth was on Richard's medium, followed by Sabouraud's and Czapek Dox media, while maximum sporulation was on Asthana and Hawker's mediuam, followed by *Trianthema* extract agar, and Sabouraud's agar. medium, Since Sabouraud's medium consistently supported good growth and sporulation, it was used for further studies. Eight different agar media amended with natural substrates *viz*., Corn starch, Wheat bran, Soybean meal, corn cob powder, mollasses, chickpea flour, maize meal and rice meal, were evaluated for growth and sporulation of *G. trianthemae*. The maximum growth (74.00 mm) was observed on wheat bran agar medium followed by corn starch agar (70.10 mm), Molasses agar (67.80 mm) and corn cob agar (62.40 mm). Minimum growth (48.10 mm) occurred on soybean agar followed by maize agar (45.90 mm), chick pea flour agar (42.60 mm) and rice agar (38.70 mm). The lowest growth was on rice agar. Chickpea flour supported maximum sporulation (155.2×10^4 mm^2), followed by maize agar (42.40×10^4 mm^2), while minimum sporulation was observed on rice agar($7.2x10^4$ mm^{-2}). Based on these results, chickpea flour was used in our further studies also. Better sporulation of fungi on various natural products has been reported, like that of *Phaeoramularia* sp. that grew best on potato dextrose Agar (PDA) and carnation leaf piece Agar and sporulated best on PDA and *A. adenophora* decoction Agar. (Wang *et al.*, 1997) [10].

The Seven different micronutrients evaluated for enhancing the growth and sporulation of *G. trianthemae*, the maximum and significantly high growth occurred at higher dose of ferric nitrate 500 mg, followed by copper sulphate recorded 64.10 mm and 36.80 mm colony diameter ,and cobalt chloride showed 61.10 mm and 52.20 mm colony diameter. Although the mycelial growth at lower and higher dose of these micronutrients was significantly less than control Sabouraud's Agar medium but some of these showed significantly increased sporulation over it. The maximum sporulation $151.8\times10^4/mm^2$ was recorded at lower dose (25 mg) of ammonium molibdinate, followed by $87.20 \times 10^4/mm^2$ in higher dose (50 mg) of zinc sulphate while on Sabouraud's Agar 79.80×10^4 conidia/mm^2 were recorded. Medium amended with copper sulphate at 25 mg/lit. Yielded 67×10^4 conidia/mm^2 spores, the sporulation was 23.0×10^4 conidia/mm^2 in zinc sulphate at 10 mg/lit. 13.4×10^4 conidia/mm^2 with Ferric sulphate at 5 mg/lit. For *Colletotrichum coccodes*, a biocontrol agent for velvet leaf (*Abutilon theophrasti*), several micronutrients were tested, and based on the results, incorporation of calcium chloride (0.5 g/l) and Copper sulphate (0.05 g/l) alongwith soy protein (5 g/l), KNO3 (5 g/l), K2HPO4 (5g/l) and MgSO4 (2 g/l) was selected as low cost and effective medium (Yu *et al*., 1996).

Temperature is one of the important external factors which influence biological system, particularly of microorganisms. *G. trianthemae* could grow over a wide range of temperature ranging from 15 to 35° C. The maximum growth (62.10 mm) was observed at 35° C followed by (30° C, 25° C and 20° C) 57.40 mm, 54.20 mm and 52.60 mm respectively. The least growth (30.60) mm was observed at 15° C. Maximum sporulation ($269 x10^4$ mm^{-2}) was observed at 30° C followed by 25° C, 20° C, and 15° C, where sporulation was $189x10^4$ mm^{-2}, $164x10^4 mm^{-2}$ and $153x10^4 mm^{-2}$, respectively. The least sporation ($138.60x10^4$ mm^{-2}) was observed at 35° C. *Gibbago trianthemae* was able to grow a wide range of pH 4.5 to 7.0. The maximum dry mycelial weight 394 (mg) was observed at 5.5 pH followed by pH 4.5, 5.0, 6.0, 7.0 and 6.5 with dry mycelia weight being 376 mg, 370 mg, 325 mg, 348 mg and 340 mg, respectively.

The present findings are in agreement with result obtained by other workers. Prashanthi and Kulkarni (2003) [5] reported that three mycoherbicides of *Chromolaena odorata viz., Alternaria alternata, Collectotrichum gloeosporioids, Aureobasidum pullulans* exhibited optimum growth at 5.5, 6.5, and 6 respectively. The optimum temperature for growth of *Alternaria alternata* was 25°C where as the optimum temperature for both C*olletotrichum gloeosporioides* and *Aureobasidium pullulans* was 30°C. Wang *et al.* (2006) [11] studied the optimum light temperature, pH, Carbon and nitrogen sources, moisture and medium for mycelial growth, sporulation and spore germination of *Cercospora piaropi* isolate WH9BR. The best cultural condition for mycelial growth and sporulation was 24 h darkness white the optimum treatment of light for germination was 24 h illumination. The

optimum temperature for mycelial growth, sporulation and spore germination were 25°C and 25-30°C respectively. The best mycelial growth and spore germination were respectively obtained at pH 8.6 and 7.0 while highest number of spore was produced at 8.0-9.0. The optimum carbon and nitrogen sources were mannitol and urea, respectively. For mycelial growth glucose and sucrose were the best carbon source for sporulation of WH9BR and $NaNO_3$ was the optimum nitrogen source, the highest spore germination rate was obtained when the spores were in drips cornmeal, peptone, glucose and sucrose could promote spore germination.

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