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Research Article

Host extracts, sugars and amino acids concentrations enhanced growth of *Macrophomina phaseolina*

M. Surya Prakash Reddy, M. Santha Lakshmi Prasad, Ashwini Kumar, K. T. Ramya, J. Jawaharlal

Abstract

Macrophomina phaseolina is a soil inhabitant and it is pathogenic to a wide range of host crops. In the present investigation, *M. phaseolina* was grown in an enriched medium using semi mature stage of plant parts i.e. capsule/pod/head, leaf, stem, root *Viz*., castor, safflower, mustard, sunflower, groundnut, soybean, sesame crop parts were used at different concentrations. Furthermore, various carbon and amino acid sources were assessed at different concentrations to investigate their impact on the fungus growth under controlled in-vitro conditions. Results showed oil seed crop extracts significantly enhanced mycelial growth was recorded at all concentrations except castor stem extract which recorded the lowest mycelial growth over control, therefore carbon sources @ 250 ppm and 500 ppm of galactose, mannose, inositol, glucose, fructose, dextrose, and mannitol significantly influenced mycelial growth of *M. phaseolina*. All eight carbon sources have shown almost similar trends of mycelial development and amino acid sources @ 2500 ppm, 5000 ppm, 7500, and 10000 ppm showed maximum mycelial growth was recorded at 2500 ppm, 5000 ppm, 7500 ppm as increasing concentration reduced growth of mycelium however L-Lysine monohydrate recorded lowest mycelial growth at all concentrations.

Keywords amino acid sources, carbon, *Macrophomina phaseolina*, oilseed crops

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Introduction

Sesame is known as the Queen of oilseeds and the oldest edible oilseeds crop, grown in India. It is commonly called gingelly and til belonging to the family Pedaliaceae which consists of many species and genera but *Sesamum indicum* has 2n=26 chromosomes and is well known as cultivated species. A good amount of antioxidants like seasmin, seasminol, sesamolin in sesame seeds increase their medicinal value [1]. It is an abundant source of edible oil (50%), oleic acid (47%), linolenic acid (39%), proteins (20%), and also rich in vitamins A, B, and E, minerals like Calcium, Phosphorus, Iron, Copper, magnesium, Zinc and Potassium [2-3]. *Macrophomina phaseolina* (Tassi.) Goid. and its sclerotial phase is named as *Rhizoctonia bataticola* [4]. *Macrophomina* is a single genus and species known as phaseolina [5]. The fungus involves two parasitic phases, one is saprophytic called as *Rhizoctonia bataticola* produces microsclerotia

and the pathogenic phases involve *Macrophomina phaseolina* mostly produces pycnidia, which moreover absent in sexual stages called mycelia sterlia. The *M. phaseolina* absorbs nutrition from crop root exudates like carbon source, and amino acid and it acts as the saprophytic phase in the degradation of crop tissue then host root appear white mycelial mat and also tissue black dot-like appearance due to microsclerotia it having high melanin content formed by high temperature varies of 28-35 °C to low moisture level [6-8] and degradation of host tissues involved toxins viz; asperlin, phomalactone, phaseolinic acid, phaseolinone, and botryodiplodin depends upon particular crop and environmental conditions it varies Pathogenicity severity and crop nutritional value [9-12,]. Due to the involvement of two stages of the pathogenic and saprophytic stage, the fungus infects a broad spectrum of crops [14]. *M. phaseolina* is a soil-borne inhabiting fungus; it poses a greater problem in managing the disease. In India, the disease was distributed in all sesame growing areas. *M. phaseolina* disease incidence was recorded from Madhya Pradesh [10] Bihar [5] and Madras [13-14] growing sesame fields and also recorded about root rot incidence in fields varied from 6.0 to 71.5 in Delhi, Haryana, Uttar Pradesh, Karnataka, and Tamil Nadu for percent varies depending on the date of sowings and soil conditions. In the present investigation, *M. phaseolina* was tested in oilseed extracts, synthetic sugars as well as amino acids growth of colony diameter under In vitro conditions.

Methodology

The present experiment findings at the ICAR-Indian Institute of Oilseeds Research (IIOR), Rajendranagar, Hyderabad, Telangana, and Department of Plant Pathology, JNKVV, Jabalpur during 2018-2021 under laboratory conditions and statistical design applied Two factorial RBD employed through OPSTAT for statistical analysis.

Crop extracts (Oilseeds crop)

The semi-mature stage of crop extracts i.e. capsule/pod/head, leaf, stem, root of different oilseeds crops *Viz*., castor, safflower, mustard, sunflower, groundnut, soybean, sesame at 50, 200, 300, 400, 500 grams per liter (Table 1). Plant parts were collected cut into pieces and boiled in distilled water and the extracts received were mixed with dextrose agar for further studies. Plant extract dextrose Agar (PEDA) and PDA served as control (without plant extracts) plates were incubated at 25 ± 10C for replicated thrice. The mycelial growth was measured radial (mm) at different intervals 48 hours to 72 hours after incubation [19].

Table 1. List of Plant Extracts used in vitro

Crops Extracts	Parts used	Quantity used (g/lit)
Castor, Safflower	Capsule	50g/L
Mustard, Sun	Leaf	200g/L
Flower, Soybean	Stem	300g/L
Sesame	Root	400g/L
Ground nut	Leaf and Root	500g/L

Carbon sources

This experiment was conducted to find out the source of carbon that can be most efficiently utilized by the fungus for its growth. The culture of *M. phaseolina* was used from infected root and stem rot. The eight different carbon sources *viz* sucrose, dextrose, fructose, galactose, mannitol, mannose, inositol, glucose at @ 250 ppm and 500 ppm were incorporated into modified elad chat solid media composed with chemical composition with peptone-8.0, KH_2PO_4-2, $MgSO_4$-1, $Feso_4$-250 ppm,

rose Bengal-0.5; chloramphenicol-0.1; agar-agar-20. The media without a carbon source was considered as control and plates were incubated at room temperature (28+2 °C) for 72 hours after incubating the plates with *M. phaseolina*.

Amino acids source

The source of amino acids that can be most efficiently utilized by the fungus for its growth. The thirteen different amino acids source *viz*. Lysine monohydrate, Phenylalanine, Valine, Glutamic Acid, Leucine, Asparagine, Methionine, Arginine, Serine, Threonine, Proline, Tryptophan, Glycine at @ 2500, 5000, 7500 and 10000 ppm were incorporated with modified elad chat medium.

Results and Discussion

Influence of Oilseed crop extracts on the growth of M. phaseolina

The evaluation of seven crop extracts and their parts *viz*; root, stem, leaf, capsule or pod were amended @ 50g/L, 200g/L, 300g/L, 400g/L, and 500g/L with Dextrose Agar (DA) and observations were recorded at 48 hrs. and 72 hours after incubation. Data is presented in Figure 1.

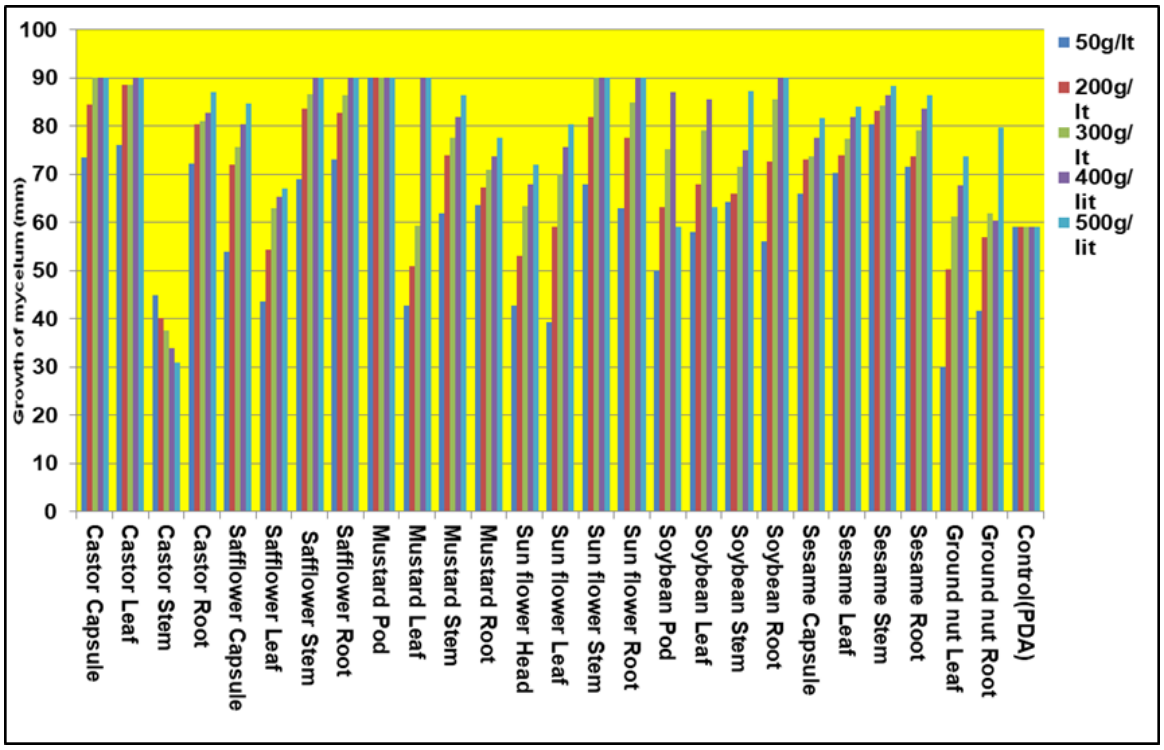

Figure 1. Influence of oilseed crop extracts on the growth of *M. phaseolina* at 72 hrs.

50 gram/L

Data presented in Table 2 and Figure 1, depicted that the crop extracts @50gm/lit amended with dextrose agar which influenced colony diameter, maximum was recorded on pod of mustard (90 mm) followed by Stem of sesame (80.33 mm), leaf of castor (76.16 mm) and lowest colony diameter was recorded with leaf of groundnut (30 mm), leaf of sunflower (39.33 mm) and root of groundnut (41.66 mm) after 72 hours. The remaining crop extracts have also shown significant growth of colony diameter. The crop extracts have been shown to significantly influence the growth of mycelium from 48 hrs. to 72 hrs. intervals when compared with control-PDA (59 mm).

Table 2. Influence of oilseed crop extracts on the growth of *M. phaseolina* @ 50g/L

Plant parts (50g/L)	Treatments	Parts	Radial growth (mm) after hrs.	
			48 hours	72 hours
Castor	T1	Capsule	*40.66	73.5
	T2	Leaf	52.83	76.16
	T3	Stem	23.00	45.00
	T4	Root	35.66	72.33
Safflower	T5	Capsule	33.33	54.00
	T6	Leaf	23.00	43.66
	T7	Stem	45.33	69.00
	T8	Root	40.00	73.00
Mustard	T9	Pod	63.66	90.00
	T10	Leaf	27.66	42.66
	T11	Stem	41.33	62.00
	T12	Root	39.66	63.66
Sunflower	T13	Head	31.00	42.66
	T14	Leaf	31.66	39.33
	T15	Stem	51.00	68.00
	T16	Root	42.00	63.00
Soybean	T17	Pod	30.66	50.00
	T18	Leaf	42.33	58.00
	T19	Stem	48.33	64.31
	T20	Root	48.33	56.00
Sesame	T21	Capsule	33.00	66.00
	T22	Leaf	31.33	70.33
	T23	Stem	43.66	80.33
	T24	Root	32.50	71.66
Groundnut	T25	Leaf	22.00	30.00
	T26	Root	30.66	41.66
PDA	T27	Control	35.66	59.00
Crop extracts (A)(P< 0.05)	**C.D.3.81**	**SE(m)1.35**		
Hours (B) (P< 0.05)	**C.D.1.03**	**SE(m)0.36**		
Crop extracts X Hours(A X B)	**C.D.5.38**	**SE(m)1.91**		

*Mean of three replication

200 gram/L

Data presented in Table 3 and Figure 1, revealed that crop extracts @ 200gm/lit amended with dextrose agar significantly influenced colony diameter. A maximum was recorded on the pod of mustard (90 mm) followed by a leaf of castor (88.50 mm), a capsule of castor (84.50 mm), and the lowest colony diameter was recorded on the stem of castor (40.20 mm) and leaf of groundnut (50.30 mm) after 72 hours. The rest of the crop extracts also have shown a significant effect on growth colony diameter. Most of the crop extracts have shown significant growth of mycelium from 48 hrs. to 72 hrs. intervals as compared to control-PDA (59 mm).

300gram/L

Data presented in Table 4 and Figure 1, revealed that the crop extracts @ 300gm/L amended with dextrose agar recorded maximum mycelial growth with a capsule of castor (90mm), a pod of mustard (90 mm), the stem of sunflower (90 mm) and the leaf of castor (88.66 mm) followed by the root of safflower (86.33 mm). The remaining crop extracts have also shown significant growth in colony diameter. The crop extracts have shown a significant effect on the growth of mycelium from 48 hrs. to 72 hrs. intervals as compared to control-PDA (59 mm).

Table 3. Influence of oilseed crop extracts on the growth of *M. phaseolina* @ 200g/L

Plant parts (200g/L)	Treatments	Parts	Radial growth (mm) after hrs.	
			48 hours	72 hours
Castor	T1	Capsule	*44.00	84.50
	T2	Leaf	60.33	88.50
	T3	Stem	27.33	40.20
	T4	Root	40.66	80.30
Safflower	T5	Capsule	42.00	72.00
	T6	Leaf	32.66	54.30
	T7	Stem	56.00	83.70
	T8	Root	53.00	82.70
Mustard	T9	Pod	67.66	90.00
	T10	Leaf	39.33	51.00
	T11	Stem	50.33	74.00
	T12	Root	43.00	67.30
Sunflower	T13	Head	36.50	53.00
	T14	Leaf	45.66	59.00
	T15	Stem	65.66	82.00
	T16	Root	54.66	77.70
Soybean	T17	Pod	41.33	63.30
	T18	Leaf	45.00	68.00
	T19	Stem	46.33	66.00
	T20	Root	52.00	72.70
Sesame	T21	Capsule	38.66	73.00
	T22	Leaf	35.00	74.00
	T23	Stem	47.00	83.30
	T24	Root	37.33	73.70
Groundnut	T25	Leaf	36.66	50.30
	T26	Root	39.00	57.00
PDA	T27	Control	35.66	59.00
Crop extracts (A)(P< 0.05)	**C.D.3.19**	**SE(m)1.13**		
Hours (B) (P< 0.05)	**C.D.0.86**	**SE(m)0.3**		
Crop extracts X Hours(A X B)	**C.D.4.51**	**SE(m)1.6**		

*Mean of three replication

Table 4. Influence of oilseed crop extracts on the growth of *M. phaseolina* @ 300g/L

Plant parts (300g/L)	Treatments	Parts	Radial growth (mm) after hrs.	
			48 hours	72 hours
Castor	T1	Capsule	*63.66	90.00
	T2	Leaf	64.33	88.66
	T3	Stem	28.33	37.66
	T4	Root	49.33	81.00
Safflower	T5	Capsule	45.66	75.66
	T6	Leaf	37.66	63.00
	T7	Stem	57.00	86.66
	T8	Root	62.66	86.33

Continued Table 4.

Mustard	T9	Pod	70.33	90.00
	T10	Leaf	49.33	59.33
	T11	Stem	53.66	77.66
	T12	Root	46.33	70.83
Sunflower	T13	Head	49.66	63.33
	T14	Leaf	60.33	70.00
	T15	Stem	71.00	90.00
	T16	Root	65.33	85.00
Soybean	T17	Pod	46.33	75.33
	T18	Leaf	54.33	79.00
	T19	Stem	50.66	71.66
	T20	Root	59.00	85.66
Sesame	T21	Capsule	41.66	73.66
	T22	Leaf	37.33	77.33
	T23	Stem	51.66	84.33
	T24	Root	41.33	79.00
Groundnut	T25	Leaf	41.50	61.33
	T26	Root	47.00	62.00
PDA	T27	Control	35.66	59.00
Crop extracts (A)(P< 0.05)	**C.D.3.13**	**SE(m)1.11**		
Hours (B) (P< 0.05)	**C.D.0.85**	**SE(m)0.5**		
Crop extracts X Hours	**C.D.4.43**	**SE(m)1.57**		

*Mean of three replication

400gm/L

Data presented in Table 5 and Figure 1, revealed that crop extracts @ 400gm/L amended with dextrose agar significantly influenced maximum mycelial growth with capsule of castor(90 mm), pod of mustard (90 mm), leaf of castor (90 mm), leaf of mustard (90 mm), stem of safflower (90 mm), stem of sunflower (90 mm), root of safflower (90 mm), root of sunflower (90 mm), root of soybean (90 mm) followed by pod of soybean (87 mm) and stem of sesame (86.33 mm). The remaining crop extracts also have shown significant growth colony diameter. The crop extracts have shown significant growth of mycelium from 48 hrs. to 72 hrs. intervals as compared to control PDA (59 mm).

500 grams/L

Data indicated (Table 6 and Figure 1) that the crop extracts @ 500gm/L amended with dextrose agar influenced the mycelial growth of *M. phaseolina*. Maximum mycelial growth was recorded on capsule of castor (90 mm), pod of mustard (90 mm), leaf of castor (90 mm), leaf of mustard (90 mm), stem of safflower(90 mm), stem of sunflower(90 mm), root of safflower(90 mm), root of sunflower(90 mm), root of soybean (90 mm) followed stem of sesame (88.33 mm), stem of soybean (87.33 mm), root of castor(87 mm). The lowest mycelial growth was recorded on the stem of the castor (31 mm). The rest of the crop extracts have also shown significant colony diameter. The crop extracts have shown significant growth of mycelium from 48 hrs. to 72 hrs. intervals as compared to control-PDA (59 mm). Pandhare et al., [20] evaluated eight culture media that Sorghum stem extract agar and Sorghum root extract agar on morphological and cultural characters of *M. phaseolina*. Sayyad et al., [21] studied host leaf extract agar on cultural characters of *Macrophomina phaseolina*, and reported also maximum mycelial growth. Suryawanshi et al., [22] evaluated that sunflower root extract agar and sunflower stem extract agar showed cultural and morphological characters of *M. phaseolina*. Osman et al., [23] found that the host extracts namely tomato, aubergine, wheat flour, dukhun, and pigeon pea on *Aspergillus niger*, *Curvularia lunata*, *Aspergillus flavus*, and *Fusarium oxyaporum* exhibited very

good mycelial growth.

Table 5. Influence of oilseed crop extracts on the growth of *M. phaseolina* @ 400g/L

Plant parts (400g/L)	Treatments	Parts	Radial growth (mm) after hrs	
			48 hours	72 hours
Castor	T1	Capsule	*67.33	90.00
	T2	Leaf	74.00	90.00
	T3	Stem	20.00	34.00
	T4	Root	54.00	82.66
Safflower	T5	Capsule	47.00	80.33
	T6	Leaf	40.00	65.33
	T7	Stem	65.66	90.00
	T8	Root	68.00	90.00
Mustard	T9	Pod	71.00	90.00
	T10	Leaf	64.33	90.00
	T11	Stem	62.00	82.00
	T12	Root	52.00	73.66
Sunflower	T13	Head	51.66	68.00
	T14	Leaf	63.00	75.66
	T15	Stem	75.66	90.00
	T16	Root	70.66	90.00
Soybean	T17	Pod	57.66	87.00
	T18	Leaf	60.66	85.66
	T19	Stem	51.33	75.00
	T20	Root	62.33	90.00
Sesame	T21	Capsule	44.66	77.66
	T22	Leaf	41.66	82.00
	T23	Stem	56.66	86.33
	T24	Root	48.00	83.66
Groundnut	T25	Leaf	44.00	67.66
	T26	Root	55.00	60.33
PDA	T27	Control	35.66	59.00
Crop extracts (A)(P< 0.05)	**C.D.2.37**	**SE(m)0.84**		
Hours (B) (P< 0.05)	**C.D.0.64**	**SE(m)0.23**		
Crop extracts X Hours	**C.D.3.35**	**SE(m)1.19**		

*Mean of three replication

Table 6. Influence of oilseed crop extracts on the growth of *M. phaseolina* @ 500g/L

Plant parts (500g/L)	Treatments	Parts	Radial growth (mm) after hrs.	
			48 hours	72 hours
Castor	T1	Capsule	*71.33	90.00
	T2	Leaf	75.66	90.00
	T3	Stem	20.33	31.00
	T4	Root	64.66	87.00
Safflower	T5	Capsule	52.33	84.66
	T6	Leaf	43.66	67.00
	T7	Stem	72.33	90.00
	T8	Root	72.33	90.00
Mustard	T9	Pod	73.00	90.00
	T10	Leaf	71.16	90.00
	T11	Stem	68.00	86.33
	T12	Root	60.33	77.66

Continued Table 6.

Sunflower	T13	Head	55.00	72.00
	T14	Leaf	69.00	80.33
	T15	Stem	77.00	90.00
	T16	Root	72.00	90.00
Soybean	T17	Pod	43.33	59.00
	T18	Leaf	40.83	63.13
	T19	Stem	62.66	87.33
	T20	Root	66.00	90.00
Sesame	T21	Capsule	54.33	81.66
	T22	Leaf	44.66	84.00
	T23	Stem	61.33	88.33
	T24	Root	55.66	86.33
Groundnut	T25	Leaf	46.33	73.66
	T26	Root	56.33	79.66
PDA	T27	Control	35.66	59.00
Crop extracts (A)(P< 0.05)	**C.D.2.67**	**SE(m)0.95**		
Hours (B) (P< 0.05)	**C.D.0.72**	**SE(m)0.25**		
Crop extracts X Hours	**C.D.3.77**	**SE(m)1.34**		

*Mean of three replication

Influence of various carbon sources on radial growth of M. phaseolina

The data on different carbon sources @ 250 and 500 ppm amended with modified elad chat medium influenced radial growth of *M. phaseolina* at 72 hours is presented in Table 7 and Figure 2. Carbon sources @ 250 ppm amended in modified Elad chat medium significantly influenced the mycelial growth of *M. phaseolina* varied from 76.83 mm to 90 mm. Maximum mycelial growth (90 mm) was recorded on galactose, mannose, inositol, and glucose followed by Fructose (84.50 mm) and mannitol (84.00 mm). Minimum mycelial growth of *M. phaseolina* recorded on control (62.67 mm). All eight carbon sources was significantly influenced the mycelial growth of *M. phaseolina* after 72 hours of inoculation. Channakeshava and Pankaja [24], reported that glucose recorded maximum dry mycelial weight and was followed by sucrose. Minimum dry mycelial weight was recorded in lactose. Gaikwad et al., [25] studied carbon sources of dextrose, sucrose, and cellulose for growth and sclerotial production of *Rhizoctonia bataticola*.

Table 7. Influence of various carbon sources (sugars) on radial growth of *M. phaseolina*

Carbon source	**Radial growth (mm)**	
	250 ppm	**500 ppm**
T1Sucrose	*76.83	89.67
T2Dextrose	81.50	90.00
T3Fructose	84.50	90.00
T4Galactose	90.00	90.00
T5Manitol	84.00	90.00
T6Mannose	90.00	90.00
T7Inositol	90.00	90.00
T8Glucose	87.33	90.00
T9Control	62.67	62.50
Carbon source (A)(P< 0.05)	**CD. 1.58**	**SE(m) 0.54**
Ppm (B)(P< 0.05)	**CD. 0.74**	**SE(m) 0.25**
AXB(P< 0.05)	**CD. 2.23**	**SE(m) 0.77**

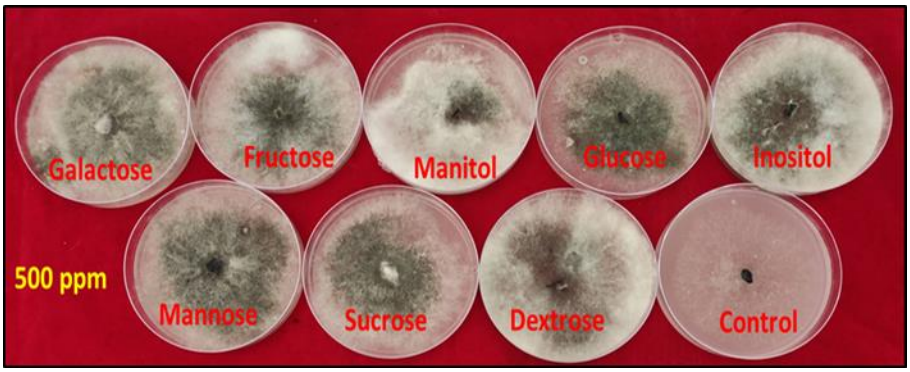

Figure 2. Influence of sources of carbon on radial growth of *M. phaseolina* at 250 and 500 ppm at 72 hrs.

Influence of amino acid sources on radial growth of M. phaseolina

Thirteen amino acids at four concentrations @ 2500 ppm, 5000 ppm, 7500 ppm, 10000 ppm amended with elad chat medium significantly influenced the growth of *M. phaseolina* at 72 hours incubation and the data is presented in Table 8 and Figure 3. Influenced growth of the mycelium of test fungus ranged from 57.33 to 90 mm @ 2500 ppm concentration. Highest mycelial growth was recorded on Asparagine (90 mm), Methione (90 mm), Threonine (90 mm), Proline (90 mm) and Tryptophan (90 mm) followed by Serine-(89.66 mm), Phenylalanine (89.33 mm), Valine and Glutamic Acid (88 mm), L-Arginine (86.66 mm) and L-Leucine (85.33 mm) when compared to control (57.33 mm). Mostafa and Mohamed [26] reported that glutamine, glycine, and tryptophan recorded the maximum mycelial dry weight of *Fusarium spp*. Upamanyu and Gupta [27], observed that Methionine and Glycine were responsible for the maximum growth of *Rhizoctonia solani*.

Table 8. Influence of different sources of amino acids on the radial growth of *M. phaseolina*

Amin Acids	**Radial growth (mm)**			
	2500 ppm	**5000 ppm**	**7500 ppm**	**10000 ppm**
L-Lysine monohydrate-T1	*77.66	40.67	23.33	20.00
L-Phenylalanine-T2	89.33	89.33	84.67	86.67
L-Valine-T3	88.00	88.67	89.33	82.33
L-Glutamic Acid-T4	88.00	76.00	74.33	72.67
L-Leucine-T5	85.33	86.00	87.00	89.33
L-Asparagine-T6	90.00	90.00	90.00	83.67
L-Methione-T7	90.00	86.33	83.67	75.00
L-Arginine-T8	86.66	76.00	66.00	38.33
L-Serine-T9	89.66	90.00	90.00	83.00
L-Threonine-T10	90.00	90.00	87.67	84.00
L-Proline –T11	90.00	90.00	87.67	74.00
L-Tryptophan-T12	90.00	90.00	90.00	87.00
Glycine-T13	90.00	89.00	89.33	90.00
Control-T14	57.33	57.33	57.33	57.33
Mean-B	**85.85**	**81.38**	**78.60**	**73.10**
Amino acid (A)(p<0.05)	**CD. 0.93**	**SE(m) 0.33**		
Hours (B) (p<0.05)	**CD. 0.49**	**SE(m) 0.17**		
Amino acid X hours (p<0.05)	**CD. 1.85**	**SE(m) 0.66**		

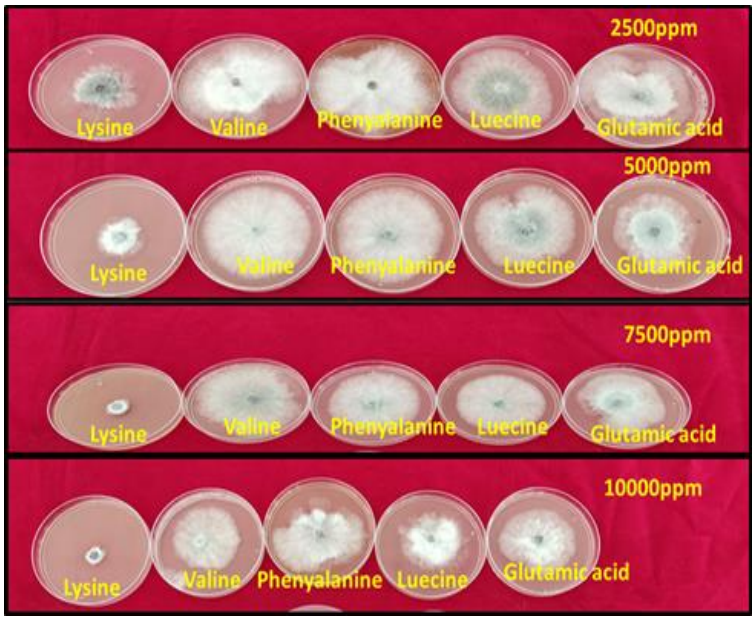

Figure 3. Influence of different sources of amino acids on the radial growth *M. phaseolina*

Conclusion

In the present research, results have been recorded regarding the impact of oilseed crop extracts, carbon sources, and amino acid sources on enhancing the mycelial growth of *M. phaseolina*. Natural host extracts will be utilized in the detection of microsclerotia, as well as in molecular and biochemical studies, in comparison to conventional Potato Dextrose Agar (PDA) and other synthetic media for future research directions. It's worth noting that the concentration of carbon and amino acids released by host plant roots can significantly influence the mycelial growth of *M. phaseolina* under field conditions; however, the specific concentrations that enhance growth under in-vitro conditions should be determined through experimental testing.

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